Handbook of Dietary Phytochemicals

Urvashi Swami

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ABA	Abscisic Acid
ACSO	S-Allyl-L-Cysteine Sulfoxide
AGEs	Advanced Glycation End-Products
AIDS	Acquired Immune Deficiency Syndrome
AMPK	Activated Protein Kinase
ARVs	Antiretroviral Drugs
ASCO	American Society of Clinical Oncology
BITC	Benzyl Isothiocyanate
C (PKC)	Protein Kinase
CE	Capillary Electrophoresis
CHLN	Chlorophyllin
COX	Cyclo-Oxygenase
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
DADS	Diallyl Disulfide
DAS	Diallyl Sulfide
DATS	Diallyl Trisulfide
DM	Diabetes Mellitus
DMF	Dimethylformamide
EBV-DP	Epstein–Barr Virus DNA Polymerase
EGC	Epigallocatechin
EGCG	Epigallocatechin Gallate
EO	Essential Oils
FRAP	Ferric Reducing Antioxidant Power
GC	Gas Chromatography
GLS	Glucosinolates
H_2O_2	Hydrogen Peroxide

HBV	Hepatitis B Virus
HCA	Heterocyclic Amines
HCIO	Hypochlorous Acid
HCN	Hydrogen Cyanide
HDL	High Density Lipoprotein
HDL-C	High Density Lipoprotein Cholesterol
HIV	Human Immunodeficiency Virus
HPFS	Health Professionals Follow-Up Study
HPLC	High-Resolution Liquid Chromatography
HSV	Herpes Simplex Virus
HTN	Hypertension
HTS	High Throughput Screening
IFN-γ	Interferon Gamma
IR	Infrared
IRS	Infrared Spectroscopy
ITCs	Isothiocyanates
ITP	Isotachophoresis
LDL	Low-Density Lipoprotein
LOL II	Lathyrus Ochrusisolectin II
LOX	Lipoxygenase
MAE	Microwave-Assisted Extraction
MAPK	Mitogen Activated Protein Kinase
MBC	Minimum Bactericidal Concentration
MCSO	S-Methyl-L-Cysteine Sulfoxide
ME	Metabolizable Energy
MEP	Molecular Electrostatic Potential
MFC	Minimum Fungicidal Concentration
MIC	Minimum Inhibitory Concentration
MMP	Metalloproteinase
MO	Methylene Oxindole
MS	Mass Spectroscopy
NDGA	Nordihydroguaiaretic Acid
NF	Nuclear Fragmentation
NHPs	Natural Health Products

NIR	Near Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide
NPQ	Nonphotochemical Quenching
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
NSP	Non-Starch Polysaccharides
OSI	Oxidative Stress and Inflammation
PCSO	S-Propyl-L-Cysteine Sulfoxide
PDGF	Platelet-Derived Growth Factor
PE	Plant Extracts
PEITC	Phenethyl Isothiocyanate
PHITC	Phenyl Hexyl Isothiocyanate
PHT	Phenylheptatriyne
PI3	Para-Influenza Virus Type 3
PIN	Prostate Intraepithelial Neoplasia
PLWBC	People Living With And Beyond Cancer
PPARγ	Peroxisome Proliferator-Activated Receptor-Γ
PSA	Polar Surface Area
RA	Rheumatoid Arthritis
RDA	Recommended Dietary Allowance
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RSV	Respiratory Syncytial Virus
SAMC	S-Allylmercaptocysteine
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	Severe Acute Respiratory Syndrome-Associated Coronavirus
SH	Sulfhydryl
SOD	Superoxide Dismutase
SRUC	Scotland's Rural College
TG	Triglycerides
TLC	Thin-Layer Chromatography
TLE	Thin-Layer Electrophoresis
TNF	Tumor Necrosis Factor

TPC	Total Phenolic Content
TPCSO	S-Trans-1-Propenyl-L-Cysteine Sulfoxide
TRAMP	Transgenic Adenocarcinoma of Mouse Prostate
UFH	University of Fort Hare
u-LC	Capillary liquid Chromatography
u-PA	Urokinase Plasminogen Activator
UV	Ultra Violet
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor
VZV	Varicella-Zoster Virus

GLOSSARY

A

Alkaloids – are a class of basic, naturally occurring organic compounds that contain at least one nitrogen atom

Alzheimer – is a progressive neurologic disorder that causes the brain to shrink (atrophy) and brain cells to die

Anethole – is an organic compound that is widely used as a flavoring substance

Anthocyanins – are water-soluble vacuolar pigments that, depending on their pH, may appear red, purple, blue or black

Anti – Aging – (of a product or technique) designed to prevent the appearance of getting older

Anticarcinogen – is a substance that counteracts the effects of a carcinogen or inhibits the development of cancer

Antidepressant – are medications used to treat major depressive disorder, some anxiety disorders, some chronic pain conditions, and to help manage some addictions

Antihypertensive – are a class of drugs that are used to treat hypertension

Anti-Inflammatory – is the property of a substance or treatment that reduces inflammation or swelling

Antimicrobial – is an agent that kills microorganisms or stops their growth

Antioxidants – are compounds that inhibit oxidation, a chemical reaction that can produce free radicals and chain reactions that may damage the cells of organisms

Antiparasitic – are a class of medications that are indicated for the treatment of parasitic diseases, such as those caused by helminths, amoeba, ectoparasites, parasitic fungi, and protozoa, among others

Antiplatelet – are medicines that stop cells in the blood (platelets) from sticking together and forming a clot. A blood clot can lead to a heart attack or stroke

Asymptomatic – (of a condition or a person) producing or showing no symptoms

Autotrophic – an autotroph or primary producer is an organism that has complex organic compounds using carbon from simple substances such as carbon dioxide, generally using energy from light or inorganic chemical reactions

B

Berberine – is a quaternary ammonium salt from the protoberberine group of benzylisoquinoline alkaloids found in such plants as Berberis, such as *Berberis vulgaris, Berberis aristata,* and *Mahonia aquifolium*

Biosynthesis – is a multi-step, enzyme-catalyzed process where substrates are converted into more complex products in living organisms

C

Calicheamicin – calicheamicins are a class of enediyne antitumor antibiotics derived from the bacterium *Micromonospora echinospora*, with calicheamicin γ1 being the

most notable
Carcinogenic Process – also called oncogenesis or tumorigenesis, is the formation of a cancer, whereby normal cells are transformed into cancer cells
Cardioprotective – Cardioprotective drugs are important in the treatment of patients at risk for or with documented cardiovascular disease (CVD)
Cardiovascular disease – is a class of diseases that involve the heart or blood vessels
Carotenoids – also called tetraterpenoids, are yellow, orange, and red organic pigments that are produced by plants and algae, as well as several bacteria, and fungi
Cellulose – is a molecule, consisting of hundreds – and sometimes even thousands – of carbon, hydrogen and oxygen atoms
Cervix – is the lower portion of the uterus, an organ of the female reproductive tract
Chemoprevention – refers to the administration of a medication to prevent disease or infection
Chlorophyllin – is a chemical that is made from chlorophyll. It is sometimes used as medicine
Chromatography – is a laboratory technique for the separation of a mixture
Chronic Diseases – is a human health condition or disease that is persistent or otherwise long-lasting in its effects or a disease that comes with time
Curcumin – is a bright yellow chemical produced by plants of the Curcuma longa species
Cyclic Compounds – is a term for a compound in the field of chemistry in which one or more series of atoms in the compound is connected to form a ring
cytotoxic – toxic to living cells
D
Diabetes – is a disease that occurs when your blood glucose, also called blood sugar, is too high
Diastole – is the part of the cardiac cycle during which the heart refills with blood after the emptying done during systole
E
Ebola – also known as Ebola virus disease (EVD) or Ebola hemorrhagic fever (EHF), is a viral hemorrhagic fever of humans and other primates caused by ebolaviruses
Electrophoresis – is a laboratory technique used to separate DNA, RNA, or protein molecules based on their size and electrical charge
Ellagitannins – are a diverse class of hydrolyzable tannins, a type of polyphenol formed primarily from the oxidative linkage of galloyl groups in 1,2,3,4,6-pentagalloyl glucose
Equisetopsida – is one of the four subclasses of Polypodiopsida, a group of vascular plants with a fossil record going back to the Devonian
F
Flavonoids – are a class of polyphenolic secondary metabolites found in plants and thus commonly consumed in diets
Flexitarian – a semi-vegetarian diet, also called a flexitarian, is one that is centered on plant foods with the occasional inclusion of meat

G

Gallo tannins – are formed by galloyl transfer from 1-O-galloylglucose to the galloyl residues of the central glucose leading to meta-depside bonds

H

Hemicelluloses – is one of several heteropolymers, such as arabinoxylans, present along with cellulose in almost all terrestrial plant cell walls

Hemiterpenoids – are the simplest among the terpenoids

Herpes – is a virus causing contagious sores, most often around the mouth or on the genitals

I

Insulin – is a peptide hormone produced by beta cells of the pancreatic islets; it is considered to be the main anabolic hormone of the body

Isoprenoids – any of a class of organic compounds composed of two or more units of hydrocarbons, with each unit consisting of five carbon atoms arranged in a specific pattern

K

Keratinocytes – are the primary type of cell found in the epidermis, the outermost layer of the skin

L

Limonoid – are phytochemicals of the triterpenoid class, which abundant in sweet or sour-scented citrus fruit and other plants of the families Cucurbitaceae, Rutaceae, and Meliaceae

Lymphocytes – are white blood cells that are also one of the body's main types of immune cells

M

Metabolites – are the intermediate products of metabolic reactions catalyzed by various enzymes that naturally occur within cells.

Microphylls – in plant anatomy and evolution, a microphyll is a type of plant leaf with one single, unbranched leaf vein

Morphine – is a pain medication of the opiate family that is found naturally in a dark brown, resinous form, from the poppy plant

Mycorrhiza – the term mycorrhiza refers to the role of the fungus in the plant's rhizosphere, its root system

Myosmine – is an alkaloid found in tobacco and other plants

N

Nicotine – is a chiral alkaloid that is naturally produced in the nightshade family of plants and is widely used recreationally as a stimulant and anxiolytic

Nutrigenomics – also known as nutrigenomics, is a science studying the relationship between human genome, human nutrition and health

Nutriproteomics – is a nascent research arena, exploiting the dynamics of proteomic tools to characterize molecular and cellular changes in protein expression and function on a global level as well as judging the interaction of proteins with food nutrients

O

Organosulfur – compounds are organic compounds that contain sulfur

P

Parasite – is an organism that lives on or in a host organism and gets its food from or at the expense of its host

Pectin – is a structural acidic heteropolysaccharide contained in the primary and middle lamella and cell walls of terrestrial plants

Pharmaceuticals – are substance used in the diagnosis, treatment, or prevention of disease and for restoring, correcting, or modifying organic functions

Pharmacologic – relating to the branch of medicine concerned with the uses, effects, and modes of action of drugs

Pharmacology – is the study of how a drug affects a biological system and how the body responds to the drug

Phenols – in organic chemistry, phenols, sometimes called phenolics, are a class of chemical compounds consisting of one or more hydroxyl groups bonded directly to an aromatic hydrocarbon group

Phenylpropanoids – are a diverse group of natural products composed of thousands of different compounds, synthesized from the primary metabolites, phenylalanine or tyrosine amino acids, through a series of enzymatic reactions

Phospholipids – also known as phosphatides, are a class of lipids whose molecule has a hydrophilic "head" containing a phosphate group and two hydrophobic "tails" derived from fatty acids, joined by a glycerol molecule

Physiology – is the branch of biology that aims to understand the mechanisms of living things, form the basis of cell function at the ionic and molecular level to the integrated behavior of the whole body and the influence of the external environment

Phytochemistry – is a peer-reviewed scientific journal covering pure and applied plant chemistry, plant biochemistry and molecular biology

Pigmentation – refers to the coloring of the skin

Pollination – is the transfer of pollen from a male part of a plant to a female part of a plant, later enabling fertilization and the production of seeds, most often by an animal or by wind

Pyridine – is a primary heterocyclic organic compound with the chemical formula C_5H_5N

Pyrrolidine – also known as tetrahydropyrrole, is an organic compound with the molecular formula $(CH_2)_4NH$

Q

Quercetin – is a plant pigment (flavonoid). It is found in many plants and foods, such as red wine, onions, green tea, apples, berries, Ginkgo biloba, St. John's wort, American elder, and others

Quinoline – is a heterocyclic aromatic organic compound with the chemical formula C_9H_7N

R

Resveratrol – is a stilbenoid, a type of natural phenol, and a phytoalexin produced by several plants in response to injury or when the plant is under attack by pathogens, such as bacteria or fungi

Retrovirus – is a type of virus that inserts a copy of its RNA genome into the DNA of a host cell that it invades, thus changing the genome of that cell

S

Saponins – also referred to selectively as triterpene glycosides, are bitter-tasting usually toxic plant-derived organic chemicals that have a foamy quality when agitated in water

Spectroscopy – is the study of the interaction between matter and electromagnetic radiation as a function of the wavelength or frequency of the radiation

T

Tannins – are a class of astringent, polyphenolic biomolecules that bind to and precipitate proteins and various other organic compounds, including amino acids and alkaloids

Terpenoids – also known as isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from the 5-carbon compound isoprene and the isoprene polymers called terpenes

Terpenoids – also known as isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from the 5-carbon compound isoprene, and the isoprene polymers called terpenes

Tetranortriterpenoid – is a lass of chemical compounds most noted for the chemical azadirachtin, extracted from the neem tree that displays insecticidal properties

PREFACE

This book takes the readers through the importance of phytochemicals, how it is important and implies in various aspects from curing diseases' to playing a major role while being a compound of plants or used as a harvest in medicinal plants. This book sheds light on the chemistry and classification of dietary phytochemicals, their pharmacology, its antimicrobial and antiviral properties.

The first chapter stresses the basic overview of phytochemicals, metabolism, and phytochemistry, defining the families and structures of phytochemicals to their vital role in the prevention of diseases and different food items. This chapter will also emphasize its presence in plants as compounds and the various methods used in bioactive compounds.

The second chapter takes the readers through the functions of phytochemicals in diseases, the use of allelochemicals as phytochemicals, the harvest of medicinal plants. This chapter will provide highlights on the defense mechanism against free radicals, the role of flavonoids on the health of humans, and the effects of dietary plant extracts.

Then, the third chapter explains the chemistry and classification of dietary phytochemicals, its classifications as phenolic compounds, tannins, alkaloids, saponin, lectins, etc. It also explains the complex carotenoids, their physiology, structure, and biochemistry, the chemical properties of phytochemicals. This chapter also sheds light on the significance of the physicochemical properties of dietary phytochemicals.

The fourth chapter introduces the readers to the pharmacology of phytochemicals and the potential use of phytochemicals like alkaloids in plants. This chapter also explains the importance of the prevention and treatment of antioxidant phytochemicals for chronic diseases. Its use in cancer and circulatory system diseases.

The fifth chapter throws light on the antimicrobial and antiviral properties of phytochemicals. This chapter marks the history of natural products as antiviral drugs, the classification of antiviral phytochemicals, plant antimicrobials, and the food industry and the different antimicrobial properties in *Clitoria ternatea*.

The sixth chapter takes the readers through the concept of dietary phytochemicals in the treatment of diseases like obesity, cancer, and diabetes, the cardiovascular diseases. The readers are then told about the various functions of dietary phytochemicals, the isothiocyanates, and the anti-cancer drugs, biosynthesis and antitumor activity and its mechanisms.

The last chapter of this book sheds light on the dietary phytochemicals in health and nutrition. This chapter also mentions the benefits of phytochemicals on health, their

various role, and it talks about food and dietary supplements, their functions in curing different diseases.

This book has been designed to suit the knowledge and pursuit of the researcher and scholars and to empower them with various aspects of phytochemicals so that they are updated with the information. I hope that the readers find the book explanatory and insightful and that this book is referred by scholars across various fields.

CHAPTER 1

INTRODUCTION TO PHYTOCHEMICALS

CONTENTS

1.1. INTRODUCTION

Having been derived mainly from plants, phytochemicals have biological activity. Plants are the primary source for obtaining varied active ingredients in the pharmaceutical industry. Pharmacological effects are exhibited by these substances that can be applied for the treatment of fungal and bacterial infections as well as chronic-degenerative diseases like cancer and diabetes.

New ways of obtaining it are the next step that science has to take. Two case studies are presented in this chapter, along with a discussion about the phytochemicals' main groups. Amongst the current secondary metabolites, a natural compound of the taxoid family, Taxol is one of the most important ones.

This is known for its efficacy in antitumor activity with respect to cancer located in lungs, breasts, and prostate, as well as Kaposi's sarcoma. Taxol, which has been extracted from plant species yet to be explored, and the production thereof by its endophytic fungi, are the subject matters of the case studies.

Plants have for the entire history of humanity shown their presence as a source of health. Through a practical manner, the healing properties exhibited by plants and knowledge related to the same have been transmitted.

At the same time, man has been interested in knowing the source from which the properties of plants have been derived. Many methodologies have been developed in this process of knowledge generation to understand the organic compounds that are responsible for the plant-based healing properties.

This has basically resulted in phytochemistry which is defined as science through which the compounds that are present in plants can be studied. Numerous techniques have been developed in this field for the elucidation of organic structures ranging from sophisticated techniques and preparing the samples of plant tissue.

Continual optimization is required of the ongoing process related to the search for new products that can be used in the agrochemical and pharmaceutical industries. Earlier, one commercial product was derived from the screening of about 10,000 natural products. This relationship has undergone a change with the advent of combinational chemistry.

At present, less than one commercial product is yielded in a year as a result of screening 10,000 structures from combinatorial chemistry along with the natural products that have been screened (F. Hansske, pers. comm.).

At the cost of about $350 M, its development takes around twelve years. An enormous amount of potential for new products is offered by fungi as out of 20 medications that are prescribed commonly, six have a fungal origin, and there is a description for only about five percent of the fungi.

A polyphyletic group of extremely diverse fungi that are mainly ascomycetous, namely endophytic fungi, are functionally defined with their presence within the plants' asymptomatic tissues. These are found in the tissues above ground of hornworts, lycophytes, ferns, liverworts, mosses, equisetopsida, and seed plants from the tropics to the arctic and from the tropical forests that are biologically diverse to the agricultural fields.

Over the last four decades, there has been a growing enthusiasm in the field of these fungi, which are not well known due to their ecological importance, cryptic lifestyle, and richness within solo plants along with the now established ecological importance that they have but which has been overlooked in the past.

A large amount of enthusiasm has been generated for understanding the diversity in endophytes as a result mainly of the discussion generated by David Hawksworth on the estimates of fungal diversity at a global level.

Fungal symbiosis with plants that comprise a range of interactions varying from antagonism to mutualism are determinants of nutrient cycling, ecosystem productivity, and biomass in terrestrial habitats from the equator to the poles.

The fruit bodies that are produced by the fungi in association with the hosts have helped to recognize and catalog most of the fungi that are plant-based (e.g., mycorrhizal fungi, plant pathogens). All the same, fungi penetrate and continue to stay inside healthy tissues that are aboveground like the leaves and form a cryptic symbiosis with plants in all major lineages, which includes mosses, confers, liverworts, seed-free vascular plants, and angiosperms.

An aspect of plant biology that is often overlooked is the foliar fungal endophytes (namely microphylls or endophylls) even they are fundamental: in the photosynthetic tissues, one or more endophytic symbionts are harbored in all the plant species that have been surveyed so far. With interactions at different levels, plants live associating with microorganisms.

Insights on plant microbiome are providing insights through this assumption where the microbiome is the collective genome of microorganisms that live in association with plants, and by considering the basic role performed by the fungal endophytes that are associated with them, there has

been the development of new concepts in the evolution of plants. Bioactive compounds are produced by endophytic fungi that may have a role in the protection of plants against pests and pathogens in the yet unexplored niche of chemo diversity. A large number of benefits may accrue to the host plants through colonization by endophytes which produce varied metabolites that promote vegetative or plant growth, protect against an attack by pathogens, induce resistance, show herbicide activity, and improve the yield of crops.

Anti-insect properties have known to be exhibited by numerous endophytes and currently in agriculture natural products based in fungi are used as active ingredients of varied bio formulates. The use of bio insecticides is showing a steady increase and these are quite fascinating compounds even though they form a small part of the market share.

Whilst searching for a paclitaxel producing bacterium or fungus, the microbial endophytes of yew tree *Taxus brevifolia* from Northwest Pacific was studied by researchers commencing 1991. The secondary metabolism of plant pathogenic bacteria and fungi had rich cataloguing in literature around that time but very few reports existed in which the chemistry of plant endophytes was described.

For centuries, plant pathogenic microorganism produced secondary metabolites have been studied as initiators of diseases in plants that are susceptible and as virulence factors. Three of the examples which caused severe blight disease of plants that are economically important are well known as the three different species of Cochliobolus producing host-specific toxin.

HC-toxin, which is host-specific is produced by *C. carbonum* (*Helminthosporium carbonum*) is the cause for the Northern leaf blight of maize and it inhibits histone deacetylase of maize. The Victorian race of oats was developed by plant breeders so that they could produce crown rust-resistant oats and victorin produced by *C. victoriae* caused a devastating epidemic in this race.

Under most circumstances, plant endophytes coexist with their hosts and rarely cause any problems being quite subtle in nature. A widely accepted and inclusive definition of endophytes was given by Braun and Hirsch wherein they stated that these are microbes that do not cause any overt negative effects that are immediate and they colonize internal, living plant tissues.

Secondary metabolites may be produced by them even though by nature they are usually nonpathogenic, which helps them to survive in the

competitive interstitial space of the plant world without causing any harm to the host. In most ecosystems the ecological niches of the microorganisms are defined by the ability they have to use only their chemical arsenal and membranes or cell walls to defend themselves whilst they control fellow microbes.

To date many important chemotherapeutics used have been provided by these chemical arsenals. Actinomycetes produce both the anticancer agent calicheamicin and the antibiotic streptomycin whilst griseofulvin, the potent antifungal agent has fungal origin.

Until the time the fungus producing Taxol was discovered in the needles and bark of the yew tree from the Northwest Pacific, little attention had been paid to plant endophytes. A review of the anticancer or cytotoxic compounds produced by plant endophytes was published in the year 2011.

From the endophytic fungi, more than a hundred compounds had been isolated that demonstrated anticancer activity or cytotoxicity, which included numerous compounds that had originally been isolated from plants that were higher.

From the coniferous species, isolation of less than ten percent of these compounds was done. The fungal endophytes of conifers have been shown in work to be strong produces of secondary metabolites that are bioactive.

There were two reasons behind the commencement of this research project, namely: reduction in tree cutting and application of biotechnology for endophytic microorganisms'-based production of taxanes and bringing about a reduction in a kind of cancer by the production of secondary metabolites for pharmaceutical application.

1.2. METABOLISM

Cells of living beings carry out a series of chemical reactions for the synthesis of simpler substances into complex ones or conversely for obtaining the simple compounds by degradation of the complex ones. There are two metabolisms in autotrophic organisms or plants, one that exists in all living beings and the primary one. The second allows them to produce and accumulate compounds of varied chemical nature, which is the secondary metabolism.

Common molecules in all cells have most of the nitrogen, carbon and energy that is essential for their own as well as their host organisms' functioning. Performing the same function and being present in all plants

these are nucleotides, lipids, amino acids and sugars and go by the name of primary metabolites.

A large variety of organic molecules are synthesized through the assimilation of a significant amount of energy and carbon allocated by the plants and these do not have a direct role to play in the respiratory process, protein synthesis or solute transport, photosynthesis, lipids or carbohydrates and these are known as secondary metabolites (also called natural products, by-products).

Superior plants have the characteristic of possessing secondary metabolites. Superior plants are characterized by the essential feature of flower and seed possession. They reproductive mechanism differs from the one of the inferior plants. Due to the visibility of their reproductive organs, they are also called spermatophytes and they are subdivided into angiosperms and gymnosperms.

Biological properties and their uses in different applications like medicines, herbicides, dyes or perfumes, insecticides, etc. characterize natural products. Usually, specific stages of development in plants and the stress periods result in the biosynthesis of secondary metabolites.

Sometimes, when plants interact with the environment (protection against pathogens, environment related stress or predators), important secondary metabolites are produced by certain plant cells which may also happen due to the plant's reproductive mechanism (for the purposes of pollination plants attract insects).

1.3. PHYTOCHEMISTRY

The main objective of the discipline of phytochemistry is the study of the plants' chemical constituents. The study of these kinds of compounds consists of metabolism (degradation and biosynthesis), their chemical structure, biological function, quantitative-qualitative evaluation, extraction and natural distribution.

There should be a sufficient preparation of the plant material prior to commencing any kind of a phytochemical analysis. Application of heat treatment for instance, in an oven where 60 degrees is the reference temperature until constant weight is reached by the sample is a practical and straightforward way of stabilization whereby it can be ensured that the condition for the compounds that are to be analyzed are optimal.

Several aspects are included in the phytochemical research of plants as enumerated below:

1. The compounds that are to be analyzed have to be isolated from the specimen or sample.
2. Their isolation or separation.
3. The isolated compounds are characterized or/and identified.
4. The biosynthetic routes of a specific molecule are investigated.
5. Quantitative assessment or determination.

Based on structural analogies between the solvent used for the purpose of extraction and the substance to be extracted, certain rules are followed by which through the use of solvents the organic compounds are extracted and purified.

When a solute's solubility is considered in a solvent that is given, another element taken into account is the compound polarity. Hence, highly polar or ionic solutes are dissolved by strongly polar solvents whereas ionic solutes cannot be dissolved by low-polar solvents which can dissolve solutes with low-polarity.

Water is the most polar solvent and until this is reached solvents are used consecutively from a low polarity and extraction of the vegetal material is done.

Through celite filtration is done by a vacuum pump followed by concentration under reduced pressure for clarifying the extracts that have been obtained. A rotary evaporator is usually used for this whereby until the volume reduces the solutions are concentrated at temperatures varying from 30 to 50°C. The concentrated extracts have to be stored under refrigeration.

Varied techniques have been developed for the isolation and identification in the process of natural product's separation and identification. A summary of the main techniques has been given in Table 1.1.

Table 1.1. Separation and Identification Techniques

Chromatography	Thin-layer Chromatography (TLC)
	Gas Chromatography (GC)
	High-resolution Liquid Chromatography (HPLC)
	Capillary liquid Chromatography (u-LC)

Electrophoresis	Thin-layer Electrophoresis (TLE)
	Isotachophoresis (ITP)
	Capillary Electrophoresis (CE)
Spectroscopic Techniques	Infrared Spectroscopy (IR)
	UV Spectroscopy
	Near-Infrared Spectroscopy (NIR)
	Nuclear magnetic resonance Spectroscopy (NMR)
	Mass Spectroscopy (MS)

1.4. FAMILIES OF PHYTOCHEMICALS

On the basis of specific characteristics such as their biosynthetic origin, the solubility properties and the structural characteristics that are common, these compounds are classified to establish some kind of order.

Certain large groups of secondary metabolites are sulfur and nitrogen compounds which in their structure have sulfur or/and nitrogen that is of diverse biosynthetic origin and solubility but has their main sources in amino acids.

Nitrogen compounds and cyanogenic glycosides are some examples of these compounds which degrade upon the plant being crushed releasing substances like hydrogen cyanide (HCN) which are toxic volatile substances even though by themselves, these compounds are not toxic. Amygdalin (in the figure shown below) is an example of this kind of compound and the same can be found in the seeds of apricot, almond, peach or cherry.

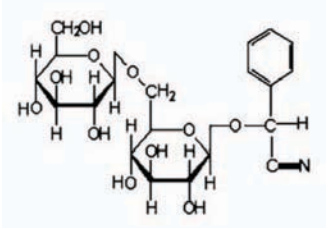

Figure 1.1. Structure of the amygdalin.

Alkaloids are other products that are naturally nitrogenated and they have a significant biological activity. Figure 4 shows some of the alkaloid examples. Three characteristics are common in the large family of alkaloids which consists of more than 15,000 secondary metabolites, namely: in their molecule, there is at least one nitrogen atom, they exhibit biological activity

and are soluble in water. Some of them are aliphatic or noncyclic nitrogen compounds like colchicine or mescaline, though they are heterocyclic.

1. In the chemical structure of the phenolic compounds one or more aromatic rings are attached with one hydroxyl group. Most of these are derived biosynthetically from shikimic acid and are water soluble.
2. The isoprene molecule is the structural unit in terpenoids which, depending upon the class of terpenoids being considered, is biosynthetically associated to the glyceraldehydes phosphate-pyruvic acid pathway or mevalonic acid pathway and is liposoluble.

The classification of the terpenoids is shown in Table 1.2. with regard to the isoprene units and their number contained by them along with an example of each kind of terpenoid. Some structures of terpenoids are shown in the Figure 1.2 mentioned below.

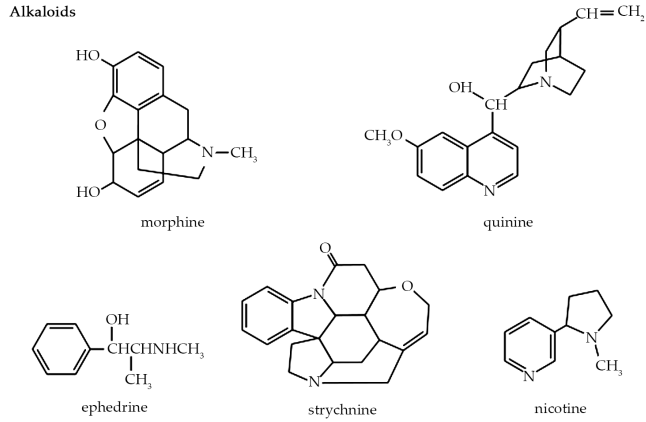

Figure 1.2. Alkaloid structures.

Table 1.2. Classification of Terpenoids

Isoprene units *n*	Carbon Atoms *n*	Name
1	5	Hemi-terpenes
2	10	Mono- terpenes

3	15	Sesqui- terpenes
4	20	Di- terpenes
6	30	Tri- terpenes
8	40	Tetra- terpenes
9 – 30,000	> 40	Poly- terpenes

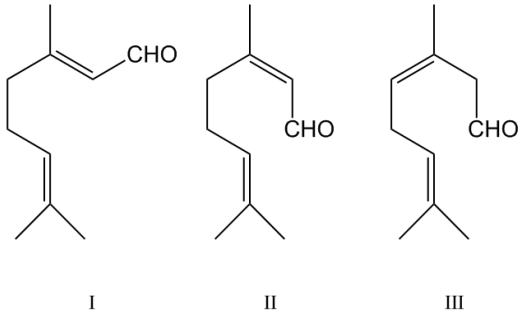

Figure 1.3. Examples of terpenoid structures.

1.5. PHYTOCHEMICALS AND DISEASE PREVENTION

A significant role in the prevention of human disease is played by phytochemicals, the plant derived non-nutritive chemical compounds. Antioxidants and secondary metabolites are examples of phytochemicals that possess important medicinal properties.

The role that phytochemicals play in the prevention of diseases along with their source shall be briefly covered in this chapter. The accumulation of bioactive chemicals in vegetables and fruits and certain stress conditions result in the production of phytochemicals in plants.

Under a stressed environment allelochemicals are produced the phytochemicals in the rhizosphere of the plant and its neighboring plants which has an immense amount of ecological role and this chapter discusses this as well. However, the role that phytochemicals play in preventing major diseases and providing a general description of these is the primary purpose of this chapter.

Figure 1.4. Phytochemicals and disease prevention.

1.5.1. Role of Phytochemicals in Disease Prevention in Human

Medicinal plants have a number of phytochemicals like tannins, phenols, carotenoids, flavonoids, alkaloids, steroids, saponins, etc. which possess activities for the prevention of a number of diseases. Important preventive activities are performed by these plant-derived chemical compounds, which are mainly anti-diabetic, anti-parasitic, antioxidant, anti-inflammatory, wound healing, anti-aging, antidepressant, antimicrobial and anticancer. They also play a significant role wherein many critical bioactive compounds in vegetables and plants are accumulated, and the plants can tolerate stress.

The most commonly found bioactive compounds in medicinal plants are flavonoids. These flavonoids have a number of activities that prevent human diseases like the capacity to heal wounds, antimicrobial, anti-inflammatory, anticancer and antioxidant. A number of vegetables and fruits have flavonoids that are anti-carcinogenic. Berries and apples have been known to have properties that are cardio-protective and this impacts the blood pressure in a positive way.

Cell vacuole have an abundance of the flavonoid- anthocyanins due to which pigmentation occurs in fruits, vegetables and flowers and these are by and large produced under environmental stress in plants. In the cell culture systems like liver, breasts, keratinocytes, colon and leukemic

cells anthocyanins have shown anti-oxidative activities *in vitro* studies. In vegetables and fruits the potential natural antioxidant showing its presence is the carotenoids. Carotenes and xanthophylls are included in this wherein they scavenge peroxyl radical. Berries and tomato commonly have lycopene whereas dark-green and yellow-orange leafy vegetables have the orange-colored carotenoids, the β-carotenes, in abundance.

1.5.2. Allelochemicals as Phytochemicals in the Plant Rhizosphere and Its Ecological Role

To protect itself from the various kinds of stresses due to the environment like drought, chemical pollution, disease infection and pest, submergence, UV exposure and other conditions that are unfavorable a number of phytochemicals are released by plants.

A number of bioactive compounds and secondary metabolites are produced by plants through this process, and these have potential anti-oxidative roles. Generally, in the natural ecosystem, a number of chemicals are released by the body of the plants into the environment, which helps to maintain their development and normal growth. All the same, under conditions of environmental stress, a series of allelochemicals/chemicals are produced by the plant. For the plants and the species in its neighborhood, both auto-toxic and hetero-toxic conditions are created by the allelochemicals that are released. Plants produce a number of allelochemicals under recycled hydroponics and replanting conditions due to which their development and growth get the s inhibited, and the same has been seen in beans, strawberry, a number of leafy vegetables, taro, ornamentals and lettuce. However, a significant role is played by these allelochemicals by way of which weeds, plant diseases, and pests can be controlled.

1.6. FOODS CONTAINING PHYTOCHEMICALS

Plants produce the compounds—phytochemicals wherein the word “phyto” means “plants.” Fruits, beans, grains, vegetables and other plants contain them. Certain phytochemicals are believed to have anti-cancer properties wherein they prevent cell damage.

As per the belief of certain scientists, cancer risk can be reduced by about 40% by the consumption of more fruits, vegetables and other plants which have some phytochemicals. Certain phytochemicals have been shown by research to:

1. Help in the stopping of potentially cancer-causing substances from forming up (carcinogens).
2. Help in the prevention of cells being attacked by carcinogens.
3. Help the cells to not only stop but also wipe out any changes that are like cancer.

Certain phytochemicals that are the most beneficial are:

1. Resveratrol in red wine
2. Polyphenols that are present in tea
3. Beta carotene and other carotenoids are present in vegetables and fruits.
4. Cruciferous vegetables contain isothiocyanates (these cruciferous vegetables are members of the cabbage family, which includes bok choy, broccoli, kohlrabi, mustard greens, cauliflower, collards, Brussels sprouts, kale and turnip greens).

It is relatively easy to include these phytochemicals in the daily diet as these are present in vegetables, fruits, beans and grains that are consumed. For instance, more than a hundred phytochemicals are present in carrot.

As per the nutrition researchers, more than 4000 phytochemicals have been estimated to have been identified even though only around 150 of them have been studied in depth. As to which phytochemicals may be beneficial in the reduction of cancer-related risk needs to be researched further.

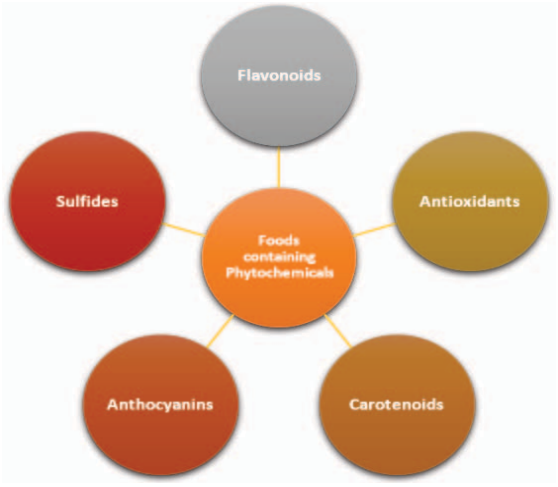

Figure 1.5. Foods containing phytochemicals.

It needs to be borne in mind that there is yet no evidence to show that taking supplements of phytochemicals may be as effective as the eating of whole fruits, beans, vegetables and grains that contain these.

As per a number of experts, the combination of the foods that are consumed and these compounds together is what keeps the body healthy. A balanced diet with myriad foods shall probably be far more effective than pills that have one or two phytochemicals where the balanced diet has at least five or more cups of vegetables and fruits from other plant sources like cereals, seeds, rice, beans, whole-grain breads, nuts and pasta.

The groups of phytochemicals that promote health have several main groups.

1.6.1. Flavonoids

A number of grains, fruits and vegetables have flavonoids. The risk of breast cancer banks on the hormone estrogen for its growth and flavonoids present in licorice, chickpeas as well as soybeans may behave a little like estrogen.

Phytoestrogens present in these plants are compounds like estrogen. However, the estrogen like activity is very weak in most of the phytoestrogens. When the natural estrogen position of the body is replaced by a weak substance that is estrogen-like, this weak substance can act as a relative anti-estrogen.

Breast cancer that depends on its growth upon estrogen may be helped by the phytoestrogens that act in this way by working against breast cancer. However, these foods have a very little amount of phytoestrogens. More can be learnt about phytoestrogens on the soy page dealing with soy foods.

Researchers are studying flavonoids to see if the risk of heart disease and some kinds of cancers can be reduced by them.

1.6.2. Antioxidants

Body's cells are protected from free radicals by antioxidants, wherein the unstable molecules created during the normal functioning of the cell are free radicals. Free radicals can be created in the body due to herbicides, pollution, cigarette smoke and radiation. The genetic parts of the cell may get damaged by free radicals and the cell may get triggered to have a growth that is out of control; as a result of these changes, cancer and other diseases may develop.

Green tea, black tea, broccoli, cauliflower, carrots, soybeans, spinach, celery, seeds, beets, strawberries, blueberries, Brussels sprouts, tomatoes, mangoes, cantaloupe, nuts, fish oil, liver, grains, red peppers, cabbage, corn, sweet potatoes, oranges, lettuce, spinach, celery and kale contain antioxidants. As compared to most vegetables and fruits dark-colored vegetables and fruits generally have more antioxidants.

1.6.3. Carotenoids

The orange color in yams, squash, carrots, apricots and cantaloupe is due to carotenoids and this may help to reduce cancer risk.

1.6.4. Anthocyanins

The dark color of grapes, raspberries, blueberries and cranberries is due to anthocyanins which in laboratories have shown to possess anti-tumor and anti-inflammatory properties.

1.6.5. Sulfides

The immune system may get strengthened with the sulfides that are present in onion and garlic.

By observing people who have mainly a plant rich diet a lot of evidence has been gathered that shows the effects generated by phytochemicals. The rate of heart diseases and certain kinds of cancer is far lower in these people.

More research is required even though the associations shown between reduction of cancer risk and certain phytochemicals is quite convincing. As of now there is no evidence available to show conclusively that phytochemicals can help get rid of cancer already existing in a human body or reduce the risk of getting cancer.

There can never be a single food ingredient that is vital and needs to be included in the diet. A balanced diet including a variety of fruits, vegetables, whole grains and legumes shall always be recommended by healthcare professionals and dieticians.

1.7. DIFFERENCE BETWEEN ANTIOXIDANTS AND PHYTOCHEMICALS

Damage to cells from “free radicals” that are highly unstable and reactive molecules is prevented through substances called antioxidants. For a healthy

living a balance between free radicals and antioxidants is essential. Cell damage resulting in a number of chronic diseases can result from free radicals that are not kept in check.

Substances that occur naturally in plant foods like vegetables, fruits, nuts, whole grains, seeds and beans are phytochemicals. Studies done in the laboratories have shown that a number of phytochemicals can perform the role of antioxidants whereby the free radicals get neutralized and their power to create damage is removed.

Mineral selenium, vitamins C and E are some nutrients that directly block free radicals inside the human body and not just in the laboratory. However, lab results have not shown to accurately depict the effects of phytochemicals in the body. Incidentally, many phytochemicals cannot be absorbed in the gut, whereas in the laboratories the results have shown high antioxidant scores. All the same, they may be broken down in the colon by healthful bacteria resulting in other compounds that are absorbable.

To protect health, phytochemicals and the compounds that result from them act in varied ways. Some of them prevent the carcinogens from starting the process of cancer development, whereas the others increase the tendency of the cancer cells to self-destruct. The development of blood vessels needed by tumors may also be developed by them and inflammation is fought by some of them.

The ability of our body to balance free radicals and antioxidants is supported by a number of phytochemicals. The antioxidant defense system of humans consists of a complex network of antioxidant nutrients that food supplies and various different compounds and enzymes that work with these antioxidants and with each other.

The bottom line of this research is that a variety of plant foods are required in our diet so that our health can be protected by the full spectrum of available phytochemicals. This is not the same as loading up one just one antioxidant or phytochemical.

1.8. FILL UP ON PHYTOCHEMICALS

Other than just the minerals, fiber and vitamins, a lot more is consumed every time we munch onto some dark leafy greens or bite into a juicy red apple. A power-packed bite of phytochemicals, the plant substances too are consumed by us. These can go a long way in keeping us healthy even though, unlike minerals and vitamins they are not known to be necessary for health.

According to the director of clinical nutrition at the Cardiovascular Disease Prevention Center at Harvard-affiliated Massachusetts General Hospital Debbie Kravitz, they may help to fight heart disease and cancer even though the science in this regard is ongoing and we are still learning about these phytochemicals.

'Phyto' means plants and phytochemicals are essentially plant chemicals. These are the compounds found in plants that contribute towards their specific taste, smell and color like in vegetables, fruits, nuts, whole grains, legumes and seeds. The vibrant orange color of the carrots, bitter taste of the Brussels sprouts, and searing bite of hot peppers is all a result of these phytochemicals. All edible plants have phytochemicals especially their peel or skin.

1.8.1. Superpowers

The study of these phytochemicals has just about started wherein they can be fully understood even though scientists have confirmed that there are more than 5000 of such compounds. The lycopene in tomatoes and carotenoids from beta carotene are probably the most studied so far. According to a professor of epidemiology and nutrition at the Harvard T.H. Chan School of Public Health, Prof Eric Rimm, the study of flavonoids has been done more during the last decade

Phytochemicals have shown positive results in the evidence garnered so far. For instance:

1. Immunity gets a boost as well as cardiovascular diseases and cancer growth get inhibited by carotenoids found in orange, green, red and yellow plants (cooked tomatoes, broccoli, squash and carrots).
2. Tumor growth and inflammation are inhibited by flavonoids found in apples, onions, coffee, berries, citrus and soybeans.
3. Lower blood pressure can be helped by anthocyanins found in red wine and berries.
4. In some animals longevity has been associated with resveratrol found in grapes, red wine, peanuts and dark chocolate.
5. Arteries function better and blood pressure can be reduced through flavanols and proanthocyanidins found in apples, red wine, grapes and cocoa.

6. "Bad" LDL cholesterol may be affected with the help of thiols and sulfides found in garlic, leek, onions, scallions and olives.
7. Cruciferous vegetables like broccoli, kale and cabbage contain isothiocyanates (sulforaphane) which may help to protect the humans against cardiovascular disease and cancer.
8. Apples, citrus fruits and onions contain quercetin that may help to decrease blood pressure and inflammation.
9. Citrus fruits and cherries that contain terpenes may help to fight viruses and slow the growth of cancer cells.
10. Eye health may get a boost through zeaxanthin and lutein contained in dark and leafy greens.

There is no conclusive evidence of these benefits as of now. These deductions have been made mainly from the study of human cells in laboratories or that of lab animals (for instance, in lab mice, resveratrol has shown its helpfulness in preventing heart diseases and cancer but the amount consumed by them was far higher than that is found in the diet of humans) and from the associations made between the outcome on health and the dietary consumption of people (where a direct cause and effect cannot be seen).

Rimm has pointed out that one thing that can be said for sure about phytochemicals is that the benefits cannot be derived from taking pills these benefits can be seen only when it is consumed in food.

1.8.2. What You Should Do?

It is difficult to select the specific compounds amongst the vast number of phytochemicals that are available which aid in protection of health. This may not even be required as loads of phytochemicals may be present in most plant foods (for instance there are more than hundreds of them in carrots).

One should try to consume a diet that has more variety. So that as many as possible phytochemicals can be taken in, "eating a rainbow" has been recommended by Krivitsky which implies different kinds of colorful vegetables, fruits, seeds, nuts and legumes. All these food items complement each other by performing different roles. Where one may interfere with the replication of cancer cells another one may block a carcinogen (cancer-causing substances).

She advises that a minimum of five to nine servings of vegetables and fruits should be consumed on a daily basis. For instance, an individual

can pick up red peppers or watermelons, which are both red in color when that individual is not fond of berries or red apples. Similarly, artichokes and lettuce can be consumed instead of broccoli or kale. Each day foods from each color category should be consumed. The potential benefits rise manifold when a variety of food items are consumed.

1.9. WHAT FOODS ARE HIGH IN PHYTOCHEMICALS?

The taste, aroma and color of plants are due to the naturally occurring compounds or phytochemicals. In addition to providing these beneficial characteristics to the plants, these compounds arm us with the necessary wherewithal in the form of antioxidant enzymes and enhancing the pathways to repair DNA to fight the harmful effects of the environment and the carcinogens that have been ingested. These compounds directly affect the fundamental hallmarks of metastasis and the progress of cancer.

Various academic bodies and the World Cancer Research Fund have not surprisingly reported that the risk of cancer or its relapse post treatment lowers when individuals consume foods rich in phytochemicals.

Polyphenols are the most well-known group of phytochemicals amongst the large range of the dietary ones that are known. The intake of polyphenols on an average daily dietary basis is about ten times higher than all the other known dietary antioxidants and other classes of phytochemicals being around one gm per day.

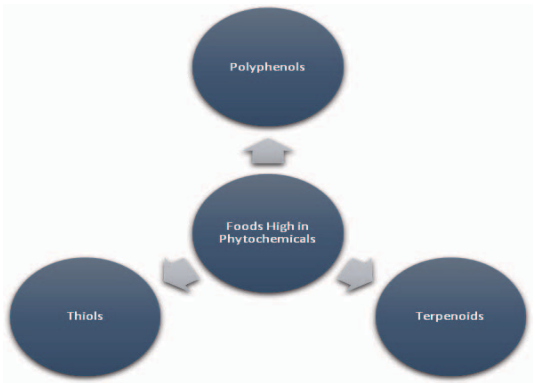

Figure 1.6. Foods that are high in phytochemicals.

Phytochemicals are of three major groups, namely:

1.9.1. Polyphenols

The three main subcategories of polyphenols are: flavonoids, phenolic acids and other non-flavonoid polyphenols.

Flavonoids are:

1. Flavones: apigenin, luteolin (herbs, chamomile, capsicum pepper, celery, parsley, rooibos tea);
2. Flavanols: Quercetin, kaempferol (kale, broccoli, red grapes, grapes, onions, tea, leeks and buckwheat);
3. Flavanones; naringenin, hesperitin (citrus fruits);
4. Isoflavones: daidzein, genistein, glycitein (soya, chickpeas, peanuts, beans, alfalfa);
5. Anthocyanidins (blueberries, strawberries, raspberries, blackberries, tea, red grapes, cherries);
6. Dihydrochalcones: phloridzin, aspalathin (rooibos tea, apples);
7. Flabanolols: silibinin, silymarin, aromadedrin (red onions, milk thistle);
8. Flavan-3-ols(tannins): epicatechin, catechins, epigallocatechin gallate (chocolate, tea, grapes).

Phenolic acids are:

1. Hydroxycinnamic acids: P-coumaric acid, ferulic acid, caffeic acid, sinapic acid (wheat bran, kiwi fruit, blueberries, cinnamon, plums, coffee);
2. Hydro benzoic acids: ellagic acid, gallic acid, vanillic acid (grape seed, blackberries, vanilla, rhubarb, tea, raspberries, pomegranate);
3. Other non-flavonoid polyphenols.

Other tannins (fruits, beans, cereals, wine, berries, cocoa, nuts) are:

1. Stilbenes: cinnamic acid, resveratrol (wine, peanuts, grapes, raspberries, blueberries);
2. Curcuminoids: curcumin (turmeric);
3. Lignans: enterolactone, secoisolariciresinol, sesamin (flaxseed, grains, sesame seeds).

1.9.2. Terpenoids

The subcategories of terpenoids are carotenoids and non-carotenoid terpenoids

Carotenoid terpenoids are:

1. Lutein (eggs, spinach, pumpkin, rhubarb, mango, corn, papaya, kale, plum, red pepper, oranges);
2. Alpha, beta and gamma carotene (carrots, kale, pumpkin, sweet potato);
3. Lycopene (watermelon, guava, tomatoes, papaya, pink grapefruit);
4. Zeaxanthin (eggs, spinach, oranges, red pepper, corn, pumpkin);
5. Astaxanthin (shrimp, crab, krill, salmon).

Non-carotenoid terpenoids are:

1. Ursolic acid (cranberries, apples, prunes, peppermint, thyme, oregano);
2. Perillyl Alcohol (caraway seeds, cherries, mint);
3. Phytosterols: natural cholesterols, stigmasterol, siosterol, campesterol (cereal grains, shoots, legumes, whole grains, vegetable oils, nuts, seeds and their oils, legumes);
4. Ginkgolide and bilobalide (Ginkgo biloba);
5. Limonene (the rind of citrus fruits);
6. Saponins (soya beans, chickpeas).

1.9.3. Thiols

Allylic sulfides, indoles that do not contain sulfur and glucosinolates comprise thiols.

Allulic sulfides are:

1. Allicin and S-allyl cysteine (leeks, garlic, onions).

Indoles are:

1. Indole-3-carbinol (Brussels sprouts, broccoli).

Glucosinolates are:

1. Isothiocyanates (sulforaphane) and di-thiolthiones (cruciferous vegetables like Brussels sprouts, broccoli, cauliflower, asparagus, radish, horseradish and mustard).

The miscellaneous category consists of the other groups of phytochemicals that have at least some of the properties within these groups and includes:

1. Chlorophylls (green leafy vegetables);
2. Peperine (black peppers);
3. Capsaicin (chili);
4. Betaines (beetroot).

1.10. WHY ARE PHYTOCHEMICALS IMPORTANT?

Medical practitioners and their patients especially the ones that have cancer and are interested in various kinds of self-help strategies are increasingly discussing the benefits of the foods rich in phytochemicals, these foods are often highlighted in the popular media as well as the medical world as they are rich in phytochemicals beneficial for health as also are their concentrated nutritional supplements.

Plant phytochemicals especially polyphenols are increasingly being shown as substances that immensely benefit humans by for instance, reducing the cancer-related risk and helping the individuals who are beyond the scope of treatment.

The guidelines from influential organizations such as the American Society of Clinical Oncology (ASCO) and the National Survivorship Initiative highlight the importance of diets rich in phytochemicals through the newly introduced living well program in the UK also highlights the importance of other factors related to lifestyle.

The beneficial anti-cancer effects get a boost by concentrating these foods or extracting the useful elements of these foods into supplements that can be added to the nutritional intake however, this has certain drawbacks. Detrimental effects have been seen in the studies conducted on concentrated vitamins, minerals and phytoestrogenic supplements. Some advantages have, however been reported of the whole non-phytoestrogenic food supplements even though no detrimental effects have been reported.

Due to the absence of randomized controlled trials conducted by academic institutions, there has been reluctance amongst the oncologist to discuss the pros and cons of the nutritional supplements in the case of patients who are sustaining beyond cancer even though there are reports that about 60% of such patients are taking these supplements.

However, as a result of the success of the Pomi-T study and various other studies being conducted which have been registered with the National Cancer Institute, it is expected that there shall be a likelihood of change in this trend.

1.10.1. Phytochemical Constituents, Antioxidant, Cytotoxicity, Antimicrobial, and Antitrypanosomal

Plants are the reservoir of bioactive compounds that are useful and considering the newer strains of malaria parasites that are resistant to drugs, there is a need to develop and discover plant-based phytochemicals that can be used as agents to fight malaria. Gas Chromatography-Mass Spectrometry (GC-MS) analysis has been used in this study to give a detailed description of the phytochemicals that are present in both methanolic and ethyl acetate extracts of *Callistemon citrinus* (*C. citrinus*).

An evaluation of both the extracts was also done for their *in vitro*antimalarial, cytotoxicity and antitrypanosomal activities against Human cervix adenocarcinoma cells (HeLa cells), *Plasmodium falciparum* (*P. falciparum*), and *Trypanosoma brucei* (*T. brucei*) parasites and through the usage of the standard methods the antioxidant and antimicrobial efficacies were determined.

A high amount of fatty acids (62.48% and 52.88%) characterized both extracts. A greater activity with minimal inhibitory concentration (MIC) values that ranged between 0.025 to 0.10 mg/ml was exhibited by the ethyl acetate extract, whereas for methanol, the range varied between 0.025 and 0.15 mg/ml. both extracts were bactericidal to *Pseudomonas aeruginosa* ACC (*P. aeruginosa*) and *Escherichia coli* ATCC 35150 (*E. coli*).

Alkaloids, saponins, triterpenoids, flavonoids, tannins, phenols, glycosides, steroids, oils and fat were seen to be present in both extracts when quantitative and qualitative phytochemical screening was done. Antiplasmodial activities with an IC50 of 8.4/13.0 µg/mL and antitrypanosomal potentials with an IC_{50} of 6.6/9.7 µg/mL was exhibited by both the crude extracts.

This plant has shown uses in folklore over a period of time, but this study gave a conclusion to show that a wide range of antitrypanosomal, antimalarial and antimicrobial activities is possessed by the extracts as well as some bioactive components that are essential in pharmaceuticals wherein for the synthesis of drugs that are required in the management of various

kinds of diseases the remarkable antioxidant capacities can be used. Several diseases can be treated with the use of plants which helps ease humans. Across the globe, conventional medicines are being substituted with traditional medication. A number of medicinal plants are being given due attention due to their antioxidant properties and researchers are looking for the varied roles they can play to fight a myriad of diseases like Alzheimer's, atherosclerosis, diabetes, cancer, hypertension and cerebral cardiovascular events, among numerous other conditions.

Plants are a reservoir for chemical compounds that are potentially valuable and can be used in the production of drugs which can be used for synthesis and current design through the lead molecules that result from them. Antimicrobial properties which are highly beneficial for therapeutic treatment make up the phytochemicals and extracts of the plants.

Secondary metabolites of the medicinal plants have most of the pharmacological activities and these secondary metabolites are much smaller molecules alongside the primary metabolites like carbohydrates, lipids and proteins.

From both the aromatic and medicinal plants' secondary metabolites such as terpenoids, saponins, cardiac glycosides, alkaloids, flavonoids and tannins synthesis of various antifungal and antimicrobial drugs can be done which have far less harmful effects on man.

In most of the developing countries across the world, the traditional practitioners use plants that have medicinal properties. *C.citrinus,* a shrub endemic to Australia belongs to the Myrtaceae family and is an aromatic plant with medicinal properties.

This plant is used locally for the treatment of pain, gastrointestinal distress and infectious diseases caused by fungi, bacteria, parasites and viruses. Its leaves have a pleasant revitalizing flavor and are used as a substitute for tea whereas the plant also serves as a herbicide. Respiratory conditions like cough and bronchitis too are treated by the extracts derived from this plant.

The essential oil from this plant is used as an antifungal and antimicrobial agent whereas this plant serves as an insecticide as well. There has been documentation of the antinociceptive and fungitoxicity activity of the leaves from this plant.

The deteriorating health of humans and animals in Africa with its adverse effects on the economy has been recorded and it has been established that trypanosomiasis of both animals and humans adversely affect this.

More and more areas of Africa that are infected with the fly are dependent on the usage of the trypanocidal drugs due to the non-availability of vaccines that are effective against trypanosomiasis and the high cost involved in the commencement and sustenance of tsetse fly control as there has been increasing resistance to this along with a high rate of toxicity, exorbitant price and inefficiency in this sphere.

In clinical use, a high degree of resistance has been shown by the parasite plasmodium falciparum that causes malaria which is a major cause of threat across many countries of the world towards most of the antimalarial drugs.

As a result, efficient, safe and inexpensive therapeutics is required that can be derived from the plant-based bioactive compounds. Alkaloids, steroids, flavonoids and terpenoids were found to be present in the leaves of this plant when a phytochemical analysis was done on it.

The antimicrobial properties of *C. citrinus* against varied pathogens of Gram-negative, fungi and Gram-positive strains have been documented by Cock. The essential oil components of the *C. citrinus* have been reported by several researchers across the world.

On the other hand, much information is not available with respect to the comparative assessment of the antibacterial, antioxidant, antitrypanosomal and antimalarial activities and more so the bioactive components of the *C. citrinus* crude extracts, which makes this study necessary.

1.11. MATERIALS AND METHODS

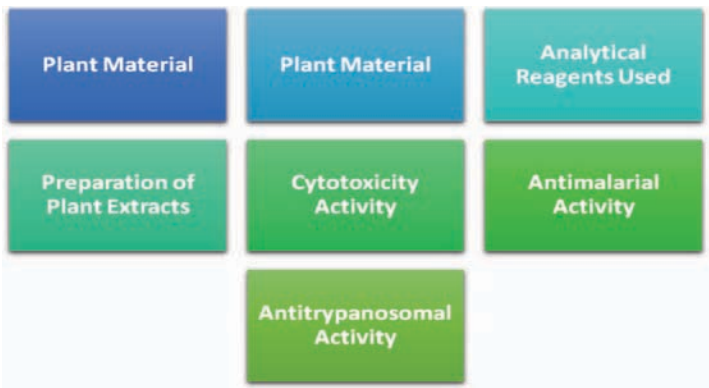

Figure 1.7. Materials and methods.

1.11.1. Plant Material

In August 2018, in the premises of the University of Fort Hare (UFH), Eastern Cape, South Africa, from the natural habitat of this plant, its fresh leaves were collected. For record purposes, a voucher sample (Larayetan 1) was kept in the Giffen Herbarium of University of Fort Hare and a taxonomist in Botany Department of the university authenticated this collection.

1.11.2. Bacteria

From the university laboratory's stock culture of AEMREG in Biochemistry and Microbiology Department six resistant bacteria and two resistant reference strains of bacteria were obtained. Until their requirement for some future use, nutrient agar was used to maintain all the cultures. The reference and laboratory strains are five Gram-negative bacteria were *Aeromonas hydrophila* (ACC), *Salmonella typhi* (ACC), *Pseudomonas aeruginosa* ACC, *Escherichia coli* (ATCC 35150) and *Vibrio alginolyticus* (DSM 2171) and three Gram-positive bacteria *Staphylococcus enteritis* (ACC), *Listeria monocytogenes* (ACC), and *Staphylococcus aureus* (ACC).

1.11.3. Analytical Reagents Used

All the reagents and chemicals that were employed were of analytical grade. The chemicals and reagents used in this research were as follows:

Mueller-Hinton agar from Oxford Ltd. (Hampshire, England); 2,2-diphenyl-1-picrylhydrazyl (DPPH),2,2-Azinobis-(3-ethylbenzothiazolin-6-sulfonic acid), potassium persulfate (PPS) and diammonium sourced from Sigma-Aldrich (St Louis, USA); methanol, ethyl acetate, and dimethyl sulfoxide (DMSO) were purchased from Fluka Chemicals (Buchs, Switzerland).

1.11.4. Preparation of Plant Extracts

At room temperature, for 21 days, fresh leaves of the plant were air-dried. A mechanical grinder, polymix (PX-MFC-90D) was used to pulverize the dried leaves, and two different extracts were prepared from the resulting powder. The same has been highlighted hereinafter.

1.11.4.1. Methanol Extract

For 72 hours, using 800 ml of methanol, 250 grams of the dried and powdered leaves was soaked; in an orbital shaker (Model 420 series, Thermo Fisher

Scientific) the mixture was shaken at 250 rpm, filtered with Whitman No.1 filtered paper (320 mm, 4 µm) and then using a rotary vacuum evaporator (bath at 40°C) it was concentrated at low pressure. Till the time it would be needed for analysis, the concentrate was labelled and preserved adequately in a vial storing it at 4°C).

1.11.4.2. Ethyl Acetate Extract

For 72 hours an equal amount of 250 grams of dried and powdered leaves was soaked in 800 ml of ethyl acetate. Dry ethyl acetate extract was obtained by following the same procedure and this too was stored in a vial under refrigeration till it would be required for further analysis.

1.11.5. Cytotoxicity Activity

By means of human cervix adenocarcinoma cells (HeLa cells), the cytotoxicity of ethyl acetate and methanolic of the crude extracts of *C. citrinus* was examined (as depicted by Keusch. These two extracts' stock solution (20 mg/ml) was diluted with a culture medium after dissolving in dimethyl sulfoxide (DMSO). Duplicate wells with around 1×10^4 HeLa cells per well at 37°C were used to incubate the resulting mixture for 48 hours in 5% CO_2. Resazurin-based reagent was used to estimate the number of cells that survived the drug contact and in a multiwall-plate reader, resorufin fluorescence was evaluated. Percentage visibility was used to express the results that were obtained.

1.11.6. Antimalarial Activity

As explained by Makler et al., parasite lactate dehydrogenase (pLDH) was used for Antiplasmodial assay on malaria parasite *P. falciparium*. At a concentration of 20 µM Sigma-Aldrich derived chloroquine was employed as positive controls. The screening of ethyl acetate and methanol crude extracts against the parasite of malaria was done with stock solutions carried out in a manner similar to the one used to describe the cytotoxicity assay.

In 96-well plates to the parasite culture, 50 µg/mL of the crude extracts were added which was then incubated at 37°C in a 5% CO_2 incubator for 48 hours. pLDH assay was carried out after the completion of 48 hours wherein 20 µL of the culture was removed from each well and in a fresh 96-well plate 125 µL of a combination of phenazineethosulphate (PES0/nitrotetrazolium blue chloride (NBT) and Malstat was added.

Further employment of the extract which could bring about an appreciable reduction in the parasite viability from the single concentration assay is done in a dose-response assay so that IC50 values can be determined.

1.11.7. Antitrypanosomal Activity

The manner in which the cytotoxicity assay was prepared was used for the stock solutions to access antitrypanosomal activity. In 96-well plates, to the *in vitro* culture of *Trypanosoma brucei* (*T. brucei*) 50 μg/mL of the two extracts was added thereafter, for 48 hours the resultant mixtures were incubated.

To calculate the number of parasites that had the capability to withstand drug contact, resazurin-based reagent was added (as earlier explained in cytotoxicity assay). Living cells usually reduce the reagent resazurin to resorufin. As a positive control, pentamidine was used.

1.12. PHYTOCHEMICAL COMPOUNDS IN PLANTS

Plants produce naturally occurring chemicals called phytochemicals. Pretty colors in plants are owing to some of the phytochemicals like for instance the red color in raspberries and the blue color in blueberries. The distinctive aroma of plants is due to certain phytochemicals. Insects get attracted to the plants due to their distinctive color or aroma (due to phytochemicals present in them), which helps pollinate and spread seeds.

When plants containing phytochemicals are consumed, they can affect health as these compounds are biologically active. Research at the preliminary level has suggested that the growth of cancer may be slowed down and through phytochemicals which may protect from cancer, regulate hormones and bring about a reduction in inflammation.

So far only a limited number of studies have been done on humans to gauge the potential properties of phytochemicals to fight cancer. More research needs to be done so that it can be found as to whether phytochemicals possess cancer-fighting benefits that are verifiable. However, research being done in the field shows that important health benefits can be derived from phytochemicals like in hormone function and reduction of inflammation.

Often, after the extraction of phytochemicals from plants is done, they are processed to be sold as supplements for diet. Regulation in the field of their dosage is limited and so is the knowledge with respect to its effectiveness even though these supplements are considered safe hence it is

always advisable to check with one's healthcare provider prior to starting with these supplements, especially if there are any existing health concerns.

1.12.1. Phytochemicals in Your Diet

All the plant-based foods that we eat have a number of different kinds of phytochemicals, and many of them have a lot of nutritional value. All the same, there are no dietary reference intakes that have been established and these are not considered as essential nutrients.

The best sources of phytochemicals are seeds, nuts, vegetables, fruits, legumes and whole grains.

Evidence exists to show that foods that are plant-based benefit the health when one consumes a diet rich in them. Fiber and essential nutrients may be the reason behind this benefit to human body. There is also likelihood that people consuming a diet rich in plant-based foods are able to manage a healthy weight. The potential health-related benefits of phytochemicals are being explored in the current researches that are being conducted so that their role in a healthy diet can also be deduced.

1.12.2. Types

Based on the chemical make-up, phytochemicals are of various groups. Carotenoids are some of the phytochemicals that are best known and these include alpha-carotene, lycopene, lutein and zeaxanthin. In the body Vitamin A can be derived from all these phytochemicals. However, beta-carotene is the primary source of Vitamin A that is plant-based.

Flavonoids family is another class of phytochemicals. Flavonoids consists of:

1. Anthocyanidins that can be found in blue, purple and red pigments of grapes and berries.
2. Isoflavones that can be found in legumes and soy.
3. Flavanols that exist in tea, berries, apples, grapes and chocolates.
4. Flavanones that can be found in citrus fruits.
5. Flavones which are present in hot peppers and celery.
6. Flavanols which are present in a number of vegetables and fruits.

One may have read of other phytochemicals which includes compounds like:

1. Chlorophyll that is found in all plants which are green in color.
2. Curcumin that is present in turmeric.
3. Indole-3-carbononol found in cruciferous vegetables.
4. Lignans which are present in whole grains and seeds.
5. Phytosterols that are used for lowering high cholesterol.
6. Resveratrol that is found in peanuts and grapes.

Fiber is sometimes classified as a carbohydrate even though it is found only in plants and can possibly be classified as a phytochemical. The number of dietary fibers is quite vast and includes cellulose, hemicelluloses, pectin, inulin, beta-glucan, resistant starch, gum and oligofructose.

The functioning of the digestive system can be improved and cholesterol levels can be kept under check by consuming a diet that is rich in fibers. Blood sugar spikes when a large amount of starch or sugar is consumed and consuming a fiber rich diet can help to slow down this sugar spike in the blood.

1.13. PHYTOCHEMICALS: EXTRACTION, ISOLATION, AND IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM PLANT EXTRACTS

Vegetables and fruits have many antioxidant compounds like carotenoids, tocopherols, phenolics and anthocyanins. Studies in the pharmaceutical field have used about 20% of the plants that are known with deep impact on the healthcare system in a positive manner like in treating harmful diseases and cancer.

Numerous bioactive compounds of a diverse nature can be produced by plants. Vegetables and fruits accumulate phytochemicals in a high concentration and these may help to protect against damage by free radicals. By acting as natural antioxidants plants consisting of phytochemicals that are beneficial can supplement the requirements of the human body.

Numerous studies have shown plants to be a rich storehouse of antioxidants. To take an example, plants have flavonoids, lignins and tannins which are vitamins A, C, E and phenolic compounds and all of these play the role of antioxidants.

Vegetables and fruits have a high nutritional value and numerous medicinal properties and a plethora of health benefits can be derived by their consumption on a regular basis. The quality and shelf life of these

foods gets extended as the oxidative damage in the foods can be controlled and reduced by inhibiting or delaying oxidation which is a result of reactive oxygen species (ROS) through antioxidants.

Aging can be delayed, cancers of certain kinds be prevented and inflammation reduced through the dynamic role played by beta carotene, many phenolics and ascorbic acid. Across the globe, many health care systems and agencies have recommended an increase in the consumption of vegetables and fruits.

The identifying, extracting and measuring the bioactive compounds of plants have been addressed in many studies related to phytochemicals and this chapter aims to review these studies. An overview of the lipid oxidation process has been included in this review along with details of plants that have been known to be sources of antimicrobial and antioxidant, phenolic compounds, antioxidants from fruits and vegetables, prevention of cancer, purification and isolation of molecules that are bioactive and techniques for these bioactive molecules' structural classification.

1.14. METHODS USED FOR BIOACTIVE COMPOUND EXTRACTION, ISOLATION, AND PURIFICATION

1.14.1. Extraction of Phenolic Compounds Using Solvents

For the purpose of extracting antioxidant from different parts of the plants like seeds and leaves, studies have been conducted by scientists and they have analyzed the impact of varied solvents like hexane, ethyl alcohol and methanol.

Different solvents with varying polarities have to be used so that various phenolic compounds can be extracted from plants with a degree of accuracy that is high. Furthermore, it has been discovered by scientists that polar solvents like methanol, as antioxidants, have high effectiveness.

N-dimethylformamide (DMF) and acetone have been reported as being fairly effective for the extraction of antioxidants by Anokwuru et al., & methanol was found to be more effective in the case of walnut fruits with a large number of phenolic contents, as was reported by Koffi.

Reports have shown that in comparison to water, acetone and methanol, phenolics extraction was higher in amount/concentration from Ivorian plants that were extracted with ethanol. To extract phytochemicals, multiple

solvents have been used and often, to eliminate water interfering with the process of extraction of bioactive compounds, dried powder of plants is employed.

Based on the polarity of the solute in question, for the extraction of plant biomolecules the solvents are chosen. The solute shall in all probability, get dissolved in a solvent that has a polarity that is similar. To ensure that the quantity of analogous compounds is limited in the desired yield, multiple solvents can be sequentially used.

The polarity of a few common solvents from the most polar to the least polar is as Water > Methanol > Acetone > Ethylacetate > Chloroform > Hexane.

Figure 1.8. The polarity of a few common solvents.

1.14.2. Microwave-Assisted Extraction (MAE)

To extract bioactive compounds from a large variety of natural residues and plants researchers have given a lot of attention to technique of MAE. The electromagnetic radiation in microwaves has wavelengths between 1cm and 1 m and occurs at frequencies between 300 MHz to 300 GHz. Both a magnetic field and an electrical field comprise electromagnetic waves.

Two perpendicular lines are used to describe these. Objects were heated up in the first application of microwaves and these objects can then covert it to heat by first absorbing a part of the electromagnetic energy. Corresponding to an energy output of 600–700 Watts, a frequency of 2450 MHz is used by commercial microwave instruments.

Without causing an increase in the time for extraction, the loss of bioactive compound has been reduced through the availability of advanced techniques in recent times. Hence in multiple fields, extraction that is assisted by microwaves has been shown to be a good technique more so in the field of medicinal plants.

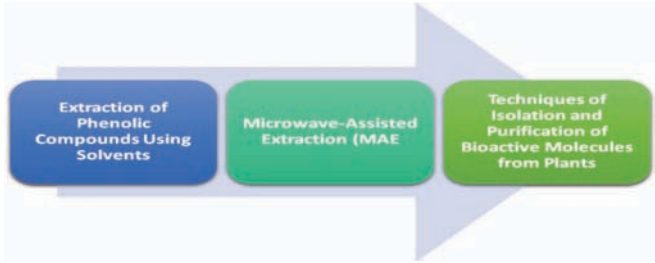

Figure 1.9. Methods used for bioactive compound extraction, isolation, and purification.

Furthermore, the loss of biochemical compounds that were extracted was also reduced through this technique. Extraction that is assisted through microwaves (MAE) reduces the volume of the extraction solvent as well as time and has been used as an alternative to conventional techniques for antioxidant extraction. Incidentally, the usage of lesser solvents for the extraction of antioxidants from plants by using the MAE for heating the solvent is the main objective of this technique's usage.

Li et al., compared to MAE, reported lesser phenolic content and antioxidant activity were presented by conventional methods that used various solvents. In a way, the finding only confirmed that the efficacy of MEA was higher for bringing about an increase in the antioxidant activity by measuring the total phenolic content (TPC), the capacity to absorb oxygen radical and ferric reducing antioxidant power (FRAP).

Certain factors like extraction time, solvent composition and extraction temperature can be used to bring about a change in the microwave extraction's efficiency. According to Tsubaki et al., for the extraction of phenolic compounds from Chinese tea the most effective temperature is 170°C. Furthermore, the extraction yield reduced if the temperature for extraction increased further than this point.

A new extraction process which used microwave-assisted extraction (MAE) was used recently by Christoohoridou et al., in which cooperation with solvents was sought for the extraction of specific compounds by converting energy to heat. Numerous advantages of MAE have been shown by William et al., which includes shorter time for extraction, lower consumption of solvent and a higher sensitivity towards the molecules that have been targeted.

1.14.3. Techniques of Isolation and Purification of Bioactive Molecules from Plants

In recent years, new development has been seen in the field of techniques used for the isolation and purification of plant derived bioactive compounds. Through this modern technique, ability has been offered to draw a parallel on the one hand between the availability and development of numerous advanced bioassays, and on the other, it has provided techniques through which separation, isolation and purification can be done precisely.

With respect to the search of bioactive compounds, the goal is to find a method that can appropriately screen bioactivity in the source material like antioxidant, cytotoxicity or antibacterial in combination with specificity, speed and simplicity.

Vis-à-vis the vivo assays, the desirability of *in vitro* methods is higher as experiments based around animals take more time, are expensive and ethical controversies are bound to crop up around them. Finalization of procedures and protocols for the isolation and characterization of some bioactive molecules is not possible due to certain factors.

One of the factors could be that plants have different parts or tissues, and different compounds shall be produced by many of them. Additionally, the fact also remains that bioactive phytochemicals have diverse physicochemical properties and chemical structures. The preliminary step for the isolation and characterization of a bioactive phytochemical is the selection as well as a collection of the plant materials.

Thereafter, in order to discern any bioactive molecules, retrieval of information that is ethnobotanical is done. Using a number of solvents, extracts can be made then so that the active compounds due to which bioactivity occurs can be isolated and purified.

For the isolation and purification of the bioactive compounds, usage of the column chromatographic techniques can be made. The purification process of the bioactive molecules can be accelerated by using developed instruments like the High-Pressure Liquid Chromatography (HPLC). The purified compounds can be identified by using a variety of spectroscopic techniques such as Infrared (IR), UV-visible, mass spectroscopy and Nuclear Magnetic Resonance (NMR).

1.15. CONCLUSION

In the conclusion, this chapter discussed about the basic significance of phytochemicals, it's metabolism and phytochemistry of various phytochemicals. In this chapter, different families of phytochemicals have also been discussed. It also discussed about the use of phytochemicals in disease prevention, difference between antioxidants and phytochemicals.

Towards the end of the chapter, various kinds of foods that are rich in phytochemicals have also been discussed such as polyphenols, terpenoids, and thiols. This chapter discusses the various constituents, antioxidants, cytotoxicity, antimicrobial, and antitrypanosomal that are present in phytochemicals.

REFERENCES

1. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., & Lightfoot, D., (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants, [online] 6(4), p.42. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5750618/ (accessed 9 September 2021).
2. Asaduzzaman, M., & Asao, T., (2018). Introductory Chapter: Phytochemicals and Disease Prevention. Phytochemicals – Source of Antioxidants and Role in Disease Prevention, [online] Available at: https://www.intechopen.com/books/phytochemicals-source-of-antioxidants-and-role-in-disease-prevention/introductory-chapter-phytochemicals-and-disease-prevention (accessed 9 September 2021).
3. Breastcancer.org, (2021). Foods Containing Phytochemicals. [online] Breastcancer.org. Available at: https://www.breastcancer.org/tips/nutrition/reduce_risk/foods/phytochem (accessed 9 September 2021).
4. Cancernet Blog, (2016). What are phytochemicals? Types and food sources – Cancernet Blog. [online] Cancernet Blog. Available at: http://blog.cancernet.co.uk/phytochemicals-types-food-sources/ (accessed 9 September 2021).
5. Harvard Health. (2019). Fill up on phytochemicals – Harvard Health. [online] Available at: https://www.health.harvard.edu/staying-healthy/fill-up-on-phytochemicals (accessed 9 September 2021).
6. Karen Collins, (2015). Antioxidants and Phytochemicals – American Institute for Cancer Research. [online] American Institute for Cancer Research. Available at: https://www.aicr.org/resources/blog/healthtalk-whats-the-difference-between-an-antioxidant-and-a-phytochemical/ (accessed 9 September 2021).
7. Larayetan, R., Ololade, Z., Ogunmola, O., & Ladokun, A., (2019). Phytochemical Constituents, Antioxidant, Cytotoxicity, Antimicrobial, and Antitrypanosomal, and Antimalarial Potentials of the Crude Extracts of Callistemon citrinus. Evidence-Based Complementary and Alternative Medicine, [online] 2019, pp.1–14. Available at: https://www.hindawi.com/journals/ecam/2019/5410923/ (accessed 9 September 2021).
8. Mendoza, N., & Silva, E., (2018). Introduction to Phytochemicals: Secondary Metabolites from Plants with Active Principles for Pharmacological Importance. Phytochemicals – Source of Antioxidants and Role in Disease Prevention, [online] Available at: https://www.

intechopen.com/books/phytochemicals-source-of-antioxidants-and-role-in-disease-prevention/introduction-to-phytochemicals-secondary-metabolites-from-plants-with-active-principles-for-pharmaco, https://www.researchgate.net/publication/328798073_Introduction_to_Phytochemicals_Secondary_Metabolites_from_Plants_with_Active_Principles_for_Pharmacological_Importance (accessed 9 September 2021).

9. Shereen Lehman, (2020). How Does Eating Phytochemicals Keep You Healthy? [online] Verywell Fit. Available at: https://www.verywellfit.com/phytochemicals-2507237 (accessed 9 September 2021).

CHAPTER 2

SOURCE OF ANTIOXIDANTS AND ROLE IN DISEASE PREVENTION

CONTENTS

2.1. INTRODUCTION

A significant role is played by the nonnutritive chemical compounds or the phytochemicals derived from plants to prevent human diseases. Antioxidants and secondary metabolites amongst the phytochemicals possess important medicinal properties.

The source of phytochemicals, the phytochemicals produced under stress conditions, the role that phytochemicals play in the prevention of diseases and the accumulation of bioactive compounds in vegetables and fruits shall be outlined briefly in this chapter.

Under conditions of environmental stress, allelochemicals are produced as phytochemicals in the plant rhizosphere and the plants in the neighborhood which has significant ecological results. The role of these phytochemicals shall also be discussed in this chapter. The role that phytochemicals play in the prevention of major diseases and a general description of these plant-based chemicals is the basis of this chapter.

2.2. ROLE OF PHYTOCHEMICALS IN DISEASE PREVENTION IN HUMAN

Medicinal plants have certain phytochemicals like alkaloids, saponins, phenols, carotenoids, tannins, steroids, flavonoids, etc. that have activities conducive to preventing several diseases. Important preventive activities are performed by these chemical compounds derived from plants like wound healing, anti-inflammatory, antiaging, antiparasitic, anticancer, antidiabetic, antioxidant, antidepressant and antimicrobial. An important role is also played by them, which helps the plants to tolerate stress and accumulate numerous bioactive compounds in vegetables and fruits.

Figure 2.1. Role of phytochemicals in disease prevention in human.

Source: *Image by Flickr.*

As far as medicinal plants are concerned, the most common bioactive compounds found in them are the flavonoids. These possess numerous activities that aid in the prevention of human diseases like wound healing, antimicrobial, anti-inflammatory and anticancer. Numerous vegetables and fruits have been reported to contain flavonoids that are anticarcinogenic. Cardioprotective properties found in berries and apples have shown positive results with respect to blood pressure.

The cell vacuole responsible for vegetable, fruits and flower pigmentation have an abundance of the flavonoid called anthocyanins, and these are generally produced when the plant is under some kind of an environmental related stress. The *in vitro* studies of the cell culture systems like colon, breast, keratinocytes, liver and leukemic cells have shown antioxidative activities of anthocyanins.

The natural antioxidants present in vegetables and fruits are potentially carotenoids. These include xanthophyll and carotenes having properties that can bring about scavenging of peroxyl radical. Berries and tomato have lycopene whereas the orange-colored carotenoids found in abundance in dark-green and yellow-orange leafy vegetables are β-carotenes.

2.3. ALLELOCHEMICALS AS PHYTOCHEMICALS IN THE PLANT RHIZOSPHERE AND ITS ECOLOGICAL ROLE

To protect itself from environment related stress like drought, chemical pollution, disease infection, pest, UV exposure and submergence plants release a number of phytochemicals. Bioactive compounds and secondary metabolites are produced by the plants through this process and these compounds have potential role as antioxidants.

A number of chemical compounds are released by the plant body into the environment under the natural ecosystem in general which helps to maintain the development and normal growth of the plants. At the same time, under conditions of environmental stress, certain allelochemicals/other chemicals too are produced by the plants.

Autotoxic and heterotoxic conditions are created for the plant and for the other plants as well in its neighborhood by the allelochemicals that are released. Plants produce numerous allelochemicals that inhibit its own development and growth under recycled hydroponics and replanting conditions.

This phenomenon has been observed in beans, strawberry, several leafy vegetables, taro, lettuce and ornamentals. These allelochemicals may play a significant role with regards to the ecology wherein weeds, plant diseases and pests can be controlled.

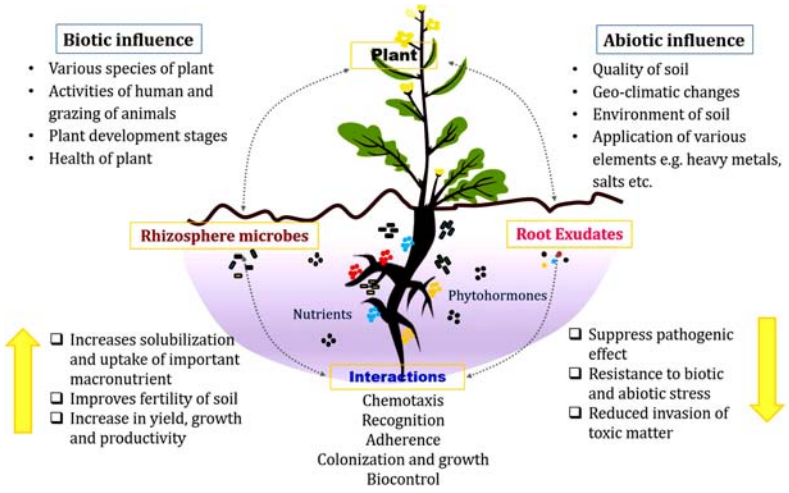

Figure 2.2. Associations in the rhizosphere between plants roots, microbes and root exudates.

Source: *Image by Wikimedia Commons.*

2.4. PHYTOCHEMICALS – GOD'S ENDOWMENT OF CURATIVE POWER IN PLANTS

The link between the knowledge of plants being a source of drugs and food for humans as a part of God's pronouncement in the Holy Bible and the availability of scientific proof that different species of plants have phytochemicals was reviewed in Phytochemicals- God's endowment of Curative Power in Plants.

The world has an abundance of plants in the form of vegetables, fruits, spices, etc., and more than 80% of their chemical composition is not known as yet, wherein lies the need to search for these phytochemicals that shall be a relatively cheaper and safer alternate drug source.

Phytochemicals like alkaloids, tannins, glycosides, saponins and anthraquinones have been reviewed in literature in the form of cyanogenetic and cardiac glycosides, carotenoids, flavonoids and phenols owing to the benefits that accrue to humans as a result of their chemical properties.

The outburst that said "the world is too much with us; late and soon, getting and spending, we lay waste our powers: Little we see in nature that is ours," was confirmed by this review. It is essential that the potential of these plant-based phytochemicals is explored at the earliest so that the numerous benefits that can be derived from their curative powers do not go to waste and humans can benefit from them.

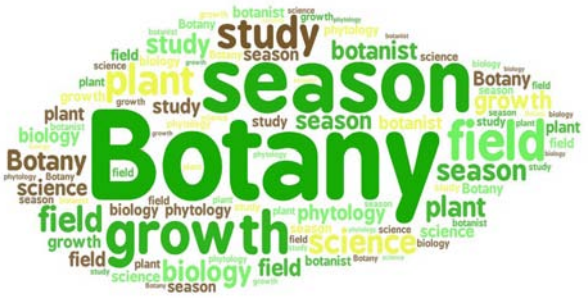

Figure 2.3. Phytochemicals – God's endowment of curative power in plants.

Source: *Image by Flickr.*

It has been recorded in the Bible in the books of Genesis and Revelation that God created plants wherein he ordered humans to make use of the trees and herbs for medicinal and food purposes. God created light on the first day (from the work of creation) and the second-day water was made which was followed on the third day by the creation of plants of various kinds, which fell in different categories like seeds that yield herbs, grass, trees that gave fruits and the seed which lay within the fruit.

The all-knowing God has given this divine order which cannot be fathomed by anyone nor can the understanding behind it be searched. God knew all the things that would be required for the creation of these plants ordained their growth only after the creation of these essential things.

Only when all these things had been created, namely the plants and other creatures God created humans so that they could rule over all these creations. He was familiar with human's frail disposition, so he ordered humans to use tree bearing fruits, herbs, other species for food and medicinal purposes. He made provision for all the things that humans would need for survival.

Phytochemicals are essentially the healing aspects of plants that were placed there by God with the purpose to serve man. Depending upon the specific diseases that they can cure, phytochemicals are of varied kinds. Some plants have a variety of these chemical constituents and secondary metabolites or the synergistic effect of these individual chemical constituents gives them abilities to cure ailments.

Synthetic drugs or orthodox medicines are often accompanied with side effects that do not occur when plant derived natural remedies are used for curative purposes and this has led to the realization that natural remedies are accompanied with their cures making the usage of plant-based healing properties preferable and recognizable.

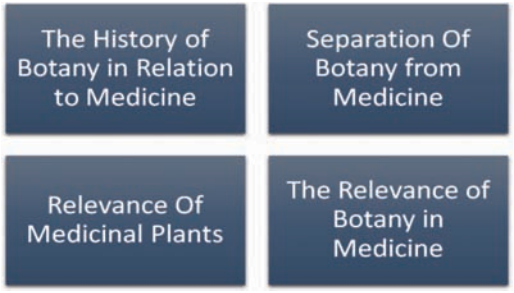

Figure 2.4. Role of botany in the field of medicine.

Wordsworth had once lamented, "the world is too much with us; late and soon, getting and spending, we lay waste our powers. Little we see in nature that is ours," and we are often reminded of this.

2.4.1. The History of Botany in Relation to Medicine

The study of plants is purely a botanical science whereas the applied science which has at its heart the study of plants for human use is botany. The most prominent fields of study in applied botany are forestry, agriculture, horticulture, weed science, plant pathology, pharmacognosy, ethnobotany and economic botany which are not included in the modern courses of botany.

The Neolithic revolution saw an increase in the knowledge of plants pertaining to their usage as food and medicines. The first prescientific written record of plants or protobotany started out as a medicinal literature in Mesopotamia, Egypt, India and China and not as a food-based literature.

Alan Morton, the botanical historian observed that the profession of the socially influential priests, physicians, shamans, apothecians and magicians was medicine whereas the uneducated and poor people practiced agriculture as their main occupation.

2.4.2. The Relevance of Botany in Medicine

As far back as 481 BC-221 BC, the concoctions of various herbs and plants for medicinal purposes were used and listed in China. Many Chinese writers contributed to the herbal pharmaceuticals' written knowledge. In c. 60AD, a Greek physician within Rome's army, Pedanius Dioscorides (c. 40–90 AD) compiled a complete synthesis of ancient Greek pharmacology, which shows that there was no neglect in the study of plants with medicinal properties.

Till the fifteenth and sixteenth centuries agriculture was at the center of the lives of the European Middle Ages after which printing came into being. However, the priority of this publishing sector was the lists of medicinal plants accompanied with their efficacy or inherent power and attributes rather than the dissertations on agriculture.

As per the records, apparently the first recorded book in ancient history was Herbal which was from a series of the ones written on plants thereby indicating that medicine included botany. The curators of the university gardens mostly contributed to the Herbal and De material Medica was the main source of the Herbal's classic texts.

2.4.3. Separation of Botany from Medicine

Rather than being medicinal, some herbals were more botanically inclined as detailed and accurate descriptions of plants were required. Herbals initiated the science of plant classification, description and botanical illusions thereby contributing towards botany.

Up till the seventeenth century, medicine and botany were essentially the same however, plant lore was omitted in the books which laid an emphasis on the aspects related to medicine and these resulted in the modern pharmacopoeias.

The ones that did not deal with the medicinal aspects were more botanical in nature and these evolved into the Flora or the modern description of plants wherein herbarium containing specimens of these plants backed the collection. The herbariums had deposits of dried plants that were described in detail in the Floras. Botany finally got separated from medicine through the transition from Herbal to Floras.

2.4.4. Relevance of Medicinal Plants

Most of the drugs that are synthesized chemically have some or the other side effects on the human body and medicinal plants provide an avenue whereby remedies can be found for health-related issues with no side effects or if et all very little effects. Hence, there is a huge amount of interest amongst people to search and use such plants for ailments.

In order to prevent the occurrence of and cure any diseases in animals and humans a large amount of interest is being shown in the use of products that are bio-friendly and eco-friendly. Natural products that are effective, affordable and safe are being increasingly looked into by the western world due to the side effects exhibited by most of the drugs that are based on chemicals.

As per the available documentation, at least 80% of the population across the globe has faith in the healing powers of traditional medicine and more so the ones that originate from plants for the maintenance of their primary health.

Traditionally, in some form or the other, medicinal plants have been used like for instance the bark of Cinchona, pawpaw leaves (*Carica papaya*), stem bark (*Azadirachta indica*), neem leaves, mango leaves and stem bark (*Mangifera indica*).

A wide array of plants in the plant kingdom is yet to be explored for the medicinal properties and potencies that they may possess. In Nigeria diseases are being treated with various parts of medicinal plants highlighting their usage.

2.5. HARVEST OF MEDICINAL PLANTS

The medicinal properties of plants fluctuate with varying seasons and should thus be harvested at the appropriate season when their benefits are the maximum. The age of the plant affects the medicinal properties and these attributes may not be present all over the plant being restricted to

a particular part or parts of the plant. To prevent any kind of changes to the phytochemical constituents, the plant or its relevant parts need to be harvested at the correct time of the year when the plant is at a particular age to be processed for the beneficial drugs. The medicinal properties of the plants are also affected by how the plant is stored whether under the sun or in the shade as well as the geographical location.

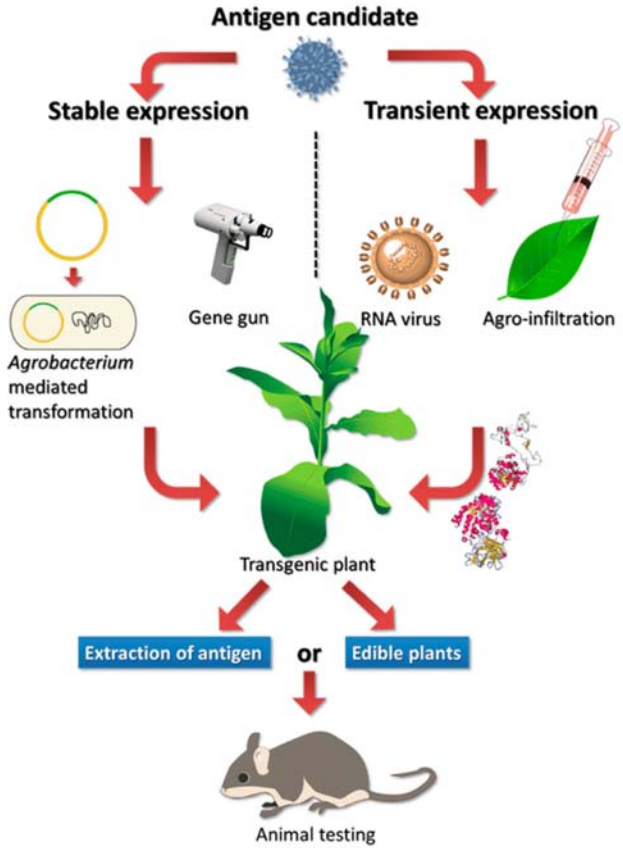

Figure 2.5. Schematic diagram of plant pharming.

Source: *Image by Wikimedia Commons.*

2.5.1. Plant-Based Drugs

Plants have been known to provide most of the therapeutic agents used in ancient times especially those derived from the higher plants and they have served humans since times immemorial. As per an estimate of the World

Health Organization, across the world about 3.5–4 billion people rely for their primary health care on the drugs derived from traditional medicines (85% of the traditional medicine derived from plant extracts caters for needs of about 80% of the people in the developing world).

In the United States, 25% of the drugs that were prescribed were derived from plants and 39% of the drugs approved between 1983 and 1994 had origins in natural resources. Amongst these were the products derived semi-synthetically from natural sources, original natural products and synthetic products that had the natural products as their base models.

A survey revealed that 62% of the 87 anticancer drugs that have been approved have origins in natural resources or they are derived by modeling the natural products of the plants. Some of the drugs that are most useful include paclitaxel, podophyllotoxin (a natural product precursor), vincristine, and camptothecin (a natural precursor for derivatives that are water-soluble).

Properties inherent to these substances are essential for the treatment in hospitals as new chemotherapeutic agents. The role played by natural products in the development and discovery of drugs cannot be replaced by the computer-based molecular modelling design or combinational chemistry that are the newer approaches for the discovery of drugs. Artemisinin, quinine and gingersome are some of the examples of drugs that are based on plants.

2.6. SYNERGY IN RELATION TO PHARMACOLOGICAL ACTION OF PHYTOMEDICINE

The idea of synergy between and within different constituents is gaining acceptance as the effect of most herbs can be seen in a number of constituents. There is no proper documentation of most herbal medicines so that it can be proved as to whether or not they are acting by way of additive effects or in a truly synergistic manner.

Without knowing the synergistic effects of herbal preparations, it is not easy to evaluate them clinically. The potency of certain active components can be enhanced by some other components they may have even though there is no biological activity in the crude drugs.

Figure 2.6. St. John's hypericum wort.

Source: *Image by Pixabay.*

St. John's wort (*Hypericum perforatum*, family Hypericaceae) is one such example which has clinically been proven to be effective as an antidepressant herb. The hypercins were quite weak and were responsible for this activity wherein the additional results were derived due to the impurities present in the fraction that was used suggesting synergistic and multiple (polyvalent) effects. Polyvalent action and synergism exhibited by the herb St. John's wort is an ideal example of a herb exhibiting such properties.

The activity of herbal drugs can be enhanced and synergism can result when herbs are combined and used. The toxic effects of the main herb can be reduced in certain cases and in others cases, the potency of the herb that has real efficacy can be enhanced by using herbs in a combination so that the resulting mixture is safe and tolerable for human consumption.

2.7. FREE RADICALS AND THE ROLE OF PLANT PHYTOCHEMICALS AS ANTIOXIDANTS AGAINST OXIDATIVE STRESS-RELATED DISEASES

Serious challenges to health can be posed by reactive oxygen species (ROS) or free radicals that various environmental sources generate, along with the ones that are generated from the cellular processes that take place within the body.

The antioxidant defense system of the body gets disrupted by these free radicals when they are present in large numbers so that the cellular macromolecules like lipids, nucleic acids and proteins or the cell membranes get damaged as a result of which uncontrolled cell division take place due to the mutations caused or the cells die.

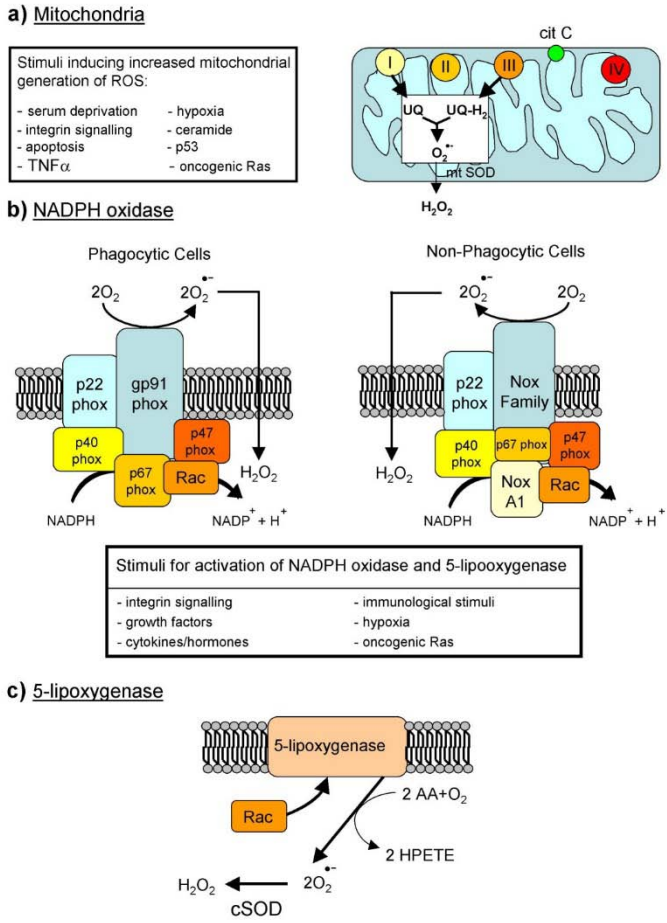

Figure 2.7. Major cellular sources of reactive oxygen species in living cells.

Source: *Image by Wikimedia Commons.*

Several diseases like diabetes, cancer, arthrosclerosis, cardiovascular diseases, etc. occur due to the oxidative stress when there is a disruption of

the cellular antioxidant system and deficiency. To supplement the antioxidant defense system of the body, external sources of antioxidants are required so that the oxidative stress of the body can be managed better.

Plants have always been seen as a major source of antioxidants due to the therapeutic benefits they have and their natural origin. Antioxidant properties have been shown both *in vivo* and *in vitro* be certain plant phytochemicals that are non-enzymatic like polyphenols, carotenoids, glutathione, hydroxycinnamates, bioflavonoids and certain vitamins as well. For the management and even prevention of diseases that are related to oxidative stress there is now the usage of these plant compounds or phytochemicals.

In order to ensure his wellbeing and survival man has always taken up a number of activities. In this process, certain free radicals or reactive substances have been released or produced by this living creature or man and these substances are then either inhaled or consumed.

In addition to this proxidants or free radicals are also generated in the body through certain physiological processes. These reactive species or free radicals are instable and deficient in electron due to which they attack lipid membranes, nucleic acids and proteins that are electron rich centers resulting in damage to the tissues and cells within the body.

Antioxidants or a group of enzymes within the body are a variety of molecules that help the human body to remove these unstable molecules. The level of free radicals gets reduced by this defense system comprising of antioxidants in the body and the homeostatic balance which is required for the body to function properly is thus maintained.

All the same a condition called oxidative stress is generated in the body when these reactive species exceed the capacity of the defense system's antioxidants due to their overwhelmingly high numbers in the body. Certain diseases like diabetes, cardiovascular diseases, atherosclerosis, cancer, etc. are characterized by this imbalance between oxidants and antioxidants.

Using exogenous antioxidants to supplement the endogenous antioxidant defense system is one of the possible ways by which this situation can be remedied. In the recent times, considerable interest has been generated in plants due to their ability to manages diseases that are related to oxidative stress due to two main reasons.

One of the reasons is that they are rich in phytochemicals that have antioxidant properties and the other reason is that in managing diseases

they have ethnopharmacological uses. An overview of the free radicals is aimed to be covered in this topic along with the sources from which they originate and the processes by which they get generated in the body and the environment.

Cellular damage and the related diseases that occur due to oxidative stress which is induced by varied mechanisms of free radicals shall also be highlighted. The contribution of plants as antioxidants as well as the different mechanisms of the antioxidant defense system: non-enzymatic as also the enzymatic antioxidants shall also be described.

Phytochemicals with antioxidant activities and certain plants shall be emphasized, laying down the method they adopt to prevent diseases that are related to oxidative stress and the manner in which they scavenge free radicals.

2.7.1. Free Radicals

The atomic orbital of certain molecular species has unpaired electrons that are capable of existing independently and these molecules are free radicals. These radicals act as an oxidant or a reductant as they are highly reactive and can either donate an electron to other molecules or extract one from another molecule.In biological systems, free radicals have a very short half-life which is less than 10–6 s even though they are highly reactive. Reactive oxygen species (ROS) are certain species of oxygen that have the capability to generate free radicals even though in their natural state they are non-reactive.

Around the twentieth century, the idea of free radicals cropped up in chemistry where initially they had several suggested definitions and were described by chemists as intermediate inorganic and organic compounds.

In 1954, based on the work of Rebecca Gersham and Daniel Gilbert a clear understanding of these radicals was proposed whereby it was suggested that an important role was played by these radicals in biological environments but at the same time they were also responsible for some deleterious processes in the cell.

It was suggested by Herman Denham (1956) that a critical role may be played by these reactive species in physiological process and specifically in the process of aging. Numerous studies and research were inspired by this hypothesis on this theory of free radicals related to aging which helped tremendously to understand free radicals and other species that were related like reactive nitrogen species (RNS), non-radial reactive species and ROS.

2.8. TYPES OF FREE RADICALS OR REACTIVE OXYGEN SPECIES

There are two main categories of compounds in the classification of ROS, which includes the non-reactive radicals and the free radicals. Table 2.1 shows the free radicals that include Hydroxyl radical (OH•), peroxyl ($ROO^{\bullet}$), nitric oxide radical (NO•), one form of singlet oxygen (O_2), alkoxyl radicals (RO•) and superoxide ion radical ($O^{\bullet}2$).

The shells around the atomic nuclei of these molecules contain at least one unpaired electron making the atomic nucleus unstable whereby they can either obtain an electron or donate one to another molecule and attain stability in free radicals. These species are thus capable of existing independently and are highly reactive.

The non-reactive radicals on the other hand are not radicals but a group of compounds that can be converted to reactive species easily and are extremely reactive. Some of the examples of these species are hydrogen peroxide (H_2O_2), hypochlorous acid (HCIO), aldehydes, organic peroxides, and O_2 an ozone (O_3).

Table 2.1. Free Radicals and Non-Reactive Radicals of Oxygen Species

Free Radicals	**Name**
Oxygen Radicals	Oxygen (Bi-radicals)
	Superoxide ion
	Hydroxyl
	Peroxyl
	Alkoxyl
	Nitric Oxide
Non-Reactive Oxygen Radical	Hydrogen Peroxide
	Organic Peroxide
	Hypochlorous Acid
	Ozone
	Aldehydes
	Singlet Oxygen
	Peroxynitrite

2.9. SOURCES OF FREE RADICALS

Physiological processes, environment or endogenous sources can be the originators of free radicals. Free radicals can originate either from the environment, physiological processes or endogenous sources.

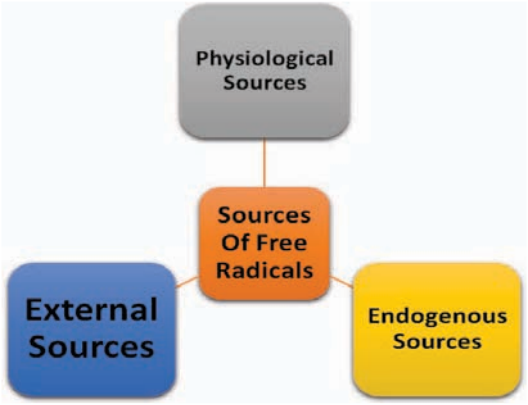

Figure 2.8. Sources of free radicals.

2.9.1. External Sources

Free radicals can be generated by some organic compounds in the atmosphere that can react with oxygen non-enzymatically. Free radicals can also be generated in the environment through reactions that are initiated by radiations that cause ionizing. Environmental pollutant, alcohol, ozone, pesticides, industrial solvents, cigarette smoke, certain drugs, radiations, anesthetics, ultraviolet light, etc., are some of the examples of free radicals' external sources.

2.9.2. Endogenous Sources

Free radicals are generated in living beings through certain processes which make enzymatic reactions necessary. Reactions involved in the cytochrome P450 system, respiratory chain, prostaglandin synthesis and phagocytosis are some of these processes.

Reactions in the phagocytes, mitochondria, arachidonate pathways, inflammation, etc. are some of the endogenous sources for the generation of free radicals. The other sources of free radicals that are endogenous are the reactions that involve iron and other transition metals, xanthine oxidase, peroxisomes, etc.

2.9.3. Physiological Sources

Free radicals can also result from some physiological conditions or processes like emotion, stress, aging, disease condition and mental status. Hyperglycemia for instance in diabetic patient is a source of free radicals through various metabolic pathways in which the flux of glucose increases through the polyol pathway, activation of protein kinase C(PKC) isoforms, advanced glycation end-products (AGEs) forming in increased quantities and activation of their receptors, decrease in the antioxidant defense and activation of hexosamine pathway.

2.10. GENERATION AND CHEMICAL REACTIONS OF FREE RADICALS

In living organisms, various physiological processes cause the generation of free radicals. To attain stability these free radicals upon generation can react with other biomolecules.

Free radicals are generated through various physiological processes in living organisms. Once generated, they can react with other biomolecules to attain stability.

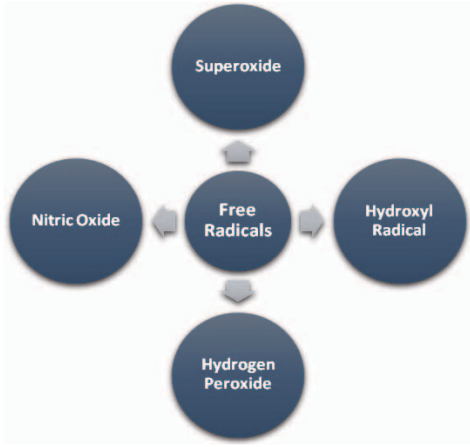

Figure 2.9. Generation and chemical reactions of free radicals.

2.10.1. Superoxide ($O^{\bullet 2}$)

When oxygen is added with a single electron, generally superoxide (O•2) is generated. Several mechanisms can result in the generation of superoxide

in living systems. In the presence of oxygen certain molecules like flavine nucleotides, glucose, thiol compounds, adrenaline, etc., get oxidized and superoxide is generated.

Metals like copper or iron when present accelerate this reaction tremendously. Oxygen gets reduced to water in the inner mitochondrial membrane during the electron transport chain as a result of which free radical intermediates are produced which react with free electrons subsequently and produce superoxide.

In the liver, enzymes like cytochrome p450 oxidase cause reactions releasing free electrons, producing superoxide by reacting with oxygen. Superoxide can also be generated during respiratory burst by phagocytic cells.

2.10.2. Hydrogen Peroxide (H_2O_2)

In the biological systems the spontaneous dismutation reaction of superoxide causes the production of hydrogen peroxide. Furthermore, H_2O_2 can be produced directly through numerous enzymatic reactions, including those catalyzed by glycolate oxidases and D-amino acid.

H_2O_2 is considered a reactive oxygen species (ROS) even though it is not a free radical as transformation of H2O2 into other free radicals like hydroxyl radical is possible. Most of the toxic effects of H_2O_2 can be mediated.

H_2O_2 can be decomposed by myeloperoxidase into hypochlorous acid and phagocytes use singlet oxygen wherein the mechanism to kill bacteria. Proteins and enzymes that contain reactive thiol groups can however be damaged directly by H_2O_2 which is essentially a weak oxidizing agent. H_2O_2 can freely traverse cell membranes and this its most important property vis-à-vis superoxide.

2.10.3. Hydroxyl Radical ($OH^{\bullet}$)

Hydroxyl radical (OH•) is reactive with most of the biomolecules including sugars, nucleotides, lipids and amino acids making it one of the most important free radicals. Usually, most of the ROS convert into hydroxyl radical which is most of the times the final mediator of a majority of the tissue damage caused by free radical.

In vivo mechanism is the most important of the various mechanisms for the generation of hydroxyl radical due to hydrogen peroxide catalyzed by transition metals and decomposition of superoxide. Transition metals are

capable of transferring a single electron as they usually contain one or more electrons that are unpaired.

The transition metals most common are copper and iron, which are quite implicated in diseases occurring within the human body and are capable enough to generate free radicals. Fenton showed that hydroxyl radical can be caused by the reaction of hydrogen peroxide with iron II (or copper I).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3}+ + OH^{\cdot} + OH^{-} \qquad E1$$

Chelates usually oxidize to biological molecules and iron to Fe^{3+} at physiological pH. There must be a conversion of iron to its reduced form Fe^{2+} for the occurrence of the Fenton reaction. Fenton reaction can be enabled by superoxide radicals that can bring about a reduction of Fe^{3+} to Fe^{2+}.

$$Fe^{3+} + O^{2-} \rightarrow Fe^{2+} + O_2 \qquad E2$$

The net reaction (Haber-Weiss reaction) is:

$$O_2^{-} + H_2O_2 \rightarrow OH^{-} + OH^{\cdot} + O_2 \qquad E3$$

2.10.4. Nitric Oxide (NO•)

The enzyme nitric oxide synthase (NOS) catalyzes one of the terminal guanido nitrogen atoms of L-arginine to produce a radical nitric oxide (NO•) which is commonly known as nitrogen monoxide [6]. L-citrulline and L-arginine, both get converted into nitric oxide. Peroxynitrite results from the further reaction of nitric oxide with superoxide.

$$NO\cdot + O\cdot_2^{-} \rightarrow ONOO-. \qquad E4$$

Cellular damage results from the oxidation of proteins and many molecules which is a result of peroxynitrite's protonated (ONOOH) form acting as a powerful oxidizing agent for the sulfhydryl (SH) group. This can cause damage to the DNA as well like breaks, nitration of aromatic amino acid residues in proteins and protein oxidation.

2.11. DEFENSE MECHANISM AGAINST FREE RADICALS

To protect itself from cellular damage human body has developed a defense mechanism as a response to the level of frees radicals that exist due to both endogenous and exogenous sources. For this purpose, the body may put in place indirect and direct mechanisms which get involved.

2.11.1. Indirect Defense Mechanisms

In order to remove or convert the free radicals into less reactive forms the indirect mechanisms do not act directly. This indirect system can albeit act in many ways. Production of ROS can be endogenous due to some regulatory mechanisms which can regulate and control the processes.

The enzymes involved in the endogenous generation of ROS may be controlled in a transcriptional manner by this mechanism. For the repair of macromolecules, transporting certain enzymes and molecules to oxidative-damage sites may be done through another indirect approach. Repair of damaged DNA, lipids or proteins may be done through this process.

Figure 2.10. An illustration of DNA.

Source: *Image by Pixabay.*

To take an example from a nucleotide sequence, a normal nucleotide base can replace damage oxidized adducts of DNA like apurinic, thiamine glycol and 8-hydroxy-2-deoxyguanosine. Sometimes damaged molecules can be donated hydrogen atoms by certain molecules that act like repair compounds.

The membrane can get repaired by molecules like tocopherol or ascorbate when a fatty acid radical on cell membrane is donated a hydrogen atom. Some surface or natural cellular barriers like the cell membrane or skin prevent certain ROS that are endogenous from reaching the macromolecules that are targeted or prevent some exogenous ROS from entering the body thus acting as an indirect defense system against ROS.

These defense systems do not act on the ROS directly and most of the times, are non-specific whilst helping against ROS even though they are indirect defense mechanisms.

2.11.2. Direct Defense Mechanism

The direct defense system acts directly on free radicals by either scavenging, decomposing or converting free radicals into forms that are less reactive. Hence, this category of the antioxidant system is the most important one of the two groups namely the non-enzymatic and enzymatic antioxidants comprise this defense mechanism.

2.12. ANTHOCYANINS-SMART MOLECULES FOR CANCER PREVENTION

Within the plant kingdom the natural pigments that are the most widespread are the anthocyanins. It is recommended that humans must consume a large number of fruits and vegetables that surround us in abundance.

Certain benefits have been evident on the daily intake of anthocyanins like cardiovascular diseases reduce as well as there is a lesser risk of arthritis, cancer and diabetes partly due to the anti-inflammatory and antioxidant properties of these compounds.

There is a need for improvement of cancer treatment which has resulted in a greater interest in the anticancer properties of anthocyanins. In the 21st century cancer has reflected as the second in the list of top ten diseases.

The growth of cancer cells can be inhibited through these colorful pigments that, through modulation of genes or direct interaction, can modulate the activity of a number of targets involved in carcinogenesis. All the same, the poor bioavailability, biodistribution, and specifically poor absorption have led to some major concerns regarding the usage of anthocyanins as cancer agents.

Anthocyanins give a variety of colors to fruits, flowers and vegetables like red, blue and purple and are abundant flavonoid constituents composing the cell vacuole. In addition to being present in the flowers and fruits, the vegetative tissues too have an accumulation of anthocyanins wherein they provide protection against various kinds of abiotic and biotic stress conditions.

Amongst the plant pigments that are water soluble, these are the most important and largest group. Some of the vegetables and fruits rich in anthocyanins are berries, grapes, apples, black soybean, black rice and purple cabbage. Due to attacks by various kinds of pathogens and herbivores species, plants are extremely vulnerable in their natural environment.

A large spectrum of secondary metabolites has shown activity against their predators. A large group of compounds that are structurally diverse or the phenolics are among this category as are some anthocyanins and flavonoids. Plants are assisted by anthocyanins in myriad ways in their defense mechanism against other organisms like visual signals and chemical repellents.

In addition to the other flavonoids, antiviral, fungicidal and antibacterial activities have also been demonstrated by some anthocyanins. It is generally accepted that the reproductive success of the plants gets enhanced due to the colors of the fruits and flowers which facilitates the plants' communication with seed- dispersers and their pollinators.

The anthocyanins exhibit the potential to *in vivo* be antioxidants *in vivo* and *in vitro* which is an additional positive property of these compounds. Cell culture lines of various kinds including colon, breast, keratinocytes, leukemic cells, ovarian and endothelial liver, have been demonstrated with the potential antioxidant anthocyanins *in vitro*.

A series of anticarcinogenic and antiproliferative effects have been exhibited by anthocyanins when applied as treatment in vitro. A great property of anthocyanins is antioxidant activity and this was demonstrated that the responsibility for this lies with their chemical structure.

2.13. CHEMICAL STRUCTURE OF ANTHOCYANINS

As glycosides, the occurrence of anthocyanins in vegetables and fruits is natural wherein to an aglycone nucleus (anthocyanidin) one or more sugar molecules are attached. The presence of a heterocyclic ring (C) and two benzyl rings (A and B) characterizes their aglycones sharing a C6-C3-C6 carbon skeleton.

There are around 25 different kinds of aglycones according to the different positions of methylations and hydroxylation on the rings. Mainly they exist combined with galactose, glucose and rhamnose in the natural products where the more common sugar moieties are attached to the aglycone.

However, other sugars too are found frequently, which as per the varied substituent groups on flavylium B-ring can be sub-divided into a minimum of six common types namely: cyaniding, petunidin, peonidin, pelargonidin, malvidin and delphinidin.

Through acylated or glycosidic bonds with other aliphatic or aromatic acids, there may be the further linkage of the sugar attached to the aglycone with other sugars. Their color changing ability depends on the pH and this is perhaps their chemical structure's one of the most striking properties.

When the pH is low, they appear pink, in neutral conditions their appearance is purple and in basics they appear greenish yellow whereas at low pH there is domination by the most stable form making them natural indicators of pH.

2.14. ANTHOCYANINS' POTENTIAL HEALTH BENEFITS

Eating habits guide the amount of anthocyanins that are consumed as a large quantity of fruits are generally consumed by humans but the daily intake may be variable. Italy was found to have the highest consumption in Europe around 64.9 mg/day whereas in United States residents were found to consume around 12.5 mg/day.

The antioxidant, anticarcinogenic and anti-inflammatory properties of anthocyanins have shown in many studies to reduce the risk of diabetes, cardio vascular disease, cancer, and arthritis partially due to the anti-inflammatory properties possessed by them.

All aerobic cells produce ROS or reactive oxygen species which are important for cell signaling, in the immune system and a series of other normal functions of the body. Degenerative diseases like cardiovascular diseases, aging, inflammation and cancer can result from ROS if they are produced in excess.

The effectiveness of anthocyanins has been tested *in vivo* and *in vitro* and they are potent antioxidants as mentioned earlier. The chain reaction that causes oxidative damage is terminated by them and they also quench the free radicals. of particular importance is their antioxidant activity at pH levels that are neutral especially due to the pH in human body.

Rats/mice were administered an anthocyanin mixture to assess the antioxidant activity of anthocyanins *in vivo* and the rats/mice were then subjected to stress psychologically. Anthocyanins were seen to have antioxidant potency similar to vitamin E (α-tocopherol).

In the human trials, dietary anthocyanins demonstrated that they could prevent cardiovascular diseases and protect against LDL oxidation as they have the potential to enhance antioxidant capacity. Atherosclerosis leading

to cardiovascular diseases can result from deposits of oxidized cholesterol on the walls of the artery.

Anthocyanins can contribute towards the prevention of type 2 diabetes as studies have shown the anti-obesity effect that anthocyanins have on diets that are high in fat. Anthocyanins in black soybean have demonstrated in a study on a group of rats with a high fat diet to effectively reverse weight gain.

Furthermore, a condition associated with insulin resistance namely type 2 diabetes showed a decrease in a couple of varied reports which was shown to be related to the consumption of polyphenol-rich fruits and vegetables. Other scientific studies also revealed that night vision can be improved through the use of anthocyanins.

2.15. ANTICANCER PROPERTIES OF ANTHOCYANINS

Across the globe cancer, a major health problem is the cause of high mortality resulting from the growth of cells this is out of control and which spreads to different parts of the body. For accurate and early diagnosis, it is essential that routine monitoring and prevention is done.

Most of the times the therapeutic options can only decelerate the progress of cancer and cannot cure it. These options have a number of side effects, and their only aim is to improve the quality of life of the patients and extend their life.

A relatively safe alternative to the drugs that have undesirable adverse and side effects has been soft fruits like berries during the last few years as these fruits have chemotherapeutic and chemopreventive properties against ailments like cancer.

Results from the animal model tumor systems *in vivo* and *in vitro* cell culture as well as the data from human epidemiology related studies, have been included in the studies recently conducted on the anthocyanins' cancer preventive activities.

Several characteristics differentiate cancer cells from normal cells resulting in different function and morphology. The uncontrolled cycle of the cells is an essential characteristic of the cancer cells that leads to proliferation and division continuously.

Anthocyanins can block the cell cycle at different stages and cell proliferation has shown inhibition through anthocyanin-rich extracts and pure anthocyanins. Through the external death receptor pathway and internal mitochondrial pathway, anthocyanins induce apoptosis of cancer cells through the external death receptor pathway and internal mitochondrial pathway.

The programmed cell death or apoptosis is usually absent in tumor cells as a result, there is no normal elimination of dead cells. To avoid apoptosis cancer cells, deregulate various genes like p53 and compared to normal cells the resistance of these cells to death is higher.

There is an increase in the mitochondrial membrane potential in the intrinsic pathway towards cytochrome c release and modulation of anti-proapoptotic and caspase-dependent proteins due to the treatment of cancer cells by anthocyanins.

Anthocyanins modulate the expression of FASL and FAS in the extrinsic pathway leading to the cancer cells' apoptosis. In recent time, through various mechanisms like tumor necrosis factor alpha (TNF-α)-induced VEGF expression in epidermal keratinocytes and inhibition of H_2O_2 as well as by causing a reduction of the VEGF receptor and VEGF expression in endothelial cells anthocyanins have shown suppression of angiogenesis.

New blood vessels are formed from the vascular network that already exists for the metastasis and growth of tumors that are malignant through the physiological process of angiogenesis. Multiple cytokines control the process of angiogenesis and the vascular endothelial growth factor (VEGF) is the most important factor thus inhibiting the receptor of angiogenesis vascular endothelial growth factor receptor (VEGFR) which can effectively inhibit the metastasis of tumors.

Cancer cell invasion was found to be inhibited through anthocyanins which reduce the expression of urokinase plasminogen activator (u-PA) and metalloproteinase (MMP) both of which as a part of the invasive process cause a degradation of the extracellular matrix and both of them counteract the action of u-PA and MMP by stimulating the inhibitors' expression.

A patient's life and health are threatened by two main aspects of cancer cells, namely: metastasis and invasion. By facilitating the degradation of extracellular matrix barriers tumor cell extravasation becomes successful. The basement membrane's degradation is determined by the balance of the naturally occurring inhibitors and the activated proteases.

2.16. ROLE OF FLAVONOIDS AS WOUND HEALING AGENT

Across the globe, the bioactive compounds found most abundantly are the flavonoids. Quite a few medical plants contain flavonoids that are used in traditional medicines as agents for healing wounds like Lam, *Moringa oleifera, Parapiptadenia rigida, Buddleja globosa, Ononis spinosa* and *Butea monosperma*.

Currently flavonoids are used in various formulations and dressings for the healing of wounds. Wound healing involves reepithelialization, proliferation and inflammation. Multiple flavonoids exist in most of the medicinal plants that are used for healing wounds, and these flavonoids act as either combined effect or synergistic effect.

The biggest challenge around the world is the care of chronic and acute wounds. Among the different kinds of wounds, burns are a significant health issue in developing countries. Different methods can be used to heal wounds like nano dressing, medicinal plants, gene therapy, Functionalized Silk Biomaterials, negative pressure, growth factors and synthetic polymers.

Different pharmacological activities are possessed by flavonoids like anticancer activities, antioxidant, anti-inflammatory, capacity to scavenge free radical, hepatoprotective, prevention of coronary heart diseases and growth regulators making it a very important bioactive source derived from plants with medicinal properties.

Edema and redness followed by pain results from wounds. Wounds result in inflammation as the most prevalent characteristic as a result of eicosanoids, reactive oxygen species, leukotrienes and prostaglandins that are released.

Anti-inflammatory and antioxidant properties were seen in recent studies done on three species from Ghana *Commelina diffusa*, *Spathodea campanulata* and *Secamone afzelii* as well as the Buddleja species. Flavonoids and certain other bioactive compounds present in these species caused the anti-inflammatory and antioxidant properties wherein they worked in combination for the healing of wounds.

2.17. WOUND HEALING MEDICINAL PLANTS

Traditional wound healers used the alternative method of using medicinal plants to heal wounds. Curcuma longa is the most commonly used medicinal plant across the world. A few more species in Malaysia have wound healing

abilities like *Centella asiatica, Aloe barbadensis, Elephantopus scaber,* and *Clinacanthus nutans*. To heal wounds a list of the famous most famous medicinal plants is quite important.

2.18. THE ROLE OF FLAVONOIDS ON HUMAN HEALTH

There are over 8000 individual flavonoids that are known. From a wide range of vascular plants flavonoids can be isolated and these are essentially phenolic substances. A wide variety of activities are attributed to them like anti-oxidant, anti-cancer, wound healing, anti-microbial and anti-inflammatory. Flavanols and flavan-3-ols amongst the wide range of flavonoids possess a wide spectrum showing synergism with antibiotics and suppression of a number of factors related to microbial virulence. There is an emerging view about flavonoids that modulatory action may be exerted by them and their *in vivo* metabolites in cells through actions at lipid kinase and protein kinase signaling pathways even though the antioxidant properties have been the source for major focus with respect to the flavonoids.

Recently, it has been reported that flavonoids and their metabolites in particular act at Akt/protein kinase B (Akt/PKB), protein kinase C (PKC), phosphoinositide 3-kinase (PI 3-kinase), mitogen-activated protein kinase (MAP kinase) and tyrosine kinases signaling cascades.

The cellular function may be greatly affected by the stimulatory or inhibitory actions at these pathways by the modulation of gene expression and alteration of the phosphorylation state of target molecules. A major role is played by flavonoids for the prevention of cancer in many molecular mechanisms by the interaction between various kinds of enzymes and genes.

There has been an identification of many mechanisms of action, which includes inactivation of carcinogens, antiproliferation, induction of apoptosis, antioxidation, arrest of cell cycle, reversal of resistance towards multidrug, angiogenesis inhibition or a combination of these mechanisms.

2.18.1. Flavonoids and Wound Healing

For the wound healing modulation that catechins have, they are the class of flavonoids that are tested the most widely. Excessive or insufficient fibroblast activity causes a delay in the healing of wounds. Some authors have suggested that for treatment of injuries of the skin, it is beneficial to use flavonoids like apigenin.

Another flavonoid present in fruits, vegetables and medicinal plants is luteolin. In various wound models too, it showed itself as a wound healing agent. Activities to heal wounds can also be found in rutin which is present in medicinal plants.

2.18.2. Flavonoids In Fruits and Vegetables

Many vegetables and fruits contain flavonoids. Anti-carcinogenic flavonoids are present in at least nine fruits and 28 vegetables. Fruits like berries, apples and onions contain properties that can protect against cardiac ailments and positively impact vascular function, levels of serum lipid and blood pressure.

In the form of vegetables and fruits, the essential bioactive compounds namely flavonoids are present in our day-to-day life. It has properties that can help heal wounds. A series of research and studies conducted over a period of time have shown the importance of flavonoids as agents for healing wounds. An example is the *Ipomoea carnea* flower that belongs to the family Convolvulaceae.

In all animal models, the wound healing activities of isolated bioactive compounds like kaempferol-3-O-β-D-glucoside and Kaempferol have been confirmed. The structure-activity relationship it has with the different models of wounds is yet to be explored. Also, the mechanism involved in the healing of wounds is not known in detail as of now. Another aspect that is yet to be analyzed is the dosage and optimal use of flavonoids for the healing of wounds.

2.19. PLANT EXTRACTS, ENERGY, AND IMMUNE MODULATION IN BROILERS

Experiments were conducted on the male Ross 308 broiler chickens and this topic deals with the information that was obtained from these experiments. The effects of a standardized combination of plant extracts (PE) like carvacrol, *Capsisum oleoresin* and cinnamaldehyde; immunomodulation and hepatic oxidation concentration on the performance of birds was observed in these experiments.

Birds were fed one of the four diets and were reared under environments recognized within the industry. Based on either maize or wheat there were two diets formulated to be iso-nitrogenic and iso-energetic. The other two control diets were supplemented with hundred grams per ton of PE.

Efficiency of the dietary feed, hepatic antioxidant contents of the birds and dietary net energy improved with the feeding of plant extracts but the dietary energy that could be metabolized (ME) did not change. On the whole, the marker CD 40 LG and the mRNA transcript levels of three cytokines (IL-12B, IFN-G, and IL-6) got reduced in tonsils when plant extracts were fed.

By reducing intestinal inflammation, the gut health could be improved by the maximization of the feed's nutritional value with dietary PE. The availability of improved dietary energy, hepatic antioxidant content and immune status in birds is what is responsible for their mode of action.

All the same the full benefit to improve the efficiency in production of broiler meat may not have been detected in the studies which are focused solely on the effect on ME of PE. A wide range of substances are included in the phytogenic which are also referred to as the phytochemicals, botanicals, plant secondary metabolites or Phyto biotics, and these are extract/products derived from plants (PE).

The ones derived from spices and herbs are included in these like oleoresins and essential oils that have been known to exhibit properties that are therapeutic or/and growth-enhancing. The initial studies were done on PE as they exhibited adverse effects on animals which had ingested them.

Only recently there has been an interest in the usage of PE as an alternate to in-fed antibiotics so that the risk of development of antibiotic resistant pathogens can be prevented and the consumer demand for a poultry chain that is free of any drug residues can be satisfied.

During the last few decades in order to bring about efficiency in production and maintain health poultry diets have been supplemented with antibiotics. The risk of bacterial disease has increased due to the withdrawal in the use of in-feed antibiotics for promotion of growth more so in poultry.

The exact manner in which the effects of PE are exerted continues to be a matter of speculation even though there is sufficient documentation of the ability of PE to contribute towards health. It is well documented that there is a vast group of diverse natural products that comprise plant extracts. Some of them have no nutritional value whereas others even possess anti-nutritional properties even though some are quite valuable as far as nutrition goes.

The variation in the PE within their chemical structures could be the likely cause for this. An explanation that is uniform for their mode of action is near impossible owing to the fact that, to quite an extent, the compounds'

chemistry has a direct impact on the effects of PE. The main benefits that can accrue from the addition to poultry diets of selective PE have been briefly outlines in this topic. The manner of action of cinnamaldehyde, capsaicin and carvacrol has been described when as a standardized commercial mix, it is fed to broiler chickens.

2.20. EFFECTS OF DIETARY PLANT EXTRACTS WHEN FED TO POULTRY

PE extracts can have an effect on the energy available through diet as well as the growth performance of the birds. In the gastrointestinal tract of poultry, the overall digestive functions can be stabilized through PE additives as per a hypothesis.

A number of trials have been reviewed with respect to feeding the birds with dietary supplements including PE. The effect of PE was detrimental in eighteen the studies reviewed, in seventeen studies it had no effect and in eleven studies it showed benefits with respect to nutrient digestibility and growth performance (this was primarily assessed as efficiency of the feed).

There is inconsistency in the information pertaining to the effects that PE has on dietary metabolizable energy (ME). The data that has been published with respect to the differences in ME and the performance on growth of dietary PE fed birds also has a discrepancy.

Studies conducted recently did not show an improvement in dietary ME even though the growth performance of birds improved when the poultry was fed with various PE. Reports are available to show that when broiler chickens were fed with a standardized commercial mix of cinnamaldehyde, capsaicin and carvacrol, there was a parallel improvement in feed efficiency and dietary ME.

2.20.1. Antioxidant Status

The interest in the studies on natural additives as potential antioxidants has been increasing as consumers are rejecting food additives that are synthetic. Research has shown the efficacy of antioxidant properties possessed by spices and herbs in retarding the lipid peroxidation process in fatty foods and oils.

The oxidative stability of products derived from animals like eggs, meat, rabbit meat, poultry and pork also improved due to the herbal phenolic compounds. Moreover, research revolving around poultry and rats showed

that the antioxidants status of animals improved with dietary phytogenic supplements wherein the integrity of the mucosal layer of the intestine was sustained and damage to the intestinal cell reduced.

These supplements influenced the animals' *in vivo* oxidant defense system and acted as effective scavengers for the free radical. Additionally, diets which were supplemented with antioxidative property possessing curcumin, turmeric, grape seed proanthocyanidins, green tea and society garlic lowered oxidative stress, brought about a reduction in the small intestinal lesion scores and during coccidial infection brought about an improvement in weight gains.

By protecting the tissues that are infected from any kind of an oxidative damage the anticoccidial activity of these compounds may be exerted which resulted in a reduction of coccidiosis severity.

2.20.2. Immune Status

When the indicators of humoral and cellular immunity and the defense factors that are nonspecific change, it is referred to as immunomodulation. Immunomodulation may occur in forms like immune-stimulation (when the components or mediators of the immune system are induced or activated by these substances) or immunosuppression (when the immune system is inhibited by these substances) as a result of which the scope, duration, competency or type of the immune response gets altered or regulated.

There is a speculation that the benefit associated with the usage of PE in animal diets is linked with a reduction in inflammation of the intestine partly from the reduction of proinflammatory cytokines. In one study it was observed that production of tumor necrosis factor (TNF), IL-1 and interleukin 6 (IL-6) which was lipopolysaccharide-induced got suppressed by cinnamaldehyde leading to the deduction that suppressive effects could be shown by cinnamaldehyde on the production of different kinds of inflammatory cytokines.

In the same way against clinical signs of experimental avian necrotic enteritis when a mixture of turmeric and capsicum oleoresins was supplied in dietary form it was found to be an effective phytonutrient. A research was conducted by Lee which suggested for shedding of an oocyst, improvement in the weight gain, increased Il-5 and interferon gamma (IFN-γ) immunomodulatory effects are responsible when birds challenged with coccidia were fed with powder from oriental plum (a phenolic compound rich plant).

Moreover, both humoral and cellular immune response showed an enhancement in chickens infected with *Eimeria tenella* when supplemented with Chinese herb extracts and mushroom.

The antimicrobial properties of PE have strong evidence which *in vitro* at minimal inhibitory concentration of 0.05–5 microliters per milliliter are able to bring about a reduction in proliferation of pathogenic organisms and in food, at higher concentrations (0.5–20 microliters per gram) they are able to bring about similar results.

The pathogens that are commonly found in poultry production can be effectively dealt with through the antimicrobial action of PE as per the available evidence even though it is not likely for the levels to be met in the feedstuffs of animals and hence in feed cannot be PE's primary use.

These pathogens include *Clostridium perfringens* and *Eschericia coli.* In the gastro intestinal tract the immune responses get triggered by the pathogenic microorganisms present in the gut. This leads to poor gut health as a result of inflammation caused to the intestine.

Direct immunomodulation properties may also be possessed by PE in addition to reducing the challenge posed by pathogens. For instance, the gene regulation activity of cinnamaldehyde is particularly known which includes antigen presentation, inflammatory diseases and humoral immune response.

2.20.3. Chemical Structure and Properties of Carvacrol, Cinnamaldehyde and Capsaicin

Several plants contain the chemical Carvacrol ($C_{10}H_{14}O$) which includes wild bergamot, pepperwort and thyme but oregano (Origanum vulgare) oil has an abundance of this chemical. The slightly spicy flavor of oregano is due to carvacrol which also makes it colorless and lends it a distinct warm odor.

Carvacrol has antibacterial, antioxidant, antiviral and antifungal impact which is quite promising. When tested against prostrate, lung, mouth and breast cancer cells, significant anti-cancer effects have been demonstrated by carvacrol.

The inner bark of many tree species belonging to the genus Cinnamomum has the naturally occurring chemical cinnamaldehyde (C_9H_80). The main component of the cinnamon bark derived essential oil is cinnamaldehydy which gives cinnamishodor and the latter is responsible for the cinnamon

spice's characteristic odor and taste. Strong antifungal, anticorrosion and antimicrobial properties are possessed by cinnamaldehyde.

In order to protect themselves from fungi and certain mammals, peppers produce capsaicinoids ($C_{18}H_{27}NO_3$). These are odorless and flavorless compounds that directly act on the throat and mouth-based pain receptors.

The most common capsaicinoid is capsaicin, and for most animals, including humans this is an irritant which produces a burning sensation in any tissues that come in contact with it. All the same, birds have no sensitivity to capsaicin and can derive benefit from the chili peppers' nutritional value. In addition, pepper fruits contain capsicum oleoresin (capsaicin is the active ingredient), which has antibacterial and antifungal activity.

2.21. CONCLUSION

In the conclusion, this chapter discussed about the role of phytochemicals in the prevention of any disease. It also discusses the significance of allelochemicals as phytochemicals in the plant rhizosphere and it's ecological role. In the chapter, production of plant-based drugs and harvest of medicinal plants have also been discussed.

Towards the end of the chapter, free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases, sources of free radicals, types of free radicals or reactive oxygen species have been discussed. This chapter discusses about the role of flavonoids on human health, anthocyanins-smart molecules for prevention of cancer, chemical structure of anthocyanins, and its potential health benefits.

REFERENCES

1. Asaduzzaman, M., & Asao, T., (2018). Introductory Chapter: Phytochemicals and Disease Prevention. Phytochemicals – Source of Antioxidants and Role in Disease Prevention, [online] Available at: https://www.intechopen.com/books/phytochemicals-source-of-antioxidants-and-role-in-disease-prevention/introductory-chapter-phytochemicals-and-disease-prevention (accessed 9 September 2021).
2. Aslam, M., Ahmad, M., Riaz, H., Raza, S., Hussain, S., Qureshi, O., Maria, P., Hamzah, Z., & Javed, O., (2018). Role of Flavonoids as Wound Healing Agent. Phytochemicals – Source of Antioxidants and Role in Disease Prevention, [online] Available at: https://www.intechopen.com/books/phytochemicals-source-of-antioxidants-and-role-in-disease-prevention/role-of-flavonoids-as-wound-healing-agent (accessed 9 September 2021).
3. Diaconeasa, Z., Frond, A., Ştirbu, I., Rugina, D., & Socaciu, C., (2018). Anthocyanins-Smart Molecules for Cancer Prevention. Phytochemicals – Source of Antioxidants and Role in Disease Prevention, [online] Available at: https://www.intechopen.com/books/phytochemicals-source-of-antioxidants-and-role-in-disease-prevention/anthocyanins-smart-molecules-for-cancer-prevention (accessed 9 September 2021).
4. Engwa, G., (2018). Free Radicals and the Role of Plant Phytochemicals as Antioxidants Against Oxidative Stress-Related Diseases. Phytochemicals – Source of Antioxidants and Role in Disease Prevention, [online] Available at: https://www.intechopen.com/books/phytochemicals-source-of-antioxidants-and-role-in-disease-prevention/free-radicals-and-the-role-of-plant-phytochemicals-as-antioxidants-against-oxidative-stress-related- (accessed 9 September 2021).
5. Ogunmefun, O., (2018). Phytochemicals—God's Endowment of Curative Power in Plants. Phytochemicals – Source of Antioxidants and Role in Disease Prevention, [online] Available at: https://www.intechopen.com/books/phytochemicals-source-of-antioxidants-and-role-in-disease-prevention/phytochemicals-god-s-endowment-of-curative-power-in-plants (accessed 9 September 2021).
6. Pirgozliev, V., Mansbridge, S., Rose, P., & Bravo, D., (2018). Plant Extracts, Energy, and Immune Modulation in Broilers. Phytochemicals – Source of Antioxidants and Role in Disease Prevention, [online]

Available at: https://www.intechopen.com/books/phytochemicals-source-of-antioxidants-and-role-in-disease-prevention/plant-extracts-energy-and-immune-modulation-in-broilers (accessed 9 September 2021).

CHAPTER 3

CHEMISTRY AND CLASSIFICATION OF DIETARY PHYTOCHEMICALS

CONTENTS

3.1. INTRODUCTION

Plants contain certain chemical compounds that occur naturally and are biologically active called the phytochemicals that have tremendous benefits for human health as nutrients and natural ingredients (Hasler & Blumberg, 1999).

The plants' color, flavor, and aroma are due to the phytochemicals that protect them from damage and disease. These chemicals protect the plants from environmental hazards like pollution, drought, pathogenic attack, UV exposure and stress (Gibson et al., 1998; Mathai, 2000). Recent studies have shown that when ingested in significant quantities, the phytochemicals also protect the health of humans (Samrot et al., 2009; Koche et al., 2010).

The 4500 phytochemicals that have been reported so far and about 350 of them have been the subject of detailed study. They are classified from the point of protective functions they perform and the chemical and physical characteristics possessed by them (Loche et al., 2010). Vegetables, fruits, whole grains, legumes, seeds, nuts, fungi, spices and herbs have a wide range of dietary phytochemicals (Mathai, 2000).

Figure 3.1. Vegetables is a rich source of phytochemicals.

Source: *Image by Flickr.*

The other sources that commonly have phytochemicals include whole wheat bread, broccoli, carrots, cabbage, garlic, onion, grapes, tomatoes, strawberries, cherries, raspberries, strawberries, soy foods and beans (Moorachian, 2000). Different parts of the plants have storehouses of phytochemicals like root, leaf, stem, seed, fruit and flower (Costa et al., 1999).

More often than not, the outer layer of various parts of a plant such as leaves, vegetables and fruits contain phytochemicals, especially the pigment molecules like flavonoids and anthocyanins. All the same, phytochemicals vary in their levels in different plants depending upon the climatic conditions under which they are growing and the variety of the plant (Rao, 2003).

Various biological activities are present in these plants like anti-microbial effect, antioxidant activity, modulation of detoxification enzymes, decrease of platelet aggregation, anticancer property, stimulation of the immune system and modulating the metabolism of hormones (Hamburger & Hostettmann, 1991). Around the same time, the anti-nutritional properties of some of the chemicals in plants were reported by Hosborne (1991).

3.2. CLASSIFICATION OF PHYTOCHEMICALS

Due to the diverse structures and forms of phytochemicals, so far, an exact classification has not been given for them. Depending upon the role played by the phytochemicals in the metabolism of plants, they are classified into primary metabolites and secondary metabolites. Common sugars, proteins, amino acids, chlorophylls, pyrimidines and purines of nucleic acids, etc. are included in the primary metabolites.

All the remaining plant chemicals like alkaloids, flavonoids, plant steroids, saponins, glucosides, terpenes, lignans, curcumines and phenolics are the secondary metabolites (Hahn,1998; Ramawar et al., 2009). The literature survey has shown that the structurally most diverse and common of the plant chemicals are the phenolics.

Figure 3.2. Classification of phytochemicals.

3.2.1. Phenolic Compounds

The most widely distributed plant chemicals in the plant kingdom falling into the phytochemicals' largest category are phenolic compounds (Walton

et al., 2003). From the hydroxyl group (-OH) of compounds, phenolics have the (-OH) group directly bonded to an aromatic hydrocarbon group. In this group of natural compounds, the simplest group is considered to be that of phenol (C_6H_5OH).

An important role is played by them as defense compounds as these are secondary metabolites. Phenolics exhibits several properties beneficial to humans and the role they play as agents that provide protection against diseases that are mediated by free radicals is due to the antioxidant properties that they possess.

The most studies and largest group of plant phenols are flavonoids (Dai & Mumper, 2010). By nature, these are found everywhere being ubiquitous and are polyphenolic compounds occurring as methylated derivatives, glucosides and aglycones.

Many of the 4000 flavonoids recognized so far occur in fruits, vegetables and beverages like coffee, fruit drinks and tea (Pridham, 1960). Since ancient times, the flavonoids played a major role in the success of the medical treatments, which continues to happen even now.

Naturally in conjugated forms, most of the flavonoids associate with sugar and they may be characterized as diglycosidic, mono glycosidic, etc. within any one class. Normally, the glycosidic linkage is located at position three or seven and Lrhamnose, glucorhamnose, arabinose, D-glucose or galactose can be the carbohydrate unit (Pretorius, 2003).

Due to the broad range of pharmacological activities and biological properties possessed by the flavonoids, they have gained a lot of attention recently. Reports have shown that multiple biological properties are exerted by flavonoids like cytotoxic, anti-tumor, anti-microbial and anti-inflammatory.

However, their capacity in acting as powerful antioxidants is the property that describes them best, and every group of flavonoids possesses this property (Shirsat et al., 2012; Teiten et al., 2013). These antioxidants have the ability to protect the body of humans from reactive oxygen species (ROS) and dangerous free radicals.

Hydroxycinnamic and hydroxy-benzoic acids, the widely distributed acids, form the diverse group of phenolic acids. There are two classes of phenolic polymers known as tannins, namely condensed tannins and hydrolysable tannins and the molecular weight of these compounds is high.

Generally, phenols that have one functional group of carboxylic acid are designated as 'phenolic acids.' Two distinctive carbon frameworks, namely hydroxybenzoic and hydroxycinnamic structures, are contained in the phenolic acids that occur naturally.

With hydroxyl carboxylic acids and glucose, hydroxycinnamic acid compounds are produced in the form of esters that are simple. Hydroxylated aromatic rings characterize the plant phenolic compounds whose molecular structure is quite different (Balsundaram et al., 2006).

The properties of these compounds against damage caused by oxidation which leads to degenerative diseases of various kinds have been mainly studied like in the case of cardiovascular diseases, cancer and inflammation.

Compared to the normal cells, cancer cells including the leukemia cells as well have reactive oxygen species that are of higher levels as a result of which their sensitivity to oxidative stress is quite prevalent (Mandal et al., 2010).

3.2.2. Tannins

Tannins encompass polymers and oligomers, some of which are quite diverse, making it quite difficult to define them (Harborne, 1999). Tannins may be considered as a group of polyphenolic compounds with a high molecular weight and are a heterogeneous group with a capacity to form mainly with proteins, alkaloids, minerals, nucleic acids and polysaccharides (hemicelluloses, cellulose, pectin, etc.) both irreversible and reversible complexes (Schofield et al., 2001).

Tannins can be divided into four major groups depending upon their structural characteristics namely: ellagitannins, Gallo tannins, condensed tannins and complex tannins.

Ellagitannins: Tannins that do not contain a catechin unit that is linked glycosidic ally and have at least two galloyl units coupled C-C.

Gallo tannins: In these tannins the meta-depsidic derivatives of the galloyl units or the galloyl units are bound to varied/ diverse catechin-, triterpenoid, polyol- units.

Condensed tannins: This group consists of all polymeric and oligomeric proanthocyanidins formed by linkage of C-4 of one catechin with C-6 or C-8 of the monomeric catechin that is next.

Complex tannins: These tannins have a glycosidic ally bound catechin unit to an ellgitannin or Gallo tannin unit.

Fruits like persimmon, grapes, blueberry, chocolate, legume trees like Sesbania spp., Acacia spp., corn, grass like sorghum, tea, legume forages, etc., commonly have tannins in them (Ginerchavez, 1996). The consumption of tannins and some epidemiological associations have been linked to a number of health benefits with the frequency of chronic diseases showing a decrease (Serrano et al., 2009).

The hike in the reporting of deadly diseases like cancer and AIDs has resulted in an enhanced interest of the scientific community in the tannins. It has become extremely important to search for novel compounds that have lead so that new pharmaceuticals products can be developed more so as the documentation of the plants containing tannins and their biological action has been done quite well (Mueller-harvey,1999).

3.2.3. Alkaloids

Heterocyclic nitrogen atom containing alkaloids are natural products that by character are always basic in nature. The root word for alkaloids is 'alkaline' which is owing to their alkaline nature and any base containing nitrogen was described through this (Muller-Harvey, 1999). A bitter taste underlines most of the alkaloids.

For instance, quinine, an alkaloid is one of the bitter-tasting substances that are known and at a molar concentration it is significantly bitter (1×10^{-5}) (Mishra, 1989). The rational classification of alkaloids is quite difficult as they involve various molecular structures and are numerous.

Grouping them into families is the best possible approach to the kind of heterocyclic ring system that makes up its molecules (Krishnan et al., 1983). Following is a list of the different classes of alkaloids as per the heterocyclic ring system which makes them up.

Pyrrolidine alkaloids: Pyrrolidine (tetrahydropyrrole) ring system makes up these alkaloids. For instance, the leaves of *Leonotis spp.,* and *Erythoxylum* spp. contain hygrine.

Pyridine alkaloids: The piperdine (hexahydro pyridine) ring system makes up these alkaloids. For instance, piperine, coniine and isop-lletierine.

Pyrrolidine-pyridine alkaloids: The heterocyclic ring system with pyrrolidi-nepyridine makes up these alkaloids. Examples include myosmine, nicotine that is present in tobacco (Nicotianatabaccum).

Pyridine-piperidine alkaloids: a pyridine ring system is joined to a ring system of piperidine in this family of alkaloids. This includes anabasine from *Anabasis aphyllan*.

Quinoline alkaloids: Quinoline the basic ring system is contained in these. The bark of the cinchona tree for example contains quinineoccurs.

Isoquinoline alkaloids: The heterocyclic ring system iso-quinoline makes up these alkaloids. Examples include opium alkaloids like papaverine, narcotine, heroine, codeine and morphine.

Alkaloids protect the plants from micro-organisms (antifungal and antibacterial activities), herbivores (feeding deterrents) and insects as well as other plants through the means of allelopathy thus making them quite important for the plant's survival (Molineux et al., 1996).

From the time that civilization started, plants containing alkaloids have been used in the form of dyes, poisons, drugs or spices. Alkaloids have shown the possession of a number of pharmacological activities which include anti-hypertensive effects (many indole alkaloids), anti-malarial activities (quinine), antiarrhythmic effect (spareien, quini-dine) and anti-cancer activities (vincristine, dimericindoles, vinblastine).

This group of plants has tremendous economic importance due to the pharmacological and other properties possessed by them. Stimulant property is present in certain alkaloids like nicotine, morphine, and caffeine, which is used as an analgesic and quinine, is used as a drug against malaria (Wink et al., 1998).

3.2.4. Terpenoids

Turpentine (balsamumterebinthinae) is the root word for the term terpenes. The "resin of the pine trees" also known as turpentine, is a balsam with a pleasant viscous smell and it flows when the new wood or bark of various species of the pine tree (Pinaceae) is carved or cut.

Some hydrocarbons and certain "resin acids" are present in turpentine and this was referred to as terpenes originally. Traditionally, isoprene subunits built up all the natural compounds and mostly this originated from the plants whereby they were dented as terpenes (Breitmaier, 2006).

The potential to manufacture terpene natural products exists in all living organisms as for various kinds of physiological functions terpenes are manufactured by all living beings. There has been a large amount of elaboration on the diversity of structures and large number of these

compounds that have been observed as the basic C5 units can combine in a number of ways and the organisms have evolved over a period of time under different selection pressures (Gershenzon and Dudareva, 2007).

Comprising of about 36,000 terpene structures (Buckingham, 2007) the largest group of natural products are the terpenes (known as isoprenoids and terpenoids as well) even though from the perspective of function very few of them have been investigated.

The number of isoprenoid units present in the terpenoid structure is the basis for the terpenoids classification. Compounds with eight (tetraterpenes), six (triterpenes), five (sesterterpenes), four (diterpenes), three (sesquiterpenes) and two (monoterpenes) isoprenoid units form the largest category (Ashour et al., 2010).

Most of the basic processes of plants have terpenoids performing roles that have been well established such as development, growth, defense and reproduction (Wink and van Wyk, 2008). Among the lower (C5-C20) terpenes that are best known, gibberellins are a large group of diterpene plant hormones that has a role to play in the control of stem elongation, germination of seed and induction of flower (Thomas et al., 2005).

The oxidative cleavage of a C40 carotenoid precursor forms abscisic acid (ABA) another terpenoid hormone so it is not really considered as a lower terpenoid (Schwartz et al., 1997). Natural products derived from the isoprene units that are five-carbon form up this class. The multi cyclic structures of most terpenoids differ from each other in their basic carbon skeletons and functional groups. All classes of living organisms have these natural lipids, which make them the largest occurring group of secondary metabolites that exist naturally (Elbein et al., 1999). These are used in fragrances and flavors that are then used in cosmetics making most of them quite viable commercially, generating interest from these industries (Horborne & Tomasbarveran 1991).

Terpenes are mainly present in plants as essential oils and are spread widely in nature. Hydrocarbon isoprene ($CH_2 = C (CH_3) – CH = CH_2$) is the building block for terpenes.

Hemiterpenoids – A single unit of isoprene comprises hemiterpenoids. Isoprene is the only hemiterpene however, phenol and isovaleric acid that are derivatives containing oxygen too fall within this classification.

Monoterpenoids – Two units of isoprene make up monoterpenoids. These may be of two kinds namely containing rings or linear (acyclic) e.g., Eucalyptol, Citral, Pinene, Camphor, Limonene and Geranyl pyrophosphate.

Sesquiterpenes – Three units of isoprene make up the sesquiterpenes. Examples are Bisabolol, Fernesol and Artmisinin; cyclic compounds like Eudesmol that is present in Eucalyptus oil.

Diterpenes – four units of isoprene comprise diterpenes. They are derivatives of geranyl-geranyl pyrophosphate. Some examples include cembrene, cafestol, taxadiene and kahweol. Diterpenes are used as the base in the biologically important compounds retinal, retinol and phytol.

Triterpenes – Six isoprene units comprise triterpenes. Some examples are Squalene and Lanosterol found in olives and wheat germ.

Tetrapenoids – Tetrapenoids comprise eight units of isoprene which may be bicyclic like beta- and alpha- carotenes, acyclic like lycopene or monocyclic like gamma-carotene.

Structurally, terpenoids among the various secondary metabolites of plants are not a very diverse group. Their main function is to as signals in indirect response for defense or in direct defense functioning as phytoalexins in the plants involving mainly their enemies and herbivores (Mccaskill & Croteau, 1998).

To be able to attract specific insects that can help in pollination, volatile terpenes are produced by certain plants. Others produce toxic or strongly bitter-tasting even though fewer volatile terpenes so that animals do not eat those plants and the plants stay protected (Degenhardt et al., 2003).

Additionally, medical properties may be present in terpenoids like anti-malarial (e.g., artemisinin), anti-carcinogenic (e.g., perilla alcohol), antimicrobial, hepaticidal, anti-malarial (e.g., artemisinin), diuretic (e.g., glycyrrhizin) or anti-ulcer activity. Anti-malarial drug artimisinin and diterpenoid anti-cancer drug taxol are some other examples (Langenheim, 1994; Dudareva et al., 2004).

3.2.5. Saponin

Stable foam is formed by most of this group's members in soap like aqueous solutions giving it the name 'saponin.' As a group, chemically saponins include compounds like steroid alkaloids, triterpenoids and glycosylated steroids.

Furostan and spirostan derivatives are the two main kinds of steroid aglycones that are known. A derivative of oleanane is the main triterpene aglycone (Bohlmann et al., 1998). One or more sugar moieties are present in the carbohydrate part which contains galactose, glucose, arabinose,

glucuronic acid, xylose or rhamnose linked to a sapogenin (aglycone) glycosidic ally.

Mono-desmosidesaponins are the saponins. At the C-3 position, one molecule of sugar is attached whereas bi-desmosidesaponins have at least two sugar molecules attached, one to the C-3 position and the other to C22 (Lasztity et al., 1998).

The anti-microbial activity is quite prevalent in many saponins wherein they protect the plant from an attack by insects and inhibit mold. A number of molecules of the plants that are protective in nature like phytopretectants or phytoanticipins and this large group of molecules include saponins that can be taken to be the defense system of the plants (Lacaille-Dubois & Wagner, 2000).

When the saponin mixtures present in the plant products and in plants, are present in animal body, their biological effects are quite diverse. The hypo-cholesterolaemic, immunostimulant, anti-carcogenic and membrane permeabolizing properties of saponins have been the subject of extensive research and they have been found to affect feed intake, growth as well as reproduction quite extensively in animals.

The other activities and properties of these structurally diverse chemicals that have been observed include their ability to kill mollusks and protozoans, act as antioxidants and antiviral as well as antifungal agents (Takechi et al., 1999; Morreissy & Osbourn, 1999; Traore et al., 2000).

3.2.6. Polyphenols

In the human diet, the most abundant antioxidants are the secondary plant metabolites, polyphenols. One or more hydroxyl moieties are carried by an aromatic ring in the design structure of these compounds. The structural elements that bind the rings and the number of these phenol rings decide the several classes that can be considered for the polyphenols.

Traditionally, two groups of polyphenols have been adopted in this context namely nonflavonoids and flavonoids. Flavonoid group consists of compounds with C6-C3-C6 structure like flavones, flavanones, flavanols, di-hydro-flavanols, isoflavones, flavan-3-ols, proanthocyanidins and anthocyanidins.

The number of carbons present classifies the nonflavonoids group and the various subgroups in this category are: simple phenols, hydrolyzable tannins, benzoic acid, acetophenons, cinnamic acids, phenylacetic acids,

coumarins, stilbenes, benzophenones, chalcones, xanthones, secoiridoids and lignans (Andrés-Lacueva et al., 2010).

3.2.7. Glucosinolates

Several vegetables contain a group of plant thioglucosides or the glucosinolates (GLS) (Larsen, 1981) which are a class of organic compounds derived from amino acids and glucose and contain nitrogen as well as sulfur (Anastas and Warner, 1998). Sinalbin, the first crystalline glucosinolate was isolated in 1831 from mustard seeds and since then more than a hundred varied GLS have been characterized.

Even though the presence of GLS has been reported in other families, mostly the order Capparales, principally in the Resedaceae, Capparidaceae and Cruciferae families contain GLS (Larsen, 1981). Some plants that contain GLS and are economically important include brown mustard, white mustard, horse radish, kohlrabi, cress, cabbages (white, red and savoy), broccoli, swede, Brussels sprout, kale, rapeseed, cauliflower and turnip (Fenwick et al, 1989)

The chemoprotective properties of GLS metabolic and hydrolysis products have been proven against chemical carcinogens wherein they block the initiation of tumors in various tissues like liver, pancreas, mammary glands and colon.

Through the induction of Phase I and Phase II enzymes, their effect is exhibited along with an inhibition of the enzyme activation, modification of steroid hormone metabolism, and protection against damage by oxidation.

Detoxification of carcinogens is facilitated by GLS through the induction of Phase I and Phase II enzymes. Glucosinolate metabolites selectively inhibit some of the enzymes of Phase I reaction which cause the activation of carcinogens (Das et al., 2000).

3.2.8. Dietary Fiber (Non-Starch Polysaccharides)

The analogous carbohydrates or edible parts which can resist digestion and consequent absorption in the small intestine are the dietary fibers and their partial or complete fermentation takes place in the large intestine. Lignin, oligosaccharides, polysaccharides and associated plant substances comprise the dietary fibers.

Physiological effects that are beneficial are promoted through dietary fibers like blood cholesterol attenuation or/and laxation or/and blood

glucose attenuation (AACC, 2001). Glycosidic linkages join the polymers of monosaccharides in dietary fibers and they are classified on the basis of structural considerations as mentioned below:

1. Monosaccharide ring forms (five membered furanose or six membered pyranose).
2. Identity of the monosaccharides that are present.
3. Configurations (a or b) of the glycosidic linkages.
4. Positions of the glycosidic linkages.
5. Absence or presence of non-carbohydrate substituents.
6. Sequence of monosaccharide residues in the chain.

Cereal cell walls usually have the following monosaccharides:

1. Acidic sugars- D-galacturonic acid, D-glucuronic acid and its 4-O-methyl ether (Choct, 1997).
2. Pentoses- D-xylose, L-arabinose
3. Hexoses- D-galactose, D-glucose, D-mannose.

In 1997 Cummings brought forth that DF provided health benefits do not cater for distinct characteristics related to the disease that can be associated with it exclusively. The closest criterion to this is fulfilled by constipation which makes it clear that specific fibers have demonstrated certain physiological and functional effects namely:

1. Stool output or fecal bulking (xanthan gum, wheat bran and ispaghula).
2. Lowering of plasma (LDL-) cholesterol (β-glucans or oat bran, psyllium, very viscous guar gum, pectins).
3. Lowering of the postprandial blood glucose response (β-glucans or guar gum that is quite viscous).

   Even though there is the availability of quite a bit of evidence from animal models or in vitro, in human subjects, the other effects have not yet been demonstrated like the colonic health effects that are related to the fermentation products (Champ et al., 2003).

3.2.9. Lectins

Lectin is derived from the word ‘lectus’ which is the past participle of legere which means to choose or select. These carbohydrate binding proteins other than antibodies or enzymes are present in most of the living organisms then

be it animals and plants or bacteria and viruses. In many species they have shown an involvement in diverse biological processes like in the circulatory system clearing the glycoproteins, adhesion of infectious agents to host cells, cell interactions in the immune system, recruitment of leukocytes to inflammatory sites, in metastasis and malignancy (Ambrosi et al., 2005).

3.2.10. Other Phytochemicals

3.2.10.1. Polyacetylenes

Due to the toxicity of polyacetylenes, they were considered undesirable in plant foods previously and they are essentially bioactive secondary metabolites (Czepa and Hofmann, 2004). The beneficial effects that these vegetables and fruits have on human health revolves can probably be explained through the important factor of taking in or consuming these "toxins' in limited amounts on a daily basis.

For instance, carrots have polyacetylenes that have shown to be extremely toxic against various cancer cell lines. From higher plants more than 1400 varied polyacetylenes and compounds related to them have been isolated.

Araliaceae and Apiaceae have a wide distribution of aliphatic C-17 polyacetylenes which are of the falcarinol kinds like falcarindiol and falcarinol (Hansen and Boll, 1986; Bohlmann et al., 1973). As a result, most of the polyacetylenes that can be found in the edible/utilized parts of the food plants of the Apiaceae are of the falcarinol kind like parsley, carrot, parsnip and celeriac.

Apiaceae, Asteraceae and Araliaceae plant families commonly have a polyacetylene called falcarinol that has anti-cancer properties (Zidorn et al., 2005). Some other plants that were found to have other polyacetylenes are *Centella asiatica, Dendranthema zawadskii* (Dendrazawaynes A and B), *Panax quinquefolium L.* (*American ginseng*) and *Bidens pilosa* (*Cytopiloyne*).

3.2.10.2. Allium Compounds

Volatile odor principles were identified by early investigators in garlic oils all the same, it was only during tissue preparation and damage that these compounds were generated. Usually, the Allium species' vegetative tissue has been observed to be free of odor, which led to the hypothesis

that nonvolatile precursor substances caused the generation of volatile compounds in Allium species.

Allin, the first stable precursor compound (+)-S-allyl-L-cysteine sulfoxide (ACSO) was identified in 1948 in the laboratory of Seebrook and Stroll. Among vegetables, this compound makes sulfur-containing molecules that are unique to garlic (Stoll and Seebeck, 1947). Cut or crushed garlic produces a series of odorous volatiles and in a majority of them the parental sulfur compound responsible for it is alliin.

Later, in the laboratory of Matikkala and Virtanen, three sulfoxides were additionally identified being present in the onion tissues. These were: (+)-S-propyl-L-cysteine sulfoxide (propiin; PCSO), (+)-S-trans-1-propenyl-L-cysteine sulfoxide or isoalliin (TPCSO) and (+)-S-methyl-L-cysteine sulfoxide (methiin; MCSO).

The source of the *A. cepa* lachrymatory factor in intact onion tissues is isoalliin which is the main sulfoxide here (Virtanen and Matikkala,1959). The most ubiquitous chemical concerning its distribution is (+)-S-methyl-L-cysteine sulfoxide in the intact tissues of *A. porrum, A. ursinum L* and *A. sativum* in varying amounts.

Allyl compounds, the oil –soluble compounds that usually account for 0.2–0.5% of the garlic extracts, upon oxidation and hydrolysis get generated like diallyl trisulfide (DATS), 5 allyl disulfide (DADS), diallyl sulfide (DAS) and other allyl polysulfides. On the other hand, allyl compounds that are soluble in water can be formed from it slowly like S-allylmercaptocysteine (SAMC) and S-allyl-cysteine (Filomeni et al., 2008).

3.2.10.3. Chlorophyll

Almost all green plants, cyanobacteria and algae contain chlorophyll or chlorophyll. It has its origins in the Greek words “chloros” meaning green and “phyllon” meaning leaf. Being critical for photosynthesis, chlorophyll is a very important biomolecule that enables plants to use light for deriving energy.

The electromagnetic spectrum’s blue portion is absorbed most strongly by chlorophyll and then comes the absorption of the red part. The green color of tissues containing chlorophyll is because chlorophyll absorbs near-green and green portions poorly (Speer, 1997). In 1817, Pierre Joseph Pelletier and Joseph Bienaimé Caventou isolated chlorophyll for the first time (Pelletier and Caventou,1951).

The unripe fruit colors of pepper vary from green, ivory to yellow. In the chloroplast, chlorophyll accumulates to give a green color and a degradation of chlorophyll is indicated by the ivory color as the ripening of the fruit takes place (Wang et al., 2005).

Mature foods that are brown or black result from pigments like anthocyanins or carotenoids which accumulate due to chlorophyll's continuous presence whilst the fruit ripens. Compared to violet fruit, in black pepper fruit, the presence of chlorophyll is higher by about fourteen times (Lightbourn et al., 2008)

3.2.10.4. Betalains

Beta vulgaris the Latin name for common beet is the genesis for the word "betalain" from which the extraction of betalains was first done. The presence of this pigment betalains gives the deep red color to amaranth, bougainvillea, beets and many cacti.

Plants of the Caryophyllales have this pigment which belongs to a class of yellow and red indole-derived pigments where anthocyanin pigments get replaced as also some fungi of the higher order (Strack and Scheliemann, 2003).

Betalains have two categories namely betaxanthins and betacyanins. Betaxanthins are the yellow appearing pigments in oranges and betacyanins, including the betalain pigments with violet and reddish color. Vulgaxanthin, portulaxanthin, miraxanthin and indicaxanthin are the betaxanthins present in plants (Salisbury et al., 1991).

Yellow and red beetroots (Beta vulgaris L.ssp. vulgaris), leafy or grain amaranth (Amaranthus sp.), Swiss chard (Beta vulgaris L. ssp. cicla) and fruits of cactus like Hylocereus gerera and Opuntia are some of the edible sources of betalains that are known (Azeredo, 2009).

3.2.10.5. Capsaicinoids

Capsaicinoids are the burning sensation causing nitrogenous compounds that are produced by the pepper fruit. The antimicrobial effects of capsaicinoids for the preservation of fruit are quite well known (billing and Sherman, 1998). Most of the time, they are used in medicines as an analgesic (Winter et al., 1995).

A number of painful conditions like arthritis, neuropathic pain and cluster headaches have been known to have been treated successfully by

capsaicinoids. Capsaicinoids' analgesic action has been described as specific for polymodal nociceptors and dose-dependent.

Cloning has been done of the gene for capsaicinoid receptor and multiple pain-producing stimuli are transduced by this receptor (Caterina et al., 1997; Tominaga et al., 1998). The volatile, acrid alkaloid responsible for the peppers' hotness is capsaicin (trans-8-N-vamllyl-6-nonenamide).

3.3. CAROTENOIDS

Most of the protection from diseases that are plant-derived in the form of their dietary intake is due to the plant-derived compounds, namely phytochemicals, a large group present in vegetables, fruits, cereals, beans, and beverages that are plant-based like wine and tea.

Scientists, producers, food manufacturers and consumers have shown quite a bit of interest in the antioxidant and phytochemicals constituents in plant material due to their essential role in maintaining health of humans.

The risk of chronic diseases can be reduced even as the health gets optimized when consumers bring about simple dietary modifications and follow the practical strategy of increasing the consumption of a range of whole grains, vegetables and fruits.

Figure 3.3. Carotenoids are found mainly on vegetables.

Source: *Image by NutaIngredients.com.*

Strong antiproliferative and antioxidant activities that the phytochemical extracts derived from vegetables and fruits and a combination of phytochemicals are responsible for a majority of the total antioxidant activity.

Carotenoids amongst the phytochemicals are considered the most important. Due to the antioxidant and provitamin role of the carotenoids they have received a huge amount of attention and this pigment is the most widespread in nature. Their usage in feed, food and cosmetics is quite extensive as they are natural, safe colorants.

For humans, these are the main dietary source and are essential for photosynthesis in plants. The primary source of provitamin A from the dietary source is derived from plant carotenoids and as it is not possible for humans to de novo synthesize vitamin A endogenous from isoprenoids precursors, these become quite important.

Carrots (Daucus carota) are the genesis for the word carotenoids whereas the Greek words xanthos (meaning yellow) and phyllon (meaning xanthos) lie at the root of the word xanthophylls which were originally known as phylloxanthins.

More than 750 varied structures have been identified for carotenoids and anthocyanins and together they are the most complex class of food colorants that are natural.

3.3.1. Physiology, Structure and Biochemistry

In tissues that are photosynthetic, the composition of plant chloroplasts is remarkably similar to that of carotenoid wherein the most abundant carotenoids are lutein which makes up for 45% of the total, β-carotene which forms up 25–30 percent, violaxanthin accounting for 10–15% and neoxanthin which are 10–15% of the total.

The photosynthetic (thylakoid) membranes have embedded in them the functional building protein structures wherein most of the carotenoids together with chlorophylls are present. Hence, photosynthesis involves various compounds of carotenoids.

Energy from the wavelength of light that is not absorbed efficiently by chlorophylls is captured by carotenoids thus assisting photosynthesis. β-carotene is a typical carotenoid in which a chain of eighteen carbon atoms connects two carbon rings with alternating double and single bonds.

Two molecules of vitamin a are produced when β-carotene is split into two halves. The pigment retinal is used in vertebrate vision and this pigment

is produced by the oxidation of Vitamin A. the vision enhancing capabilities of carrot hence become self-explanatory as they are rich in β-carotene.

The antennae of chloroplasts that harvest light have xanthophylls as an accessory pigment and these have the capability of transferring energy to the chlorophylls. Through the dissipation of excess excitation energy in a manner that is non-radiative, the triplet excited state in chlorophyll molecules are also quenched by these pigments in the process called nonphotochemical quenching (NPQ).

To protect chlorophyll from the intense light's bleaching properties, this function becomes quite crucial. Plants usually attract animals through their distinct colors and carotenoids perform this additional role in giving that color to the fruits and flowers.

Carotenoids are indispensable in chloroplasts wherein they perform essential roles in photosynthesis and on the other hand, they can be taken as secondary metabolites in chromoplasts. For the hormone abscisic acid (ABA) to be synthesized in plants, the precursors are also carotenoids.

Biosynthesis by tail-to-tail linkage of two C20 produces the isoprenoid compounds mentioned above namely the carotenoids wherein a complex 40-carbon skeleton of isoprene units is formed. The central part of the molecule in the carotenoid structure is the long stem with single and double bonds alternating with each other, which also makes this it's characteristic and most striking feature.

A conjugated system comprises this whereby over the polyene chain's entire length the π-electrons are delocalized effectively. This feature gives carotenoids a molecular shape that is distinctive, light absorbing properties and chemical reactivity.

Some of the carotenoids have keto and polar hydroxyl functionalities giving them an increased affinity towards water/lipid interfaces though generally, carotenoids are lipophilic. Hence, for the efficiency of carotenoids as antioxidants the structural traits and property to bind with keto and polar hydroxyl units of the molecules vis-à-vis lipid/water interface becomes quite important.

At both or even one end, the structure may be cyclized, it may possess functional groups that possess oxygen or may have various hydrogenation levels. Carotene is an example of cyclized carotenoids and lycopene that of a cyclized carotenoid.

Most commonly, caotenoids compounds occur in the all-trans form in nature. The central part of the carotenoids molecule is formed with a long series of conjugated double bonds and this is its most characteristic feature. Their shape, light-absorbing properties and chemical reactivity are all due to this feature. Cryptoxanthin, carotene and β-carotene can all function as provitamin A.

One double bond gets added to the molecule through each enzymatic step to lycopene from phytoene which results in lycopene, a double bond containing symmetrical molecule. After lycopene, anzymatic cyclization of the end groups takes place in the biosynthetic step resulting in β-carotene (two β- rings) and γ-carotene (one β-ring).

Xanthophylls result when the molecules are added with oxygen. The rate-limiting of various enzymes (whether they are limiting or not) in the biosynthetic cascade is deduced by the concentration of each of the carotenoids in vegetables or fruits. For instance, the enzyme activity for the conversion of lycopene to γ-carotene (i.e., insufficient cyclase activity) would lack in red tomatoes where lycopene is present in high concentration.

3.4. CHEMICAL PROPERTIES OF PHYTOCHEMICALS

3.4.1. Terpenes

A general principle is followed in the basic structure of terpenes namely: Isopreen rule 1 formulated by Ruzicka wherein the carbon skeleton of terpenes is built up by 2-methylbutane residues which though with lesser precision are often referred to as isoprene units (C5)n (1953).

The ethyl residue is defined as the tail and the head is the isopropyl part of 2-methylbutane (Breitmaier, 2006). Predominantly, in nature the occurrence of terpenes is as hydrocarbons, ethers, ketones, esters, alcohols and their glycosides, carboxylic acids and aldehydes (Breitmaier, 2006).

Terpenoid sidechains are attached to a non-terpenoid nucleus in most of the important groups of plant compounds which includes cytokinins, electron carriers that are quinine-based and chlorophylls (the ubiquinones and plastoquinones).

Movement and anchoring within the membranes is facilitated through these side chains. Control of the cell cycle (Qian et al., 1996; Crowell,

2000), transduction of abscisic acid signal (Clark et al., 2001) and nutrient allocation (Zhou et al., 1997) in plants may involve prenylated proteins.

Hemiterpene (C5) isoprene, 2-methyl-1,3-butadiene is the most abundantly emitted hydrocarbon by plants. The redox balance of the atmosphere is impacted majorly by isoprene, which gets emitted from numerous taxa and more so, the woody species affecting the levels of carbon monoxide, ozone and methane (Lerdau et al., 1997).

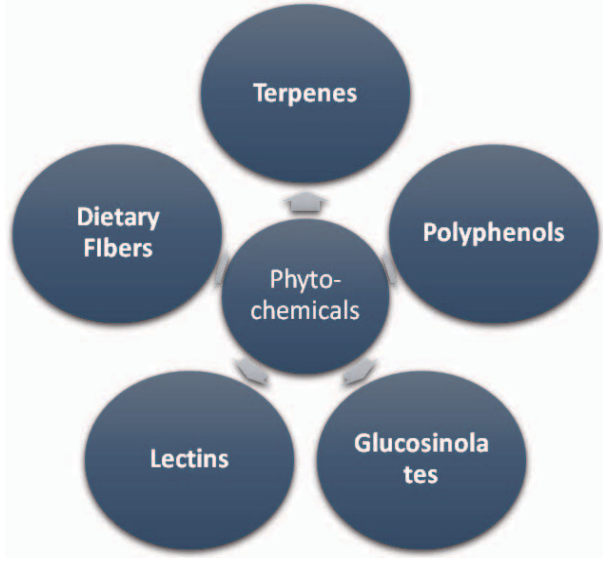

Figure 3.4. Chemical properties of various phytochemicals.

Temperature and light strongly influence the plant release of isoprene where under conditions of high temperature and high light the release rates are greatest (Lichtenthaler, 2007). For years, the direct function of isoprene in plants has stayed a mystery.

However, now there are indications that cellular damage may be prevented by them when the temperatures are high, which could be due to a reaction with free radicals that stabilized the components of the membrane (Sasaki et al., 2007).

3.4.2. Polyphenols

In one or more positions, an alcohol ring substitutes an aromatic ring in the simplest group of simple phenols (C6). In their structure, some substituent groups like alcoholic chains may be there (Andrés-Lacueva et al., 2010).

The structure simple phenols exist in phenolic acids (C6<-C1) as well and they are hydroxylated derivatives of cinnamic benzoic acids (Herrmann, 1989; Shahidi and Naczk, 1995). They attract insects and birds thus helping in the pollination through the dispersal of seeds (Harborne, 1994) and also act as support materials for the cell wall (Wallace and Fry, 1994).

The hydrolysable tannins are mostly glucose esters of gallic acid make up. These are of two kinds: ellagic acid (as the common product of degradation) producing the ellagitannins and the ones that upon hydrolysis produce only gallic acid namely the Gallo tannins (Andrés-Lacueva et al., 2010).

In phenylacetic acids to the benzene, a chain of acetic acid is linked up and acetophenones are aromatic ketones. C6-C2 structure makes up both of them. Four basic structures are there namely: caffeic, sinapic, ferulic and coumaric acid. The phenylpropanoid group (C6-C3) includes hydroxycinnamic acids.

Chlorogenic acid is the link between quinic and caffeic acids and in nature, the above group of chemicals are often found in association with other compounds like chlorogenic acid (Andrés-Lacueva et al., 2010).

The benzopyrone group of compounds consists of a benzene ring joined to a pyrone and coumarins belong to this group. In nature, they can be found as glycosides in combination with sugars. There categorization can be as simple pyranocoumarins, furanocoumarins and coumarins substituted in the pyrone ring (Murray et al., 1982).

The C6-C1-C6 structure is present in xanthones and benzophenones. Diphenyl ketone is the basic structure of benzophenone and a 10-oxy-10 H-9-oxaanthracene makes up the xanthones. Currently, nature is known to have more than 500 xanthones and mangosteen with prenyl substituents has about 50 of these (Andrés-Lacueva et al., 2010).

The basic structure in stilbenes is a 1.2-diphenylethylene (C6-C2-C6). The basic structure of the compound known most commonly namely resveratrol has three hydroxyl groups and is called 3,4,5-tri-hydroxy-stilbene.

In plants stilbenes are present as trans or cis isomers. Through UV radiation, isomerization of trans forms can be done to cis forms (Lamuela-Raventós et al., 1994). Strictly speaking, lignans are phenylpropanoid dimmers that are in the side chain linked by a C-C bond between 8 “prime” and 8.

Depending upon the substitution patterns and other linkages introduced into the original hydroxy-cinnamyl alcohol dimmer, these can be divided into sub-groups. Lignans are present in more than 55 families of plants, mainly in dicotyledonous angiosperms and gymnosperms (Dewick, 1989).

Amongst all the plant phenolics, the most ubiquitous groups constitute flavonoids. More than 8000 flavonoid varieties have been identified (De Groot and Raven, 1998). Usually, in plants, flavonoids are glycosylated with rhamnose or glucose however, there can also be linkages with arabinose, glucuronic acid, galactose, xylose and other sugars (Vallejo et al., 2004).

The basic nucleus of all flavonoids has fifteen carbon atoms: two six-membered rings linked with a unit of three carbon that may be a part of the third ring or may not even be a part of it (Middleton, 1984). A, B and C are the three rings.

A numbering system using "primed" numerals for B and ordinary numerals for C and A ring form the base for the individual carbon atoms. The derivatives of isoflavones (6): rotenoids and pterocarpans; and chalcones(2) do not use the numbering system that is primed modified.

Various classes of flavonoids are provided through the different ways in which this ring is closed that is associated with the varying degrees of oxidation of ring A. The ring that is six-membered and condensed with the benzene ring can be either a di-hydro-derivative ((flavanones (4) and flavan-3-ols (5)) of α-pyrone [(flavones (1) flavanols (3)) or α-pyrone ((flavones (1) flavanols (3)] itself.

There are two classes of flavonoids depending upon the position of benzenoid substituent, namely: iso-flavonoids (6) (3-position) and flavonoids (1) (2-position). Naturally, flavonoids occur in conjugated forms in association with sugar and they may be characterized as diglycosidic, monoglycosidic, etc. within a single class.

Usually, position 3 or 7 is the location for the glycosidic linkage and the carbohydrate unit can be D-glucose, galactose, L-rhamnose, arabinose or glucorhamnose (Tapas et al., 2008).

3.4.3. Glucosinolates

Cruciferous plants exclusively contain the amino –acid-derived secondary plant metabolites called glucosinolates. The Brassicaceae family consists of most of the plants that have been cultivated and contain glucosinolates like in cauliflower, Brussels sprouts, broccoli and cabbage.

In the diet of humans, these are the major source of glucosinolates and categorization of approximately 120 different glucosinolates has been done. Due to the anti-nutritional and nutritive properties, the characteristic odor and flavor imparted by them to vegetables and the anti-carcinogenic properties of glucosinolates and their breakdown products, they have been the subject of immense interest (Verkerk and Dekker, 2008).

A major chunk of glucosinolates containing plants that have been cultivated are from the family of Brassicaceae. Mustard seeds, commonly used as a seasoning, is derived from *B. juncea* (L.) *Coss, B. hirta* and *B. nigra* species.

Cabbage, Brussels sprouts, broccoli, turnip and cauliflower amongst the vegetable crops has these and they belong to the species- *B. oleracea L., B. campestris L., B.rapa L.,* and *B. napus L.* From the *B. oleracea* species, Kale is utilized for forage, silage and pasture.

In the human diet, glucosinolates in is mainly derived from brassica vegetables like cabbage, Brussels sprout, cauliflower and broccoli. These are often consumed by the Eastern and Western cultures (McNaughton and Marks, 2003).

Per person, more than 36 grams of Brassica is consumed every day on an average in their vegetable intake (Godeschalk, 1987). The volatiles derived from glucosinolate gives a typical flavor to the Brassica vegetables. The very fact that glucosinolates are very toxic for some insects shows the versatility of these compounds, which helps their use in a number of natural pesticides.

At the same time, glucosinolates are used by cabbage aphids and certain other insects to locate feed in the form of their favorite plants and find a congenial environment for depositing their eggs (Baker et al., 2006). Moreover, antibacterial and antifungal properties are shown by glucosinolates (Fahey et al., 2001).

Even though around 120 different kinds of glucosinolates have been characterized so far, a very limited number of these have been investigated in detail. As of now, quite a bit of data is available about the individual and total glucosinolates.

Depending upon the conditions for cultivation, variety, agronomic practice and climate, the levels of total glucosinolates present in plants vary. Within a plant too, there is a variation in the levels to which they are present in plants different parts of a particular plant.

Most of the times, irrespective of the genetic origin, in a particular sub-species glucosinolates present are the same and most of the species have a relatively high concentration of only one to four glucosinolates. Throughout the plant when the glucosinolates are separated within the subcellular compartments, they are biologically inactive and stable chemically.

When tissue damage takes place due to harvesting, pests, chewing, or food processing, contact is initiated with myrosinase- the endogenous enzyme resulting in hydrolysis wherein a broad range of products that are biologically active like isothiocyanates (ITCs), oxazolidinethiones, ionic thiocyanate and organic cyanides are released.

In higher animals a varied number of anti-nutritional and toxic effects are exerted when glucosinolates break down and the adverse effects on thyroid metabolism amongst the lot have been studied the most in-depth (Tripathi and Mishra, 2007).

In vegetables, the role played by glucosinolates and indole compounds in the formation of N-nitroso compounds have been investigated by Tiedink et al., (1990, 1991). It was revealed in the studies that the nitrosating can be done of the indole compounds that occur in Brassica vegetables whereby they become mutagenic. All the same, only when free nitrite is present in large amounts can nitrosated products be stable.

3.4.4. Dietary Fiber (Non-Starch Polysaccharides)

Amongst the biopolymers, polysaccharides are quite widespread, representing in the botanical feed, the most important group of nutrients. Carbohydrates are quite diverse, and in the nutrient category, they range from the one sugar that can be easily digested in the small intestine of animals to the variety of dietary fiber that the microbes can ferment in the large intestine.

The individual NSPs' chemical and physical properties are influenced by the plant cell wall's structure and there are variations of this between different polymers and the same polymers with different molecular weights (Choct, 1997).

The manner in which the polysaccharides' monomer units are linked together also differentiates their physical properties (Moms, 1992). Often similar physical properties are exhibited by polysaccharides when different sugars are linked together in the same manner.

Moreover, when the monomer units are linked together in different manners, the polysaccharides exhibit different physical properties even if they

are built up from the monomer units that are the same. The physicochemical properties of these compounds are responsible for the physiological effects of NSP on the nutrient absorption and digestion in monogastric animals and humans.

From the nutritional point, NSP's main physicochemical properties that have significance are:

1. viscosity;
2. properties related to the absorption of organic compounds;
3. hydration and
4. capacity for cation exchange.

The water holding as well as binding capacity are influenced by the NSP's hydration properties (Bach Knudsen, 2001). These are further dependent upon the ability of NSP to incorporate water within the molecular matrix and the molecules' physicochemical structure.

The molecular size or weight (branched or linear), the surrounding structure, ionically charged groups as well as NSP's concentration form the basis of the NSP's viscosity properties (Smits and Annison, 1996). Chelation of ions takes place due to the three-dimensional structure of the NSP molecule which causes the formation of the cation exchange capacity.

NSP has the capacity to bind small molecules through both hydrophilic and hydrophobic bond interactions resulting in properties related to organic compound absorption.

3.4.5. Lectins

The action of ricin on blood cells was reported by Stillmark six years after Weir Mitchell for the first time observed that rattle snake venom has some lectin activity (Kilpatrick, 2002) and this was followed up by a similar report on abrin shared by Helin which finally caught the medical community's attention.

From various sources this was followed by reports of hemagglutinins. Agglutins, in addition to plants can be found in bacteria, vertebrates, invertebrates, fungi and viruses. The properties of precipitation, cell agglutinations and the proteinaceous nature of lectins was established beyond doubt in this early period thereafter, for the next quarter of a century the research related to lectin was beset with difficulties and problems.

As far as the research on lectin is concerned, the proverbial "shot in the arm" was provided by the studies conducted by Jonsson, Renkonen, Boyd and Sugishita wherein they identified that lectin could have certain practical applications and the lectins were identifies as cell-recognition molecules (Kocoureck, 1986). Almost in no time this was followed by reports of mitogenicity, blood-group specificity and tumor cell-binding of lectins.

There was a rapid increase in the possibilities where lectin could be applied along with the properties that came to be known. In 1936 Sumner and Howell crystallized and characterized extensively the first lectin concanavalin A (Con A), from jack beans and also showed for the first time that its agglutination activity could be inhibited by sucrose.

Two other major discoveries gave the research that followed a direction. From Ricinus communis, the first non-toxic lectin was isolated by Funatsu and his collaborators which shattered the notion existing during the time that essentially lectins are toxic proteins (Ishiguro et al., 1964). It was further shown that several lectins were glycoproteins like the ones extracted from soybean (Lis and Sharon, 1973).

The earlier research had already started looking into the effect on different types of cells by plant lectins which resulted in an extensive search for the presence of lectins in the extracts of plants which in turn for practical purposes led to the identification of numerous lectins.

The biological function of protein was not really required for this kind of identification for the purpose as mentioned. It is only *in vivo* that clues have been found on the putative function of the plant lectins on animal or microbial cells in several cases where the biological function is proven or even hypothesized.

Even though some progress has been made on the endogenous roles of plant lectins, the research in this aspect has started quite late. The very fact that plants are available in abundance in nature makes it both feasible and practical to apply them in various areas resulting in a sustained study of the plant lectins (Komath et al., 2006).

At the cellular level a key step in a series of processes is glycosylation. There is an alteration of the oligosaccharides on the cell surface into different types of pathological conditions, which also includes the transformation of the malignant kind.

Various biological processes like fertilization, viral replication, cell-matrix interaction, enzymatic activity, cell-cell adhesion, immune defense

and parasitic infection are deeply influenced by the oligosaccharide-mediated recognition, an aspect revealed owing to the developments in the field of glycobiology to which it is related closely.

In most of these events that have been recognized, lectins were found to have played a key role. Both the ligand and the receptor corresponding to it are imposed with a stringent geometry due to the selectivity that is very strict in recognition of this kind conferring lectins with unique sugar-specificities (Sharon and Lis, 2004).

Through van der Waal interaction, hydrogen bonds, hydrophobic interaction and metal coordination bonds there can be an interaction of carbohydrates with lectins. Specific hydrogen bonding or/and metal coordination bonds with carbohydrates' key hydroxyls, resulting in selectivity, acting as both donors and acceptors of hydrogen bonds.

In these interactions, most of the time, water molecules act as bridges. In these events, the decisive player appears to be mainly the hydroxyl at the C4 position. Unwanted recognition is minimized by steric exclusions which fine tunes the lectines' saccharide specificity even further.

The binding selectivity increases manifold where possible due to subunit multivalency and subsite binding (Rinni, 1995). For carbohydrate recognition, the primary binding site seems critical in subsite binding however, the affinity of the lectin gets enhanced towards specific oligosaccharides due to the contribution of secondary binding sites.

For instance, the oligosaccharide preferences of the legume lectins Con A and Lathyrus ochrusisolectin II (LOL II) are quite different even though both of them are Man/Glc specific lectins. Con A does not have affinity for oligosaccharides that have additional a (1–6)-linked fucose residues whereas LOL II has an affinity that is higher by many times (Rinni, 1995; Weis and Drickamer, 1996).

3.5. PHYSICOCHEMICAL PROPERTIES OF DIETARY PHYTOCHEMICALS

The risk of chronic diseases like neurodegenerative diseases, cardiovascular diseases, cancer, diabetes and obesity reduces when the diet has a high intake of plant foods that are rich in phytochemicals. These chronic diseases have the common underlying factor of oxidative stress and inflammation (OSI).

The absorption and timing for the best effect of phytochemicals and their corresponding metabolites is still not understood well even though they

have shown to regulate OSI, thus positively effecting health. In vegetables and fruits in order to predict the time required for achieving maximal plasma concentration (T_{max}) of phytochemicals a model was developed.

From 31 clinical studies the training data set which contained 67 dietary phytochemicals was used for the development of the model. Thereafter, using three independent datasets that comprised 98 pharmaceutical compounds and 108 dietary phytochemicals, the model was then validated.

Over a broad range of chemical class, the T_{max} of dietary phytochemicals and pharmaceutical compounds was predicted accurately by the developed model which was based on the physicochemical properties lipophilicity, molecular mass and the forms of dietary intakes.

T_{max} of dietary phytochemicals in the human body was predicted through this direct model which was the first such model. To optimize the uptake, maximizing the biological efficacy and sustained presence of dietary phytochemicals so that OSI in chronic diseases can be managed and human health is affected positively, this model informs the frequency of clinical dosing.

Being responsible for 68% of the deaths worldwide, the leading cause of mortality is the chronic diseases. Currently, there is evidence to show that diets that have a rich intake of plant foods are linked with a reduction in the risk of chronic diseases like neurodegenerative and cardiovascular diseases, cancer, diabetes and obesity.

In healthy individuals, the daily cycles of activity (which includes exercise and digestion) can also be linked to these transient elevated states of OSI. In healthy volunteers for a high–fat meal challenge, post-prandial OSI that gets induced can be prevented by the intake of grape extracts or fruit juice rich in phytochemicals. Moreover, OSI that is linked to the exercise patterns in athletes has shown positive effects due to phytochemicals.

Phytochemicals, when consumed in diet regularly facilitate the protection of human health as they are then bio-available to the target cells and can aid in having healthy bodies. All the same, the bioavailability of phytochemicals is quite low as the body handles them as xenobiotics resulting in a transient presence in the body.

Through the small intestine, some of the phytochemicals once ingested are absorbed in the circulatory system even though all of them do not get absorbed. In the liver these phytochemicals may be metabolized and their hepatic metabolites get released back to the circulatory system.

The colon receives the phytochemicals that fail to be absorbed in the small intestine wherein colonic microbiota causes substantial modifications to the structure and their microbial metabolites are released back to the circulatory system.

The form in which the dietary intake takes place and the chemical structures are the main factors that affect the bioavailability of phytochemicals. In the body, for reaching the maximal plasma concentration (T_{max}) a broad range of associated time is required due to the dietary plants having chemical heterogeneity of key bioactive phytochemicals.

To take an example, post-ingestion of green tea within one to two hours flavan-3-ols peak in human body and gets cleared the next couple of hours whereas for tomato, only after 15–22 h (hours) of ingestion lycopene was observed and it cleared completely over a period of next few days.

T_{max} of 0.5–1 h was found to be present in ellagic acid from an extract of pomegranate when it the intake was in a liquid form, but when the ingestion was in a solid form, this increased to 2–3h. There is a possibility that the OSI-reducing effects of dietary phytochemicals may have been underestimated in the studies conducted earlier if the blood sampling was done outside the time span of T_{max} in the body.

To take an example, after a duration of either a 2-week or 1 day treatment, on the plasma biomarkers of OSI no effects were seen of vitamin C supplementation (1 g/d). All the same when two hours before exercise, a bolus dose of vitamin C was given, OSI induced due to exercise was prevented.

The blood sampling that may have mismatched the short T_{max} of vitamin C (approximately three hours) may have been the reason for the inconsistency in the findings of vitamin C's bio-efficacy. In order to understand and optimize the phytochemical derived health benefits, an important factor could be the timing of dietary phytochemical consumption relative to OSI challenges (exercise or meal for instance).

In silico modeling is used widely in drug discovery and pharmaceutical sciences and this may be applied to the oral bioavailability of phytochemicals. To predict the absorption of similar compounds in the passive absorption of drugs, these models correlate *in vivo* or/and *in vitro* with their chemical structures as described by the properties of phytochemicals.

In drug absorption, the important physicochemical properties include lipophilicity (expressed as the logarithm of the partition coefficient between

1-octanol, log P and water), polar surface area (PSA), molecular volume, molecular mass (M_r), number of hydrogen (H) acceptors and donors as well as the number of freely-rotatable bonds.

To predict the bioavailability and absorption kinetics of pharmaceutical compounds, a number of models have been developed. All the same, as of now, for predicting T_{max} of dietary phytochemicals from the properties that are physicochemical, no model exists as such.

This study aimed at determining whether it was possible to predict the dietary phytochemicals' T_{max} in individuals who are healthy based on the form of dietary intake and physicochemical properties. From clinical studies of healthy volunteers, T_{max} of the dietary phytochemicals was collected to calculate their physicochemical properties, and the training data set that was used to model this was utilized in this study.

An independent photochemical validation data set (from 34 clinical studies) was used to validate the dietary phytochemicals' predictive model, and this contained 108 phytochemicals.

REFERENCES

1. Ashour, A., El Aziz, M., & Gomha Melad, A., (2019). A review on saponins from medicinal plants: chemistry, isolation, and determination. Journal of Nanomedicine Research, [online] *7*(4), pp.282–288. Available at: https://medcraveonline.com/JNMR/a-review-on-saponins-from-medicinal-plants-chemistry-isolation-and-determination.html (accessed 9 September 2021).
2. Bouchenak, M., & Lamri-Senhadji, M., (2013). Nutritional Quality of Legumes, and Their Role in Cardiometabolic Risk Prevention: A Review. Journal of Medicinal Food, [online] *16*(3), pp.185–198. Available at: https://pubmed.ncbi.nlm.nih.gov/23398387/ (accessed 9 September 2021).
3. Deepak Koche, R. Shirsat and Mahesh V Kawale, (2016). An overview of major classes of phytochemicals: their types and role in disease prevention. [ebook] *Hislopia Journal*, pp. 1–11. Available at: https://www.researchgate.net/publication/327304552_an_overview_of_major_classes_of_phytochemicals_their_types_and_role_in_disease_prevention (accessed 9 September 2021).
4. B.K. Tiwari, Nigel P. Brunton, Charles S. Brennan, Handbook of Plant Food Phytochemicals. (2013). [online] Available at: https://onlinelibrary.wiley.com/doi/book/10.1002/9781118464717 (accessed 9 September 2021).
5. Pius O. Ukoha, Egbuonu A. C. Cemaluk, Obasi L. Nnamdi, & Ejikeme P. Madus, (2011). Tannins and other phytochemical of the Samanaea saman pods and their antimicrobial activities. [ebook] *African Journal of Pure and Applied Chemistry,* pp. 237–244. Available at: http://www.academicjournals.org/app/webroot/article/article1379425653_Ukoha%20et%20al.pdf (accessed 9 September 2021).
6. Prakash, D., & Gupta, C., (2012). Glucosinolates: The Phytochemicals of Nutraceutical Importance. *Journal of Complementary and Integrative Medicine,* [online] *9*(1). Available at: https://pubmed.ncbi.nlm.nih.gov/22850070/ (accessed 9 September 2021).
7. Selby-Pham, S., Miller, R., Howell, K., Dunshea, F., & Bennett, L., (2017). Physicochemical properties of dietary phytochemicals can predict their passive absorption in the human small intestine. *Scientific Reports*, [online] *7*(1). Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5434065/ (accessed 9 September 2021).

8. Timoshenko, A., Gorudko, I., & Gabius, H., (2014). Lectins from Medicinal Plants: Bioeffectors with Diverse Activities. *Phytochemicals – Biosynthesis, Function and Application*, [online] pp.43–56. Available at: https://www.researchgate.net/publication/278712588_Lectins_from_Medicinal_Plants_Bioeffectors_with_Diverse_Activities (accessed 9 September 2021).
9. Upadhyay, S., & Dixit, M., (2015). Role of Polyphenols and Other Phytochemicals on Molecular Signaling. *Oxidative Medicine and Cellular Longevity*, [online] 2015, pp.1–15. Available at: https://www.hindawi.com/journals/omcl/2015/504253/ (accessed 9 September 2021).
10. Zakynthinos, G., & Varzakas, T., (2016). Carotenoids: From Plants to Food Industry. *Current Research in Nutrition and Food Science Journal,* [online] *4*(1), pp.38–51. Available at: http://www.foodandnutritionjournal.org/vol4nospl-issue-carotenoids-2016/carotenoids-from-plants-to-food-industry/ (accessed 9 September 2021).

CHAPTER 4

PHARMACOLOGY OF PHYTOCHEMICALS

CONTENTS

In this chapter pharmacology of phytochemicals has been discussed in detail. The different possible uses of phytochemicals in pharmacology have been done in detail.

In this chapter, there is also a detailed discussion about the prevention and treatment of antioxidant phytochemicals for several chronic diseases. There is also discussion about phytochemicals use in the prevention of cancer. At last, the phytochemicals and cardiovascular disease have been discussed in detail.

4.1. INTRODUCTION

It takes time and practice to choose the appropriate medicinal plants. The statistical surveys that were conducted in Australia and the United States revealed that 48.5% and 34% of the population had used at least one unconventional treatment, respectively (Kim et al., 2007; Momtaz & Abdollahi, 2010).

The World Health Organization estimated that about 65–80% of the world population in the developing world relies primarily on plants for their health care due to poverty and lack of access to modern medicine (Calixto, 2005; Momtaz & Abdollahi,2010). Moreover, there is general agreement that many countries, especially in developing regions, suffer from a shortage of quality modern medicines.

Therefore, assessing appropriate plants for the treatment of diseases such as diabetes, including diabetes, is recommended (Momtaz &Abdollahi, 2010). In the last decade, several researchers have reported efficacy of herbal medicines in many diseases. According to a large number of research papers (Kim et al., 2007; Momtaz & Abdollahi, 2010), of currently prescribed synthetic drugs, approximately 25% of the active ingredients were discovered through a process of extraction and refinement from plants.

Additionally, 20,000 plants have been used for medicinal purposes, with about 4,000 of these plants being used on a daily basis. Ten percent of these items have a commercial value that is actually present (Kim et al., 2007; Momtaz & Abdollahi, 2010). Satureia was the first genus in the Lamiaceae family to be identified by Roman writer Pliny. Satureia is derived from Latin, meaning saffron.

In short, it means herb of satyrs. That is why, before the time of monasteries, herb of satyrs was illegal (Zavatti et al., 2011). This genus contains around 200 species of aromatic herbs and shrubs which grown.

Mainly in the Middle East, Mediterranean region to Europe, West Asia, North Africa, the Canary Islands, and South America.

This genus range includes several different areas in the eastern Mediterranean, as shown by the list of more than 30 species present in these regions (Cantino et al., 1992; Momtaz & Abdollahi, 2010).

Due to its sweetness and basic cultivation characteristics, they are used as flavoring compounds in fruit, pharmaceutical, and cosmetic industries. Stomach and intestinal disorders such as cramps, nausea, indigestion, and diarrhea are usually treated with herbal remedies such as coltsfoot, horsetail, and senna that are also good muscle pain relievers, tonics, and carminatives (Momtaz & Abdollahi, 2010; Zargari, 1990). The food ingredients often referred to as phytochemicals, are chemicals found in plant-based foods.

These compounds have various beneficial biological activities. Plants are considered to be the primary source in the pharmaceutical industry for different active ingredients. Additionally, they have pharmacological effects relevant to the treatment of bacterial and fungal infections and the treatment of chronic-degenerative diseases such as diabetes and cancer. However, to discover new ways to acquire it, the next step is to develop them.

Figure 4.1. Diagram showing classification of phytochemicals.

4.2. PHYTOCHEMICALS POTENTIAL USES IN PHARMACOLOGY

4.2.1. Alkaloids in Plants

Alkaloids are classified into many groups, depending on their origins, pharmacokinetics, and chemical structural makeup. The Isoquinoline alkaloids, Indole alkaloids, Pyrrolidine alkaloids, Piperidine alkaloids, Pyridine alkaloids, Methylxanthine derivatives, Vinca alkaloid, Lycopodium alkaloid, Indole βcarboline, and Erythrine byproducts are many alkaloids that are widely known and are in use in daily lives. Although the Leguminosae, Papaveraceae, Ranunculaceae, Rubiaceae, Solanaceae, and Berberidaceae are noteworthy plants that yield large amounts of alkaloids.

The only families of gymnosperms that contain negligible amounts of alkaloids are Taxaceae family, which only contains Taxaceae. However, due to the suggestion that monocotyledons do not usually contain alkaloids, some recent investigations have proven otherwise. One of the most promising families in which to look for alkaloid-producing plants is the Amaryllidaceae and Liliaceae.

It is also found in a wide variety of fungi, such as in the Claviceps purpurea fungus, the ergot fungus, (Tudzynskik et al., 1999), and in many plant sections, including seeds (Physostigma and Areca), underground stems (Sanguinaria), roots (Belladonna root), rhizomes and roots (Ipecac, Hydrastis), and in barks of Cinchona.

A heterocyclic ring identifies the true alkaloid with a nitrogen atom, which is a feature that can be found in amino acid structures. In contrast, a proto alkaloid lacks the heterocyclic ring, and their structures can be found in amino acid structures.

Pseudo alkaloids are aromatic compounds that have a heterocyclic ring with a nitrogen atom, but which are not created by the addition of simple amine-based building blocks such as steroids. Classification of alkaloids follows the structure-based classification system, dividing alkaloids into two major groupings. In other words, it is a compound that is either non-hetrocyclic, or it is standard alkaloids or biological amines.

This is a list of the several dozen known members of the Hetrocyclic and Alkaloid community that includes, in this order, Pyrrole and pyrrolidine, Pyrrolizidine, Pyridine, and piperidine, tropane, quinoline, Isoquinoline, aporphine, norlupinane, indole, indolizidine, imidazole, purine, steroid, and terpenoid. The main groups can be further explained as given below.

4.2.1.1. Isoquinoline Alkaloids

Isoquinoline is a structural isomer of quinoline, which is often referred to as a heterocyclic aromatic organic compound. The two benzo pyridines are isoquinoline and quinoline, containing a benzene ring fused to a pyridine ring.

In a more general context, the term Isoquinoline is being used to refer to Isoquinoline derivatives. The main structural backbone of naturally occurring alkaloids, like papaverine, is a 1-benzylisoquinoline. The Benzophenanthridines, Ipecacuanha alkaloids, Aporphines, Proaporphines, Homoaporphines, Homoproaporphines, Homomorphines, Amaryllis alkaloids, and Aristo lactam are the possible exceptions.

One of the major alkaloids in this class is Galantamine, Berberine, Morphine, Monta nine, and Salsoline (Jianxin et al., 2013). There are a number of plants which have large amounts of barberine, such as *Hydrastis canadensis, Coptis chinensis, Berberi saquifolium, Berberis vulgaris*, and *Berberis aristata*.

The kind of opium poppy most widely known as poppy seed (*Papaver somniferum*) is a good source of morphine. some plants, such as *Hippea strumvittatum, Salsolaop positefolia, Narcissus tazetta, Galanthus nivalis,* and *Leucojum aestivum,* from which *Montanine, Salsoline*, and Galantamine can be extracted (Acqua et al., 2013; Zhu et al., 2014; Mojarad et al., 2014).

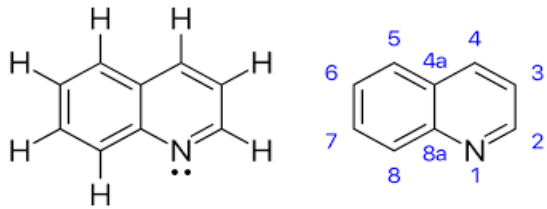

Figure 4.2. Structure of quinoline.

Sources: Image by Wikimedia

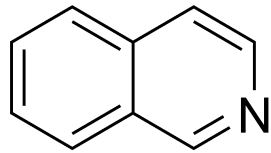

Figure 4.3. Structure of Isoquinoline.

Source: Image by snappy goat

4.2.1.2. Indole Alkaloids

Indole-containing alkaloids are categorized under this class, because of their structural moiety of indole. The plant alkaloids isoprene and secologanin tryptamine also contain indole groups and are thus referred to as terpeneindole or secologanin tryptamine alkaloids.

The world's fourth largest alkaloid class, with over 4100 different chemicals discovered, is part of a class that contains many biologically important alkaloids (Seigler et al., 2001). Indole alkaloids are made by a biochemical pathway that includes the breakdown of amino acids including tryptophan (Knunyants et al., 1988).

Geissospermum vellosii plants are a source of the compound geissospermine used in treating neurodegenerative disorders (Choudhury et al., 2014).

Figure 4.4. Diagram showing 3-D structure of Indole

Sources: Image by Wikimedia

4.2.1.3. Pyrrolidine Alkaloids

A pyrrolidine is an organic compound, which has the molecular formula $(CH_2)_4NH$, is referred to as tetrahydropyrrole. The combination is a cyclic secondary amine, which is also known as a saturated heterocycle.

The liquid is colorless and can mix with water and a wide range of organic solvents. It has a characteristic odor that has been explained as ammoniacal, fishy, and like shellfish. Not only does pyrrolidine itself exist, but several substituted pyrrolidines are identified as well.

The compound pyrrolidine is used in alcoholic drinks. Pyrrolidine is found in foods in extremely low concentrations, thought to be the product of bacterial decarboxylation that yields proline. Certain animal and plant substances in tobacco and carrot, such as milk, cheese, coffee, and some alcoholic substances, contain pyrrolidine naturally. Pyrrolidine ring structure is found in many naturally occurring alkaloids such as nicotine and hygrine. Procyclidine and bepridil are both pharmaceuticals containing the medication used in Epikri-TZ.

4.2.1.4. Piperidine Alkaloids

This organic compound has a molecular formula of $(CH_2)_5NH$ and contains a heterocyclic amine, which is made up of a six-membered ring with five methylene bridges ($–CH_2–$) and one amine bridge (–NH–). The latter compound can be categorized as a piperidine.

This is a colorless liquid with an odor defined as objectionable and characteristic of amines and the name is based on the Latin term "pepper," as it is derived from the genus name "Piper," which refers to black pepper (Senning et al., 2006).

Its therapeutic effects include antipyretic, analgesic, insecticidal, antitumor, anti-inflammatory, and antidepressant properties. The alkaloid piperine is commonly found in black pepper (*Piper nigrum*) and long pepper (*Piper longum*). Additionally, lobeline is a piperidine alkaloid found in Lobelia inflata plants (Carradori et al., 2014).

4.2.1.5. Pyridine Alkaloids

Whereas piperidine alkaloids have heterocyclic nitrogen-containing ring systems, pyridine alkaloids are similar to them in that their nucleus contains a pyridine ring. Additionally, the class comprises nicotine and anabasine,

both of which are present in plants of the genus Nicotiana, which includes tobacco.

A naturally occurring parasympathomimetic alkaloid found in the nightshade family of plants, nicotine is a stimulant and a powerful parasympathomimetic. It is also used to treat tobacco use disorders, where it is used as a smoking cessation aid and a nicotine dependency treatment for the management of withdrawal symptoms.

As a temporary help to the cigarette smoker trying to give up smoking, it is fixed to an ion exchange resin in a chewing gum foundation. Arecoline derived from *Areca catechu* nut is believed to be involved in the causation of schizophrenia (Wang et al., 2015).

4.2.1.6. Tropane Alkaloids

Ornithine, which is derived from pyrrolidine, is used to synthesize a bicyclic compound with three acetate derived carbon atoms, thereby yielding an ornithine pyrrolidone. They are both visible in the molecule due to the pyrrolidine piperidine ring mechanism (Lounasmaa et al., 1993).

Like all tropane alkaloids, Tropane does not occur naturally in its free form but is usually present as part of esters in plant species. In these plants, a primary metabolite of tropane serves as a precursor to the esters of tropane.

Belladonna root, Hyoscyamus, Stramonium, Atropine, Hyoscyamine, Scopolamine, Coca, and Cocaine are some of the major alkaloids that are included in this category. Belladonna comes from the Italian word bella means lovely, as well as donna, which means lady, is an antimuscarinic agent that has been used as a spasmolytic drug and for treatment of diarrhea, diverticulitis, and pancreatitis.

Atropines are hyoscyamine derivatives and esters, which can be prepared synthetically, but are generally obtained through plant extraction from plants belonging to the Solanaceae family, that includes *Atropa belladonna, Datura stramonium, Hyoscyamus niger,* and *Hyoscyamus muticus* also known as Henbane (Griffin and Lin et al., 2000).

4.2.1.7. Imidazole Alkaloids

The formula of the aromatic heterocycle known as $C_3N_2H_4$ has two non-adjacent nitrogen atoms, making it a member of this class. It is a white or colorless solid that is soluble in water, and in doing so, it releases a slightly alkaline solution. The ring system is present in significant biological

building blocks, such as histidine and the associated hormone histamine. The imidazole series of antibiotics contains certain antifungal drugs, as well as a certain class of antiseizure medications that includes the nitroimidazole series. The sedative midazolam also belongs to this class of medications. The following are some of the primary Imidazole derivatives: histidine, pilocarpine, pilosine, and stevensine.

4.2.1.8. Purine

Pyrimidine and imidazole heterocycles make up the purine class of organic compounds, which consists of pyrimidine and imidazole fused together. Purines are the bases found in nucleic acids; two of these, adenine, and guanine, are pyrimidines. Proteins, fats, and carbohydrates from food or from tissue turnover are metabolised by many enzymes, including xanthine oxidase, into uric acid.

If your uric acid levels are elevated, the uric acid could form crystals and deposit in your joints, leading to gout. This condition occurs only in humans and certain other animals like dogs that lack an enzyme that can further degrade uric acid.

4.2.1.9. Steroids

Although there are plenty of biological and non-biological chemicals, only a small number have four rings grouped in a particular structure, and steroids are one. The cyclical organic compounds referred to as steroids encompass a group of seventeen carbon-based compounds whose framework is a characteristic arrangement of four carbon rings, three of which are six-carbon rings connected from two five-carbon rings, and one of which is an eight-carbon side chain on a carbon-17 an extensive number of steroids are discovered in plants, animals, and fungi. Lanosterol (opisthokonts) or cycloartenol are both generated in cells from the sterols lanosterol (opisthokonts) or cycloartenol (plants).

The Squalene is converted into lanosterol and cycloartenol, which are obtained from the cyclization of the triterpene. All individuals naturally contain the hormones testosterone and oestrogen; these hormones are released naturally in the body by breaking down proteins in order to make muscle tissue. To put it in general terms, this is what takes place (Rasheed et al., 2013).

The phytosterols is a term that is used to describe steroid molecules that are known as plant sterols and stanol esters in plant cell membranes.

Since phytosterols are structurally identical to the body's cholesterol, they compete with cholesterol for absorption throughout the digestive system when they are ingested.

4.2.1.10. Flavonoids

Flavonoids are a large and diverse group of phytonutrients (plant chemicals) present in almost all fruits and vegetables. They are polyphenolic compounds with fifteen carbon atoms, consisting of two benzene rings connected by a linear three carbon chain, in which the carbon skeleton is C6–C3–C6. They are the plant pigments and are polar in nature, and they are soluble in methanol and water.

Of the several thousands of phytonutrients, flavonoids make up the largest group, and there are over 6,000 varieties. A few of the most well-known flavonoids are quercetin and kaempferol. Flavonoids were discovered by the Hungarian scientist Albert Szent-Györgyi.

Vitamin C was first discovered and described by him in 1937, which is when he received the Nobel Prize in the sciences. Szent-Györgyi was engaged in research on the isolation of vitamin C, and while doing so, he discovered the flavonoids.

Flavonoids or bioflavonoids were first used by German researchers Geissmann and Hinreiner in 1952 to describe the substance found in nature. Additionally, these plant types are organized by framework based on the basic flavonoid structure, which consists of an oxygen pyran ring. There are several different groups of plant phenols, and more research has been done on flavonoids than on any other class of phenols (Dai et al., 2010).

In the past few years, researchers have turned to a number of flavonoids to help understand some of the health benefits linked to fruit and vegetable-rich diets, as the Linus Pauling Institute points out. Flavonoids, like other phytonutrients, are effective antioxidants with anti-inflammatory and immune system benefits. A diet high in flavonoid-rich foods can sometimes be associated with various forms of cancer, neurodegenerative disease, and cardiovascular disease prevention.

But so far, the only thing that is known is that flavonoids are not directly responsible. Fruits, vegetables, herbs, and spices all contain flavonoids, found in almost all other forms of food. Besides beans and grains, flavonoids can be found in many different forms of food, such as dried legumes (responsible for the color of red and black beans) and grains (flavonoids usually color them yellow).

Generally, the most colorful parts of our food, such as the skins and rinds of fruits, contain the highest concentrations of flavonoids. Some types of flavonoids are assigned to various subgroups based on the carbon of the heterocyclic ring on which the other rings are attached. When one of the heterocyclic rings has three positions joined together by isoflavones, the flavonoids are said to be isoflavones.

These compounds in which the ring of one heterocyclic compound is connected in position 4 are known as neoflavanoids, while those in which the ring of one heterocyclic compound is linked in position 2 can be further divided into several subgroups according to the structural features of the heterocyclic ring. Flavones, flavanols, flavanonols, flavanones, flavanol or catechins, anthocyanins, and chalcones are all subgroups that exist under this category (Panche et al., 2016).

4.2.1.11. Saponins

Saponins are naturally occurring glucosides that often foam. Saponins are complex polycyclic aglycones, typically with several sugar side chains, bound to one another. There are two significant forms of sapogenin, and these are referred to as the C27 steroid sapogenin and the triterpene sapogenin (C30). Saponins are hydrophobic, so they tend to keep water from sticking to the container.

The combination of hydrophobic sapogenin and hydrophilic sugar contributes to the foaming capability. Saponins have a strong, unpleasant taste. Sapotoxin is a group of saponins that is harmful and which is called sapotoxin.

Saponins are phytochemicals that can be found in several different types of plants, including veggies, beans, and herbs. Saponins are one of the most commonly found types of saponins. Peas, soybeans, and herbs with names suggesting foaming properties such as soapwort, soaproot, soapbark, and soapberry are all sources of saponins.

The commercial saponins are primarily obtained from the plant *Yucca schidigera* and *Quillaja Saponaria* (Ozlem et al., 2007). Saponins, glycosides, which are found in all plant kingdom members, including a diverse group of compounds that are distinguished by having a steroidal or triterpenoid aglycone base and possessing one or more sugar chains, are all found in these plants. The variety of their structural design is depicted in their physicochemical and biological properties, which are taken advantage of in a range of conventional and industrial applications.

Although saponins used in food and other industrial applications, such as foaming and surface-active agents, have typically been used as antinutritional factors and at times as cautions have been raised due to their bitter taste, saponins in food have historically been called antinutritional factors and in some cases have restricted their use because of their bitterness. Therefore, much of the earlier studies on saponins focused on getting rid of saponins to make the food more edible for human consumption.

4.2.1.12. Phenolics

When it comes to the naming of phenolic compounds, several different substances have one or more hydroxyl substituent and bear an aromatic ring in general. They are thus classified as phenolic compounds.

Phenolic compounds are acidic. The most commonly occur together with sugar as glycosides, which means they are typically soluble in water due to their strong affinity for water molecules (Harborne et al., 1978). Among the simplest groups of naturally occurring substances, phenol (C_6H_5OH) is known to be the prototype.

The phenolic compound group is large and complex, containing a wide range of chemical constituents present in plants (Walton et al., 2003). These compounds are secondary plant metabolites, and they play a significant function in the plant's defensive strategies.

Often known as phenolic compounds, phenolics have many advantageous properties for humans. They have powerful antioxidant properties, which are key components in deciding their effectiveness as antioxidants in neutralizing free radicals that damage cells. of all the plant phenols, flavonoids are the largest group and are among the most researched (Dai et al., 2010).

4.2.1.13. Tannin

The word tannin was invented for the first time by Seguin in 1796. In this context, the term tannin was used to refer to the substance present in plant extracts that react with protein in animal hide, thus preventing the putrefaction of hiding and skin and converting hide and skin into leather.

Tannins are polyphenolic natural substances, and tannin is a derivative of the French tannin or tanning substance. These compounds are known as high molecular weight phenolic compounds, which are nitrogen less and non-crystalline substances.

Tannin compounds are widely distributed in many plant species, which is very common in both gymnosperms and angiosperms. Family, class, and order family, class, and order families are known for their tannins to include *Aceraceae, Actinidiaceae, Bixaceae, Burseraceae,* and *Ericaceae* (Jaiswal et al., 2018).

The Tannins can be found in various plant parts: leaf, seed, root, bud, and stem. These are commonly found in the spaces of plants, such as secondary phloem and xylem, which form a continuous band that separates the cortex from the epidermis. These growth regulators help to control the growth of these tissues. Based on the fact that the phenolic nuclei are involved and how they are joined?

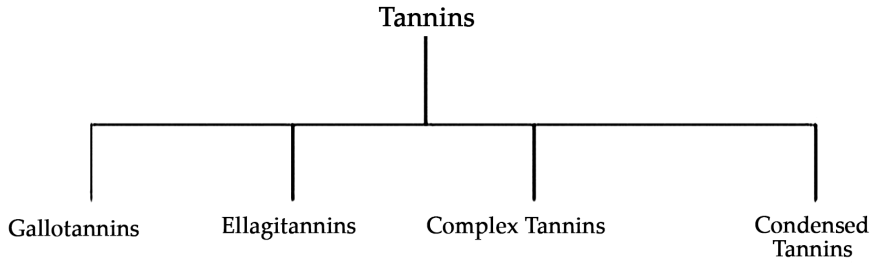

Figure 4.5. Classification of tannins.

The tannins that are obtained from plants are widely used in the treatment of diarrhea, diuretics, and particularly in Asian peoples who have tumorous growths of the intestines like Japanese and Chinese people.

The term tannins may refer to substances found in nature that can help the body when used as natural remedies (De Bruyne et al., 1999). Tannins may have anti-inflammatory, antiseptic, antioxidant, and hemostatic characteristics that can be used as a precursor for a variety of pharmaceuticals.

Additionally, dyes may use synthetic tannins or tannin compounds as harsh caustics in their processes, using these as cationic dyes (Tannin Dyes) or as aniline dyes (Aniline Dyes) (iron gallate ink). When the product has some amount of tannins, then describe it as wine, beer, or fruit juice beverages. Tannins are also widely used in other industrial applications, including in dyes for textiles, as antioxidants in the fruit juice, beer, and wine industries, and as coagulants in rubber processing (Saxena et al., 2013).

4.3. PREVENTION AND TREATMENT OF ANTIOXIDANT PHYTOCHEMICALS FOR SEVERAL CHRONIC DISEASES

When too much oxygen is produced in the human body, an imbalance is created, and this can cause oxidative damage to large biomolecules such as lipids, DNA, and proteins. This extensive damage plays a major role in the development of many human diseases, including CVD, various forms of cancer, and age-related diseases (Poulose, Miller, Shukitt-Hale, 2014; Singh, Suman, & Shukla, 2014).

Because of this, it is believed that antioxidants found in plants can help with the prevention and treatment of chronic diseases (Sung & Lee, 2010; Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005).

Research shows that phytochemicals have been found to possess antioxidant properties *in vitro* as well as in human studies. Studies have proven that the consumption of high levels of fruits and vegetables that are high in antioxidant phytochemicals increases the antioxidant potential of the blood or serum. A good example is the increased total antioxidant ability of serum experienced by elderly women who consume strawberries, red wine, vitamin C, or spinach, all of which are antioxidant-rich foods.

The Plasma vitamin C levels and serum urate levels, which are both plasma vitamin C parameters, also increase when you eat these types of food. This higher serum overall antioxidant ability cannot be solely explained by increased serum vitamin C and urate levels, as other substances have increased too (Cao, Russell, & Lischner, 1998).

The findings were in accordance with the results of another review, which showed that plasma antioxidant potential was significantly improved by eating 10 servings of fruit and vegetables per day for 15 days (Cao, Booth, & Sadowski, 1998).

A possible explanation for this increase is that there has been an increase in the concentration of α-tocopherol in the plasma. It is possible that the increased total antioxidant potential may be explained by the presence of polyphenols since a study found that nearly all of the anthocyanins in blueberries (a common type of polyphenol) were present in human serum.

This observed increase in serum antioxidant potential is likely attributed to total anthocyanins in the serum (Mazza, Kay, Cottrell, & Holub, 2002).

Additionally, polyphenols can help increase the total oxidant-scavenging capacities of human blood by binding to red blood cells (Koren, Kohen, &

Ginsburg, 2010). In short, it has been shown that the antioxidant activity of apples in general can be due to phenolics and flavonoids; however, the antioxidant activity of an apple with skin accounts for just 0.4% of the overall antioxidant activity.

Phytochemicals in fruit and vegetables, such as flavonoids, may be responsible for their antioxidant activity due to their additive and synergistic impact (Liu, 2003; Liu, 2004).

In addition to causing or aiding in the development of many chronic diseases, chronic inflammation is another factor that may exacerbate or help with the onset of these diseases, including cardiovascular disease (CVD), cancers, and type 2 diabetes (T2D) (Dahlen, Tengblad, Lanne, Clinchy, Ernerudh, Nystrom, & Ostgren, 2014; Qiao & Li, 2014; Steinberg, & Schertzer, 2014). A majority of antioxidant phytochemicals have been discovered to possess anti-inflammatory properties.

In addition to resveratrol, anthocyanins, and curcumin, phytochemicals such as resveratrol, anthocyanins, and curcumin have been shown to reduce inflammation by blocking prostaglandin development and nuclear factor-κB activity, as well as enhancing cytokine production (Costa, Garcia-Diaz, Jimenez, & Silva, 2013; Hutchins-Wolfbrandt, & Mistry, 2011).

An antioxidant phytochemical can usually be found to have high antioxidant and free radical scavenging capabilities as well as anti-inflammatory activity. This serves as the basis for additional health benefits and activities (Deng, Xu, Li, Xia, & Li, 2012).

4.3.1. Protective Action on Cardiovascular Diseases

Cardiovascular disease is responsible for the greatest share of deaths and disabilities in developing countries (Reuland, McCord, & Hamilton, 2013). Evidence gleaned from epidemiological research has shown that flavonoids are connected to a decreased rate of cardiovascular disease (CVD) among adults in Europe and the United States (McCullough, Peterson, Patel, Jacques, Shah, Dwyer, 2012; Peterson, Dwyer, Jacques, & McCullough, 2012).

There is a variety of contributing causes to cardiovascular disease, and the overproduction of oxidants is one of the major pathogenic factors. Oxidative damage can cause endothelial cell injuries and vasodilator effects that lead to deleterious outcomes. The Oxidative polyphenols have been shown to be capable of affecting molecular events, resulting in improved endothelial function, and thereby serving an important role in the prevention

of cardiovascular disease. Flavonoids from *Euterpe oleracea* pulp showed athero defensive effects *in vitro* (Costa, Garcia-Diaz, Jimenez, & Silva, 2013).

Other research found that total flavonoids in *Flos chrysanthemum* promote vasodilation and protect endothelium-derived hyperpolarizing factor sensitivity from oxidative stress, both of which are accomplished through mechanisms associated with the endothelium-derived hyperpolarizing factor (He, Ru, Wen, Jiang, Bruce, Jin, Ma, & Xia, 2012).

Macrophage scavenger receptors take up oxidized low-density lipoprotein to facilitate cholesterol ester aggregation and foam cell development, which is another factor leading to atherosclerotic disease. In animal models, quercetin attenuated atherosclerosis by interacting with foam cell formation and pro-oxidant/proinflammatory response, which were key proatherogenic activities of macrophages (Lara-Guzman, Tabares-Guevara, Leon-Varela, Alvarez, Roldan, Sierra, Londono, & Ramirez-Pineda, 2012).

Polyphenols could also protect the cardiovascular system from oxidative stress and other damage, since they possess other physiological effects, such as blood pressure reduction and inflammation decreasing action (Prahalathan, Saravana Kumar, & Raja, 2012).

Conversely, platelet-derived growth factor (PDGF) induced an inhibition of human arterial smooth muscle cell proliferation and migration, which was at least partially due to dehydroglyasperin C (an antioxidant compound of liquorice) (Kim, Cha, Park, Lim, Woo, & Kim, 2013).

Because aggregation of vascular smooth muscle cells plays a key role in the formation and development of lesions in atherosclerosis, dehydroglyasperin C maybe beneficial to CVD. Additionally, a study revealed that blueberry phenolic acids can protect against the buildup of artery-damaging fatty substances (Xie, Kang, Chen, Nagarajan, Badger, & Wu, 2011).

As a result of this platelet aggregation and adhesion under pathophysiological conditions, there is the possibility of blood clot formation in the coronary arteries, which is correlated with the development and intensity of CVD. Some polyphenolic antioxidants can influence the molecular events leading to the inhibition of platelet aggregation (Costa, Garcia-Diaz, Jimenez, & Silva, 2013).

The Stilbenoids, a type of Gnetum macro stachyum found in plants, had both antioxidant and anti-inflammation properties, and exhibited inhibition

of platelet aggregation and adhesion in humans (Kloypan, Jeenapongsa, Sri-In, Chanta, Dokpuang, Tip-Pyang, & Surapinit, 2012).

Also, phlorizin, a polyphenol contained in apples, was found to have a high antioxidant function and helped to avoid diabetic macrovascular complications in diabetic mice (Shen, You, Gao, Li, Yu, & Pei, 2012). In diabetes, complications caused by the macrovascular system, like CVD, are an essential factor.

Thus, phlorizin may be effective. Furthermore, anthocyanins have been shown to have a beneficial effect against several cardiovascular risk factors (Kruger, Davies, Myburgh, & Lecour, 2014). Aside from polyphenols, other antioxidant phytochemicals such as crocin, lycopene, and allicin are also known to show protective properties for cardiovascular disease.

Using a natural antioxidant carotenoid called crocin, it was discovered that this substance blocked platelet aggregation and protected oxidative stress-induced apoptosis of platelets (Thushara, Hemshekhar, Santhosh, Jnaneshwari, Nayaka, Naveen, Kemparaju, & Girish, 2013).

Many research studies found that people who consume a Mediterranean diet with high quantities of tomato products have a lower cardiovascular disease risk because lycopene, an antioxidant found in tomatoes, can boost endothelial function in CVD patients (Weberling, Bohm, & Frohlich, 2011; Gajendragadkar, Hubsch, Maki-Petaja, Serg, Wilkinson, & Cheriyan, 2014).

Garlic organosulfur compounds, allicin in particular, have also been found to protect the cardiovascular system, and this occurs by acting on the vasorelaxation of the blood vessels and alleviating cardiac hypertrophy, angiogenesis, platelet aggregation, hyperlipidemia, and hyperglycemia (Chan, Yuen, Chan, & Chan, 2013).

In this case, it is possible that antioxidant phytochemicals may be excellent choices for reducing CVD and treating it through antioxidants as well as other bioactives like anti-inflammation and preventing platelet aggregation and adhesion.

4.3.2. Anti-Obesity Activity

The prevalence of obesity is increasing globally, and it is becoming a huge public health burden, accompanied by immense social and economic costs. An elevated risk of mortality is often seen in people who are obese, as well as an inability to lead a healthy life due to issues such as sleep apnea, respiratory disorders, osteoarthritis, and infertility (Savini, Catani,

Evangelista, Gasperi, & Avigliano, 2013). In addition, antioxidant defenses like endogenous antioxidant compounds and antioxidant enzymes of the obese are lower compared with their average weight equivalents, which is inversely proportional to central adiposity (Alves, Enes, Martino, Alfenas, & Ribeiro, 2014).

Low-grade chronic inflammation triggered by inflammatory factors, such as tumor necrosis factor-α, interleukin-6, and monocyte chemotactic protein-1, is another important mechanism in developing obesity, which may function synergistically with oxidative stress to contribute to obesity.

Chronic inflammation starts in white adipose tissue and subsequently spreads across the entire body in obesity and type 2 diabetes. *In vitro* and *in vivo* studies have shown that the high concentrations of antioxidant phytochemicals found in fruits and plant extracts effectively reduce weight gain.

Including citrus fruits, some foods and plant parts exhibit inhibitory activity on the enzymes α-glucosidase and pancreatic lipase *in vitro* due to their high content of antioxidant phytochemicals, flavanones among them (Girones-Vilaplana, Moreno, & Garcia-Viguera, 2014).

Finally, phytochemicals from Vaccinium floribunda, also known as Chilean Wineberry, and Aristotelia chilensis, which include anthocyanins and proanthocyanidins, restrict adipogenesis and inflammatory pathways *in vitro* (Schreckinger, Wang, Yousef, Lila, & de Mejia, 2010). Additionally, the administration of Plant ago maxima extract into the diet-induced obese model led to the reversal of obesity.

This Plant ago maxima extracts provided a significant amount of antioxidant compounds, such as flavonoids, iridoids, phenol carboxylic acids, tannins, and ascorbic acid (Tinkov, Nemereshina, Popova, Polyakova, Gritsenko, & Nikonorov, 2014). Metabolic syndrome, which includes obesity, hypertension, and abnormal cholesterol levels, is often accompanied by insulin resistance, abdominal fat accumulation, and endothelial dysfunction.

Grape polyphenols with antioxidant properties may be able to reduce obesity-mediated chronic inflammation through multiple mechanisms, such as antioxidant action, attenuating endoplasmic reticulum stress signaling, blocking proinflammatory cytokines, suppressing inflammatory- or inducing metabolic-gene expression via increasing histone deacetylase activity, and activating transcription factors that antagonize chronic inflammation (Chuang, & McIntosh, 2011).

Therefore, antioxidant phytochemicals are of certain importance for obesity, particularly those with anti-inflammatory activity. Several flavonoids isolated from fruits and plant extracts demonstrated potent anti-obesity activity *in vitro* and *in vivo*. For example, studies suggest that genistein can control adipocytes' life cycle and reduce inflammation and oxidative stress associated with obesity (Behloul, Wu, & Genistein, 2013).

The effect on the development of high fat diet-induced obesity in mice was accompanied by a decrease in fat accumulation and increased insulin sensitivity and glucose intolerance (Dong, Zhang, Bian, Xu, Bao, & Liu, 2014).

To investigate this theory, researchers treated preadipocytes and mature adipocytes with quercetin to test whether AMPK and MAPK signal pathways are involved in the anti-adipogenesis effects of quercetin in preadipocytes and mature adipocytes (Ahn, Lee, Kim, Park, & Ha, 2008). Additional research shows that naringenin, luteolin, and kaempferol also display adipogenesis inhibitory activities (Williams, Edwards, Hamernig, Jian, James, & Johnson, 2013).

In addition to anti-obesity properties, several other phytochemicals have shown anti-obesity activity. Another way to say this is, for example, resveratrol inhibited adipogenesis in order to exert its anti-obesity action *in vitro* (Taing, Pierson, Hoang, Shaw, Dietzgen, Gidley, Roberts-Thomson, & Monteith, 2012).

Also, caffeic acid and chlorogenic acid were found to significantly lower body weight, visceral fat mass, and plasma leptin and insulin levels in the mice exposed to a high-fat diet, relative to the mice who were fed a standard diet. However, chlorogenic acid had a more significant effect than caffeic acid (Cho, Jeon, Kim, Yeo, Seo, Choi, & Lee, 2010).

Lastly, curcumin was shown to significantly lower obesity and limit the deleterious health effects of obesity (Bradford, 2013). Allicin exhibited anti-adipogenesis properties (Williams, Edwards, Hamernig, Jian, James, Johnson, & Tapsell, 2013).

This highlights the double role of phytochemicals: they can block the formation of new fat cells while at the same time promoting fat loss by inhibiting adipogenesis, inhibiting inflammation, and reducing oxidative stress.

4.3.3. Anti-Diabetes Activity

Diabetes is a serious global health issue. It is characterized by the long-term, persistent presence of hyperglycemia, which ultimately causes many microvascular and macrovascular complications (e.g., endothelial dysfunction and atherosclerosis).

Diabetes can be divided into type-1 diabetes (T1D) and type-2 diabetes (T2D). Diabetes is typically caused by a rise in free radicals or oxidative stress, which is attributable to the presence of elevated glucose and lipids (Barbosa, Silveira, de Menezes, Neto, Bitencurt, Estavam, de Lima, Thomazzi, Guimaraes, Quintans, et al., 2013).

It was also shown that, as a result of diabetes and its complications, plasma antioxidants such as α- and γ-tocopherol, α- and β-carotene, lycopene, β-cryptoxanthin, lutein, zeaxanthin, retinol, and ascorbic acid, all had a substantial decrease (Dembinska-Kiec, Mykkanen, Kiec-Wilk, & Mykkanen, 2008).

Studies conducted on cohorts demonstrated improved metabolic homeostasis and postponed or avoided the development of type 2 diabetes and its complications due to the regular consumption of whole grain foods (Belobrajdic, Bird, 2013).

A study found that the antioxidant properties of brown seaweed *Ascophyllum nodosum* (a brown seaweed) were associated with the phenolic content, while the α-glucosidase inhibitory activity was found to have a similar pattern to that of the phenolic content observed *in vitro* (Apostolidis, Karayannakidis, Kwon, Lee, & Seeram, 2011).

In this way, the anti-diabetic activity could be the product of the antioxidative effects. Also, an investigation found similar findings (Girones-Vilaplana, Mena, Moreno, & Garcia-Viguera, 2014). Lime is a candidate for being used to treat diabetes and for lipolysis inhibition (e.g., α-glucosidase and lipase inhibition), which were associated with flavone material.

Also, it is believed that maqui berry polyphenols from blackthorn can be advantageous for people with diabetes and those who prefer foods that are easy and delicious (Girones-Vilaplana, Mena, Moreno, & Garcia-Viguera, 2014).

Polyphenolic compounds prominent in Lactuca sativa (also known as lettuce) are associated with antidiabetic activity in mice (Cheng, Pogrebnyak, Kuhn, Krueger, Johnson, & Raskin, 2014). A second study found that the aqueous extract of Chrysobalanus icaco was very potent in

the antioxidant capacity and was also shown to reduce blood glucose levels in rats (Barbosa, Silveira, de Menezes, Neto, Bitencurt, Estavam, de Lima, Thomazzi, Guimaraes, Quintans, et al., 2013).

Also, the juices from the roots of raphanus, ajwan, sowa, and amaranthus leaves contained antioxidants and could counteract the impact of a starch-based meal on blood glucose levels and limit the magnitude of the postprandial blood glucose increase in rats (Tiwari, Jyothi, Tejeswini, Madhusudana, Kumar, Zehra, & Agawane, 2013).

Besides, Aloe was found to contain several antioxidant phytochemicals and is confirmed to have therapeutic actions for diabetes in the way that it helps to lower blood glucose levels (Botes, van der Westhuizen, & Loots, 2008).

The production of obesity-related insulin resistance often depends on both the presence of low-grade inflammation and a lack of clinically relevant inflammation. Polyphenols in grape products can help to reduce obesity-mediated chronic inflammation, which helps to prevent metabolic diseases.

They exert antidiabetic activity by acting as an antioxidant, blocking pro-inflammatory cytokines and endotoxin-mediated kinases and transcription factors to exert its activity (Chuang, & McIntosh, 2011).

Additionally, curcumin was seen as having the potential to aid in the prevention and alleviation of diabetes, thanks to its anti-inflammatory and anti-oxidant properties (Meng, Li, & Cao, 2013). Butein, an antioxidant polyphenol, protected pancreatic β-cells from cytokine-induced toxicity by inhibiting the production of nitric oxide, and may therefore be used to help prevent the development of type 1 diabetes (Jeong, Lee, Song, Park, Kang, Lee, Kwon, & Kim, 2011).

Additionally, in streptozotocin-induced diabetic rats, ferulic acid demonstrated hypoglycemic activity, and this synergistic effect was greater when used in combination with hypoglycemic drugs (Prabhakar, Prasad, Ali, & Doble, 2013).

Also, kaempferol has been shown to promote β-cell survival, enhancing insulin secretion, and reducing hyperglycemia in the T2D mice (Zhang & Liu, 2011). Daidzein has also been demonstrated to promote glucose absorption, improve glucose homeostasis, and lower glucose levels in T2D mice (Cheong, Furuhashi, Ito, Nagaoka, Yonezawa, Miura, & Yagasaki, 2014). Furthermore, naringenin was found to act as an antioxidant and enhance diabetes-induced memory dysfunction by inhibiting elevated cholinesterase

activity in T2D rats (Rahigude, Bhutada, Kaulaskar, Aswar, & Otari, 2012) and genistein could help prevent diabetes (Fu, Gilbert, Pfeiffer, Zhang, Fu, & Liu, 2012).

Older adults with type 2 diabetes mellitus (T2DM) benefit greatly from PYC, a combination of procyanidins (made up of catechin and epicatechin subunits) with varying chain lengths, since the supplementation lowers blood glucose levels.

These findings indicate that the supplement could have anti-diabetes properties in patients with T2DM (D'Andrea, Pycnogeno). After going over all it is apparent that phytochemicals can combat diabetes by modulating α-glucosidase and lipase activities, lowering postprandial glycemic levels, having anti-inflammatory activity, enhancing pancreatic function, and working together with hypoglycemic drugs.

4.4. PHYTOCHEMICALS AND CANCER

Natural products consisting of isoprenoid units, which accounts for over 40,000 distinct compounds, make up the second-largest natural product class, after phenols (Thoppil and Bishayee, 2011). Natural products have been studied in the production of cancer-preventive anti-cancer agents. As a result, a number of terpenoids were identified, which inhibit cancer cell proliferation.

These terpenoids are considered good candidates for anti-cancer chemopreventive regimens (Huang et al., 2012). Currently, a variety of triterpenoids are being investigated in a number of phase I/II clinical trials to test the chemopreventive properties and anticancer effects of these chemicals (Liby et al., 2007; Fulda, 2009).

Although epidemiological and experimental research suggests that mono-, di- and tri-terpenoids may be beneficial in preventing and treating many cancers, such as breast, skin, lung, digestive tract, colon, pancreatic, and prostate tumors, this has not yet been substantiated in the clinic (Kris-Etherton et al., 2002; Gould, 1997; Reddy et al., 1997; Vigushin et al., 1998; Crowell, 1999; Burke et al., 2002; Carvalho and Fonseca, 2006; Barile et al., 2008; Corea et al., 2009).

While a variety of prior animal trials have proved effective in demonstrating the anti-cancer and pro-cancer properties of both naturally occurring and synthetic derivatives of triterpenoids, there is currently only one human trial assessing the ability of these compounds to prevent and treat

skin, prostate, and colon cancers (Liby et al., 2007; Chaturvedi et al., 2008; Rabi and Gupta., 2008; Mull-auer et al., 2010; Bishayee et al., 2011; Patlolla and Rao, 2012; Lanzotti et al., 2012; Zolfaghari et al., 2013).

A complete Chinese traditional medicine scheme includes terpenoids derived from the species of Rhizoma Curcumaeas that have been shown to contain anti-cancer therapeutics for use in Chinese medicine (The State Pharmacopoeia Commission of P.R. China, 2005).

Studies that have examined terpenoids derived from Rhizoma Curcumae, including b-elemene, d-elemene, furanodiene, furanodienone, curcumol, and germacrone, have shown that these are correlated with the inhibition of cell cycle arrest, activation of apoptosis, and the inhibition of metastasis or tumor metastasis (Lu et al., 2012).

Elemene has also been approved by China's State Food and Drug Administration as an anti-cancer adjuvant drug and has been prescribed as a part of several cancer treatment regimens in China. As reviewed elegantly by Lu et al., (2012) b-elemene exhibits broad-spectrum anti-cancer activity against several forms of cancer cells, including leukemia, brain, breast, prostate, ovarian, cervical, colon, laryngeal, and lung carcinoma cells. Also, it has been stated that b-ELEMENTE has very low toxicity to normal cells (Li et al., 2005a,b,c; Wang et al., 2005).

Furanodinene, a sesquiterpene, exhibits growth inhibitory properties on the following cell lines: HeLa, Hep-2, HL-60, PC-3, SGC-7901, MCF-7, MDA-MB-231, and HT-1080 (Zhong et al., 2012; Sun et al., 2009).

Furthermore, it was shown to hinder tumor growth in mice (Zheng et al., 2008). Curcumol, another sesquiterpenoid, blocks the proliferation of MCF-7, MM-231, HeLa, and OV-UL-2 cancer cells, as well as normal breast cells, and does not have much of an impact on proliferation in the cells that it targets (Xu et al., 2005).

Inhibition of the complete RNA synthesis is dramatically reduced in MCF-7, MM-231, and HeLa cells after treatment with 50 mg/ml of curcumol (Xu et al., 2005). Curcumol has been reported to cause cell death that is concentration-dependent in human lung adenocarcinoma ASTC-a-1 cells and to arrest the cell cycle in G2/M process (Zhang et al., 2011).

At inhibiting the incidence and mean number of visible hepatocyte nodules as well as the size of complete, permanent, and remodelling p-gene-positive preneoplastic lesions, the sesquiterpene farnesol had an impact (Ong et al., 2006).

This daphnane-type diterpenoid, known as GNIDIMACRIN, was reported to suppress the growth of protein kinase CbII gene-transfected human hepatoma HLE cells during the G2 process by arresting cell growth (Yoshida et al., 2009).

Another study isolated oridonin, a diterpenoid present in *R. rubescens-necens*, which possesses cytotoxic activity against H.G2 cells. Additionally, this study discovered that oridonin increased the number of apoptotic cells and the production of ROS (ROS radicals) (Huang et al., 2008).

He-dyotic diffusa and Radix actinidiae, which are widely used in the treatment of colorectal cancer in China, are used in Chinese traditional medicine for this condition. This herbaceous triterpene has three-ring compounds known as ursolic acid; an active compound found in these herbs. Many studies found that Ursolic acid was able to have significant inhibitory effects on various cancer cell lines (Subbaramaiah et al., 2000; Shishodia et al., 2003).

Inhibiting the EGFR/MAPK pathway reduces proliferation and induces apoptosis in HT-29 human colon cancer cells, which involves interfering with the EGFR/MAPK pathway (Shan et al., 2009). Inhibiting the growth of HT-29 cells in dose- and time-dependent manners was another effect of the toxin.

These median inhibition concentrations (IC50) for treatment at 24, 48, and 72 hours yielded IC50 values of 26, 20, and 18 L mol/L, which led to apoptotic rates up to 40.5% for the highest dose. Recently, a transgenic adenocarcinoma of mouse prostate (TRAMP) mouse study was conducted in order to find out whether a stage-specific chemopreventive activity has Ursolic acid was added to feed containing 1% w/w to see whether or not this food enriched with 1% w/w Ursolic acid has chemopreventive activity against prostate cancer (Shanmugam et al., 2012).

It was stated that TRAMP mice fed with a diet containing UA for eight weeks had delayed the development of prostate intraepithelial neoplasia (PIN). The mice in a different study that were fed urosolic acid diet for six weeks had a decrease in the progression of PIN to adenocarcinoma as shown by hematoxylin and eosin staining.

TRAMP mice fed a tri-terpinoid diet for 12 weeks showed significantly reduced tumor development, even though the mice gained less weight than mice who received the same diet but were not treated with the compound. On the other hand, a study investigated the potential influence of ursolic acid on the growth of the BGC-803 gastric cancer cell line and hepatobiliary

cancer cell line H22 xenografts (Wang et al., 2011). It was proven in *in-vitro* studies that the agent has an inhibitory effect on the growth of BGC-803 cells and this can be seen by the fact that it can also inhibit the progression of tumor cells which are currently in the G0/G1 phase. *In vitro* and *in vivo*, there was a substantial increase in the apoptotic rate in tumor cells treated with urosolic acid.

The treatment causes DNA fragmentation in BGC-803 and drives the expression of activated caspase-3, -8, and -9 as well as Bcl-2 to decrease while also inducing increased expression of activated caspase-3, -8, and -9 in BGC-803 cells.

Furthermore, the expression of caspase-3 and -8 was elevated in tumor cells that were treated with a tri-terpenoid compound that was xenografted into a different animal. Researchers found that18F-FLT PET-CT imaging confirmed the efficacy of the chemopreventive terpenoid in combatting tumor formation.

Additional studies showed that ursolic acid prevented the proliferation of different colon cancer cell lines, which correlated with inhibition of constitutive NF-jB activation and down-regulation of cell survival (Bcl-xL, Bcl-2, cFLIP, and survivin), proliferative (cyclin D1), and metastatic (VEGF, MMP-9, and ICAM-1) proteins.

The results were extrapolated in animal research. . The animal model was employed when assessing in orthotopic nude mouse model, ursolic acid inhibited tumor volume, ascites development, and distant organ metastasis (Prasad et al., 2012).

The results of the study have found that urosolic acid supports the therapeutic effects of capecitabine (chemosensitization) by reducing the level of multiple biomarkers that are associated with inflammation, proliferation, invasion, angiogenesis, and metastasis.

The most important benefit of urosolic acid was that it potentiated the apoptotic effects of thalidomide and bortezomib in multiple myeloma cells, demonstrating its translational importance as an adjuvant treatment (Pathak et al., 2007).

Treatment for many types of cancer relies on taxanes, which are potent cytotoxic terpenes extracted from natural products. Taxanes have been studied in clinical trials and laboratory studies and show promising results in preventing cell division in a wide range of cancers, such as breast, ovarian, lung, prostate, pancreas, gastric, and head and neck cancer (Tannock et al.,

2004;Khan et al., 2003; Roth and Ajani2003; Nabell and Spencer2003). When looking into Taxol and Taxotere as well as other taxanes homologs, which are made from natural sources, Fauzee et al., (2012) found that most taxanes included Paclitaxel (Taxol) and Docetaxel (Taxotere).

The popular Taxol is derived originally from Taxus Brevifolia (bark of Pacific yew/Western yew conifers) (Wani et al., 1971) while docetaxel is an esterified derivative of10-deacetylbaccatin-III (10-DAB) extracted from Taxus Baccata(needles of European yew tree) (Biss-ery et al., 1991).

When Abraxas Bioscience (now Abraxis Biomed) introduced their latest drug, Abraxane, in 2005, it was a game changer for patients with breast metastases and patients with lung cancer. Both patient populations were followed by subsequent clinical trials in June 2010, which confirmed Abraxane's efficacy (Fauzee et al., 2012; Pazdur2005; Gen News Highlights, 2010).

4.5. PHYTOCHEMICALS AND CIRCULATORY SYSTEM DISEASE

Many epidemiological studies have shown a link between vegetable and fruit consumption and coronary heart disease, even though the apparent connection is difficult to attribute to macronutrients and known vitamins and minerals. It can be deduced from this finding that other components of plants can play a significant role in lowering the risk of cardiovascular disease.

Also, it has been said that ingredients in the supposition, which is intended to reduce the severity of cardiovascular and other chronic diseases, has in fact brought about stories of miraculous ingredients that have beneficial effects on cardiovascular disease and other chronic diseases. The two primary fields of scientific research have accumulated significant amounts of evidence and they are plant sterols, flavonoids.

4.5.1. Plant Sterols

The plant kingdom contains several sterols that differ from cholesterol by having ethyl or methyl groups or unsaturation in the side chain. While both sitosterol, stigmasterol, and campesterol are present in Western diets in almost equal quantities to dietary cholesterol, the predominant ones can be found in the diet (and have been found in there) in lower concentrations (T Miettinen, et al, 1990). The most common of these sterols are 24-carbon-

based β-sitosterol, which varies from cholesterol in that it has an ethyl group at carbon 24 of the side chain.

At the dawn of the 1950s, researchers discovered that feeding sitosterol to cholesterol-fed chickens or rabbits had the side effect of lowering both animal's and human cholesterol levels. The herb even helped protect against atherosclerosis in rabbits (Pollak, Kritchevsky & Sitostero, 1981).

Extensive research in the use of soy sterols and mixtures of soy sterols was performed between the years of 1950 and 1960 to evaluate their cholesterol-lowering capabilities (Lees, Mok, Lees, McCluskey, & Grundy, 1977). When we mixed the ingredients, the expected level of cholesterol reduction was approximately 10% (Vahouny & Kritchevsky, 1981).

This is based on data that indicates that the mode of action includes inhibition of cholesterol absorption, while plant sterols are only slightly absorbed (Tilvis & Miettinen, 1986). Since crystallization and co-precipitation appear to be involved in cholesterol absorption inhibition, this mechanism is most likely to be at work.

In addition to the 38% reduction in cholesterol absorption in the food containing 500 mg of cholesterol, 1 g of β-sitosterol decreased cholesterol absorption by 42% in the meal (Mattson, Grundy, & Crouse, 1982).

There has likely been an increase in LDL receptor activity, and as a result, the decreased cholesterol. However, the reduction in plasma cholesterol is relatively less than the decrease in absorption, possibly because of a compensatory rise in cholesterol synthesis. This part of the environment should be studied again with more advanced technology.

It was shown in the decade of 1980 that a 5-α saturated sitosterol analogue, sitostanol, had a greater impact on intestinal absorption of cholesterol and serum cholesterol than did its saturated cousin, sitosterol, and did so at doses that were lower than that of sitosterol (Heinemann, Leiss, & von Bergmann, 1986).

In a recent study (Miettinen, Puska, Gylling, Vanhanen, & Vartiainen, 1995), sitostanol was interesterified with margarine, and the resulting product (1.9 to 2.6 g sitosterol per day) had a hypocholesterolemic impact in a population with moderate hypercholesterolemia.

The mean decreases in plasma cholesterol of 10.2% occurred over a year. The sitostanol failed to be absorbed, and hence it is unlikely to have interfered with fat-soluble vitamin absorption. The idea that Squalene, a sterol precursor that is also present in plants, may have a cholesterol-

lowering effect had already been put to the test on animals and was ultimately found to have no beneficial effect on atherosclerosis (Pollak, Kritchevsky, & Sitosterol, 1981).

Sitosterol's and squalene are found in both monounsaturated and polyunsaturated vegetable oils, and this may help to explain some of the variable cholesterol-lowering effects that have been observed in studies that use these products; this may also explain the discrepancies between different types of olive oil and degrees of refinement.

Other cholesterol-lowering alcohols found in rice bran oils include esters of triterpene alcohols that inhibit hepatic cholesterol esterase and tocotrienols that inhibit HMG Co-A reductase (Rukmini, Raghuram, 1991).

Although on the one hand, there is evidence to support this claim, on the other, the research seems to provide a contradicting view (Lichtenstein, Ausman, Carrasco, Gualtieri, Jenner, Ordovas, Nicolosi, Goldin, & Schaefer, 1994).

Cafestol is a terpene found in coffee, in particular decaf coffee. This has been proposed in various studies, but there is no consistency between them, and it appears that coffee consumption may have no relationship with changes in plasma cholesterol that the presence of this compound could explain. Some say that the preparation method can influence the effects of coffee, while others say that the filtering method may help extract cholesterol-raising compounds.

4.5.2. Flavonoids

Flavonoids are 2-phenyl-1-benzopyran-4-one derivatives used in various plant foods such as fruits, vegetables, nuts, and seeds (Kuhnau, 1976; Cook, & Samman, 1996). Flavonoids are the largest flavonoid category, containing flavanols, flavones, catechins, flavanones, and anthocyanins. Many of these compounds are obtained from dietary sources such as tea, onions, soy, and wine.

The most important flavonoid in onions is quercetin glucoside, while the most important flavonoid in tea is quercetin rutinoside. Fruit and vegetable intake has been inversely associated with cardiovascular disease in a number of studies, including the Zutphen Elderly Study (Hertog, Feskens, Hollman, Katan, Kromhout, 1993), the Seven Countries Study (Hertog, Kromhout, Aravanis, Blackburn, Buzina, Fidanza, Giampaoli, Jansen, Menotti, Nedeljkovic, Pekkarinen, Simic, Toshima, Feskens, Hollman, & Katan, 1995), and a cohort study in Finland (Knekt, Jarvinen, Reun). In the Zutphen

Elderly Study (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993), in which 0 to 19.0 mg/d of flavonoid intake was associated with a coronary heart disease mortality rate of 18.5 per 1000 person-years, compared to a rate of 7.8 among those who consumed more than 29.9 mg/d (30 mg/d represents approximately five to six cups of tea per day), a flavonoid intake of 0 to 19.0 mg/d was associated with a coronary heart disease mortality rate of 18.5 per 1000 person-years, while the rate among those who consumed more than 29.9 mg/d was 7.8 per 1000.

It should be noted that certain flavonoids are harmful (or maybe) in large quantities, such as when ingested, or when applied to the skin or mucous membranes in very large amounts. Therefore, it is necessary to undertake systematic study on the primary groups of flavonoids in order to better define their structure, effectiveness, and possible side effects.

Although it has yet to be proven, the link between flavonoids and atherosclerosis is theorized to be due in part to the observed fact that certain flavonoids possess antioxidant properties and have been demonstrated to be effective inhibitors of LDL oxidation *in vitro*. For example, red wine phenolic compounds such as Rosmarinus acid can inhibit the oxidation of human LDL (Frankel, Kanner, German, Parks, & Kinsella, 1993).

Some flavonoids have also been shown to suppress platelet aggregation and adhesion, which may be another way they lower the risk of heart disease. In addition, lowering plasma cholesterol and having estrogen-like effects have also been documented in studies on the properties of isoflavones in soy (Dwyer, Goldin, Saul, Gaultieri, & Bakarat, 1994).

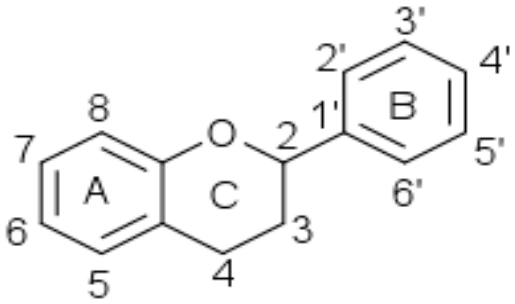

Figure 4.6. The basic structure of flavonoid.

Sources: Image by Wikimedia.

4.6. CONCLUSION

Chronic diseases are responsible for the most significant number of deaths and disabilities. The reactive oxygen or nitrogen species under specific conditions can cause an imbalance and lead to oxidative damage to large biomolecules such as lipids, DNA, and proteins.

It is thought that chronic diseases are caused by the chronic inflammation and oxidative stress that many people experience. Therefore, antioxidant phytochemicals are among the most effective therapeutic agents for treating chronic disease.

Often referred to as the biomedical benefits of an herb, an herb's biological activities and health benefits include antioxidant and free radical scavenging abilities, anti-inflammatory activity, anticancer, anti-aging, and protection from diabetes, obesity, and neurodegenerative diseases.

To be even more specific, numerous antioxidants, such as resveratrol, have more than one property; for example, they are also protective against cardiovascular disease, tumors, aging, obesity, diabetes, and Alzheimer's disease. Therefore, as a dietary recommendation, it is advised to eat fruits, vegetables, and grains and certain medicinal plants more frequently since they are loaded with antioxidants phytochemicals.

REFERENCES

1. Egamberdieva, D., (2017). Phytochemical and Pharmacological Properties of Medicinal Plants from Uzbekistan: A Review Plants from Uzbekistan: A Review. [online] Scholarworks.umass.edu. Available at: https://scholarworks.umass.edu/cgi/viewcontent.cgi?article=1117&context=jmap (accessed 9 September 2021).
2. Howard, B., & Kritchevsky, D., (1997). Phytochemicals and Cardiovascular Disease. Circulation, *95*(11), 2591–2593.
3. Mendoza, N., & Silva, E., (2018). Introduction to Phytochemicals: Secondary Metabolites from Plants with Active Principles for Pharmacological Importance. Phytochemicals – Source of Antioxidants and Role in Disease Prevention.
4. Sharma, D., Shah, K., & Dave, R., (2020). Phytochemicals: Promising Potential Uses In Pharmacology. [online] Research Gate. Available at: https://www.researchgate.net/publication/341678677_Chapter_-6_phytochemicals_promising_potential_uses_in_pharmacology (accessed 9 September 2021).
5. Tepe, B., & Cilkiz, M., (2015). A pharmacological and phytochemical overview on Satureja. *Pharmaceutical Biology, 54*(3), 375–412.
6. Ullah, M., Bhat, S., Husain, E., Abu-Duhier, F., Hadi, S., Sarkar, F., & Ahmad, A., (2014). Cancer chemopreventive pharmacology of phytochemicals derived from plants of dietary and non-dietary origin: implication for alternative and complementary approaches. Research Gate.
7. Zhang, Y., Gan, R., Li, S., Zhou, Y., Li, A., Xu, D., & Li, H., (2015). Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules*, *20*(12), 21138–21156.

CHAPTER 5

ANTIMICROBIAL AND ANTIVIRAL PROPERTIES OF PHYTOCHEMICALS

CONTENTS

This chapter revolves around the antimicrobial and antiviral properties of phytochemicals. It begins with illustrating the history of natural products used as antiviral drug. Then it goes on to explain the classification of antiviral phytochemicals, the flavonoids.

It also sheds light on the antimicrobial properties of different classes of phytochemicals. It further explains the plant antimicrobials and the food industry such as the simple phenolics, flavones and flavonoids. Then towards the end it goes on defining the antimicrobial properties in *Clitoria ternatea*.

5.1. INTRODUCTION

A virus is a small infectious agent that is mainly made up of RNA or DNA gene strands, a protein shell may envelop it. Bacteria are free-living entities, viruses on the contrary use the environment of their host cells to spread new viruses.

Then, the newly produced viruses leave the host cell, sometimes killing them and infecting other cells. Viral infections can cause mild illnesses, such as colds or fatal diseases, such as The Bloody African fever.

Lassa Fever, Ebola, Acquired Immune Deficiency Syndrome (AIDS) caused by a retrovirus are easily spreading diseases caused by viral infections that threaten human life and health very rapidly sometimes, and there is no cure or vaccine for these (Jassim and Naji, 2003). One of the major causes of global morbidity and mortality today is viral infection (Golean et al., 2009).

Viruses have multiple invasion strategies; every virus strain has its own unique surface molecular configuration. These molecules act as the key to the lock and allow the virus to invade the host by fine-tuning the molecules on the surface of the target cell (Jassim and Naji, 2003).

As viruses and hosts are closely connected, it has proven challenging to develop effective antiviral drugs that inhibit viral enzymes or replication without affecting host cells (Golean et al., 2009; Chattopadhyay et al., 1999). Public health policies and prophylactic vaccines are still essential tools used by society to control the spread of viral infections.

In the last 20 years, advances in molecular biology and reverse genetics have clearly shown that many viruses have unique characteristics in their structure or replication cycle, which are their potential targets. As viral enzymes are essential for disease progression and virus replication, viral enzyme inhibitors have been the most ideal combat viruses.

Most of the well-studied inhibitors against HIV, herpes simplex virus (HSV) or the influenza viruses target the host cell binding (T-20, betulinic acid, etc.), uncoating of capsid (amantadine derivatives, pleconaril), replication (reverse transcriptase inhibitors like zidovudine or abacavir, nevirapine, etc.), integrase inhibitors, DNA or RNA polymerase inhibitors (acyclovir, cidofovir, ribavirin, etc.), proteinase involved in viral polyprotein precursors and assembly/maturation inhibitors (indinavir, ritonavir and rimantadine, etc.). Based on this strategy, a variety of compounds have been tested on multiple viruses in the past few decades, but there are still less than 40 approved antiviral drugs on the market (Chattopadhyay et al., 2009).

Viruses have developed a resistance towards the current antiviral drugs, this has increased the demand for new compounds that are effective against viral infections. At present, some new antiviral compounds are under preclinical or clinical evaluation, and there is a promising prospect of finding interesting new antiviral drugs (Chattopadhyay et al., 2009). There are several natural compounds isolated from plants used in traditional medicine among these antiviral agents.

According to statistics, in the last 20 years, natural products have become one of the most important resources for the research and development of new drugs, especially for the treatment of infectious diseases. Among the antiviral drugs approved between 1981 and 2006, approximately 44% were natural substances, semi-synthetic analogues of natural substances, or synthetic compounds based on natural-product pharmacophores (Newman and Cragg, 2007).

Plant extract drug screening has resulted in evaluating their *in vitro* antiviral activities (Jassim and Naji, 2003; Mukhtar et al., 2008). Furthermore, the use of new technologies and methods, such as high throughput screening (HTS) and molecular biology, has increased the likelihood of discovering valuable new bioactive plant extracts or compounds (Li and Vederas, 2009).

5.2. HISTORY OF NATURAL PRODUCTS AS ANTIVIRAL DRUGS

The history of medicinal plants can be traced back to the origin of human civilization on earth (Mukhtar et al., 2008). Earlier, natural products were used to treat all diseases, including viral ones directly. Natural products have contributed significantly to human health greatly, many effective constituents of natural substances have been identified, and their mechanism of action has been elucidated.

In the past 60 years, the inhibitory effect of medicinal plant extracts on the replication of multiple viruses has been reported. In 1952, Boots Pharmaceuticals (Nottingham, England) tested 288 plants for anti-influenza activity. Twelve of these plants are effective against influenza viruses in embryonated eggs (Chantrill et al., 1952).

The antiviral activity of extracts from grapes, apples, strawberries, and other juices against HSV, poliovirus type 1, Coxsackievirus B5, and echovirus was reported in the 1970s (Konovalchuk and Speirs, 1976, 1978a, b).

In 1995, 100 kinds of medicinal plants in British Columbia also showed antiviral activity, 12 of which showed antiviral activity against corona viruses, respiratory syncytial virus (RSV), para-influenza virus type 3 (PI3), herpesvirus type 1 (HSV-1), and rotavirus (McCutcheon et al., 1995). In 1998, more than 800 common Chinese herbal medicines were discovered to have antiviral effects and more than 100 plants showed anti-HIV activity (Lou, 1998).

In 2005, more than 200 Chinese herbal medicines were tested for antiviral activity against severe acute respiratory syndrome-associated coronavirus (SARS-CoV), and four of them displayed intense antiviral activity (Li et al., 2005a).

Furthermore, herpes simplex virus type 2 (HSV-2) (Debiaggi et al., 1988), HIV (Asres and Bucar, 2005; Vermani and Garg, 2002), hepatitis B virus (HBV) (Huang et al., 2003, 2006; Quan et al., 2005), dengue virus type 2 (DEN-2) (Parida et al., 2002; Reis et al., 2008), and emerging viral infections related to poxvirus and severe acute respiratory syndrome (SARS) (Kotwal et al., 2005) is strongly inhibited by various plant extracts. Most of these studies have used water-soluble or alcoholic herbal extracts, and limited efforts have been made to identify active natural ingredients exhibiting antiviral effects.

5.3. CLASSIFICATION OF ANTIVIRAL PHYTOCHEMICALS

5.3.1. Flavonoids

The structure of flavonoids, primarily a polyphenol, made up of 15 carbon atoms skeleton (C6-C3-C6 system), is the largest source of antiviral agents in the entire plant kingdom. In some compounds, the C2 carbon atom is directly attached to oxygen, forming a furan-like molecule called Auron.

The oxidation and substitution pattern of the ring C is used to subclassify flavonoids further. The biochemical effects of flavonoids can be achieved by inhibiting a variety of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca+2-ATPase, lipoxygenase, cycloxygenase, etc. Besides the regulatory role on different hormones like estrogens, androgens, and thyroid hormone. When evaluating the activity of flavonoids against the herpes simplex virus (HSV), Thomas et al., observed that flavanols are more active than flavones (galangin > kaempferol > quercetin). A flavonoid-based polymer (MW 2100 Daltons) is effective against herpes simplex types 1 and 2.

According to the evaluation of the flavonoid subgroup, Gerdin et al., found that Flavan-3-o1 is more effective in selectively inhibiting the human immunodeficiency virus (HIV)-1, -2 and similar immunodeficiency virus infections. Chalcone with the general formula Ar CH = CHC (=O) Ar forms the core of many critical biological compounds. These compounds are considered to be the precursors of flavonoids and iso- flavonoids, which are widely found in edible plants and have a variety of pharmacological effects. Deng et al., reported the excellent antiviral activity of chalcones 3 and 4, which use pharmacophore models to identify chemical characteristics important for antiviral activity.

Dihydrochalcones (obtained by reducing the double bond of chalcone) are a derivative of Millettia leucantha KURZ (Leguminosae) and have shown activity against herpes simplex virus (HSV). Flavones are structurally characterized by the 2-phenylchromen-4-one skeleton, and exist in Lamiaceae, Apiaceous, and Astraea families.

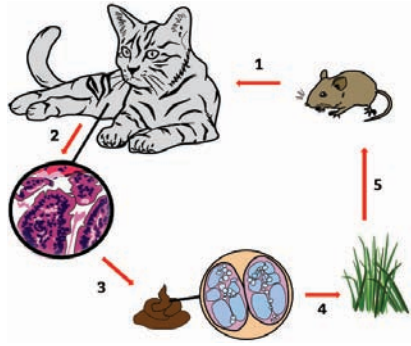

Figure 5.1. An illustration of the Toxoplasmosis cycle.

Source: *Image by Wikimedia Commons.*

Based on molecular electrostatic potential (MEP) maps, Mishra et al., proposed that the anti-picornavirus activities of flavones are related to negative MEP values in two regions, one near the 3-methoxy group the other in a diagonally opposite region near the substituent attached to the C7 atom of the molecules, based on molecular electrostatic potential (MEP) maps.

5.3.2. Alkaloids

Alkaloids are synthesized by plants from amino acids that contain nitrogen in a heterocyclic ring. The *in vitro* activities of 36 alkaloids isolated from Catharanthus plants or *C. lanceus* against vaccinia virus and polio type III virus were tested. Nine of these alkaloids proved to be effective antiviral drugs, of which Percaline is the most effective. Houghton et al., tested numerous naturally occurring chromone alkaloids (derived from the rootbark of *Schumanniophyton magnificum*) for the inhibition of HIV and HSV infections in C8166 and Vero cells in order to gain SAR data.

The free hydroxyl groups and the piperidine ring in the molecule seem to promote anti-HIV activity. Irreversible binding to GP 120 is believed to be responsible for the anti-HIV activity.

5.3.3. Terpenoids

Terpenoids, sometimes called isoprenoids, are a large variety of natural phytochemicals derived from five-carbon isoprene units, composed and modified in thousands of ways. Multiple phytocompounds were tested for anti-severe acute respiratory syndrome-associated coronavirus (SARS-CoV) activity using a cell-based assay measuring the SARS-CoV-induced cytopathogenic effect on Vero E6 cells, and compounds (30–32) demonstrated excellent activity.

Using a cell-based test to measure the cytopathogenic effects of SARS-CoV on Vero E6 cells, the activity of more than 220 plant compounds (including 10 diterpenoids, two sesquiterpenes, and two triterpenoids) against SARS-CoV was tested. Abietane-type and labdane-type diterpenes, sesquiterpenes, and lupane-type triterpenes were among the bioactive compounds with anti-SARS-CoV activity in the μM range.

5.3.4. Carotenoids

Carotenoids are considered the backbone of the compound's structure; they belong to the tetraterpenoids (hydrocarbons formed by several isoprene

units). Most carotenoids are derived from 40 carbon polyene chains that sometimes end in a ring.

Carotenoids can be Xanthophylls (oxygen-containing molecules), such as lutein and zeaxanthin, and carotenes(the unoxygenated or oxygen-free carotenoids). Plasma carotenoids (α-carotene, β-carotene, lutein/zeaxanthin, and lycopene concentrations have been linked to an increased risk of death during HIV infection in Ugandan infants.

5.3.5. Organosulfur Compounds

All vegetables in the cruciferous family have sulfur-containing compounds. Furthermore, allium family plants represent an important class of antiviral agents. There are several typical examples of organosulfur antivirals, namely cauliflower, cabbage, kale, bok choy, brussels sprouts, radish mustard, and water garden cress, are rich in organosulfur compounds.

Several asymmetric aralkyl disulfides have been synthesized and oxidized to study the relatively unobserved class of thiosulfates. Because of the strong odor and chemical instability of these compounds, animal studies are difficult; thus, structural modifications were made. Multiple derivatives of sulfamate (based upon brassinin and sulforaphane structures) have been synthesized and screened for their HSV activities.

5.3.6. Vitamins

Studies have shown that vitamin C can enhance the host's immune response and provide protection against infectious diseases. Vitamin E supplementation can effectively treat chronic hepatitis B. The term "Vitamin E" refers to a group of eight fat-soluble compounds known as tocopherols (methyl derivatives of tocopherol) and tocotrienols.

5.3.7. Selenium Compounds

A large number of studies have shown the importance of selenium compounds as effective antiviral agents. The data obtained from experiments in various animal models and *in vitro* models show that selenium has a significantly positive effect on various viral infections.

Cermelli et al., examined the antiviral effects of three selenium compounds on Coxsackie B5 virus replication. Selenite has been shown to reduce virus replication during Coxsackie B5 virus replication, but selenate and selenomethionine do not show any significant antiviral activity.

Waotowicz et al., synthesized and tested the activity of various ebselen analogues in an *in vitro* antiviral test. Some of the tested analogues showed significant inhibition of the cytopathic activity of HSV-1 and encephalomyocarditis virus-EMCV.

5.3.8. Miscellaneous

Curcumin is obtained from turmeric, an important food ingredient in the Indian subcontinent, and has shown high activity against HIV-1 integrase. Chlorophyllin (CHLN) is a synthetic chlorophyll derivative that has been tested for its ability to prevent nuclear fragmentation (NF) in HEP-2 cells infected with poliovirus.

Carboxymethyl chitin is a polysaccharide polymer with partially deacetylated amino sugar, it has been shown to significantly inhibit Friend murine leukemia helper virus (F-MuLV) and HSV. Seven ellagitannins isolated from *Phyllanthus myrtifolius* and *P. urinaria* (Euphorbiaceae) have been shown to be active against Epstein–Barr virus DNA polymerase (EBV-DP).

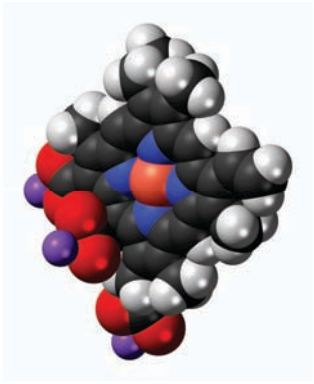

Figure 5.2. Structure of chlorophyllin.

Source: *Image by Wikimedia Commons.*

Polyacetylene is a hydrocarbon that can strongly absorb long-wave ultraviolet rays. The therapeutic activity of these compounds is changed by exposure to light (light activation). Phenylheptatriyne (PHT) from the leaves of *Bidens pilosa* is one of the polyacetylenes that have been extensively studied for its antiviral effect that can be enhanced by exposure to ultraviolet light. Polyacetylenes are one of the few natural substances that inhibit

CMV. CMV is a herpes virus that causes diseases in people with weakened immunity. Importantly, unlike other herbal photoactivated substances, such as furanocoumarins found in Umbelliferae plants, these polyacetylenes do not cause DNA changes, and the action seems to be mediated by cell surface activities, implying a higher level of safety for their use.

The highly sulfated red alga polysaccharide ($Cn(H_2O)n$) is extracted from *Gelidium cartilagineum*. It can provide protection against animal viruses, influenza B viruses and mumps. Red microalgae Porphyridium sp. has a sulfated polysaccharide cell wall and has shown impressive antiviral activity against herpes simplex virus types 1 and 2 (HSV1,2) and varicella-zoster virus (VZV).

One of the main categories of phytoestrogens are Lignans. Phytoestrogens are chemical substances similar to estrogen that also act as antioxidants. Nordihydroguaiaretic acid (NDGA), a lignan found in the perspired resin of *Larrea divaricata* leaves, inhibits several viruses in vitro, including HIV, HSV-1 and -2, and human papilloma.

Retrojusticidin B, an anti-HIV reverse transcriptase agent isolated from *Phyllanthus myrtifolius*, has been studied in rats for its pharmacokinetics and metabolism. Chrysophanic acid (1,8-dihydroxy-3-methylanthraquinone) is isolated from *Dianella longifolia*, a native medicinal Australian plant, and can inhibit poliovirus types 2 and 3 (*in vitro* SARS-CoV) from replicating.

The spike (S) protein is a type I membrane-bound protein that is required for the virus to bind to the angiotensin-converting enzyme 2 receptor on the host cell (ACE2). Emodin, derived from Rheum and Polygonum, has been shown to significantly inhibit the interaction of S protein and ACE2 in a dose-dependent manner.

It also prevents the S protein-pseudo-typed retrovirus from infecting Vero E6 cells. This demonstrates that emodin has the potential to be a lead therapy for the treatment of SARS. Gingerols, (derived from ginger, a common South Asian spice) is traditionally used to treat colds and throat infections, it is an important component of Ayurvedic medicine. Several studies have been conducted to demonstrate the effectiveness of such medicines as antiviral agents.

Salicylic acid (C_6H_4 (OH) CO_2H) can stimulate the inhibition of the three major stages of viral infection: replication, cell-to-cell transmission, and long-distance transmission. Evidence has shown that Salicylic Acid can stimulate the downstream pathway, resulting in the induction of RNA interference-based resistance mechanisms.

5.4. ACTIVITY OF EXTRACTS/MIXTURES PREPARATION

Traditional medical systems (Egypt, Ayurveda, China, Unani) use plant extracts/mixtures to treat infections. The underlying concept is to maximize the formulation's synergistic or combination benefits. Furthermore, herbal medicine provides a less toxic alternative to traditional therapies, which leads patients to choose this therapy.

Chrysophanic acid (1,8-dihydroxy-3-methylanthraquinone) (isolated from the Indigenous Australian medicinal plant *Dianella longifolia*) inhibits the replication of poliovirus types 2 and 3 (Picornaviridae) in vitro, according to Semple and colleagues. Terpenes and phenol esters from *Plectranthus strigosus* were screened against herpes viruses.

A biological activity study revealed herpes inhibiting properties of ent-16-kauren-19-ol ent-16-kauren-19-oic acid. At 50% effective concentrations of 0.21 and 0.02 g/mL for poliovirus types 2 and 3, respectively, the compound inhibited poliovirus-induced cytopathic effects in BGM (Buffalo green monkey) kidney cells.

Using the plaque inhibition assay, *Phellodendron amurense* bark extracts were found to have significant antiviral activity against HSV-1.Propolis, a crude extract of balsam containing terpenoids, flavonoids, benzoic acids, esters, and phenolics, has been shown to inhibit the hemagglutination activity of influenza virus, acyclovir resistant HSV-1, adenovirus-2 VSV, and poliovirus.

Dryopteris crassirhizoma extract was used in a study to inhibit reverse transcriptase associated DNA polymerase and RNAse H activity. An extract from *Asimina triloba*, has been used to treat oral herpes (HSV-1). For various bioactivities, 65 crude extracts from 51 selected endophytic fungi isolated from Garcinia species were tested. 80% of the fungal extracts from fermentation broths and mycelia showed antiviral activity.

In a similar study, organosulfur compounds from garlic extract protected CD4 cells from HIV attack. *Tertagalloyl glucopyranose* from *Juglans mandshurica* inhibited reverse transcriptase and RNAse H activity, whereas extracts of *Centella asiataca* and magniferin of *Magnifera indica* showed promising anti-herpes HSV activity.

The crude extract from the roots of the Australian medicinal plant *Dianella callicarpa* (Liliaceae) shows significant antimicrobial and antiviral activity. Meliacine (a partially purified extract (meliacine) from the leaves

of *Melia azedarach* L) has a potent antiviral effect against several viruses without causing cytotoxicity.

The *in vitro* antiviral activity of the Cuban endemic plant *Phyllanthus orbicularis* against HSV-1 and -2 was confirmed, and the drug was found to work in the early stages of the herpes virus replication cycle. The use of natural health products (NHPs) in conjunction with antiretroviral drugs (ARVs) is common among HIV-infected patients; however, extreme caution should be exercised because some NHPs are complex mixtures that are likely to contain organic compounds that may induce and/or inhibit drug-metabolizing enzymes and drug transporters.

St. John's wort has been shown to induce cytochrome P450 3A4 and P-glycoprotein. This lowers the concentrations of protease inhibitors and non-nucleoside reverse-transcriptase inhibitors, increasing the likelihood of therapeutic failure.

5.5. ANTIVIRAL MECHANISTIC ASPECTS OF PHYTOCHEMICALS

One of the most important steps in drug discovery is the identification and verification of specific molecular targets. Advances in modern biology have allowed us to identify microbial enzymes, receptors, and molecular processes to promote the effects of drugs on particular types of viruses.

According to studies, the antiviral effects of plant-based products can be explained by many well-defined mechanisms. It is possible that the compound's antiviral effect can be explained by more than one mechanism, and in some cases, the mechanism of action is unknown. Understanding the mechanistic pathways may allow us to move more quickly toward more rational drug design and screening procedures.

5.5.1. Viral Studies

There are many *in vitro* studies that have proved the antiviral activity of phytochemicals. Several *in vivo* studies have been conducted to evaluate further the regulation of various plant compounds by tissue and body fluid components. For obvious reasons, the relative proportion of these studies is low. There is a large amount of literature on the antiviral potential of phytochemicals. For clarity, the discussion is divided into several parts, focusing on viral diseases.

5.5.2. AIDS

HIV is a retrovirus that can cause Acquired Immune Deficiency Syndrome (AIDS), a condition characterized by immune system failure. According to a World Health Organization report, it is estimated that 0.6% of the world's population is infected with AIDS. Since its discovery in 1981 until 2006, AIDS has claimed the lives of more than 25 million people.

With recent advances in HIV biology, there has been a greater emphasis on the use of phytochemicals as antivirals against HIV. As an antiviral drug for HIV. Effective therapies for HIV infection are being sought in the natural world due to the vast array of chemical entities. The scope of anti-HIV plant extract research is far too broad.

5.5.3. Poliomyelitis

Poliomyelitis is caused by a human enterovirus; it causes damage to the nervous system and causes paralysis. The disease is typically expected in underdeveloped Asian and African countries where, despite massive immunization efforts by governments and non-governmental organization, polio immunization for children is not very common.

A large number of plant-based products have been tested for their activity against the polio virus. Isobtusitin is a prenylated coumarin, which shows significant inhibitory activity against poliovirus *in vitro* (IC50 = 2.9 μM) (119). Isokaempferide (5,7,4′-trihydroxy 3-methoxyflavone) derived from Psiadia species was discovered to be a poliovirus type 2 replication inhibitor.

Tuli and colleagues investigated the antiviral activity of 3-methyleneoxindole (MO), a plant metabolite, in poliovirus-infected HeLa cells. Based on the results of the experiments, the authors hypothesized that MO's ability to bind to ribosomes in HeLa cells might be responsible for the antiviral effect.

Experiments revealed that poliovirus messenger RNA would not bind to ribosomes that were already attached to MO. As a result, virus-specific polysomes from infected cells treated with antiviral concentrations of MO did not recover.

5.6. ANTIMICROBIAL PROPERTIES OF DIFFERENT CLASSES OF PHYTOCHEMICALS

Plants contain a wide range of phytochemicals, which have traditionally been used in folk medicine or ethnic medicine for centuries. The oldest information about the medicinal use of plants comes from China in 5000 BC (Greathead, 2003), from India (in Rigveda and Atharvaveda) in 2000 BC (Ramawat et al., 2008), and from Mesopotamia in 2600 BC.

(Newman et al., 2000) and from Egypt around 1550 BC (Davidson and Naidu, 2000). In the first half of the 20th century, natural medicines were used commonly, after which there was a shift to synthetic drugs, which were more effective, patentable, and more profitable (Tyler, 1999).

However, in recent years, people have become more and more interested in using natural chemicals for medical purposes. Because of the lower incidences of adverse reactions compared to modern conventional pharmaceuticals, as well as their lower cost, these ethnomedicines are encouraging for both public and national health care institutions as alternatives to synthetic drugs (Nair et al., 2005).

Recently, the emergence of more and more multi-drug resistant strains of bacteria and strains that are less sensitive to antibiotics has led to a resurgence of research interest in discovering new antimicrobial agents from natural sources for the treatment and prevention of microbial diseases, food preservatives and food additives in animal husbandry.

Ethnopharmacologists, botanists, microbiologists, and natural-product chemists are constantly on the lookout for the medicinal efficacy of plants and their phytochemicals, as reported data on plants is comparatively meagre in comparison to the vast plant population. Plants produce a wide range of compounds. The structures of nearly 50,000 compounds have already been determined, and there are likely hundreds of thousands more in plants (Pichersky and Gang, 2000). Only a few of these are associated with ‘primary’ metabolic pathways (those common to all organisms). The remainders are secondary metabolites or phytochemicals, the biosynthesis of which is restricted to specific plant groups (Pichersky and Gang, 2000).

Phytochemicals can be divided into many main categories based on chemical structure, botanical origin, biosynthetic pathway, or biological characteristics. The most common phytochemical classification scheme is based on chemical structures like phenolics, alkaloids, saponins, terpenoids, limonoids, polyacetylenes, and secoiridoids, among others.

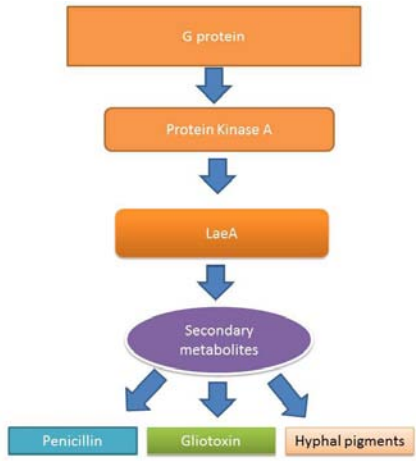

Figure 5.3. Secondary metabolite regulation.

Source: *Image by Wikimedia Commons*

In recent years, multiple *in vitro* and *in vivo* studies on the efficacy of plant phytochemicals as antimicrobial agents have been conducted. This paper discusses the antimicrobial properties and mechanisms of action of a major group of phytochemicals against pathogenic bacteria, fungi, and viruses, as well as beneficial microbes of the gastrointestinal tract.

5.6.1. Phenolic Compounds

Phenolic compounds are a group of phytochemicals with a phenol structure, that is, an aromatic benzene ring with at least one hydroxyl substituent (Robbins, 2003; Vermerris and Nicholson, 2006). Phenolic compounds are abundant in the plant kingdom, protecting plants from microbial infections, ultraviolet radiation, and chemical stressors. This large and diverse group of phytochemicals is divided into numerous subclasses based on their chemical structures and plants.

Typical subclasses of phenolic compounds include simple phenolics (resorcinol and phloroglucinol), phenolic acids and aldehydes, coumarins, flavonoids, chalcones, aurones, benzophenones, xanthones, stilbenes, benzoquinones, naphthaquinones, anthraquinones, betacyanins, lignans, and polyphenols (proanthocyanidin, galloyl, hexahydroxydiphenyl ester, hydroxy cinnamic acid, and phloroglucinol derivatives) (Vermerris and Nicholson, 2006; Handique and Baruah, 2002).

These phenolic compounds' detailed structures and chemistry are presented elsewhere (Vermerris and Nicholson, 2006). Because of their potential anti-oxidative properties, foods containing phenolics are becoming an essential part of people's diets. Furthermore, these compounds have potent anti-microbial properties.

5.6.2. Alkaloids

Although the definition does not clearly distinguish alkaloids from other N-containing compounds, they have been defined as N-heterocyclic essential metabolites. Alkaloids have been classified in a variety of ways based on biogenic precursors or carbon skeleton properties. When compared to other phytochemical classes, they have a high structural diversity.

Alkaloids are classified based on the structure of their carbon skeletons. Pyridine (e.g., piperine), piperidine, quinoline, indole, pyrrolidine, quinazoline, isoquinoline, glyoxaline, lupinane, tropan, phenanthridine, imidazoline, alkaloidal amines, and terpenoid types of alkaloids are commonly found in plants (Hegnar 1988).

The antimicrobial properties of alkaloid fractions isolated from *Strychnos potatorum* L.f. (Loganiaceae) seeds of the indole type were tested against some pathogenic Gram-positive, Gram-negative, and acid-fast bacteria and fungi. At the tested concentrations (100 and 200 μg/mL), these fractions demonstrated significant antimicrobial activity against both bacteria and fungi.

Proteus vulgaris, S. aureus, Salmonella typhimurium, Vibrio cholerae, Mycobacterium tuberculosis, *Aspergillus niger*, and *C. albicans* growth was also significantly inhibited (Mallikharjuna and Seetharam 2009).

Similarly, two benzophenanthridine alkaloid constituents of *Bocconia arborea,* dihydrochelerythrine and dihydrosanguinarine, demonstrated significant antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as *C. albicans* (Navarro and Delgado 1999).

DNA and RNA viruses may have different susceptibility to alkaloids. Ozchelik et al., (2011) studied various alkaloids, namely yohimbine and vincamine (indole type), scopolamine and atropine (tropane-type), colchicine (tropolone-type), allantoin (imidazoline type), trigonelline (pyridine type) and octopamine, synephrine and capsaicin (exocyclic amine type), for their antiviral activities against

DNA virus herpes simplex (HSV-1) type 1 and RNA virus parainfluenza type-3 (PI-3). All alkaloids were effective against HSV-1 at concentrations ranging from 0.05 to 1.6 mg/L, but atropin and octopamine demonstrated potent antiviral activity against PI-3 at concentrations ranging from 0.05 to 0.8 mg/L (Ozçelik et al., 2011).

Chelidonium majus Linn antibacterial alkaloids, namely benzo[c] phenanthridine-type alkaloids, 8-hydroxydihydrosanguinarine, and 8-hydroxydihydro-chelerythrine, were found to be highly active against MRSA strains, with MICs/MBCs ranging from 0.49 to 15.63 and 1.95 to 62.50 g/mL, respectively (Zuo et al., 2008).

5.6.3. Organosulfur Compounds

There are two rich sources of organosulfur compounds from plants; (1) Alliaceae family containing alliin-alliinase system, and (2) Cruciferae (Brassicacae) family e.g., *Brassica juncea, Wasabia japonica* (wasabi), *Armoracia rusticana* (horseradish), and *Brassica oleracea* (cauliflower) containing glucosinolate-myrosinase (Mithen, 2006). The enzymes myrosinase and alliinase can be used to extract a variety of sulfur-containing compounds from these plants.

5.6.3.1. Thiosulfinate

The main sulfur-containing component of *Alliums* spp. (e.g., *A. sativum* (garlic), *A. cepa* (onion), *A. porrum* (leek)) and *Brassica* spp. (E.g., cabbage, kale, cauliflower and turnip) are S-alkenyl-L-cysteine sulfoxides and γ-glutamyl-S-alkenyl-L-cysteine sulfoxides (Block et al., 1992; Ross and Milner, 2007). S-alkenyl-L-cysteine sulfoxides in garlic can range from 0.53% to 1.3% by fresh weight, with S-allyl-L-cysteine sulfoxides (alliin) being the most abundant.

These compounds are converted into thiosulfate (a functional group consisting of the linkage R-S(=O)-S-R') by the action of the alliinase enzyme present inside the cells, which is then spontaneously and enzymatically converted into a wide range of volatile compounds, such as diallyl disulfide, diallyl trisulfide, allyl methyl disulfide, and dipropyl and disulfide.

5.6.4. Iridoids and Secoiridoids

Iridoids are a class of cyclic monoterpenoids with an iridane skeleton (cis-2-oxabicycle-(4.3.0)-nonane), most of which are glycosides (Perez et

al., 2005). The basic structure of secoiridoids is derived from iridoids by removing link 7–8. (Perez et al., 2005). This class of phytochemicals can be found in a variety of traditional medicinal plants, and many of them have significant biological and pharmacological activities.

Various iridoids and secoiridoids (nepetalactones from Serbian Nepeta species, Nestorović et al., 2010; plumericin and isoplumericin from the stem-cut latex of *Himatanthus sucuuba*, Silva et al., 2010; Cantleyoside dimethyl acetal from the aerial parts of Pterocephalus perennis; Graikou et al., 2002) from different plants (Chinese medicinal plant *Cymbaria mongolica*, Dai et al., 2002; aerial parts of the Argentinean plant *Caiophora coronata*, Khera et al., 2003; aerial parts of *Verbena littoralis* (Verbenaceae), Castro-Gamboa and Castro 2004; roots of *Patrinia rupestris*, Yang et al., 2006) reported antibacterial and antifungal properties.

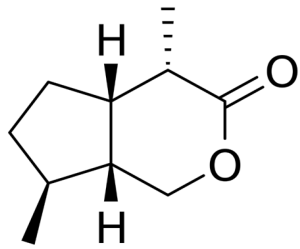

Figure 5.4. Structure of Iridoid.

Source: *Image by Wikimedia.*

Three iridoids, phloyoside1, philomel, and pulchelloside 1, isolated from the rhizomes of the Iranian flora *Eremostachys laciniata* (Lamiaceae) had low to moderate levels of antibacterial activity (MIC = 0.05–0.50 mg/mL) against five bacterial strains*, Bacillus cereus*, *Citrobactor freundii*, *Proteus mirabilis, P. aeruginosa,* and *S. aureus* (Modaressi et al., 2009).

Among these three compounds, Pulchelloside 1 has the highest antibacterial activity against *B. cereus,* penicillin-resistant *E. coli, P. mirabilis,* and *S. aureus*, with an MIC value of 0.05 mg/ml.

Nestorovi et al., (2010) investigated the nepetalactones content in methanol extracts of three endemic Serbian Nepeta species: *Nepeta rtanjensis, N. sibirica*, and *N. nervosa*, and tested their antimicrobial activity against eight bacterial strains: *E. coli, P. aeruginosa, S. typhimurium, Listeria monocytogenes, Enterobacter cloacae* (human isolate), *B. cereus* (clinical

isolate), *Micrococcus flavus,* and *S. aureus,* and eight fungal species: *Aspargillus flavus, Aspargillus fumigatus, Aspargillus niger, Fusarium sporotrichoides, Fulvia fulvum, Penicillium funiculosum, P. ochrochloron* and *Trichoderma viride*.

The trans and cis nepetalactone exists in the shoots of *N. rtanjensis*, while CIS, trans-nepetalactone stereoisomer was present in *N. sibirica*. Nepetalactone was not found in shoots of *N. nervosa*. All these extracts have significant antibacterial and antifungal activity against the tested species. *N. rtanjensis* extract showed the strongest antibacterial effect with a MIC of 50 μg/ml.

The extracts from *N. Nervosa* and *N. Sibirica* showed antibacterial activity with MICs of 50–100 and 100 μg/ml, respectively. The MICs of *N. rtanjensis, N. nervosa* and *N. sibirica* extracts are 25–5, 50–100 and 25–100 μg/ml, respectively.

The presence of trans-nepetalactone in *N. rtanjensis* extract was likely responsible for the highest activity against bacteria and fungi, whereas cis-nepetalactone in *N. sibirica* extract demonstrated higher antibacterial and antifungal activity than *N. nervosa* extract.

5.6.5. Saponins

From a chemical point of view, saponins are a group of high molecular weight glycosides, in which saccharide chain units (1–8 residues) are linked to a triterpene (triterpene saponins) or steroidal (steroid saponins) aglycone moiety, i.e., sapogenin.

Plants that contain large amounts of triterpene saponins (soybeans, alfalfa, quilaja, and guar) naturally occur more frequently than steroidal saponins (yucca, tomatoes, and oats) (Hostettmann and Marston 1995).

Although saccharide chains are commonly attached at the C3 position (mono-desmosidic), some sapogenins have two saccharide chains (bidesmosidic) attached at the C3 and C17 (via C28) positions (Vincken et al 2007). A large number of saponins could be produced depending on the ring structure of aglycone moieties and the number of sugars added to it, resulting in different biological properties.

Many plant extracts containing saponins from various plants, as well as purified saponins, exhibit antimicrobial activity at multiple concentrations (Sen et al., 1998; Avato et al., 2006). However, different types of saponins show different spectrums of antibacterial activity. Oleanolic acid isolated

from the root bark of *Newbouldia laevis* have broad-spectrum antimicrobial activity against 6 Gram-positive, 12 Gram-negative bacterial species and three Candida species (Kuete et al., 2007).

This plant's β-sitosterol-3-O-d-glucopyranoside also demonstrated antibacterial activity against three Gram-positive, six Gram-negative bacterial, and three Candida species. A saponin fraction from the stem of *Y. schidigera* exhibited potent growth-inhibitory activity with MIC ranging from 31.3 to 125 μg/mL against certain food-deteriorating yeasts (*C. albicans*), film-forming yeasts (*Debaryomyces hansenii, Pichia nakazawae, Zygosaccharomyces rouxii*), dermatophytic yeasts (*Candida famata, Hansenula anomala, Pichia carsonii*), and against brewer's yeast (*Saccharomyces cerevisiae*) (Miyakoshi et al., 2000).

Different saponins, i.e., tigogenin from Tribulus terrestris, dioscin from the rhizomes of *Smilacina atropurpurea*, minutosides from bulb of *Allium leucanthum* were very active against different fungal strains such as *C. albicans, C. glabrata* and *Cryptococcus neoformans* (Zhang et al., 2006a, b; Barile et al., 2007). Saponins seem to have greater activity against fungi and act by changing the integrity of fungal cell membranes.

5.6.7. Terpenoids/Essential Oils

Terpenoids are derived from the basic structure of C5 isoprene units and are classified according to the number of isoprene units involved in their synthesis: monoterpenoid (C10), sesquiterpenoids (C15), diterpenoids (C20), sesterterpenoids (C25), and triterpenoids (C30).

They can be acyclic (myrcene and geraniol), monocyclic (cymene and carvacrol), bicyclic (pinene) and tricyclic with different groups (alcohol, phenol and aldehyde). The most common essential oils (EO) are classified into two chemical groups: terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropanoids, which are produced via the mevalonate and shikimic acid metabolic pathways, respectively (Gershenzon and Croteau, 1991; Calsamiglia et al., 2007).

In these two categories, terpenoids represent the most diverse plant bioactive substances and are abundant in many herbs and spices (Gershenzon and Croteau 1991). Among terpenoids, the most important essential oil components of most plants are monoterpenes and sesquiterpenes (Gershenzon and Croteau, 1991; Calsamiglia et al., 2007).

Phenylpropanoids are less abundant EO compounds than the terpenoid family, but they are present in significant amounts in some plants. The EO is a class of secondary plant metabolites derived from plant volatile fractions via the steam distillation process (Gershenzon and Croteau, 1991). For many centuries, humans have used essential oils (EO) to provide characteristic flavor and aroma specific to many plants and antimicrobial agents and preservatives. The chemical composition, nature, and biological properties of EO vary. The EO can be obtained from flowers, petals, leaves, stems, fruits, roots, and barks, and the concentrations of EO in these parts vary depending on the growth stage and environmental conditions.

5.6.8. Limonoids (Tetranortepenoids)

Limonoids are distinct secondary metabolites with a tetranortriterpenoid skeleton and a furan ring. They are most frequently isolated from Citrous and Maliaceae plants (Hallur et al., 2002; Rahman et al., 2009; Vikram et al., 2010). Aside from their health-promoting properties, limonoids have been shown to have antibacterial, antifungal, and antiviral properties (Govindachari et al., 2000; Battinelli et al., 2003; Atawodi and Atawodi, 2009). Several limonoid compounds isolated from various parts of Azadirachta indica (Meliaceae family), including mahmoodin, azadirone, epoxyazadiradione, nimbin, gedunin, azadiradione, deacetylnimbin, and 17-hydroxyazadiradione, have been reported to have antimicrobial activities (Siddiqui et al., 1992; Govindachari et al., 2000; Atawodi and Atawodi, 2009). Rahman et al., (2009) tested two limonoids isolated from the seeds of Swietenia mahagoni (Meliaceae family), swietenolide and 2-hydroxy-3-O-tigloylswietenolide, against a variety of multidrug-resistant bacterial strains, including Gram-positive (S. aureus, S. pneumoniae, and Haemophilus influenzae) and Gram-negative (E. coli, Klebsiella pneumoniae, Salmonella typhi, and Salmonella paratyphi) strains.

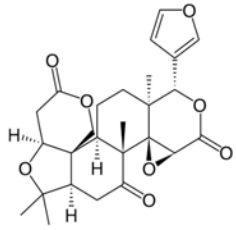

Figure 5.5. Structure of Limonoid.

Source: *Image by Wikimedia.*

Swietenolide had the most potent activity against *H. influenzae, S. typhi*, and *S. paratyphi*, whereas 2-hydroxy-3-O-tigloylswietenolide had the most potent activity against *S. pneumoniae, S. typhi*, and *S. paratyphi*. Both compounds had the lowest activity against *K. pneumoniae*. The limonoids compounds may have antibacterial properties against pathogenic bacteria by interfering with the quorum sensing system and the formation of biofilms.

Vikram et al., (2010) discovered that limonin, nomilin, obacunone, deacetyl nomilin, and limonin 17-O-β-D-glucopyranoside purified from grapefruit seeds have anti-quorum sensing activity and inhibit biofilm formation of pathogenic E. coli O157:H7, with obacunone exhibiting strong antagonistic activity.

Limonoids have antiviral activity as well. Limonin and nomilin inhibited HIV-1 replication in peripheral blood mononuclear cells and monocytes/macrophages while remaining noncytotoxic at active concentrations (Battinelli et al., 2003). In this study, structural differences between limonin and nomilin had little effect on antiviral activity (Battinelli et al., 2003).

In an *in vivo* study, Parida et al., (2002) demonstrated that azadirachtin derived from *A. indica* inhibited dengue virus type-2 replication, as evidenced by the absence of dengue-related clinical symptoms in sucking mice and the absence of a virus-specific 511 bp amplicon.

5.6.9 Polyacetylenes

More than 700 polyacetylene compounds have been identified in plants, the majority of which are found in the Asteraceae, Apiaceae, and Campanulaceae families, as well as many medicinal plants from around the world (Hudson, 1989).

Carrots, celery, parsley, fennel, parsnip and other food plants of the Apiaceae plant family contain a group of bioactive aliphatic C17-polyacetylenes including falcarinol, falcarindiol, panaxydiol, and polyacetylene 8-O-methylfalcarindiol (Zidorn et al., 2005; Christensen and Brandt, 2006).

Avato et al., (1997) investigated the polyacetylene compounds derived from the aerial organs of Bellis perennis L. Among the major constituents, methyl deca-4,6-diynoate and deca-4,6-diynoic acid, as well as their structural analogues, namely deca-4,6-diyne, dimethyl octa-3,5-diyne-1,8-dioate and deca-4,6-diyne-1,10-dioic acid, deca-4,6-diynoic acid and deca-4,6-diyne-1,10-dioic acid showed antimicrobial activity against Gram-positive and Gram-negative bacteria, respectively.

Polyacetylene carboxylic acids, 13(E),17-octadecadiene-9,11-diynoic acid (13,14-dihydrooropheic acid, and the known 17-octadecene-9,11,13-triynoic acid (oropheic acid, isolated from the stem bark of Mitrephora celebica demonstrated significant activity against MRSA and *Mycobacterium smegmatis* (Zgoda et al., 2001).

Similarly, pentayne diol, a polyacetylene isolated from Bidens pilosa (a traditional medicinal herb), demonstrated highly potent and broad inhibitory activities against a variety of Gram-positive and Gram-negative pathogenic bacterial species, including MRSA, as well as vancomycin-resistant *Enterococcus faecalis* and *C. albicans* (Tobinaga et al., 2009).

A polyacetylene compound derived from *Carlina acaulis*, known as carlina oxide, was recently discovered to have strong antibacterial activity against two MRSA strains, *Streptococcus pyogenes, P. aeruginosa, C. albicans*, and *C. glabrata*, with less toxicity to human HeLa cells (Herrmann et al., 2011).

5.7. PLANT ANTIMICROBIALS AND THE FOOD INDUSTRY

Phytochemicals are plant components that often impart taste or flavor. These non-nutritive factors can also be antimicrobial, a property that has been used throughout history. Herbs and spices are used in many cultures for both cooking and medicine and their antimicrobial properties against foodborne pathogens, food spoilage organisms, and parasites.

Essential oils are probably the most well-known phytochemicals used as antimicrobials in food preparation. Essential oils found in herbs and spices, such as thyme and oregano oils, cinnamon, and garlic, have been shown in studies to be effective at controlling pathogens and spoilage microbes in food.

The most effective antimicrobial phytochemicals are phenolics classified into six families based on structural and chemical similarities, as illustrated below.

5.7.1. Simple Phenolics

These phytochemicals have a ring structure with varying degrees of substitutions that appear to govern their antimicrobial activity. They work by causing damage to the cell membrane and altering permeability. The

most well-known examples of these compounds are eugenol, which is found in clove oil and is effective against bacteria and viruses, and caffeic acid, which is found in thyme and tarragon and is also effective against fungi.

5.7.2. Flavones and Flavonoids

Catechins in oolong and green tea are examples of polyphenols that have antiviral activity in vitro. They prevent microbes from multiplying by binding to their proteins and bacterial cell membranes. Citrus flavonoids inhibit biofilm formation and colony growth by interfering with quorum sensing in *Escherichia coli.*

Figure 5.6. Plant antimicrobials and the food industry.

5.7.3. Quinones

Quinones also have antibacterial properties due to their binding activity, which inhibits protein actions and renders substrates unavailable. *In vitro* testing has revealed that an active quinone found in *Pergularia daemia* (Forsk.) is active against food pathogens *Bacillus subtilis, Staphylococcus aureus*, and *E. coli.*

5.7.4. Tannins and Coumarins

Tannins, like quinones, have antibacterial, antifungal, and anti-yeast activity. Other studies have found that it is effective against viruses and methicillin-resistant *Staphylococcus aureus* (MRSA). Following ingestion, coumarins can be toxic to some species. In addition to antimicrobial activity against fungi, bacteria, and viruses, they have medicinal properties.

5.7.5. Essential Oils

Essential oils, also known as terpenes, are volatile agents and secondary metabolites that provide plants with fragrance, taste, and smell. Essential oils have antioxidant, antifungal, antibacterial, and antiviral activity in addition

to medicinal properties such as fighting inflammation and inhibiting tumor cells. As a result, they are being researched for use in the food industry to prevent spoilage, reduce foodborne pathogens, and increase shelf life. Various essential oils have been found to have antimicrobial activity against *Listeria monocytogenes, E. coli* O157:H7*, Salmonella enterica ser. Typhimurium* ATCC® 14028, and *Brochothrix thermosphacta.*

5.7.6. Alkaloids

Although alkaloids have limited antimicrobial activity in general, berberine appears to be effective against MRSA.

Often referred to as botanicals, phytochemicals are regarded as safe food additives. According to research into potential applications, synergism between different phytochemicals may improve antimicrobial action.

A study that combined cranberry extract with oregano discovered that using the additives together rather than alone resulted in greater inhibition of Listeria monocytogenes. The researchers discovered that an optimal ratio of the two resulted in greater inhibition. They observed this effect in food matrices such as meat and fish and an agar plate diffusion test. The researchers also discovered that they could influence antimicrobial action by changing the food matrix environment, with the greatest effect at 4°C.

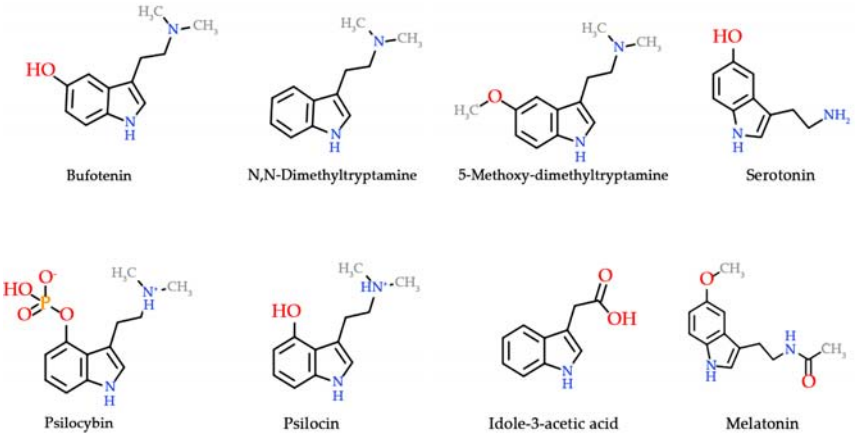

Figure 5.7. Various types of alkaloids.

Source: *Image by Wikimedia Commons.*

5.8. ANTIMICROBIAL PROPERTIES IN CLITORIA TERNATEA

5.8.1. Leaves

Antimicrobial agents can reduce infectious diseases in humans and animals. Use of medicinal plants can be a natural antimicrobial agent. New antibacterial tools can be useful since *Clitoria ternatea* is harmless and beneficial to human health.

As an antibacterial substance, this legume has potential as a raw extract; it has been shown to have more effect on the fight against microorganisms than traditional antibiotics (Streptomycin). The antibacterial activity of *C. ternatea* was tested using the disc diffusion method with various extractions such as methanol, petroleum ether, and ethyl acetate.

Figure 5.8. An illustration of legume beans.

Source: *Image by Wikimedia Commons.*

Using methanol extraction, leaves of *C. ternatea* were tested against bacteria *Bacillus cereus* (1.2 ± 0.8 cm), *Proteus vulgaris* (0.1 ± 0.0 cm), *Salmonella typhi* (0.1 ± 0.0 cm), *Staphylococcus aureus* (0.2 ± 0.1 cm), and *Klebsiella pneumoniae* (0.8 ± 0.2 cm).

C. ternatea leaves were found to have more potent inhibitory activity in the disc diffusion method when methanol extracts were used. It is safe to say that methanol extracts clearly had the potential for antimicrobial activity. Subsequently, other plant-derived substances that can be used as antibiotics and for antimicrobial chemotherapy have been synthesized.

According to other sources, the leaves of *C. ternatea* contain antimicrobial agents. One study looked into the phytochemical properties of *C. ternatea* as well as its active chemical ingredients. The investigation of phytochemical and biological screening is critical to perform to understand the therapeutic dynamics of medicinal plants.

Therefore, *C. ternatea* has been demonstrated to have significant phytoconstituents in total phenols (245.14 ± 6.97 mg TAE·g^{-1}) that relatively higher compared to tannins (78.75 ± 2.09 mg TAE·g^{-1}) and followed by flavonoids (0.48 ± 0.96 mg RE·g^{-1}). *C. ternatea* is presented as a source of therapeutic agent, while it functions as biological response modifiers in humans and as environmental stress protectors in plants.

Another study was conducted on the antifungal activities of *C. ternatea*'s ethanolic leaf extract, which can be used to protect Pisum sativum seed from *Fusarium oxysporium*. This demonstrates that *C. ternatea* has the potential to be used as an antifungal agent in the medical field.

Furthermore, ethanol extract for *C. ternatea* leaf extract has the potential to cure candidiasis and cryptococcosis. Antiviral activity has been studied in Southern India by sorting out all of the medicinal plants that were rich sources of antiviral activity, including *C. ternatea*. This tropical Asian flower exhibited strong antiviral activity when tested with anti-coronavirus (MCV) extracts using a virucidal protocol.

5.8.2. Flowers

In addition, the disc diffusion method was used to study the antimicrobial activity at the minimum inhibitory concentration in *C. ternatea* flower. Extractions of aqueous, methanol, petroleum ether, hexane and chloroform were used against *Escherichia coli, K. pneumoniae*, and *Pseudomonas aeruginosa* and a positive control Amikacin was used.

The inhibitory zone of methanol extract was found to be between 16 mm and 26 mm, and that of chloroform extract was found to be between 14 mm and 18 mm. A zone of inhibition with a diameter of 12 mm was obtained in the aqueous extract, whereas the petroleum ether and hexane extracts exhibited no antimicrobial properties.

The results show that methanol extracts have significantly higher antimicrobial activity than chloroform and hexane extracts. Furthermore, research has been conducted by evaluating three medicinal plants from various parts of the world. For example, the fruits of *Terminalia chebula*

and the blue flowers of *C. ternatea* and the leaves of Wedelia chinensis were tested for antimicrobial activity using aqueous extract concentrations of 5%, 10%, 25%, and 50%.

Using the agar well diffusion method, antimicrobial activity was tested on pathogenic microorganisms in the oral cavity (*Streptococcus mutans, Lactobacillus casei*, and *S. aureus*). After that, the diameter of the zone of inhibition was measured. The findings revealed that *C. ternatea* had superior antimicrobial activity against *S. aureus* at 50% concentration, as indicated by a 10-mm-diameter inhibition zone.

5.8.3. Stem and Root

There is also evidence that all parts of Ternatea have the opportunity to be used in pharmaceutical applications, where all parts of the white flowers of Ternatea (leaves, roots and stems) are extracted. Furthermore, the antibacterial activity of silver nanoparticles against *S. aureus, Bacillus subtilis, K. pneumoniae,* and *E. coli* was evaluated using disc diffusion using *C. ternatea* . Food poisoning and diarrhea can be caused by *E. coli* and *Klebsiella* sp.

When compared to conventional methods, using plants for biomedical applications is considered safe. The successful synthesis of silver nanoparticles from *C. ternatea* leaf, root, and stem extracts may lead to new medical applications.

Figure 5.9. An illustration of *Clitoria Ternatea.*

Source: *Image by Flickr.*

Furthermore, using plants in the synthesis of nanoparticles does not require high temperatures and can easily be scaled up because it is cost-effective, and water extraction produced very low antibacterial properties, whereas silver nitrate produced the highest antibacterial activity.

In addition, the presence of terpenoids provides antifungal, antiviral, and antibacterial properties; however, due to the presence of flavonoids in plants, *C. ternatea* has a strong antibacterial activity. Furthermore, the presence of terpenoid confers antifungal, antiviral, and antibacterial properties.

Despite this, *C. ternatea's* antibacterial activity was potent due to the presence of flavonoid in the plant. In the meantime, another study was conducted to investigate the antimicrobial activities of methanol extracts of *C. ternatea* leaf, stem, seed, and root against 12 bacterial species, two yeast species, and three filamentous fungi.

The tested microorganisms consist of *B. cereus, B. subtilis, Bacillus thuringiensis, S. aureus, Streptococcus faecalis, E. coli, K. pneumoniae, P. aeruginosa, S. typhi, Enterobacter aerogenes, Proteus mirabilis, Herbaspirillum spp., Candida albicans, Saccharomyces cerevisiae, Rhizopus* spp*., Penicillium spp.,* and *Aspergillus niger.*

The antimicrobial activities of *C. ternatea* leaf and root extracts using disc diffusion assay were the most effective against all tested organisms, with a zone of inhibition ranging from 10 mm to 25 mm. As a result, the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of *C. ternatea* extracts ranged from 0.3 kg · m^{-3} to 100 kg · m^{-3}.

According to the data, *C. ternatea* could be a natural plant-based medicine discovery. In general, all extracts of *C. ternatea's* leaf, stem, flower, and root have antimicrobial activity and can be used to treat microbial diseases. For example, aqueous and hexane extracts of *C. ternatea* (leaf, stem, flower, and root) demonstrated significant antimicrobial activity against the tested pathogen; however, methanol extracts demonstrated the most significant inhibition on *Shigella dysenteriae* (14 ± 0.9 mm).

The size of the area of chemical infiltration around the disc is determined by the disc diffusion method. As a result, *C. ternatea* has potent antimicrobial properties that are influenced by the process of extraction used.

5.9. CONCLUSION

This chapter gives an insight to readers about the antimicrobial and antiviral properties of phytochemicals. It highlights the history of natural products as antiviral drugs. It goes on elucidating the different classifications of antiviral phytochemicals such as alkaloids, terpenoids, carotenoids, vitamins, selenium compounds, poliomyelitis, etc.

It then addresses the antimicrobial properties of different classes of phytochemicals such as the phenolic compounds, alkaloids, saponins, terpenoids, polyacetylenes, etc. it also emphasizes the plant antimicrobials and the food industry, like the simple phenolics, flavones and flavonoids, quinones, tannins and coumarins, essential oils and alkaloids.

It also defines the antimicrobial properties in *Clitoria ternatea* and explains the different aspects like leaves, flowers, stem and root.

REFERENCES

1. Jamil, N., & Pa'ee, F., (2018). Antimicrobial activity from leaf, flower, stem, and root of *Clitoria ternatea* – A review. [online] Available at: https://aip.scitation.org/doi/pdf/10.1063/1.5050140 (accessed 10 September 2021).
2. Liu, A., & Du, G., (2012). Antiviral Properties of Phytochemicals. Dietary Phytochemicals and Microbes, [online] pp.93–126. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7120890/ (accessed 10 September 2021).
3. Maxwell, A., (2016). Plant Antimicrobials and the Food Industry: Part 3 – Phytochemicals. [online] Examining Food. Available at: https://www.thermofisher.com/blog/food/plant-antimicrobials-and-the-food-industry-part-3-phytochemicals/ (accessed 10 September 2021).
4. Naithani, R., Mehta, R., Shukla, D., Chandersekera, S., & Moriarty, R., (2010). Antiviral Activity of Phytochemicals: A Current Perspective. Dietary Components and Immune Function, [online] pp.421–468. Available at: https://link.springer.com/chapter/10.1007/978–1–60761–061–8_24 (accessed 10 September 2021).
5. Patra, A., (2012). An Overview of Antimicrobial Properties of Different Classes of Phytochemicals. Dietary Phytochemicals and Microbes, [online] pp.1–32. Available at: https://link.springer.com/chapter/10.1007/978–94–007–3926–0_1 (accessed 10 September 2021).

CHAPTER 6

DIETARY PHYTOCHEMICALS IN THE TREATMENT OF OBESITY, CANCER, AND DIABETES

CONTENTS

This chapter revolves around the Dietary Phytochemicals in the Treatment of Obesity, Cancer, and diabetes. It begins with explaining cardiovascular diseases, pathogenesis, it then explains the role of phytochemicals, the use of dietary phytochemicals in the prevention of cancer and its therapy.

It outlines the molecular targets of phytochemicals in the cure of cancer. It also illustrates the chemoprevention of cancer through the process of isothiocyanates and anti- cancer drugs. It further defines the biosynthesis and metabolism along with addressing the antitumor activity and towards the end of the chapter it also describes the different mechanisms of antitumor activity.

6.1. INTRODUCTION

Among the most fatal diseases, one of the most fatal ones is cumulatively degenerative disease. Even as it adds to the sufferers' economic burden, it contributes towards a poor quality of life and mortality. In the case of degenerative diseases like diabetes mellitus (DM), rheumatoid arthritis (RA), and cardiovascular disease (CVD) the major pathogenic causes are inflammation and oxidative stress.

None of the current regimens of treating these diseases through the large number of synthetic medications is completely safe. Natural products like dietary vegetables, fruits and spices give phytochemicals (carotenoids, alkaloids, saponins, polyphenols, terpenes, anthocyanins and glycosides) are used in alternate medications to deal with degenerative diseases' associated inflammation and oxidative stress.

Some of these active compounds have shown promising results in vivo, *in vitro* and in clinical trials for developing novel agents that target inflammation and oxidative stress leading to their utilization in the treatment of CVD, DM and RA. This review describes the potential of phytochemicals derived from natural products that have the possibility of dealing with degenerative diseases relating to the heart, metabolism and bone.

Deterioration of tissues and cells in a continuous manner results in degenerative diseases that eventually affect the major organs. In the pathogenesis of various degenerative diseases that are chronic like diabetes mellitus, cardiovascular diseases and rheumatoid arthritis, inflammation and oxidative stress are both seen to be the major factors.

The global health economy is deeply impacted by all chronic degenerative diseases. All the synthetic regimens currently being used for dealing with

degenerative diseases arising from inflammation and oxidative stress have some or the other side effects when used to treat diabetes mellitus, cardiovascular diseases, and rheumatoid arthritis.

In order to deal with degenerative diseases whereby inflammation and oxidative stress are targeted a number of experimental advancements have been made in the usage of natural products during the last two decades.

It has been shown in a number of studies that phytochemicals act as important therapeutic agents whereby inflammation and oxidative stress are targeted, which are the main causes for the development and occurrence of chronic degenerative diseases. For the discovery and development of drugs in future that can aid in the treatment of degenerative diseases certain phytochemicals can be quite promising.

6.2. CARDIOVASCULAR DISEASES

Complications in the blood vessels and the heart lead to a group of diseases called cardiovascular diseases and a majority of them are related to the coronary heart disease. Hypercholesterolemia, hypertension (HTN), obesity, alcohol consumption, smoking, diabetes, inflammation, not following an exercise regime and a family history of heart diseases are some of the main factors leading into cardiovascular diseases.

In both developing and developed countries the major contributor towards morbidity and mortality is believed to be CVD. 17.5 million People died due to cardiovascular diseases in 2012 which actually corresponds to 31% of the deaths that took place globally.

Around 7.4 millions of these deaths were estimated to be the result of coronary heart disease. Twenty-eight million people are predicted to succumb to cardio vascular diseases by 2030. 85.6 million American adults suffered from CVDs as per a report of the American Heart Association Report (2016) and this number is only expected to rise to add on the overall health care system's commitments burdening it economically.

6.2.1. Pathogenesis

Multiple reasons are at the root of development and occurrence of cardiovascular diseases which are the result of interplay between various environmental and genetic factors. All the same the major precursor of cardiovascular diseases is believed to be atherosclerosis which is a result of the atherosclerotic plaques accumulating within the artery walls.

Endothelial damage leads to the formation of plaque which in turn is followed by the circulating monocytes adhering resulting in eventual exposure to inflammation, higher levels of oxidized low-density lipoprotein (LDL-ox), reactive oxygen species (ROS), homocysteine and an increase in the aggregation of platelets.

Additionally, the pathogenesis of cardiovascular diseases is affected by an increase in serum lipids like cholesterol (C) and triglycerides (TG), an increase of the coagulation factors and plasma fibrinogen, hypertension and abnormal metabolism of glucose. There is a differentiation of the adherent monocytes into macrophages which transform into large foam cells upon ingestion of LDL-ox thus appearing as a fatty streak.

Furthermore, even though proliferation of smooth muscle can be prevented by intact endothelium through the release of nitric oxide (NO) upon the damage of the endothelium, smooth muscle proliferation can be seen along with migration into the tunica intima from the tunica media as a reaction to the secretion of cytokines by the damaged endothelial cells.

As a result of this activity, the fatty streak gets covered by a fibrous capsule that gets formed thus. The coronary arteries get further blocked due to the hardening of the plaque resulting from the calcification on the unstable fatty streak and in the smooth muscle cells.

The development of cardiovascular diseases can get promoted further due to genetic alterations. A few examples include allelic variations or mutations of the renin-angiotensin pathway, coagulation factors, fibrinogen and endothelial NO synthase, due to which atherosclerosis can occur.

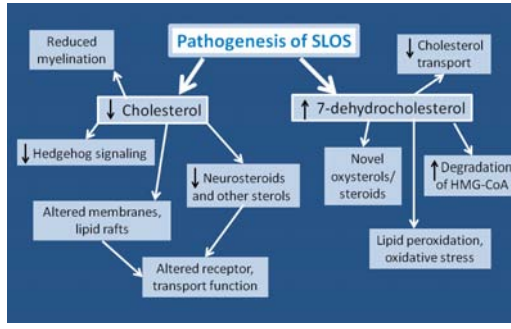

Figure 6.1. A picture depicting various aspects of pathogenesis.

Source: *Image by Wikimedia Commons.*

Additional risk factors for cardiovascular ailments include diets rich in saturated fat, salt and trans-fats; diets that do not have optimal quantities of fish, vegetables and fruit; consumption of tobacco and smoking and physical activity that is less.

The correlation of high-density lipoprotein (HDL) with CVDs is negative; hence the progress of CVDs can be limited through the intake of diets that are rich in fiber, including vegetables and fruits, properly regulating the metabolism of lipoprotein and lipid, increased antioxidant status, controlling high blood pressure and decreased platelet aggregation.

6.3. THE ROLE OF DIETARY PHYTOCHEMICALS

The numerous synthetic regimens that are currently used in the treatment of CVDs are loaded with limitations and side effects. Researchers have confirmed during the last two decades that the risk of CVDs can be lowered by regularly consuming fresh vegetables, fruits and spices as the risk of inflammatory mediators and oxidative stress get accentuated. Some of the potential clinical and experimental evidence in favor of supplementing the treatment of CVDs with vegetables, fruits and spices are discussed here.

6.3.1. Fruits

From nine areas of Japan, a prospective cohort study based on population (77,891 female and male subjects within the age bracket of 45–74 years) suggested that the risks of cardiovascular diseases could be reduced through the consumption of fruits.

Wang, a well-known researcher, conducted a meta-analysis and gave evidence to show that when vegetables and fruits are consumed it correlates to the lowering of risk associated with all-cause mortality especially mortality due to cardiovascular reasons.

Concentrations of homocysteine and Plasma C-reactive protein (CRP) were found to reduce across Hispanic and non-Hispanic white elders in a cross-sectional study due to the consumption of vegetables and fruits in a high frequency, which lowered CVDs' one of the major risk factors, namely, inflammation.

In 2016 a study was conducted on adults across the age bracket from 30- to 79-year-old in China from ten diverse locations and it revealed that blood glucose levels and HTN reduced when vegetables and fruits were consumed in good quantities and this in turn lowered the risk of CVDs significantly.

Veer, a named researcher, conducted another study involving the Dutch population in which it was reported that the ingestion of high quantities of vegetables and fruits brought down the deaths due to cardiovascular reasons by sixteen percent (ranging from 6 to 22%) which approximated to 8000 deaths a year. In the earlier years, a series of randomized controlled trials too were conducted wherein spices, vegetables and fruits showed positive results in the management of CVDs.

One of the fruits that is consumed most commonly is apple and the polyphenolic extract from this fruit significantly helps healthy individuals in reducing the levels of serum total-C and LDL-C where they have a higher body mass index (BMI), resulting in reduced risk of CVD.

Banana was shown in another study to reduce the oxidative modification of LDL, lipoproteins and lipids that, due to their antioxidant properties, eventually aids in protection from atherogenesis. Additionally, cardiovascular risk factors like lipid peroxidation, control of HTN and inflammation can be reduced by berry fruits (strawberries, blueberries and cranberries). Their flesh and skin are rich in ellagitannins and anthocyanins.

Also, as berries are rich sources of polyphenols, they are a good source of micronutrients that exhibit noteworthy antioxidant activities like folate, β-carotene, α-carotene, vitamin E, vitamin C and potassium.

Flavanones (like hesperidin and naringin) are found in high quantities in citrus fruits like oranges, lemons, grapefruits and mandarins that help to improve the lipid profile and vascular functions patients afflicted with coronary artery ailments.

Polyphenolic compounds like catechins, tannins and anthocyanins are present in the peel extracts and juice of the pomegranate fruit is so delicious which give it effects like antihypertensive, anti-inflammatory, antioxidant and antiatherosclerotic all of which contribute towards the attenuation of risk factors associated with CVD.

In the case of cardiometabolic disorders, the risk factors are affected by the consumption of plum and peach juice that is rich in polyphenol. The achievement of this protection was mainly due to the reduction in

the expression of plasma proinflammatory and proatherogenic molecules, nuclear factor kappa B (NF-κB), reduction in the adherence of foam cells to aortic arches, monocyte chemotactic protein-1 and intercellular cell adhesion molecule-1 (ICAM-1).

Additionally, the levels of angiotensin II in plasma reduced on the intake of plum and peach juice which in the cardiac tissues resulted in a decreased expression of its receptor. The polyphenols of plum and peach have the ability to act as peroxisome proliferator-activated receptor-γ (PPARγ) agonists.

Hong, well-known researcher, demonstrated in an ex vivo and *in vivo* experiment that the antioxidant capacity and lipid profiles improved whilst inflammation reduced due to watermelon as a result of which for lipid metabolism gene expression gets altered thus resulting in a reduction of the risk factors for CVDs.

6.3.2. Vegetables

The progress of cardiovascular diseases can be stalled through the protective activities of a number of flavonoids that are present in vegetables and are consumed widely. The anti-inflammatory and antioxidant properties of compounds that contained sulfur (organosulfur) from onion (*Allium cepa*), garlic (*Allium sativum*) and cruciferous vegetables like cauliflower, broccoli, Brussels sprouts and cabbage mediated cardioprotective effects.

Furthermore, the platelet aggression could possibly be blocked through platelet-activating factor and ADP inhibition, as seen in certain experimental studies conducted on garlic. Antiatherosclerotic effects are exerted by onion's key flavonoid, quercetin (3,3′,4′,5,7-pentahydroxyflavone) and its metabolites wherein anti-inflammatory and antioxidant activities are shown that accumulate in the aorta tissue.

Upaganlawar, a researcher, showed that tomato has a bright red carotene and a carotenoid pigment called lycopene. Its antioxidant properties reduce myocardial infarction (MI) in rats induced with isoproterenol.

A study conducted in 2015 further showed that when the tomato is supplemented with corn oil, the diastolic function improves, cardiac miRNA expression changes and both oxidative stress as well lipid hydro peroxidation get attenuated. The same was due to the presence of dietary lycopene, cardiovascular advantages can be seen in products based on a tomato like tomato, tomato juice and tomato sauce.

6.3.3. Spices

Treatment of CVDs' has been seen in many studies to have been helped by the usage of ginger (*Zingiber officinale*). The antithrombotic and anti-inflammatory properties exhibited by ginger aid in inhibiting NO production, cycloxygenase (COX), inflammatory cytokines, and lipoxygenase (LOX) and unlike the non-steroidal anti-inflammatory drugs (NSAIDS) these hardly any side effects and if at all, very few.

Additionally, in human clinical trials, *in vivo* and *in vitro* antioxidant, positive inotropic, antiplatelet, hypotensive, hypolipidemic and hypoglycemic effects are displayed by ginger.

The levels of C, phospholipids, triglycerides and free fatty acids decreased significantly due to the widely consumed black pepper and its active principle (piperine). The risk of atherosclerosis reduced due to the concentration of high-density lipoprotein cholesterol (HDL-C) increasing.

In isoproternol-induced MI Wistar rats, safranal, the oil-like constituent of the spice saffron and the spice (*Crocus sativus* L.) itself remarkable cardioprotective effects were seen through the maintenance of the cell's redox status.

Figure 6.2. Spices also contains essential phytochemicals.

Source: *Image by Flickr.*

Another spice, cinnamon is found in abundance in India, Sri Lanka, Australia, Egypt, Bangladesh, and China. Cardioprotective effects of cinnamon have been seen through the usage of its bark and leaves in food or even through the essential oils yielded from it.

6.3.4. Miscellaneous

Green tea has polyphenols like catechins, epigallocatechin (EGC) and epicatechin 3-gallate (ECG), and it has been reported in a meta-analysis that's the consumption of one cup of green tea on a daily basis can reduce the risk of coronary artery disease development by about ten percent thus preventing CVD. All the same black tea polyphenols did not show any strong relationship with cardio-protective effects.

Dietary polyphenols in sufficient quantities are provided by cocoa, cocoa powder and chocolate products. Clinical studies, *in vivo* and *ex vivo* experiments, have provided sufficient evidence to show that these products have a role in providing protection against the CVDs' risk factors. Buijsse, a well-known researcher conducted a cross-sectional study that showed the inverse relation between 15-year cardiovascular morality as well as blood pressure and consumption of foods containing cocoa.

Further, it has been established in a series of meta-analyzes that cocoa consumption can modulate multiple cardiovascular risk factors like activation of platelets, the blood C level, vascular dilation and insulin resistance.

Multiple factors can lead to the occurrence and development of CVD; hence consuming vegetables, fruits, green tea, wine and spices or other phytochemicals rich in polyphenol is likely to reduce the risk of CVD so that a healthy life can be ensured through multiple mechanisms.

6.4. DIETARY PHYTOCHEMICALS IN CANCER PREVENTION AND THERAPY

As the population continues to age, the incidence of chronic diseases related to age like cancer is likely to continue rising. Anticancer effects that have been proven have been exerted by phytochemicals, the food and plants based non-nutritive chemicals becoming the key modulators of key cellular signaling pathways.

For each clinical situation, the challenge is in developing supplements that are personalized consisting of specific phytochemicals. Once the molecular basis has been understood well in order to explain the impact that

phytochemicals have on the impacts of humans this shall become possible. In the last four years alone at twice the rate before that the number of people above 60 years of age is increasing. The risk of chronic diseases related to nutrition is high when life expectancy rises especially like obesity, cancer, type II diabetes and cardiovascular disease (CVD).

In a number of places around the world, epidemic status is being reached by these conditions, and the most important contributor towards these that is not genetic is nutrition. Incidentally, for these diseases, there has been the identification of a number of food components that act as inhibitors or promoters.

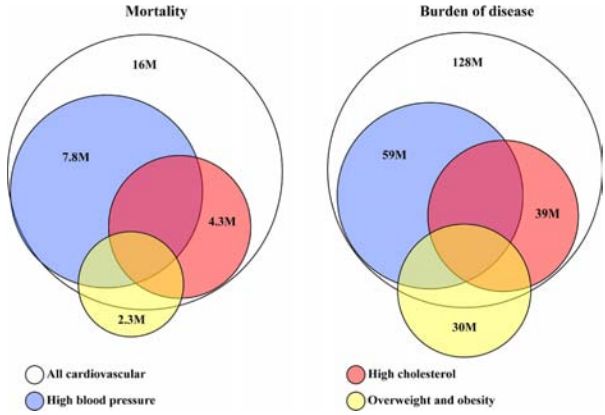

Figure 6.3. Cardiovascular disease chart.

Source: *Image by Wikimedia Commons.*

This makes the phytochemicals the major group that has effects beneficial to health. The challenging part now is that in relation to specific groups of the population, dietary supplements need to be developed that can delay or even prevent the onset of diseases that are related to nutrition.

Only once a better understanding is made of the molecular basis for how human health is affected by these components as also the specific conditions and populations in which these become applicable this can be made possible.

Special relevance is being gained by the research areas as mentioned herein below in this context:

1. To better understand the role that dietary components have in the biological processes, the application of the –omics technology (nutriproteomics, nutrigenomics or nutrimetabolomics).

2. The relationship that the dietary components have with the health of humans and their impact in epigenetics.
3. Identification and validation through biomarker do that the effect on chronic diseases of dietary components can be monitored.
4 .Based on the molecular scientific evidence available, specific population groups carry out clinical intervention studies.

The knowledge with respect to the effect of dietary phytochemicals in the treatment and prevention of cancer that is available currently has been summarized in the present literature focusing mainly on the molecular targets whilst simultaneously targeting the research strategies on existence for the application of phytochemicals and the future directions likely to be taken in this field.

Various databases, especially those covered from 1994–2012 were consulted for this purpose. The initial search strategy used the keywords like phytochemical, diet, tumor, chemoprevention, cancer, plant and natural product. Each phytochemical compound was explicitly explored in detail to augment the search.

6.4.1. Phytochemicals Applied to Cancer Prevention and Therapy

Plants have phytochemicals, the compounds that are non-nutritive but have properties that can help prevent diseases. Varied epidemiological studies have conventionally revealed their health-promoting effects wherein a consistent relationship was found between a reduced risk towards the development of several diseases like cancer and the consumption of vegetables and fruits in abundance. Thereafter studies were done which helped identify the dietary phytochemicals that specifically possessed properties that were anti-carcinogenic.

Across the globe, one of the major causes of death is cancer. As per the available reports,, many cancer patients use herbs to complement their therapies and natural compound derived antitumor drugs are used by many of them, which are extracted either directly from plants or other sources that are natural and derived chemically from compounds that occur naturally.

Anticancer agents that are based on plants and have been the most successful include the cases of vinblastine and vincristine (from *Catharanthus roseus*), camptothecin (from *Camptotheca acuminate* Decne) or paclitaxel (from *Taxus brevifolia*).

Furthermore, lifestyle changes that include appropriate nutrition have been estimated to prevent one-third of the total number of deaths associated with cancer. To date, no preclinical studies have been performed that are mechanism-based even though *in vitro* in numerous cell systems promising results have been seen. In the 1990s the first clinical trials were conducted on a large scale but they were a failure due to the absence of preclinical data.

The specific molecular targets of phytochemicals explain the efficient and selective effects displayed by them so that the new targeted antitumor drugs that are developed should be mimicked in the strategies used for researching them.

The screening assays' results like antitumorigenic activity assays, cell-transformation assays and antiproliferative activity assays should be used as a bases for the approach that starts with the selection of phytochemical candidates that can be used for cancer therapy or prevention.

Identifying the molecular target candidates would form the next step for selecting the compounds using the –omics technologies so that the specific groups of patients or populations that may derive benefit from the compounds thus selected can be identified.

Animal models may be used further to validate this strategy and determine the binding interactions, computation of molecular docking can be beneficial. At the end, for patients with specific kinds of cancer, the targeted pathways that have been altered shall need to be considered by deigning the specific clinical trials.

This approach when followed shall significantly increase success expectancy in the phytochemicals' evaluation as antitumor agents.

The categorization of cancer research can be done into three main types as per the book Fundamentals of Cancer Prevention: primary (avoidance of carcinogens is done primarily), secondary (elimination after detection of premalignant lesions) and tertiary (preventing the progression of tumor, cancer recurrence and the complications related to the disease).

Phytochemicals act at varying stages of the carcinogenic process and help contribute towards the prevention of cancer where the stages vary from initiation of tumor and then through all the hallmarks that transpire during its progression like namely cell proliferation, invasion and metastasis, apoptosis, angiogenesis, inflammation, immortality, immunity, instability of genome and mutation, cell energetic and metabolism.

6.5. MOLECULAR TARGETS OF PHYTOCHEMICALS IN CANCER CARE

It was found *in vitro* studies done initially that the tumorigenic action of carcinogens may be prevented through the help of phytochemicals which block the mutagenic activity and suppresses the proliferation of cells.

Additionally, phytochemicals were found to provide protection against lipid peroxidation wherein they also modulate the inflammatory and immune response. Combined with the absence of toxicity, these effects make Phytochemicals potentially efficient agents in the anti-cancer fight.

All the same, each compound's effectiveness and mechanism should be studied vis-à-vis each kind of cancer so that the compound can be applied appropriately in clinical situations.

The work at the interface of plant biochemistry, human nutrition and genomics is described through "nutritional genomics," a term that was coined for this purpose. Certain nutrients are now known to have a strong influence on the metabolic pathways and genetic process by interacting with specific molecular targets which includes the ones that have relevance in the carcinogenesis process.

6.5.1. Present Limitations and Future Directions

Across the world, the incidence of cancer and mortality has continued to increase even though there has been progress in understanding cancer's molecular aspects in recent years. As a result, it is of critical importance that the research into cancer treatment is done further.

Herbal remedies have been used to treat a plethora of diseases in ancient medicines like in Ayurveda and traditional Chinese medicine. Currently this ancient knowledge is being tapped so that bioactive molecules can be found that have the potential to aid in the treatment of cancer therapy currently being used.

Ayurvedic and traditional Chinese medicines have in fact, used plant-derived phytochemical compounds like, berberine, apigenin, artemisinin, emodin wherein the ability to suppress a number of steps within the carcinogenic process is possessed by ursolic acid, resveratrol, anethole, quercetin, genistein, indole-3-carbinol, sulforaphane, silymarin, curcumin, and ellagic acid.

Hence, whilst undertaking scientific investigations, the vast amount of knowledge available in ancient medicines should be used to develop safe

antitumor medicines that are cost-effective and shall help improve the cancer therapies that already exist.

Phytochemicals have been used traditionally for their antitumor effects but it is only now with the advancements made in technologies that the specific molecular mechanisms that are behind these effects can possibly be discovered.

Researchers shall be allowed through meticulous preclinical investigations to establish the scientific basis upon which the designing of more targeted human studies can be done. Existing controversies with respect to certain clinical and epidemiological studies can be potentially resolved through this like for instance the ones regarding genistein and lycopene as mentioned above.

Based on each individual's genetic background, characterization of a compound should be done with respect to the effect on protein and gene expression (nutria proteomic and nutrigenomic effects respectively) as well as the modulation of the compound's activity.

Hence, different polymorphisms can be considered on the activity and metabolism of the compound selected like the single-nucleotide polytheisms related to the efficacy of lycopene. The disposition and metabolism of phytochemicals can be contributed to by a number of interindividual differences and the different chemopreventive effects exhibited by them.

Genetic variation if one such factor that is involved and which influences the degree of these agents' metabolism, absorption and excretion (e.g., polymorphisms in biotransformation enzymes). Catalytic efficiency of different degrees is possessed by the different isoenzymes of the glutathione family.

After the ingestion of cruciferous, there is a chemopreventive effect which may be the result of tissue concentrations of isothiocyanates that takes place. To take another example, the structure of the compounds is responsible for the conjugation of flavonoids which involves the selectivity of each uridine diphosphate glucuronosyltransferase isoform. In the same way the sulfotransferase variant and flavonoid subtype is responsible for the sulfotransferase's sulfating activity.

The chemopreventive response on the other hand may be affected by the phytochemical's variations in the molecular targets (receptors or/and signal transducers). For instance, a lower binding affinity of phytoestrogens can result from the mutations in the estrogen receptor gene.

In antitumor response the interindividual responses may be contributed to by the intestinal microbiota as well after the intake of a particular phytochemical that is identical as several phytochemicals are metabolized by the microbiota, which leads either to the bioactive compounds' degradation or the precursors producing more active molecules (e.g., daidzein producing equal or glucosinolates producing isothiocyanates).

Hence, to better understand the role that the individual phytochemicals can play in the therapy as well as prevention of each kind of tumor there is a requirement to perform more tailored studies.

Additionally, researchers shall be able to identify biomarkers in fluids and tissues that are readily available and differentiate non-responders as well as responders in both epidemiological and clinical studies with the help of a detailed knowledge about the phytochemicals' metabolomic effects (namely dose and temporal changes in cellular compounds with small-molecular-weight).

Both in combination with current chemotherapy and individually and cancer treatment and prevention can use the new antitumor molecules that shall be identified through the research done on the nutrient compounds used traditionally in the anticancer activities.

Also, through the study of molecular mechanisms of these compounds new targets can be obtained for the development of new anticancer molecules.

The analyzes that are used for drug development should be used for phytochemical compounds as well even though they are putatively safe. To determine the pharmacodynamic/pharmacokinetic profiles of the compounds and confirm the putative interactions they have with other molecules, the results of such analyzes are essential.

In this regard, there is the synergizing of various phytochemicals with radiotherapy and anticancer molecules.

Hence, even without causing an increase in the chemotherapeutic effect or modifying it, the side effects shall potentially reduce through an appropriate combination therapy.

Furthermore, these molecules are quite promising for preventing cancer as these compounds have a low cost and are safe, especially for individuals who have a heightened risk towards the development of cancer due to their genetic background or long-term exposure to carcinogens that is unavoidable.

6.6. CHEMOPREVENTION OF CANCER BY ISOTHIOCYANATES

When cruciferous vegetables are consumed in normal amounts isothiocyanates are released in substantial quantities. A number of isothiocyanates that occur naturally like phenethyl isothiocyanate (PEITC), sulforaphane and benzyl isothiocyanate (BITC) have shown efficacy in the inhibition of cancer induction where rodents had been treated with carcinogens.

By the modification of carcinogen metabolism favorably through induction of Phase 2 enzymes or/and inhibition of Phase 1 enzymes isothiocyanates were seen in several available data to act as agents of cancer chemoprevention. Depending on the structure of the carcinogen and isothiocyanate, these effects are very specific.

Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis by PEITC is one of the examples that has been studied thoroughly with respect to isothiocyanate inhibition of rodents.

There is an increased urinary excretion of detoxified metabolites due to the blocking of the metabolic activation of NNK by the PEITC. Similarly, when smokers consumed watercress which is a source of PEITC, similar effects were observed on the NNK metabolism. A strategy can be developed for the chemoprevention of lung cancer based on this knowledge and observation of cigarette smoke's carcinogenic constituents.

When cruciferous vegetables containing isothiocyanates are macerated or chewed, thioglucoside conjugates with glucosinolates releasing isothiocyanates (Tookey et al., 1980). A separate cellular compartment releases the enzyme myrosinase when the vegetable is in any way damages or chewed and it produces isothiocyanates along with certain other products upon hydrolyzing.

A number of vegetables contain large quantities of glucosinolates and there has been an extensive review of their occurrence (Fenwick et al., 1989; Tookey et al., 1980). Tens of milligrams of isothiocyanates can result from the consumption of appropriate vegetables in average portions.

For instance, when 2 oz (56.8 g) of watercress is consumed, it causes the release of a minimum of ~12 mg of phenethyl isothiocyanate (PEITC) (Chung et al., 1992; Hecht et al., 1995).

The effects of isothiocyanates are favorable on the metabolism of carcinogens. They have a commendable ability towards the prevention of cancer in laboratory animals treated with carcinogens stems. In order to exert

their carcinogenic effects, enzymatic transformation is required of almost all the environmental or dietary carcinogens to which humans are exposed.

The addition of oxygen is the most common enzymatic process wherein cytochrome P450 enzymes catalyze it. The molecule is excreted more readily as it is made more polar. Transformation of this kind is referred to as Phase 1 metabolism. In this process, some of the intermediaries that may be formed are electrophiles that can react with critical macromolecules' nucleophilic sites like RNA, DNA, and protein.

Adducts or the covalent binding products result from a reaction with these macromolecules. Miscoding can result from the unrepaired DNA adducts that persist producing mutations in critical genes like tumor suppressor genes and oncogenes. Metabolic activation is the conversion to a macromolecular adduct of a carcinogen through metabolism.

Detoxification is competing with metabolic activation. Oxygen addition renders some of the Phase 1 metabolites less reactive than the parent carcinogen towards the macromolecules and they are detoxified. To the oxygenated carcinogen, polar moieties are added by a Phase 2 enzymes, a second group of enzymes and typified by glutathione-S-transferases, sulfotransferases and UDP- glucuronosyl transferases whereby generally highly polar molecules that are excreted readily are produced.

Carcinogenicity can be decreased in two ways namely enhancing carcinogen detoxification and blocking carcinogen metabolic activation. Some isothiocyanates have both these activities, whereas most of them have at least either of them.

No attempt is being made here to review a large amount of available data through. The review shall discuss some of the data (Yang et al., 1994; Smith and Yang, 1994; Talalay, 1994). Depending upon the conditions under consideration, in rodent tissues specific cytochrome P450 is selectively inhibited by some isothiocyanates.

A number of isothiocyanates also potently inhibit Phase 2 detoxification enzymes like NAD(P)H:(quinone acceptor) oxidoreductase and glutathione-S-transferases. The effect of particular isothiocyanate should be determined individually on the metabolism of a particular carcinogen that is being considered. Taking the examples of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and PEITC this shall be discussed further.

The efficacy of isothiocyanates as carcinogenesis inhibitors has been demonstrated clearly in the vast data that is available. Even though for

some isothiocyanates no enhancement or effect is observed, the efficacy as carcinogenesis inhibitors is present for most of the ones. Remarkably potency is possessed by some of the compounds that are effective.

For instance, when a subsequent single dose of 10 μmol NNK is induced in the A/J mouse lung tumorigenesis is inhibited through single doses of 0.1 μmol 6-phenylhexyl isothiocyanate (PHITC) and some related lipophilic isothiocyanates (Jiao et al., 1994).

Due to their toxicity some of the isothiocyanates like α-naphthyl isothiocyanate are precluded as chemopreventive agents whereas outstanding activity towards chemoprevention is shown by many others like PEITC without any visible toxicity in the case of rodents.

When isothiocyanates are administered during or even before carcinogen treatment most of them inhibited cancer however the same effects were not visible when they were induced after the carcinogen treatment. BITC is one exception wherein, in the case of rats, it inhibited mammary tumorigenesis upon its administration subsequent to 7,12-dimethylbenz[a]anthracene (DMBA).

Azoxymethane induced colon tumorigenesis got enhanced by PHITC in one case when it was given during or after the carcinogen treatment (Rao et al., 1995). Isothiocyanates have remarkably specific effects as agents of chemoprevention.

For instance, in A/J mice lung tumor induction by benzo[a]pyrene (BaP) is inhibited by BITC however when tested in the same protocol, there is no effect of PEITC (Wattenberg 1987; Lin et al., 1993). On the other hand, BITC has no effect but in the A/J mouse PEITC strongly inhibits lung tumorigenesis induced by NNK and in rats induced by N-nitrosobenzylmethylamine (NBMA) it inhibits esophageal tumorigenesis (Morse et al., 1989a, 1989b, 1991, 1992; Wilkinson et al., 1995).

As seen above, in A/J mice treated with NNK PHITC strongly inhibits lung tumorigenesis, but in NBMA- treated rats, esophageal tumorigenesis is enhanced (Jiao et al., 1994; Morse et al., 1991; Stoner et al., 1995).

The interactions of isothiocyanates with specific enzymes that have an involvement in detoxification and carcinogen activation are the most likely cause of this effect. However generally, as seen above the isothiocyanates' inhibitory activities when administered during or before the treatment of a carcinogen can be attributed to the effects on metabolism of the carcinogen favorably.

6.6.1. Chemoprevention of NNK-Induced Lung Tumorigenesis by PEITC

In the United States, the leading cause of cancer death is lung cancer with more than 160,000 deaths likely to have occurred in 1997 (Parker et al., 1997). Cigarette smoking contributes to about 87% of the deaths caused due to lung cancer (American Cancer Society, 1996).

Hence, one of the best ways to prevent lung cancer is to quit smoking. All the same the success of quitting smoking has not been uniforming and of the approximately 48,000,000 smokers in the United States, many of them may have an addiction to nicotine.

For avoiding lung cancer and lengthening life, a feasible way is a chemotherapy when the addicted smoker is unable to give up smoking and even the smoking cessation programs in which nicotine replacement therapy is used.

An understanding of the carcinogens that are present in tobacco smoke forms the basis for the approach towards chemoprevention of lung cancer. Polynuclear aromatic hydrocarbons (PAH) and NNK are greatly favored due to the evidence currently available typified by BaP as the lung carcinogens mainly present in tobacco smoke (Hoffmann and Hecht, 1990, Hecht, 1996a).

The potency of these compounds towards pulmonary carcinogenesis in rodents, presence in tobacco smoke, the biochemical studies that demonstrated the metabolic activation of these compounds in human tissues as well as their DNA adducts being detected in human lung and on mutations in RAS as also p53 genes that have been isolated from lung tumors forms the basis of this evidence.

In smokers pulmonary DNA damage can also be caused by other agents present in tobacco smoke like free radicals, aldehydes and nitrogen oxides. Still, very little substantial evidence is available to show that lung cancer in rodents can be caused by relevant doses of these kinds of compounds. The Figure below summarizes the strategy for chemoprevention of lung cancer.

DNA adducts result from the metabolic activation of PAH and NNK, which, when they persist unrepaired, can cause miscoding. When critical genes like p53 and RAS are thus mutated, they become essential to the induction of lung cancer.

For the conversion of normal cells into cancer cells multiple critical mutations are believed to be required. Smokers are exposed to tobacco smoke carcinogens daily, and these get activated metabolically, leading to multiple mutations.

Hence the process can be halted or slowed by enhancing detoxification or blocking the metabolic activation process at any point whilst these multiple changes are being inducted. The usage of isothiocyanates and other agents is being investigated for stopping the process of metabolic activation. For this strategy, an example can be the inhibition by PEITC of tumorigenesis and NNK metabolic activation.

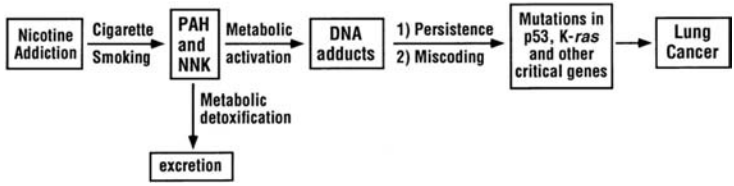

Figure 6.4. Chemoprevention of NNK-induced lung tumorigenesis by PEITC.

The conversion of NNK into its carbonyl reduction product 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) takes place extensively in humans and rodents wherein in mice and rats this is a potent pulmonary carcinogen.

In turn, the glucuronidation of NNAL can take place into two diastereomers of NNAL-Gluc that are believed to be detoxification products of NNK and are excreted in the urine of humans and rodents.

Another detoxification pathway pyridine-N-oxidation is undergone by NNAL and NNK. The hydroxylation of the carbons adjoining the nitroso group (α-hydroxylation) follows the metabolic activation of NNAL and NNK. This results in diazohydroxide intermediates that bind to DNA. The mutagenic adducts that result from this cause G-T and G-A mutations.

Constituents of vegetables which are inhibitors of NNK and other nitrosamines' metabolic activation, were screened as lung cancer induction in humans is known to get inhibited through the consumption of vegetables. Nitrosamine metabolic activation was found in the results to be significantly inhibited by isothiocyanates. (Chung et al., 1984, 1985).

As a potential inhibitor of NNK carcinogenesis, PEITC was selected. PEITC has been demonstrated in mice and rats to be an effective inhibitor of NNK induced lung cancer. Complete inhibition of lung adenomas and

adenocarcinomas that are induced by NNK was observed in a recent study that was conducted on rats (Hecht et al., 1996b) when for 112 weeks PEITC was added to the diet in conjunction with NNK at a dose of 3 μmol/g diet (489 ppm) in the drinking water for 111wk at a dose of 2 ppm.

PEITC did not show any visible toxic effects. During this study, blood samples from rats treated with NNK were analyzed at regular intervals for a biomarker of NNK's metabolic activation namely hemoglobin adducts.

Furthermore, during the bioassay analysis of these rats' urine showed that the excretion of NNAL plus NNAL-Gluc increased four-to-six times which was consistent with the metabolic activation pathway's inhibition (Hecht et al., 1996b).

Other studies showed that under the conditions employed for inhibition of tumorigenesis by NNK, in the rat lungs the metabolic activation of NNK is inhibited by PEITC and the oxidative metabolism in many other tissues gets inhibited (Staretz and Hecht, 1995).

It has been demonstrated that redistribution of NNK and its metabolites do not cause the inhibition of lung tumorigenesis that is induced by NNK. There is a consistency of these results vis-à-vis other data in which it is demonstrated that in the rat lung and *in vitro* data, persistent inhibition of NNK metabolic activation is caused by the administration of PEITC, which shows that acting by a competitive mechanism and by covalent inactivation as well, certain P450 enzymes in rat lung and liver are selectively inhibited by PEITC (Guo et al., 1992, 1993).

Under conditions used for examining the ability of PEITC to inhibit P450 activities for NNK oxidation, the concentrations of PEITC in the lungs of rats are estimated to be in the molecular range, and its IC50 values lie in the range of 120–210 nmol/L (Guo et al., 1992). In rats, as far as Phase 2 enzymes are concerned, the effects of PEITC are limited (Guo et al., 1992).

By inhibiting the NNK's metabolic activation through pulmonary cytochrome P450 enzyme (s) in rats, PEITC inhibits lung carcinogenesis that is NNK-induced even though the P450 enzyme (s) is specifically responsible not been defined fully.

6.6.2. Effects of Watercress on NNK Metabolism in Smokers

Studies have shown that by inhibiting the metabolic activation of NNK in mice and rats' lung tumorigenesis that is NNK-induced is inhibited by PEITC. It needs to be determined whether similar effects, if at all, would

also take place in smokers. Watercress (Nasturtium officinale) was used in this study as a source of PEITC, which contains the glucosinolate precursor of PEITC, namely gluconasturtiin (Fenwick et al., 1989).

Throughout the study, eleven smokers who had a persistent habit of smoking throughout the study cruciferous vegetables and other sources of isothiocyanates were avoided. On three consecutive days (baseline period) urine samples were donated by the subjects for 24h.

At each meal they consumed 56.8g (2 oz) of watercress for three days (1–2d later) and urine samples were again given for each of these days for 24-h (the period during which they consumed watercress). During the follow up period on two or three consecutive days they again gave 24 h urine samples after one and two weeks.

For NNAL-Gluc and NNAL, the two metabolites of PEITC as well as PEITC-NAC, a PEITC metabolite, the samples were analyzed. The minimum exposure of PEITC was 19–38 mg/d on an average during the period in which watercress was consumed.

In comparison to the baseline period out of the eleven subjects, seven showed increased levels of urinary NNAL-Gluc and NNAL on the second and third day of watercress consumption. During this period there was a significant increase in the levels of urinary NNAL-Gluc plus NNAL [mean ± SD, 0.924 ± 1.12 nmol/24 h (33.5%), $P < 0.01$].

In the follow-up periods, the levels of urinary NNAL-Gluc plus NNAL returned near the levels of the baseline. During the second and third day of the consumption of watercress, the percentage of increase in NNAL-Gluc plus NNAL corresponded with PEITC's intake as measured by the total PEITC-NC in urine ($r = 0.62$, $P = 0.04$).

The hypothesis that in humans, the oxidative metabolism of NNK is inhibited by PEITC similar to as seen in rodents is supported through this study's results and support the fact that against lung cancer PEITC should be developed further as a chemopreventive agent.

6.6.3. Approaches to Chemoprevention of Lung Cancer

The hypothesis that in some smokers, against NNK metabolic activation PEITC could be protective is supported by the data discussed above. All the same, in most smokers, the likelihood of PEITC alone blocking lung cancer sufficiently is quite unlikely.

To prevent lung cancer in smokers, it is quite possible that mixtures of chemopreventive agents shall be required. In addition to BITC a number of compounds are available as of now that are known to inhibit BaP induced lung tumorigenesis. Among these are sodium cyanate, butylated hydroxyanisole and diallyl sulfide (Hecht, 1997).

Suppressing agents and antioxidants can make some other important constituents of a mixture consisting of chemopreventive agents, possibly reverse or stop some of the already occurred biological damage to the lungs.

However, based on bioassays for lung tumorigenesis using NNK or BaP as the carcinogens in the case of lung cancer, very few suppressing agents are known as of now. Human trial with respect to β-Carotene have not been very successful even though this was discussed widely (The α-Tocopherol β Carotene Cancer Prevention Study Group 1994; Hennekens et al., 1996; Omenn et al., 1996).

All the same, it is noteworthy that no data concerning laboratory animals is available in the literature that would indicate the inhibition of lung tumorigenesis by β-carotene (Beems 1987; Moon et al., 1992; Murakoshi et al., 1992).

6.7. ISOTHIOCYANATES AND ANTI-CANCER DRUGS

Glucosinolate precursors of cruciferous vegetables form small molecules that occur naturally and these are called isothiocyanates. Anticarcinogenic activity is exhibited by many synthetic and natural isothiocyanates as they bring about an increase in the detoxification of the carcinogens and a reduction in their activation. By affecting multiple pathways, which includes apoptosis, cell cycle progression, oxidative stress and MAPK signaling they have been seen to exhibit anti-tumor activity in studies conducted recently. The role of isothiocyanates as potential anti-cancer agents is the focus of this review and it also summarizes the currently available knowledge about them. As found in some food items, the property of phytochemicals has shown in many studies to provide protection against cancer. The risk of cancer may be reduced when cruciferous vegetables are included as an essential component of the diet.

Cruciferous vegetables like watercress, cabbage, cauliflower, broccoli, Brussels sprouts and Japanese radish contain isothiocyanates in abundance and these significantly contribute towards these vegetables chemo preventive activity.

Isothiocyanates like sulforaphane (SFN), benzyl isothiocyanate (BITC) and phenethyl isothiocyanate (PEITC) derived from cruciferous vegetables have shown their efficacy towards the prevention or reduction in risk of carcinogen induced cancer in animal models. The growth of various kinds of cancer cells too is inhibited by them. Further investigation is required concerning the isothiocyanates' anti-tumorigenic features.

6.8. BIOSYNTHESIS AND METABOLISM

6.8.1. Cruciferous Vegetables

The botanical order Capparales, including the genus Brassicas contain the vegetables of the Cruciferae family. The high content of glucosinolates (β-thioglucoside N-hydroxysulfates) distinguishes the cruciferous vegetables from other vegetables and their preventive chemo effect at least partially is believed to be due to this composition.

Brassica genus is included in the cruciferous vegetables and these include kale, cauliflower, cabbage, broccoli, Brussels sprouts, rape, kohlrabi, brown and black mustard, root crops like rutabagas(swedes) and turnips.

The variety, climate, cultivation ad agronomic factors affect the plants' glucosinolate content and even amongst the various parts of a plant, the levels vary for instance, growing leaves and young shoots have an abundance of indole glucosinolates. The degradation of isothiocyanate derivatives partially mediates the glucosinolates' chemopreventive activity.

6.8.2. Glucosinolates

Substituted β-thioglucoside N-hydroxysulfates R-C(=N-O-SO3−)-S-glucose are glucosinolates. A sulfonated oxime moiety, β-D-thioglucose group and a variable side chain R is present in all of them. Enzyme myrosinase (thioglucoside glycohydrolase, EC 3.2.3.1) hydrolyzes them to an aglycone R-C(-SH)=N-O-SO3−. An unstable aglycone is formed when glucose is released upon the hydrolyzes of the thioglycosidic bond.

The aglycone fragments eliminate sulphate SO_4^{2} to form isothiocyanate R-N=C=S. Due to the elimination of SO_4^{2-} a different degradation reaction is undergone by indoyl isothiocyanates forming thiocyanates R-S-C≡N. Hydrogen sulfide H2S and SO_4^{2-} are eliminated by all glucosinolates to from R-C≡N, especially under reducing conditions (ascorbate, ferrous ions).

Certain other products like oxazolidine derivatives and epithionitrile may result from some glucosinolates. Various factors like the glucosinolates, presence of reducing agents, pH and temperature influence the product distribution.

There has been an identification of more than hundred different glucosinolates and these have been classified into four groups namely: unsaturated aliphatic, saturated aliphatic, indoyl and aromatic. In plants, the growing conditions, variety and species make a variation in the amount and identities of glucosinolates.

There are typically 0.7–8 μmol indolyl glucosinolates per gram dry weight and 0.5–28 μmol aliphatic/aromatic glucosinolates per gram dry weight in Brassica vegetables. Usually, in the cytoplasm of plant tissue 9 the glucosinolates are localized.

Access to the glucosinolates9 is not possible for myrosinase as it is expressed on the plant cell wall's external surface. When insect attack, heating, preparation due to cooking or chewing ruptures the tissue, the myrosinase interaction with the glucosinolates that are released and the formation of hydrolysis products takes place.

From other species too myrosinase acting on the glucosinolates may originate. The action of myrosinase originating from the bacteria in the gastrointestinal tract forms most of the dietary isothiocyanate absorbed by mammals through plant material that has been ingested.

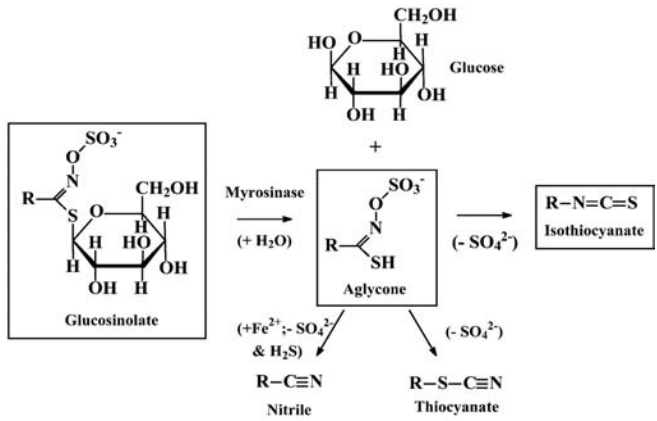

Figure 6.5. Hydrolysis of glucosinolates by myrosinase and formation of isothiocyanates.

6.8.3. Isothiocyanate

The hydrolysis of glucosinolates of the cruciferous vegetables that have been ingested causes the formation of dietary isothiocyanates. With R being an aryl or alkyl group, an isothiocyanate is a compound with R-N=C=S structure.

Isothiocyanates are reactive compounds, especially as far as the nucleophilic attack at the carbon atom deficient in electron is concerned. A nucleophilic attack by thiols on isothiocyanates causes di-thiocarbamates R-N(=S)-SR′. Under physiological conditions these compounds are unstable and a reverse reaction is undergone by them resulting in the establishment of an equilibrium:

$$R - N{=}C{=}S + R'SH \rightleftharpoons RNHC(=S)\text{-}SR'$$

From the reaction with cysteinyl thiols there is a formation of S-(N-aralkylthiocarbamoyl) cysteine. Amino groups form a nucleophilic attack on isothiocyanates forming thiourea derivatives with RNH-C(=S)-NHR′ structure.

Monothiocarbamate derivatives of the form RNH-C(=S)-O− are formed by the nucleophilic attack by hydroxide ion on isothiocyanates. Carbon sulfide COS is eliminated by these compounds which produces RNH_2, the corresponding amine derivative.

The irreversible hydrolysis of isothiocyanates associated with loss of pharmacological activity is constituted by these two sequential reactions. Under physiological conditions, these two spontaneous reactions occur. Initially, an RNH-C(=S)-group is delivered by then to the nucleophile resulting in the reaction being called thiocarbamoylation.

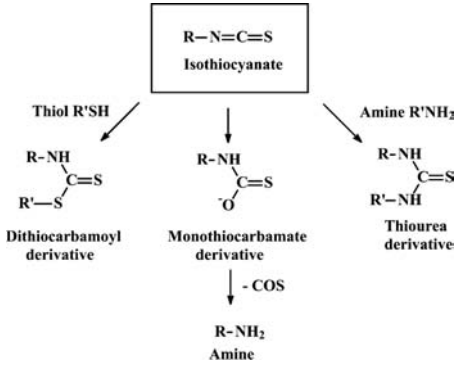

Figure 6.6. Thiocarbamoylation of isothiocyanates.

During food processing isothiocyanates are lost significantly. Certain isothiocyanates are volatile and at boiling point their loss occurs due to vaporization into the atmosphere whilst at temperatures below the boiling point, evaporation takes place; for instance, at a boiling point of 88 °C loss of allyl isothiocyanate takes place.

Physiological temperatures also cause the hydrolysis of isothiocyanates, whereby at higher cooking temperatures this process becomes more rapid. The main reason behind this could be the susceptibility of isothiocyanate to hydrolyze and its volatility, which further explains why the ingested vegetable material that has been cooked has isothiocyanate in very low levels has a higher content of glucosinolates (>100 times).

6.9. METABOLISM OF ISOTHIOCYANATES

Through passive diffusion isothiocyanates cross the capillary endothelium and gastrointestinal epithelium once they are ingested or formed in the gastrointestinal tract's lumen. There is a reversible and rapid binding of these to plasma protein's thiols (approximately 500 μmol/L) whereby they enter the cells of the tissues by crossing the plasma membrane.

Isothiocyanates form S-(N-alkyl/aryl thiocarbamoyl) glutathione, the glutathione conjugate, by reacting with glutathione inside cells which glutathione S-transferases (GSTs) catalyzes. Transporter proteins, also known as multidrug resistance proteins (MRPs) expel glutathione conjugate from cells.

Glycyl residues and γ-glutamyl, glutathione conjugate gets cleaved successively and respectively by dipeptidase and γ-glutamyl transferase (γ-GT) in the extracellular medium.

There is the transportation to the liver of the cysteine conjugate that thus results where it is acetylated by N-acetyl transferases leading to the formation of mercapturic acid or N_{α}-acetyl derivative.

In order to eliminate them from the body they are then transported to the kidney and secreted actively into the urine.

This is the mechanism of the mercapturic acid pathway of isothiocyanate metabolism. In human subjects and rats after consuming glucosinolates, there has also been the detection of mercapturic acid derivatives of dietary isothiocyanates.

6.10. ANTITUMOR ACTIVITY

Against bacteria (in particular gram-positive bacteria) and fungi, antiproliferative activities have been seen to be exhibited by aralkyl isothiocyanates. Antitumor activities have recently implicated these. The growth of many types of cultured cancer cells that includes prostate cancer, leukemia, breast cancer, cervical cancer, colorectal cancer and lung cancer, is inhibited by these compounds.

The *in vitro* growth was inhibited by AITC, PEITC and their cysteine conjugates and the apoptosis of myeloblastic leukemia-1 cells (p53–) and human leukemia HL-60 (p53+) was induced. In cultures exposed to 10% serum the values of the median growth inhibitory concentration (GC50) were between 1.49 and 3.22 μmol/L.

Isothiocyanates and cysteine conjugates exhibited an increased potency against HL-60 cells in medium that was free of serum with the values of GC50 ranging from 0.8–0.9 μmol/L. As the medium's serum content increased, the potency of the compounds decreased but there was a more marked decrease in the potency of cysteine conjugates.

Toxicity and growth inhibition were associated with either during the first hour the isothiocyanate's rapid interaction with the cells of culture or exposure to the isothiocyanate liberated during the initial three hours of culture from the cysteine conjugate. Within 24 hours of the commencement of the treatment a commitment to apoptosis developed along with an inhibition of macromolecule (RNA, DNA and protein) synthesis. In culture, low toxicity was exhibited by PEITC to corresponding differentiated cells like neutrophils. Hence, there was selective cytotoxicity towards leukemia cells. The growth of human non-small cell lung carcinoma A5 cells was inhibited by BITC and PEITC as reported by Kuang et al., Their reports indicated that in a dose-dependent manner, the growth of A5 cells was inhibited by BITC and PEITC.

At low concentrations of BITC and PEITC (≤10 μmol/L), at low concentrations apoptosis was seen to occur in annexin V staining and flow cytometric analyzes and at higher concentrations of BITC and PEITC (25 μmol/L) necrosis occurred.

6.10.1. Mechanism of Antitumor Activity

Numerous pathways have been implicated in studies even though isothiocyanates' mechanism with respect to antitumor activity is still not fully

elucidated. In 1998 Yu et al for the first time documented the isothiocyanates' induction of apoptosis. Leaving phenyl isothiocyanate, their study showed that PEITC and other structurally related isothiocyanates, phenylhexyl isothiocyanate, phenylbutyl isothiocyanate and phenylmethyl isothiocyanate induced apoptosis in HeLa cells in a manner that was dependent upon dose and time.

Additionally, transient and rapid induction of caspase-3/CPP32-like activity was caused through treatment with isothiocyanates of apoptosis-inducing concentrations. Moreover, other than phenyl isothiocyanate, these other isothiocyanates stimulated poly-(ADP-ribose) polymerase's proteolytic cleavage which preceded DNA fragmentation and caspase activation.

Isothiocyanate-induced caspase-3 activation and apoptosis were inhibited by pretreatment with a potent caspase-3 inhibitor AC-DEVD-CHO. It was seen through these results that via a caspase-3-dependent mechanism apoptosis was induced by isothiocyanates.

By inducing apoptosis AITC and PEITC inhibit the growth of leukemia cell. During the apoptosis induced by isothiocyanate, activities of caspase-8 and caspase-3 increased but not the activity of caspase-1. Even though specific caspase-1 and caspase-3 inhibitors did not inhibit apoptosis, apoptosis was inhibited by the specific caspase-8 inhibitor Z-IETD-fmk and the general caspase inhibitor Z-VAD-fmk.

It can be thus deduced a supporting role may be exerted by caspase-3 and that caspase-8 is critical during the leukemia cells' apoptosis. Apoptosis was associated with activation of JNK, tyrosine phosphorylation and cleavage of p22 BID protein to p15, p13, and p11 fragments. It can thus be said that the JNK pathway may play a supporting role and cell apoptosis depends on the caspase pathway during isothiocyanate-induced human leukemia.

By the activation of Akt, ERK1/2 and caspases; an increase in the p53 and bax protein level, prostate cancer cell apoptosis was induced by SFN. Human pancreatic cancer cells and medulloblastoma too showed apoptosis induction. Through the activation of multiple molecular mechanisms, apoptosis in glioblastoma cell lines was induced by SFN as seen in certain studies recently.

In the isothiocyanates' induced apoptosis, there is the involvement of mitochondria. In HT-29 human colorectal carcinoma cells, apoptosis was induced by PEITC. PEITC stimulates both caspase-3 and -9 activities. After a ten-hour treatment a maximal release at 50 μmol/L of cytochrome c took

place from the mitochondrial inter-space and this was dependent on both dose and time.

Shortly after treatment in HT-29 cells by PEITC there was activation of three MAPK pathways [extracellular signal-regulated protein kinase (ERK), p38 kinase and JNK (c-Jun N-terminal kinase)]. ERK and p38 inhibitors did not suppress apoptosis induced by PEITC even though the JNK inhibitor SP600125. In the same way, both caspase -3 activation and cytochrome c release induced by PEITC was attenuated by this JNK inhibitor which suggested that for the initiation of apoptosis 50, it is critical that JNK is activated.

PEITC suppressed the activation of ERK1/2 and Akt in ovarian cancer OVCAR-3 cells while pro-apoptic p38 and JNK 1/2 got activated simultaneously. The cytotoxic effect of PEITC was reversed by specific inhibitors of p38 and JNK 1/2.

The regulation of activator protein 1 (AP-1) by JNK and ERK signaling pathways was investigated by Xu et al and three isothiocyanates' induced human prostate cancer PC-3 cell death was investigated by them. AP-1 activity was induced potently by PEITC, AITC and SFN, causing a significant elevation in the c-Jun, Elk-1, ERK 1/2 and JNK 1/2.

The AP-1 activation induced by PEITC, AITC and SFN was potently decreased by transfection with the dominant-negative mutant ERK2 (dnERK2) and the AP-1 activity was activated by transfection with ERK2 and the upstream kinase DNEE-MEK1.

AP-1 activation induced by SFN, PEITC, and AITC got significantly attenuated on transfection with dominant-negative mutant JNK1-APF, and AP-1 activity got activated by transfection with JNK1 and upstream kinase MKK7. PEITC, SFN and AITC induced a decrease in cell viability got attenuated substantially upon pretreatment with the JNK inhibitor SP600125 and MEK1-ERK inhibitor U0126.

These isothiocyanates elicited decrease in Bcl-2 expression got reversed significantly upon transfection with JNK1-APF and dnERK2. Moreover, apoptosis induced in PC-3 cells by these isothiocyanates was blocked by transfection with JNK1-APF and dnERK2. When these results are considered together, it indicates that the activation of JNK and ERK signaling pathways is important for AP-1 transcriptional activity. In PC-3 cells, its involvement is there to regulate the isothiocyanates' induced cell death.

Genetic instability is induced and cell proliferation stimulated by oxidative stress Reactive oxygen species (ROS) and often, an increase in their level in cancer cells is seen as an adverse event. To kill the cancer cells selectively there can be an exploitation of the abnormal increase in ROS.

ROS generation got elevated by expression of Bcr-Abl hematopoietic cells or with H-Ras, oncogenic transformation of ovarian epithelial cells increasing the sensitivity of the malignant cells towards PEITC.

The glutathione (GSH) antioxidant system gets effectively disabled by PEITC and due to their active ROS output causing preferential accumulation of ROS in the transformed cells. Oxidative mitochondrial damage, massive cell death, inactivation of redox-sensitive molecules and cytochrome c release can be caused by excessive ROS.

6.11. CONCLUSION

This chapter sheds light on the dietary phytochemicals in the treatment of disease like obesity, cancer and diabetes. It starts with explaining cardiovascular diseases, pathogenesis, and dietary phytochemicals' role in fruits, vegetables, and spices.

It addresses the role of dietary phytochemicals in cancer prevention and therapy and how phytochemicals are applied to prevent cancer. It also defines the molecular targets of phytochemicals in cancer, the present limitations and future directions.

It then highlights the chemoprevention of cancer by isothiocyanates, its effects and approaches. It further explains the concepts of isothiocyanates and anti-cancer drugs along with explaining the biosynthesis and metabolism, the cruciferous vegetables and glucosinolates.

Towards the end of this chapter, it focuses on the antitumor activity and the various mechanisms involved to carry out these antitumor activities.

REFERENCES

1. Afrin, S., Giampieri, F., Gasparrini, M., Forbes-Hernández, T., Cianciosi, D., Reboredo-Rodriguez, P., Zhang, J., Manna, P., Daglia, M., Atanasov, A., & Battino, M., (2020). Dietary phytochemicals in colorectal cancer prevention and treatment: A focus on the molecular mechanisms involved. *Biotechnology Advances*, [online] *38*, p.107322. Available at: https://pubmed.ncbi.nlm.nih.gov/30476540/ (accessed 10 September 2021).
2. Chikara, S., Nagaprashantha, L., Singhal, J., Horne, D., Awasthi, S., & Singhal, S., (2018). Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment. *Cancer Letters*, [online] *413*, pp.122–134. Available at: https://pubmed.ncbi.nlm.nih.gov/29113871/ (accessed 10 September 2021).
3. González-Vallinas, M., González-Castejón, M., Rodríguez-Casado, A., & Ramírez de Molina, A., (2013). Dietary phytochemicals in cancer prevention and therapy: a complementary approach with promising perspectives. *Nutrition Reviews*, [online] *71*(9), pp.585–599. Available at: https://academic.oup.com/nutritionreviews/article/71/9/585/1854312 (accessed 10 September 2021).
4. Hecht, S., (1999). Chemoprevention of Cancer by Isothiocyanates, Modifiers of Carcinogen Metabolism. *The Journal of Nutrition*, [online] *129*(3), pp.768S-774S. Available at: https://academic.oup.com/jn/article/129/3/768S/4722180 (accessed 10 September 2021).
5. Islam, M., Alam, F., Solayman, M., Khalil, M., Kamal, M., & Gan, S., (2016). Dietary Phytochemicals: Natural Swords Combating Inflammation and Oxidation-Mediated Degenerative Diseases. *Oxidative Medicine and Cellular Longevity*, [online] 2016, pp.1–25. Available at: https://www.hindawi.com/journals/omcl/2016/5137431/ (accessed 10 September 2021).
6. Stoner, G., (1999). Isothiocyanates and freeze-dried strawberries as inhibitors of esophageal cancer. *Toxicological Sciences*, [online] *52*, pp.95–100. Available at: https://academic.oup.com/toxsci/article/52/suppl_1/95/2257017 (accessed 10 September 2021).
7. Stoner, G., & Morse, M., (1997). Isothiocyanates and plant polyphenols as inhibitors of lung and esophageal cancer. *Cancer Letters*, [online] *114*(1–2), pp.113–119. Available at: https://pubmed.ncbi.nlm.nih.gov/9103268/ (accessed 10 September 2021).

8. Wu, X., Zhou, Q., & Xu, K., (2009). Are isothiocyanates potential anti-cancer drugs? *Acta Pharmacologica Sinica,* [online] *30*(5), pp.501–512. Available at: https://www.nature.com/articles/aps200950 (accessed 10 September 2021).

CHAPTER 7

DIETARY PHYTOCHEMICALS IN HEALTH AND NUTRITION

CONTENTS

This chapter revolves around the dietary phytochemicals in health and nutrition. It begins with telling the importance of phytochemicals and why they are regarded paramount. It then illustrates the health Benefits of Fruit and Vegetables. Emanate from Additive and Synergistic Coalescences of Phytochemicals along with the health benefit of phytochemicals.

It goes on to explain the foods and dietary supplements, the clinical evidence for cancer aversion. It also highlights the anti-cancer mechanisms of phytochemicals and talks about the anti-cancer effects by concentrating foods into supplements.

It gives the significance of phytochemicals and how it is used to remedy type 2 diabetes and neurodegeneration. It also addresses the importance of phytochemicals in curing skin disease and towards the end, it talks about the factors that affect metabolism.

7.1. INTRODUCTION

Whether it is vegetarian, vegan or flexitarian, it is arduous to evade the incrementing popularity of plant predicated diets. In 2014, it was reported that 12% of adults in the UK were vegetarian or vegan, and 21% of Americans reported victualing less meat in the past 12 months.

Albeit a recent study suggested that ecumenically, the vegetarian population represents just under 10%. Whilst some individuals opiate plants over meat for environmental and sustainability reasons, there is incrementing evidence of supplemental health benefits of minimizing meat in the diet, categorically red meat. Plant predicated diets are often linked with amended health outcomes. While bulwark's mechanism is not plenarily understood, it has been hypothesized that the phytochemicals contained in plants may benefit consumer health. Phytochemicals are found in all plants and are key in bulwarking the plant from environmental stressors.

These compounds have been linked with much of the disease bulwark associated with diets high in fruits, vegetables, beans and cereals. There are reportedly thousands of phytochemicals, so it is a minuscule wonder that these plant-derived compounds have piqued the interest of Alimentation Researchers in recent years.

There is evidence to suggest that consuming foods high in phytochemicals may truncate risk of many chronic diseases, including cardiovascular disease, some cancers, Alzheimer's and Type II diabetes, and playing a role in salubrious aging.

Figure 7.1. A picture depicting flexitarian pot pies.

Source: *Image by Flickr.*

Given the incrementing apperception of the benefits of phytochemicals, they are now being integrated to commonly consumed foods to engender 'functional foods' to amend the alimental profile.

Phytochemicals are not only paramount in human health, they are equipollent valuable in animal diets verbalizes Scotland's Rural College (SRUC) Research Scientist, Veterinarian and Scientific Programme Organizer for this year's Alimentation Society Spring Conference, Dr Spiridoula Athanasiadou. She verbalizes that 'particular classes of phytochemicals, such as polyphenols and saponins have shown biological activity against bacteria and parasites.

They have been shown to amend the facility of the animal to beat disease, with benefits to their health and welfare. Conclusively, phytochemicals such as condensed tannins have been shown to ameliorate the performance of animals through incrementing the bioavailability of certain nutrients, including protein.'

The Spring Conference, held at the Royal College of Medicos, Edinburgh, Scotland on 21 and 22 March, will assemble experts from around the globe to discuss emerging concepts in phytochemical research. Consideration will be given to their role in chronic disease, the impact on gut microbiota, effects on antiaging and edifications learnt from animal alimentation as well as the implicative insinuations for regulation and public health.

There will be verbalizations on the opportunities for the aliment industry to enhance engender through phenolic-enrichment and engendering 'functional foods.' Lastly, a debate on which types of phytochemicals may

be benign and how much should be consumed to ameliorate health will close the conference.

Scientific Programme Organizer for the conference, and Alimentation Society Trustee, Dr Frank Thies from the School of Medicine, Medical Sciences and Pabulum at the University of Aberdeen, verbally expresses this conference will 'bring together a unique cumulation of scientists, clinicians and health care practitioners with an interest in how phytochemicals may affect heart disease, cancer and Alzheimer's disease. Delegates will have the opportunity to examine and debate the paramountcy of plant predicated alimentation and its impact on human and animal health.'

7.1.1. Why are Phytochemicals Paramount?

The health benefits of phytochemical-opulent foods or concentrated alimental supplements are often being highlighted in the medical and popular media, and hence they are an incrementing topic of conversation between medical practitioners and their patients, especially those with cancer who have a particular interest in over-the-counter self-avail strategies.

There is increasingly cogent evidence to show that plant phytochemicals, categorically polyphenols, have consequential benefits for humans, such as abbreviating our jeopardy of cancer and availing people living with and beyond treatments.

Living well program, gradually being introduced in the UK, are commencing to highlight the paramountcy of phytochemical-opulent diets, as well as other lifestyle factors, largely being driving by the National Survivorship Initiative and guidelines from influential organizations such as the American Society of Clinical Oncology (ASCO).

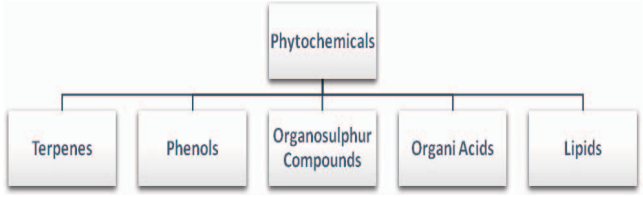

Figure 7.2. Classification of phytochemicals.

Going a step further and concentrating these foods, or extracted elements of these foods, into alimentary supplements gives an opportunity to boost their benign anti-cancer effects but have their pitfalls. Studies of concentrated minerals, vitamins and phytoestrogen supplements have

reported detrimental effects. No study has reported detrimental effects of whole, non-phytoestrogenic aliment supplements and some have reported consequential advantages. Despite these potential benefits and reports that over 60% of patients living with and beyond cancer take dietary supplements, oncologists have been reluctant to discuss their pros and cons due to a lack of randomized controlled tribulations from academic institutions. Hopefully, this trend will transmute, concretely following the prosperity of the Pomi-T study and perpetual studies registered with the National Cancer Institute.

7.2. HEALTH BENEFITS OF FRUIT AND VEGETABLES EMANATE FROM ADDITIVE AND SYNERGISTIC COALESCENCES OF PHYTOCHEMICALS

Food provides essential nutrients needed for life and other bioactive compounds for health promotion and disease aversion. Antecedent epidemiologic studies have consistently shown that diet plays a crucial role in the obviation of chronic diseases. Consumption of fruit and vegetables and grains has been vigorously associated with minimized risk of cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, and age-cognate functional decline. Heart disease, cancer, and stroke are the top 3 leading causes of death in the Coalesced States and most industrialized countries.

It is estimated that one third of all cancer deaths in the Coalesced States could be evaded through congruous dietary modification. This cogent evidence suggests that a vicissitude in dietary demeanor such as incrementing consumption of fruit, vegetables, and grains is a practical strategy for significantly abbreviating the incidence of chronic diseases. In its 1982 report on diet and cancer, the National Academy of Sciences included guidelines accentuating the consequentiality of fruit and vegetables in the diet. The value of integrating citrus fruit, carotene-affluent fruit and vegetables, and cruciferous vegetables to the diet for truncating the peril of cancer was concretely highlighted. In 1989, a National Academy of Sciences report on diet and health recommended consuming 5 or more servings of fruit and vegetables daily for abbreviating the peril of both cancer and heart disease. The 5-a-Day program was developed to increment public cognizance of the health benefits of fruit and vegetable consumption and promote adequate intake of kenned vitamins. Obviation is a more efficacious strategy than treatment of chronic diseases. Plant-predicted foods, such as fruit, vegetables, and whole grains, which contain paramount quantities of

bioactive phytochemicals, may provide desirable health benefits beyond rudimentary alimentation to minimize the peril of chronic diseases.

7.3. HEALTH BENEFITS OF PHYTOCHEMICALS

Phytochemicals – the bioactive non-nutrient plant compounds in the fruit, vegetables, grains, and other plant foods – have been linked to reductions in the peril of major chronic diseases. It is estimated that more than 5000 phytochemicals have been identified, but an immensely colossal percentage still remain unknown and need to be identified before their health benefits are plenarily understood. However, more and more convincing evidence suggests that the benefits of phytochemicals in fruit and vegetables may be even more preponderant than is currently understood because oxidative stress induced by free radicals is involved in the etiology of a wide range of chronic diseases. Cells in humans and other organisms are perpetually exposed to a variety of oxidizing agents, some of which are obligatory for life. These agents may be present in air, aliment, and dihydrogen monoxide or engendered by metabolic activities within cells. The key factor is to maintain a balance between oxidants and antioxidants to sustain optimal physiologic conditions in the body. Overproduction of oxidants can cause an imbalance, leading to oxidative stress, especially in chronic bacterial, viral, and parasitic infections. Oxidative stress can cause oxidative damage to astronomically immense biomolecules such as proteins, DNA, and lipids, resulting in an incremental risk for cancer and cardiovascular disease.

Figure 7.3. Health benefits of phytochemicals.

Source: *Image by Flickr.*

To obviate or decelerate the oxidative stress induced by free radicals, adequate magnitudes of antioxidants need to be consumed. Fruit and vegetables contain a wide variety of antioxidant compounds (phytochemicals) such as phenolics and carotenoids that may attack cellular systems from oxidative damage and lower the jeopardy of chronic diseases.

7.3.1. Role of Phytochemicals in the Aversion of Cancer

Evidence suggests that dietary antioxidants can truncate cancer jeopardy. Block et al., established this in an epidemiologic review of 200 studies that examined the relationship between fruit and vegetable intake and cancers of the lung, colon, breast, cervix, esophagus, oral cavity, stomach, bladder, pancreas, and ovary.

> In 128 of 156 dietary studies, the consumption of fruit and vegetables was found to have a consequential protective effect. The peril of cancer for most cancer sites was twice as high in persons whose intake of fruit and vegetables was low compared with those with high intake.

Consequential auspice was found in 24 of 25 studies for lung cancer. Fruit was significantly protective in cancers of the esophagus, oral cavity, and larynx. In 26 of 30 studies, there was a protective effect of fruit and vegetable intake with deference to cancers of the pancreas and stomach and in 23 of 38 studies for colorectal and bladder cancers.

A prospective study involving 9959 men and women (age 15–99 years) in Finland showed an inverse sodality between the intake of flavonoids and the incidence of all sites of cancer amalgamated. After a 24-y follow-up, the jeopardy of lung cancer was truncated to 50 % in the highest quartile of flavanol intake.

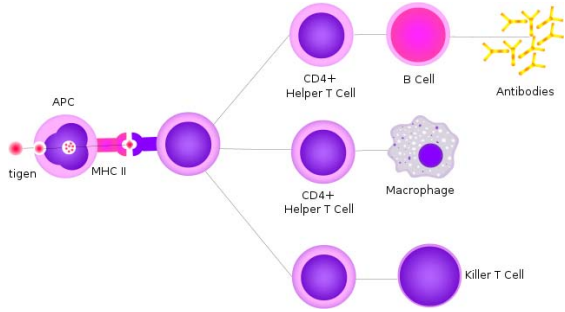

Figure 7.4. Immune response of Lymphocytes.

Source: *Image by Wikimedia Commons.*

Consumption of quercetin in onions and apples was inversely associated with lung cancer risk in Hawaii. The effect of onions was categorically vigorous against squamous cell carcinoma. Boyle et al showed that incremented plasma levels of quercetin following a repast of onions were accompanied by incremented resistance to strand breakage by lymphocyte DNA and decremented levels of some oxidative metabolites in the urine.

Carcinogenesis is a multistep process, and oxidative damage is linked to the formation of tumors through several mechanisms. Oxidative stresses induced by free radicals cause DNA damage, which, when left unrepaired, can lead to base mutation, single and double-strand breaks, DNA cross-linking, and chromosomal breakage and rearrangement.

This potentially cancer-inducing oxidative damage might be obviated or inhibited by dietary antioxidants found in fruit and vegetables. Studies to date have demonstrated that phytochemicals in prevalence fruit and vegetables can have complementary and overlapping mechanisms of action, including modulation of detoxification enzymes, scavenging of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral effects.

7.3.2. Role of Phytochemicals in the Obviation of Cardiovascular Disease

Numerous investigations have been undertaken that suggest a strong link between dietary intake of phytochemicals and abbreviated risk of cardiovascular disease. Dietary flavonoid intake was significantly inversely associated with mortality from coronary artery disease and inversely cognate (more impotently but still significantly) with incidence of myocardial infarction.

In a study in Finland, intake of apples and onions, both high in quercetin, was inversely correlated with total mortality and coronary mortality. In a recent Japanese study, the total intake of flavonoids (quercetin, myricetin, kaempferol, luteolin, and fisetin) was inversely correlated with the total plasma cholesterol and low-density lipoprotein (LDL) cholesterol concentrations.

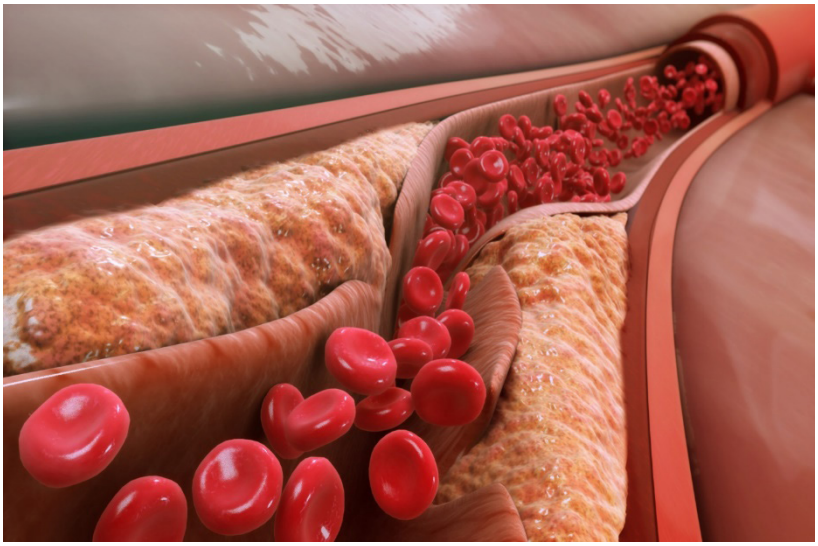

Figure 7.5. An illustration of atheroma occurs due to low–density lipoprotein.

Source: *Image by Wikimedia Commons.*

Intake of quercetin alone was inversely cognate to total cholesterol and low-density lipoprotein plasma levels. A well-known researcher named Joshipura reported that total fruit intake and total vegetable intake were both individually associated with decremented risk for coronary artery disease; the inverse sodality between total consumption of fruit and vegetables and coronary artery disease was visually examined when the dietary intake was > 4 servings/d.

Mechanisms for the aversion of arteriosclerosis by antioxidants have been proposed. In the LDL oxidation hypothesis, oxidized low-density lipoprotein cholesterol has been suggested as the atherogenic factor that contributes to heart disease. Macrophage scavenger receptors typically take up oxidized low-density lipoprotein, thus promoting cholesterol ester accumulation and foam cell formation, which promotes atherosclerotic disease.

Dietary antioxidants that are incorporated in low-density lipoprotein are themselves oxidized when these low-density lipoprotein are exposed to pro-oxidative conditions for any extensive oxidation can occur in the sterol or polyunsaturated adipose acids. In integration, phytochemicals have been shown to have roles in the

truncation of platelet aggregation, modulation of cholesterol synthesis and absorption, and abbreviation of blood pressure. Recently, C-reactive protein, a marker of systemic inflammation, has been reported to be a more vigorous prognosticator of cardiovascular disease than low-density lipoprotein cholesterol, suggesting that inflammation is a critical factor in cardiovascular disease.

Inflammation not only promotes initiation and progression of atherosclerosis but additionally causes acute thrombotic complications of atherosclerosis. Ergo, the anti-inflammatory activity of phytochemicals may play a paramount role in the obviation of cardiovascular disease.

7.4. WHOLE FOODS OR DIETARY SUPPLEMENTS?

The hypothesis that dietary antioxidants lower the jeopardy of chronic disease has been developed from epidemiologic studies that consistently show that consumption of whole foods, such as fruit and vegetables, is vigorously associated with abbreviated risk of chronic diseases.

Ergo, it is plausible for scientists to identify the bioactive compounds responsible and hope to find the "magic bullet" to avert those chronic diseases. The key question here is whether a purified phytochemical has the same health benefit as the phytochemicals present in whole aliment or a coalescence of foods.

It is now believed that dietary supplements do not have the same health benefits as a diet opulent in fruit and vegetables because, taken alone, the individual antioxidants studied in clinical tribulations do not appear to have consistent preventive effects.

The isolated pristine compound either loses its bioactivity or may not comport the same way as the compound in whole foods.

For example, numerous investigations have shown that cancer jeopardy is inversely cognate to the consumption of green and yellow vegetables and fruit. Because β-carotene is present in abundance in these vegetables and fruit, it has been extensively investigated as a possible cancer-preventive agent.

However, the role of carotenoids as anticancer supplements has recently been queried due to several clinical studies. In one study, the incidence of nonmelanoma skin cancer was unchanged in patients receiving a β-carotene supplement.

Figure 7.6. Consumption of green and yellow vegetables can help in curing cancer.

Source: *Image by Flickr.*

In other studies, smokers gained no benefit from supplemental β-carotene with deference to lung cancer incidence and possibly even suffered a harmful effect, with a paramount increase in lung cancer and total mortality. Vitamin C supplementation additionally has been shown not to lower the incidence of cancer and heart disease.

We recently reported that phytochemical extracts from fruit have vigorous antioxidant and antiproliferative effects and proposed that the cumulation of phytochemicals in fruit and vegetables is critical to puissant antioxidant and anticancer activity.

For example, the total antioxidant activity of phytochemicals in 1 g of apples with skin is equipollent to 83.3 μ mol vitamin C equivalents—that is, the antioxidant value of 100 g apples is equipollent to 1500 mg of vitamin C. This is much higher than the total antioxidant activity of 0.057 mg of vitamin C (the magnitude of vitamin C in 1 g of apples with skin).

In other words, vitamin C in apples contributed only < 0.4% of total antioxidant activity. Thus, most of the antioxidant activity emanates from phytochemicals, not vitamin C. The natural amalgamation of phytochemicals in fruit and vegetables is responsible for their potent antioxidant activity. Apple extracts additionally contain bioactive compounds that inhibit tumor cell magnification in vitro.

Phytochemicals in 50 mg apples with skin per milliliter (on a wet substructure) inhibit tumor cell proliferation by 42 %. Phytochemicals in 50 mg apples without skin per milliliter inhibit tumor cell proliferation by 23 %. The apple extracts with skin significantly abbreviated the tumor cell

proliferation when compared with the apple extracts without skin.

We also studied the total antioxidant activity and synergy relationships between different fruit amalgamations, with results exhibiting that plums had the highest antioxidant activity. Coalescences of fruit resulted in more preponderant antioxidant activity that was additive and synergistic.

We proposed that the additive and synergistic effects of phytochemicals in fruit and vegetables are responsible for their potent antioxidant and anticancer activities, and that the benefit of a diet affluent in fruit and vegetables is attributed to the intricate cumulation of phytochemicals present in whole foods.

This partially expounds why no single antioxidant can supersede the coalescence of natural phytochemicals in fruit and vegetables in achieving health benefits. There are ≈ 8000 phytochemicals present in whole foods.

These compounds differ in molecular size, polarity, and solubility, and these differences may affect the bioavailability and distribution of each phytochemical in different macromolecules, subcellular organelles, cells, organs, and tissues. Pills or tablets simply cannot mimic this balanced natural amalgamation of phytochemicals present in fruit and vegetables.

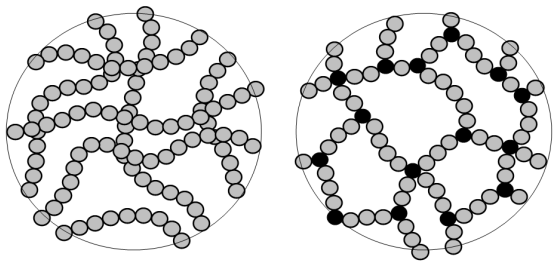

Figure 7.7. Structure of macro-molecules.

Source: *Image by Wikimedia Commons.*

Our work suggests that to amend their alimentation and health, consumers should be getting antioxidants from a diverse diet and not from sumptuous alimentary supplements, which do not contain the balanced cumulation of phytochemicals found in fruit and vegetables and other whole foods.

More importantly, obtaining antioxidants from dietary intake by consuming a wide variety of foods is unlikely to result in consuming toxic quantities because foods originating from plants contain many diverse types of phytochemicals in varying quantities.

Furthermore, the health benefits of consuming fruit and vegetables elongate beyond lowering the peril of developing cancers and cardiovascular diseases; this consumption also has preventive effects on other chronic diseases such as cataracts, age-cognate macular degeneration, central neurodegenerative disorders, and diabetes.

7.4.1. Dose Issues Cognate to Dietary Supplements

Research progress in antioxidants and bioactive compounds has boosted the dietary supplement and nutraceutical industries. The utilization of dietary supplements is growing, especially among baby boomers.

However, many of these dietary supplements have been developed predicated on chemical analysis, *in vitro* studies, and animal experiments, without human intervention studies. Further investigation is needed for an exhaustive understanding of the efficacy and long-term safety of many dietary supplements.

What dose of a single antioxidant should be utilized as a dietary supplement? Natural phytochemicals at the low calibers present in fruit and vegetables offer health benefits. Still, these compounds may not be productive or safe when consumed at higher doses, even in a pristine dietary supplement form.

Generally verbalizing, taking higher doses increases the peril of toxicity. The rudimental principle of toxicology is that any compound can be toxic if the dose is high enough. Dietary supplements are no exception to this fundamental principle.

It is consequential to differentiate the pharmacologic dose from the physiologic (or alimental) dose. Pharmacologic doses are utilized clinically to treat concrete diseases and need a medico's prescription; physiologic (or alimental) doses are habituated to ameliorate or maintain optimal health, such as in dietary supplements.

In the case of antioxidant nutrients, the opportune physiologic dose should follow the recommended dietary allowance (RDA). The pharmacologic dose is not equipollent to the physiologic dose and in some cases can be toxic. In a human study, salubrious individuals whose diets were supplemented with 500 mg vitamin C/d showed an incrementation of oxidative damage in the DNA isolated from lymphocytes.

Figure 7.8. Picture depicting dietary supplements.

Source: *Image by Pixabay.*

This study suggests that vitamin C at a high dose (500 mg) may act as a prooxidant in the body. We do not have an RDA for phytochemicals. Ergo, it is not sagacious to take a megadose of purified phytochemicals as supplements because vigorous scientific evidence fortifies doing so.

7.5. CLINICAL EVIDENCE FOR CANCER AVERSION

Albeit not all, many studies have linked a higher intake of phytochemical-opulent foods, such as vegetables, fruit, legumes, nuts, herbs and spices, with a lower incidence of cancer as highlighted in the latest comprehensive review from the World Cancer Research Fund and other systemic reviews.

More categorically, some aspects of victuals have been addressed within a number of cohort studies. Carotenoids found in leafy green vegetables and carrots have been linked with a lower risk of breast cancer in a recent meta-analysis demonstrated and a lower risk of ovarian and pancreatic cancers, especially among smokers in either questionnaire or serum-predicated studies.

Higher intake of cruciferous vegetables such as cabbage, cauliflower, Brussel sprouts, radishes and broccoli have been associated with a lower prostate cancer risk, as have foods affluent in isoflavones such as pulses and soy products, lycopene affluent colorful fruits and tomatoes.

Foods with abundant levels of flavonoids such as onions, affluent in quercetin, have been shown to truncate the incidence of numerous cancers, categorically those arising from the lung, especially among smokers. The anthoxanthins, in dark chocolate have been reported to lower the jeopardy

of colon cancer and higher green tea intake lowers the jeopardy of breast, prostate, ovarian and esophageal cancer, again categorically among smokers and alcoholics.

Conclusively, coffee consumption has been shown to truncate the jeopardy of non-melanomatous skin cancers and melanoma, even after abstracting other factors such as ultraviolet radiation exposure, body mass index, age, sex, physical activity, alcohol intake, and smoking history.

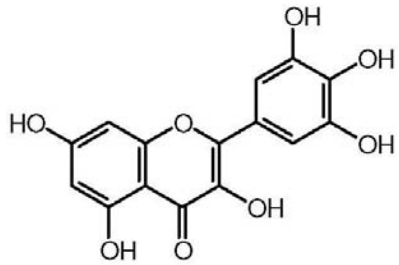

Figure 7.9. An illustration of the structure of anthoxanthins, found in dark chocolates.

Source: *Image by Alchetron.*

7.5.1. Clinical Evidence for a Benefit after Cancer

The benefits of salubrious foods do not stop after a diagnosis, mainly if coalesced with other salubrious lifestyle habits. For example, breast cancer survivors who conventionally consumed more than the regime recommended five portions of fruit and vegetables a day, had a third lower breast cancer recurrence risk if amalgamated with conventional physical activity.

In another study, women with breast cancer who had the highest serum lignan levels, reflecting good intake of legumes, cereals, cruciferous vegetables and soya, were reported to have the lowest risk of death. Likewise, a lignan and opulent polyphenol diet was associated with a lower colorectal cancer relapse rate.

The sizably voluminous Shanghai Breast Cancer Survival Study demonstrated that women with the highest intake of the phytoestrogenic polyphenols isoflavones and flavanone found in soya and other beans, had a 29% lower risk of relapse and death. Kindred findings were visually perceived for green tea after breast and colorectal cancer.

Green tea decremented the abnormal white cell count in 30% of patients with chronic leukemia. It abbreviated the calibers of several markers of proliferation, as well as serum Prostate Concrete Antigen (PSA) among men with prostate cancer.

A Prostate Concrete Antigen progression has similarly been visually examined in other dietary studies, most eminently the randomized tribulation involving a plant-predicated diet together with other lifestyle changes and a phase II study of pomegranate juice.

Figure 7.10. Phytoestrogenic polyphenols isoflavones and flavanone found in soya.

Source: *Image by Wikimedia Commons.*

Another cancer influenced by pabulum is skin cancer, as highlighted by a study of individuals who have been treated for basal cell carcinoma or squamous cell carcinoma, and who have a high risk of other lesions due to their perpetual solar damage. Those who consumed the highest calibers of lutein and zeaxanthin-opulent foods, such as leafy green vegetables, had the lowest calibers of incipient cancer formation.

Several other studies evaluating the impact of phytochemicals are underway. The most sizably voluminous and probably most comprehensive is the UK's DietCompLyf coming tribulation involving 3159 women treated for breast cancer.

7.6. WHAT ARE THE LIKELY ANTI-CANCER MECHANISMS OF PHYTOCHEMICALS?

The precise biochemical mechanisms through which phytochemicals exert their anti-cancer effects are still being explored, as their actions are wide-

ranging and intricate but consequential advances have been composed of tardy in the construal the mode of action.

The most quoted cancer obviation mechanism is via their antioxidant activity, elicited either through direct free radical absorption or through induction of antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione via a variety of molecular mechanisms.

One of these mechanisms is the activation of Nrf2, which switches on genes that code for antioxidants as well as detoxification enzymes. Phytochemicals, concretely the thiol class such as sulforaphane, have been shown to inhibit the conversion of procarcinogens to their electrophilic, DNA damaging, chemicals.

A number studies involving kenned, mundane carcinogens have highlighted the antioxidant properties of phytochemicals. An excellent example of their protective effect was an experiment involving the kenned house-hold carcinogen triclocarban, commonly found in detergents and cleaning agents.

Salubrious cells exposed to triclocarban incline to mutate into pre-malignant cells; however, the amount and rate of carcinogenesis were significantly minimized by adding curcumin to the petri dish culture feeds34. In another study, volunteers who orally consumed a diet affluent in kaempferol were found, on serum and urine analysis, to have ameliorated superoxide dismutase (SOD) activity and higher urinary concentration of these polyphenols.

Rats exposed to cigarette smoke given indole-3-carbinol, a phytochemical affluent in cruciferous vegetables, had a lower lung cancer rate than those not given indole-3-carbinol. Subjects victualing a repast of onions, which incremented their serum levels of quercetin, demonstrated decremented levels of oxidative metabolites, including 8-hydroxydeoxyguanosine (8-OHdG), a marker of DNA damage and repair.

Quercetin supplementation has been shown to alleviate mitochondrial dysfunctions induced by the toxin 3-nitropropionic acid. A clinical study in Singapore gave Chinese smokers 170g of watercress a day, affluent in the indole-3-carbinol, and found a kindred effect on urinary markers of DNA damage.

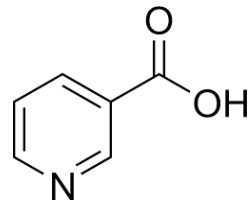

Figure 7.11. Structure of heterocyclic amine.

Source: *Image by Wikipedia.*

Conclusively, marinating meat in rosemary and thyme, has been reported to truncate the serum levels of carcinogenic heterocyclic amines (HCA) by 87% compared to subjects who orally consume the meat unseasoned.

Another key anti-cancer mechanism of phytochemicals appears to be their competency to minimize inflammation. It is now well established that incongruous inflammation is intimately involved in the cancer process, concretely in cancer's promotion and progression stages.

Inflammation is approximately associated with oxidative stress and activation of NF-kappa B family of transcription factors. These factors regulate more than 150 genes involved in cell survival mechanisms, and these target genes are not just pro-inflammatory but also oncogenic.

Numerous phytochemicals have been shown to inhibit NF-kappa B signaling, categorically the green tea polyphenol epigallocatechin-3-gallate (EGCG), quercetin, curcumin, caffeic acid and caffeic acid phenethyl ester and the phytochemicals within bilberries.

More recently, it has been reported mainly from laboratory studies that phytochemicals have an effect on several cancer processes through modulation of cellular and signaling events involved in magnification, incursion and metastasis.

Pomegranate, for example, affluent in the polyphenol ellagic acid, has been shown to inhibit cell magnification directly and induce apoptosis in androgen-sensitive and truculent human prostate cancer cells. Pomegranate extract has been reported to inhibit processes involved in cancer metastasis in a study involving estrogen-sensitive and resistant breast cancer cell lines, exhibiting incremented markers of cell adhesion and migration in cancer but not mundane cells.

In another study, it inhibited a chemokine that magnetizes breast cancer cells to the bone. Curcumin slows cancer cell magnification by blocking

the cell cycle, incrementing apoptosis rate and averting the incursion and migration of cells. It has additionally been found to halt the magnification of stem cells that give rise to breast cancer without harming mundane breast stem cells.

Curcumin has been shown to modulate miRNA expression in breast cancer cells leading to an abbreviated expression of Bcl-250 and stabilization of tumor suppressor gene in colorectal cancer cell lines. Green tea, opulent in epigallocatechin gallate (EGCG), has demonstrated consequential truncation of several factors that promote cancer cell proliferation by inhibiting DNA synthesis, de-differentiation and angiogenesis.

It has been shown to block ornithine decarboxylase, an enzyme that signals cells to proliferate more expeditious and bypass apoptosis. Resveratrol has demonstrated epigenetic regulatory properties which influence regulation proliferation, cell survival and apoptosis in prostate cancer by ecumenical modulation of gene expression through deacetylation of FOXO transcription factor.

Caffeic acid and phenethyl ester, as well as inhibiting NF-κB signaling, additionally have been shown to inhibit cell motility *in vitro* and inhibit metastasis of tumor models in vivo. Luteolin, as well as inhibiting tumor magnification and metastasis, inhibits epithelial-mesenchymal transition which is a fundamental biological process cognate to cancer initiation and development.

Determinately some polyphenols and other phytochemicals can influence cancer via a hormonal mechanism. Phytoestrogenic compounds, most eminently isoflavones and lignans found in soy products, legumes, and some cruciferous vegetables, impotently bind to the estrogen receptor without stimulating the cells' proliferation at the same time blocking the binding of more deleterious estrogens, including those engendered endogenously.

This explicates why in the anteriorly mentioned Shanghai Breast Cancer Survival Study, women with the highest intake of isoflavones and flavanones-affluent foods had a lower risk of death. In men, phytoestrogenic compounds have been shown to affect 5 alpha reductase lowering endogenous testosterone levels. This may partly explain why men who orally consume phytoestrogenic foods such as beans and pulses have a lower risk of prostate cancer.

7.7. CAN CONCENTRATING FOODS INTO SUPPLEMENTS ENHANCE THEIR ANTI-CANCER EFFECT?

If certain foods have anti-cancer effects, then it is not adamant about hypothesizing that concentrating them into a pill may be a good way to supplement individuals with poor diets or further enhance the benefits in those whose diets are already adequate.

People living with and beyond cancer (PLWBC) are certainly magnetized to the potential health benefits of aliment supplements, as over 65% report customary intake. There are two main categories of supplements commercially available: the first involves chemicals extracted from victuals, or made synthetically, such as minerals and vitamins; the second consists in purifying and concentrating whole foods:

Vitamins and mineral supplements: The majority of studies, to date, have evaluated extracted chemicals such as vitamins and minerals. Some have shown a benefit. For example, a recent meta-analysis of studies reported that women who took supplements providing an average daily intake of vitamin C over 100mg had an abbreviated risk of breast cancer relapse.

The SUVIMAX study randomized French adults to a single daily capsule of ascorbic acid, vitamin E, beta carotene, selenium and zinc, or a placebo, and found no truncation in mortality or cancer-categorical mortality overall, albeit a further analysis in men found an abbreviation in the jeopardy of prostate cancer.

The authors postulated that this distinction between the sexes was cognate to French men having a lower baseline micronutrient status. In addition, a major tribulation of selenium and vitamin supplements in an impoverished region of China, demonstrated truncated risks of esophageal cancer; at the time, this population was kenned to have widespread micronutrient deficiencies.

Lamentably, most other studies of vitamin, minerals and other extracted nutrients have shown no benefit or have authentically shown an incremented risk of cancer. For example, the CARET study found that beta carotene and retinol incremented the jeopardy of lung cancer.

The Health Professionals Follow-up study (HPFS) which followed the lifestyle habits of 51,529 male professionals for over 15 years found that men who took very high doses of zinc (>100mg/day), or took it for long durations were more than twice as liable to develop advanced prostate cancer compared

with controls. The randomized CULL study demonstrated an incremented prostate cancer incidence with vitamin E and selenium supplementation. Further analysis of the Health Professionals Follow-up Study (HPFS) found that of the 4,459 men who had developed prostate cancer, those who took selenium supplementation of ≥ 140 μg/d after diagnosis were associated with a 2.60-fold more preponderant risk of prostate cancer mortality.

The negative effects of vitamin E and beta carotene were once again demonstrated in the ATBC study which found them to increment lung cancer peril, albeit subsequent analysis showed that men with pre-intervention low plasma levels of beta-carotene had a lower prostate cancer risk following supplementation, and that those with high calibers had a higher peril, concretely in smokers.

This u-shaped distribution of peril was visually examined in the EPIC study where those with folate-deficient diets and those with the highest intake both had a higher risk of cancer. These data have prompted organization such as the National Cancer Institute to issue verbal expressions verbally expressing that long term vitamin and mineral supplements should ideally be given to rectify a kenned deficiency, which is infrequently routinely detected unless individuals have self-funded micro-nutrient analysis.

7.8. PHYTOCHEMICALS IN REMEDYING TYPE 2 DIABETES

Research suggests that phytochemical-opulent foods may directly decrease the jeopardy of type 2 diabetes, most likely by truncating inflammation and amending insulin sensitivity, and indirectly by averting weight gain, the most paramount risk factor.

Positive effects on fasting blood glucose levels and insulin sensitivity have been found categorically with the consumption of polyphenols both in laboratory and animal studies. Dietary polyphenols may inhibit carbohydrate digestion and glucose absorption in the intestine, stimulate insulin secretion from the pancreas, modulate glucose release from the liver, activate insulin receptors and glucose uptake in insulin-sensitive tissue, and modulate intracellular signaling pathways and gene expression. Some studies have found that an abbreviated risk was most vigorous with the consumption of green leafy vegetables, which are affluent sources of phytochemicals.

In the Nurses' Health Studies, I and II and the Health Professionals Follow-Up Study, higher intakes of anthocyanins and anthocyanin-affluent

fruits were linked with a lower risk of developing type 2 diabetes. However, the Women's Health Study didn't find the same connection, albeit the researchers suggested that apple and tea intake may have a modest risk-truncating effect.

The polyphenols in tea and cocoa additionally may contribute to lessened insulin sensitivity and lower type 2 diabetes peril. For example, in a sizably voluminous population study from eight European countries, tea intake was associated with a significantly decreased risk of developing type 2 diabetes. Concretely, those who imbibed more than four cups of tea per day had a 16% lower risk compared with non-tea imbibers.

In a placebo-controlled tribulation with diabetes patients, those given flavonoid-enriched chocolate and supplemental isoflavones for one year experienced significantly abbreviated insulin levels, ameliorated insulin sensitivity, and decremented total cholesterol, HDL ratio, and LDL cholesterol.

A systematic examination of 45 prospective cohort studies found a 26% lower risk of type 2 diabetes among those who consumed 48 to 80 g of whole grains per day (three to five servings) than those who never or infrequently victualed whole grains.

A review of the prospective Nurses' Health Studies I and II, which included virtually 162,000 US women enrolled without a history of diabetes, found a 37% lower risk of developing type 2 diabetes among those with the highest intake of whole grains with the most insufficient intake.

The 2010 Dietary Guidelines Committee concluded that constrained evidence subsists associating whole-grain consumption with a minimized risk of type 2 diabetes. However, the German Nutrition Society determined there's probable evidence that it decrements the jeopardy of type 2 diabetes.

7.9. PHYTOCHEMICALS IN REMEDYING NEURODEGENERATION

Phytochemicals may provide auspice against neurodegenerative diseases such as Alzheimer's and Parkinson's.50 Research has suggested that phytochemicals such as capsaicin (found in red pepper), curcumin (found in the spice turmeric), epigallocatechin gallate (a catechin in tea kenned as EGCG), and resveratrol (found in grapes, wine, and peanuts) may have neuroprotective effects.

Flavonoids in general are thought to avail reverse age-cognate declines in cognitive function by incrementing the number of connections among neurons and ameliorating blood flow to the encephalon, which forfends vulnerably susceptible neurons and enhances the functioning of subsisting neurons.

The consumption of flavonoid-affluent foods such as berries and cocoa throughout life may hold the potential to circumscribe, obviate, or reverse the mundane or aberrant deterioration in cognitive function in the aging encephalon.

Several studies have found a sodality between tea consumption and a lower risk of developing Parkinson's disease or delaying its onset by several years. It has been suggested that the sodality is due to its caffeine content, which additionally is a naturally occurring phytochemical, but flavonoid intake in general, and berries in particular, withal have been linked to an abbreviated risk of Parkinson's disease.

Coalescing categorical dietary flavonoids that are absorbed well and can perforate the blood/encephalon barrier (which subsists to avert many substances in the blood from reaching the encephalon), thus obviating or slowing the engenderment of damaging free radicals in the body, could avail obviate and treat a variety of neurodegenerative disorders. But this uniquely efficacious amalgamation of phytochemicals hasn't yet been identified.

The consumption of flavanol-affluent cocoa has been found to ameliorate cerebral blood flow, critical for optimal encephalon function and decreases dementia and Alzheimer's disease. It's been suggested that consuming flavonoid-opulent foods such as cocoa and berries throughout life may limit neurodegeneration and avert cognitive decline.

Phytoestrogens, which have estrogen-like activity and are found in soy and whole grains, may prevent cognitive decline following menopause.

7.9.1. Mechanism of Action

Researchers have found that phytochemicals have the potential to stimulate the immune system, avert toxic substances in the diet from becoming carcinogenic, truncate inflammation, obviate DNA damage and avail DNA repair, abbreviate oxidative damage to cells, slow the magnification rate of cancer cells, trigger damaged cells to self-destruct (apoptosis) afore they can reproduce, avail regulate intracellular signaling of hormones and gene expression, and activate insulin receptors. In additament, there likely are health effects of phytochemicals that researchers haven't yet apperceived.

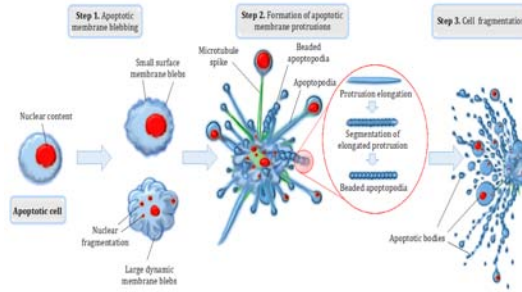

Figure 7.12. Apoptotic cell disassembly.

Source: *Image by Wikimedia commons.*

Much laboratory research has fixated on the antioxidant function of phytochemicals. However, their antioxidant activity is abbreviated in the body during metabolism, and the calibers present in blood and tissue are fleeting and quite low.

For many of the phytochemicals in the pabulum, their antioxidant effects on cell signaling and gene expression may be more paramount for health benefits than direct antioxidant activity, which can be visually perceived even with low concentrations phytochemicals in plasma and tissues.

In integration to being abundant sources of phytochemicals, plant foods withal are sources of fiber, vitamins, and minerals whose mechanisms have been more limpidly elucidated. But identifying which individual compounds are responsible for the benefits associated with phytochemical-opulent foods is arduous, if not infeasible, because of the interactions that occur with vitamins, minerals, and fiber as well as among the phytochemicals themselves.

The unique cumulation of these compounds may be the key to abbreviated disease peril, but that formula hasn't yet been identified and tested.

7.10. PHYTOCHEMICALS IN SKIN DISEASES

According to the European Medicines Agency (http://www.ema.europa.eu/ema/), several studies analyze in-depth the real efficacy of herbal medicinal products and derived molecules. Herbal products can be sorted according to their clinical use in several diseases such as appetite disorders, slumber disorders, pain and inflammation control, ocular perceiver disorders, gastrointestinal disorders, and others.

We focus here the attention on the benign utilization of herbal medicinal products in skin disorders, with a categorical attention on pathologies often cognate to the skin-aging process. Since, in most cases they have been studied or proposed through topical applications, they are often referred to as oils or herbal preparations whose relegation in terms of phytochemical content is rather involute.

Therapeutic applications to skin pathologies are proposed for *Agrimoniae herba* (Agrimonia eupatoria; in minor inflammation and superficial wounds), *Echinacea purpurea* (in diminutive superficial wounds and mild acne), *Soiae oleum* (Glycine max; in mild recurrent eczema), *Juglandis folium* (Juglans regia; in minor skin inflammation), *Matricariae aetheroleum* (in anous and genitals exasperation), *Matricariae flos* (*Matricaria recrutita*; in mild skin inflammation and sunburns and superficial wounds), *Melaleuca spp.* (in insects bites, mild acne, itching, minor skin inflammation), *Meliloti herba* (*Melitotus officinalis*; in minor skin inflammation), *Origani dictamni* herba and *Origani majoranae* herba (Origanum spp.; in minor skin inflammation and exasperation), *Rosae flos* (Rosa spp.; in skin and mouth inflammation), and *Solani dulcamarae stipites* (Solanum dulcamara; in itchy and rash from mild eczema)

The European Medicines Agency official list of herbal substances, preparations, and cumulations for utilize as traditional herbal medicinal products contain 12 substances, according to the European Community decision reported at the EU site.

Figure 7.13. An illustration of *Foeniculum vulgare.*

Source: *Image by Wikimedia commons.*

The 12 herbal substances are as follows: *Calendula officinalis, Echinacea purpurea, Eleutherococcus senticosus, Foeniculum vulgare, Hamamelis virginiana, Melaleuca* spp.*, Mentha* spp.*, Pimpinella anisum, Sideritis scardica, Thymus vulgaris, Valeriana officinalis*, and *Vitis vinifera.*

Their exordium within such an official list is sustained by scientific reports demonstrating their therapeutic efficacy in different pathological settings. Thus, we accumulated the scientific literature reported in PubMed for each of these substances co-occurring in the ALL fields with any of the mundane skin diseases reported in the following list: acne, allergy, basal cell carcinoma, blister, carbuncle, cellulitis, chickenpox, dermatitis, eczema, hives, impetigo, lupus, measles, melanoma, psoriasis, ringworm, rosacea, squamous cell carcinoma, vitiligo, and wart.

The investigated phytotherapeutic agents are mostly associated with allergy, dermatitis, and melanoma, cognate in most cases to either Melaleuca, Mentha, *Thymus vulgaris* or *Vitis vinifera.*

More in detail, local application of *Melaleuca alternifolia* derived oils has been consistently reported to achieve a paramount amendment of acne lesions, according to several independent studies. The clinical efficacy of Melaleuca oil is likely cognate to its kenned antibacterial action as well as anti-inflammatory activity.

The essential oil from *Melaleuca alternifolia* additionally shows antioxidant activities potentially utilizable in dermatitis and skin cancers. Extracts from *Melaleuca quinquenervia* have been shown to inhibit melanin content in mouse melanoma cells, thus exerting potential cosmetic applications. Minor side effects are associated with Melaleuca such as burning, scaling, itch, redness, dryness, pruritus, and stinging.

Contact allergy to Melaleuca oil has been reported in some cases and dermatitis reactions designating the essential oil from Melaleuca as a sensitizer and potentially irritant agent. Mentha-derived oil is utilized to assuage skin inflammation and pruritus; nevertheless, allergic reactions have been reported in some cases.

Determinately, regarding Vitis vinifera, a meta-analysis recently published demonstrates *Vitis vinifera* as one of the efficacious components of medical contrivances utilizable in atopic dermatitis local treatment. Intriguing results have been amassed in oncological conditions; *Vitis vinifera* has shown some efficacy in abbreviating radiotherapy-induced dermatitis and inhibiting cell proliferation in melanom and skin non-melanoma cancer designating grape seed proanthocyanidin as an apoptosis and autophagy

inducer. Recherche allergic reactions are reported for *Vitis vinifera*. Hundreds of other phytochemicals are reported in the literature with potential effects on the skin, such as anti-age activity, photoprotection, wound rejuvenating, and anti-infection.

7.11. FACTORS THAT AFFECT METABOLISM

The bioavailability of phytochemicals varies greatly and can range from less than 0.03% of what's consumed (certain flavanols) to 50 % (isoflavones). While the evidence is circumscribed regarding how phytochemicals are stored, research suggests there are no long-term stores of polyphenols in the body.

Figure 7.14. Factors that affect metabolism.

Source: *Image by Flickr.*

Aside from innate differences in the bioavailability of these compounds, absorption withal is affected by the gut microflora and individuals' genetic makeup, both of which vary considerably. In additament, processing, such as steaming, drying, freezing, and boiling, can abbreviate the calibers of some phytochemicals found in the final victuals product.

7.11.1. Unidentified Therapeutic Intakes

Albeit many phytochemicals are believed to have disease-averting properties, the lack of aliment composition data and the incomplete understanding of their absorption, metabolism, and interaction have averted the Institute of Medicine from engendering a Dietary Reference Intake (DRI) for any of them. In additament, unlike vitamins and minerals, these compounds aren't essential and are ergo not considered nutrients.

The research regarding phytochemicals is perplexed by the fact that the effects of consuming phytochemical-affluent foods may be most propitious for people with more rigorous metabolic abnormalities, such as ascended blood lipids, type 2 diabetes, or exorbitant corpulence, and may not be as ostensible in otherwise salubrious populations.

The involution of the family of phytochemicals, their potential interactions, and the possible variations in levels found in any given aliment make it currently infeasible to issue concrete phytochemical guidelines.

In additament, people orally consume various foods and nutrients every day, each coalescence holding the potential for unique interactive effects, again making it astronomically arduous to link a particular pabulum, nutrient, or phytochemical to a concrete health or disease outcome.

More information is needed afore dietary recommendations can be made. However, researchers have suggested that once ample data are established, a DRI should be developed for one or more of the groups of flavonoids.

7.12. CONCLUSION

This chapter sheds light on the dietary phytochemicals in health and nutrition. It gives an insight to the reader about the importance and benefits of phytochemicals by illustrating how they help cure several diseases and provide with the health benefits.

It also revolves around dietary supplements and foods and explains the clinical evidence for cancer aversion, the concept of anti-cancer mechanisms. It defines the role of phytochemicals in the aversion of cancer and obviation of cardiovascular disease.

It emphasizes the vitality of phytochemicals and how it is used to cure type 2 diabetes and acts as a remedy for neurodegeneration, skin diseases, the mechanism of action and the factors that affect metabolism along with elucidating unidentified therapeutic intakes.

REFERENCES

1. Cancernet Blog, (2016). What are phytochemicals? Types and food sources – Cancernet Blog. [online] Cancernet Blog. Available at: http://blog.cancernet.co.uk/phytochemicals-types-food-sources/ (accessed 9 September 2021).
2. Densie Webb, (2013). Phytochemicals' Role in Good Health. [online] Todaysdietitian.com. Available at: https://www.todaysdietitian.com/newarchives/090313p70.shtml (accessed 9 September 2021).
3. Forni, C., Facchiano, F., Bartoli, M., Pieretti, S., Facchiano, A., D'Arcangelo, D., Norelli, S., Valle, G., Nisini, R., Beninati, S., Tabolacci, C., & Jadeja, R., (2019). Beneficial Role of Phytochemicals on Oxidative Stress and Age-Related Diseases. BioMed Research International, [online] 2019, pp.1–16. Available at: https://www.hindawi.com/journals/bmri/2019/8748253/ (accessed 9 September 2021).
4. Liu, R., (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. The American Journal of Clinical Nutrition, [online] 78(3), pp.517S-520S. Available at: https://academic.oup.com/ajcn/article/78/3/517S/4689990 (accessed 9 September 2021).
5. Robert Thomas, Elizabeth Butler, Fabio Macchi & Madeine Williams, (2015). Phytochemicals in cancer prevention and management? | British Journal of Medical Practitioners. [online] Bjmp.org. Available at: https://www.bjmp.org/content/phytochemicals-cancer-prevention-and-management (accessed 9 September 2021).

Index

Handbook of Dietary Phytochemicals

This book takes the readers through the importance of phytochemicals, how it is important and implies in various aspects from curing diseases' to playing a major role while being a compound of plants or used as a harvest in medicinal plants. This book sheds light on the chemistry and classification of dietary phytochemicals, their pharmacology, its antimicrobial and antiviral properties. The first chapter stresses the basic overview of phytochemicals, metabolism, and phytochemistry, defining the families and structures of phytochemicals to their vital role in the prevention of diseases and different food items. This chapter will also emphasize its presence in plants as compounds and the various methods used in bioactive compounds. The second chapter takes the readers through the functions of phytochemicals in diseases, the use of allelochemicals as phytochemicals, the harvest of medicinal plants. This chapter will provide highlights on the defense mechanism against free radicals, the role of flavonoids on the health of humans, and the effects of dietary plant extracts. Then, the third chapter explains the chemistry and classification of dietary phytochemicals, its classifications as phenolic compounds, tannins, alkaloids, saponin, lectins, etc. It also explains the complex carotenoids, their physiology, structure, and biochemistry, the chemical properties of phytochemicals. This chapter also sheds light on the significance of the physicochemical properties of dietary phytochemicals. The fourth chapter introduces the readers to the pharmacology of phytochemicals and the potential use of phytochemicals like alkaloids in plants. This chapter also explains the importance of the prevention and treatment of antioxidant phytochemicals for chronic diseases. Its use in cancer and circulatory system diseases. The fifth chapter throws light on the antimicrobial and antiviral properties of phytochemicals. This chapter marks the history of natural products as antiviral drugs, the classification of antiviral phytochemicals, plant antimicrobials, and the food industry and the different antimicrobial properties in Clitoria ternatea. The sixth chapter takes the readers through the concept of dietary phytochemicals in the treatment of diseases like obesity, cancer, and diabetes, the cardiovascular diseases. The readers are then told about the various functions of dietary phytochemicals, the isothiocyanates, and the anti-cancer drugs, biosynthesis and antitumor activity and its mechanisms. The last chapter of this book sheds light on the dietary phytochemicals in health and nutrition. This chapter also mentions the benefits of phytochemicals on health, their various role, and it talks about food and dietary supplements, their functions in curing different diseases.

This book has been designed to suit the knowledge and pursuit of the researcher and scholars and to empower them with various aspects of phytochemicals so that they are updated with the information. I hope that the readers find the book explanatory and insightful and that this book is referred by scholars across various fields.

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