PHYTOCHEMISTRY OF CHIA SEEDS (SALVIA HISPANICA), BERRIES AND MEDICINAL PROPERTIES

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Contents

Summary

This chapter discusses the phytochemistry of Chia Seeds (Salvia hispanica), and different types of berries which have remarkable medicinal properties. It is the outcome

of the effort by author to encapsulate the voluminous knowledge and information on phytochemistry and role of phytochemical constituents of these in human health.

The preventive and curative bioactive roles of Chia seeds in the case of certain deadly diseases; Polyphenols and flavonoids- vital phytochemical constituents, of different berries, their potential role in the treatment of diseases, the pharmacological mechanism of action and medicinal properties have long been a requirements in the curricula of various universities across the world for future studies and research to enable us with enhanced knowledge.

according to ethnopharmacology, phyto-pharmaceuticals can help us in the development newer drugs to manage many health issues such as cancer, tumor, cardiovascular disorders (hypertension, stroke, myocardial necrosis, chronic kidney disorder), obesity/metabolic dysfunction, diabetes mellitus, bacterial infection, viral infection, gastrointestinal disorders, inflammation, neuro-pharmacological disorders (depression, Alzheimer, Parkinson disorder) and in obtaining detoxifying agents etc.

There is an extensive body of research that has now firmly established that the intake of berry fruits and Chia seeds in daily lifestyle has a positive impact on human health. In the past, pharmacognosy was considered as the study of drugs that originate from natural sources along with the physical, chemical, biochemical, and biological properties of bioactive components/phytochemical compounds by performing their phytochemistry analysis by different methods. New drugs are created by a new approach which unfolds the medicinal properties of berries and Chia seeds which show different bio-nutrients and bio phytochemical compounds with their precious role in managing different types of diseases.

This chapter first takes up a study of Chia Seeds which includes the historical background, phytochemical constituents, and therapeutic effects for several chronic diseases. Next, berries are studied for their types, (red raspberry, cranberry, blueberry, lingonberry, goji berry, strawberry, blackberry, black raspberry etc) phytochemistry aspects, bio-nutrients, therapeutic and pharmacological properties against chronic diseases.

The chapter briefly discusses the mechanism of action of flavonoids and polyphenols which are the potential phytochemical constituents at molecular or cellular levels and also includes applications in food industry and health care.

The chapter also presents a brief account of recent researches on and future perspective of, Chia seeds and different berries both in combination and individually for their extracted phytochemical constituents. The chapter is addressed to audience with a reasonable background of the subject.

1. Introduction

In the early part of the 19th century of rational drug discovery for the treatment of diseases, plants have been an integral part of pharmacotherapy. The natural products from herbs, plants and their parts found a very vital role in the new drugs discovered for

the formation bioactive entities which have pharmacological properties against certain chronic diseases. Pharmacognosy was established to bring renaissance with its conventional botanical approaches. These approaches have been accepted for the screening of their bioactive components and phytochemicals. They have been analyzed at molecular and metabolomics levels by various researches in which the phytochemical constituents such as alkaloids, polyphenols, glycosides, terpenes and primary and secondary metabolites which are found in crude drugs after their extraction show pharmacological aspects and molecular mechanisms of action following biochemical pathways which induce signals and reduce the effects of carcinogenic, mutagenic, inflammatory, bacterial, viral, hyperlipidemic, cardiovascular diseases, oxidative stress, diabetes etc.

At present, medicinal research is based on the new drug discovery from natural products because they have fewer side effects as compared to synthetically derived drugs. It led to the development of a large number of herbal products which are used not only as food supplements, complementary, nutraceuticals and alternative medicines but also in the discovery of potential bioactive lead chemicals or drug compounds along with their mechanism of pharmacological actions on the human body. The extracted drugs needed to go through numerous preclinical and clinical studies in the sequential order, that is from "bench to bedside", meaning that the efficacy of a herbal compound against certain diseases, is indicated by its traditional use and thereafter proved by pharmacological investigations- a process known as "reverse pharmacology" which is considered as a very strong and useful strategy for identification of new lead compounds for better prophylactic and therapeutic activities on human subjects. This necessarily follows a phytochemical extraction procedure for herbs and synthesized products (see Shah, 2009).

1.1. Phytochemistry and its General Methods

Phytochemistry is the branch of pharmacognosy which deals with the study of phytochemicals, (Greek word *phyton* means plants) which are chemicals and bioactive compounds derived from plants and natural products. It aims to describe the structural organization and molecules of a large number of primary and secondary metabolites - compounds found in plants along with their functions and biosynthesis of these compounds in human and plant biology. The phytochemicals are also referred to as phytonutrients which are found in fruits, vegetables, whole grains, legumes, beans, herbs, spices, nuts, and seeds. They were classified according to their chemical structure and functional properties in therapeutic activity. The plants synthesize phytochemicals for several reasons, including defense against insects, birds and animal attacks, growth regulation, and defense of plant cells against different pollution, stress, drought, UV exposure, predators and diseases due to bacterial, fungal, viral and pathogenic infections. They help to maintain and balance between color, aroma and flavor of the compounds. Phytochemistry is a sub-field of botany (due to its plant origin) or chemistry (dealing with chemical composition and structural aspects). Phytochemicals which are synthesized by food plants are often active in human body and produce several health benefits. The phytochemicals found in plants are of various kinds but four major biochemical classes are alkaloids, glycosides, polyphenols and terpenes.

It is known that phytochemicals show valuable prophylactic and therapeutic activities for human health in several pharmacological aspects by enhancing their phytochemistry features and the related research is known as ethnopharmacology because it can determine the medicinal aspects of the plant-derived products along with their pharmacological actions. Applications of these are from pharmacognosy or the new drug discovery for the aid of plant physiology studies.

Over 4000 phytochemicals have been so far identified, catalogued and classified based on their protective, functional, physical and chemical characteristics and about 150 phytochemicals have been studied in detail.

Name of the Group of Phytochemicals	Members of the group
Alkaloids	• Ajmaline, • Bernerine, • Caffeine, • Cocaine, • Hyoscyamine, • Morphene, • Nicotine, • Oxymorphone
Allium compunds	• Menthiin, • Propiin, • Isoalliin
Betalains	• Betaxanthins, • Vulgaxanthin, • Miraxanthin, • Portulaxanthin, • Indicaxanthin
Capsaicinoids	• Capsicin, • Dihydrocapcaicin, • Nonivamide
Carotenoids	• Beta-carotene, • Lutein, • Zeaxanthin
Chlorophyll	
Glucosinolates	• Progoitrin, • Sinigrin, • Gluconapoleiferin, • Glucobrassicin, • Glucosinalbin, • Gluconasturtin, • Glucotropacolin
Lectins	• Ricin, • Peanut • agglutinin, • Concanavalin A
Polyacetylenes	• Falcarinol, • Falcarindiol, • Panaxydiol, • Oenanthetol

Name of the Group of Phytochemicals	Members of the group
Polyphenols	• Flavonones, • Flavones, • Anthocynadins, • Proanthocynadins, • Phenols, • Flavon-3-ols, • Isiflavones, • Coumarines, • Stilbenes, • Lignans, • Secoridoids, • Xanthones, • Benjoic acid, • Hydrolyzable tannins
Polysacharides	• Cellulose, • Hemicellulose, • Inulin, • Methyl cellulose, • Pectin, • Gums, • Mucilages, • Polydextrose, • Arabinogalactans
Terpenes	• Cinerin 1, • Geraniol, • Strigol, • Squalane, • Farnesane, • Calotropin

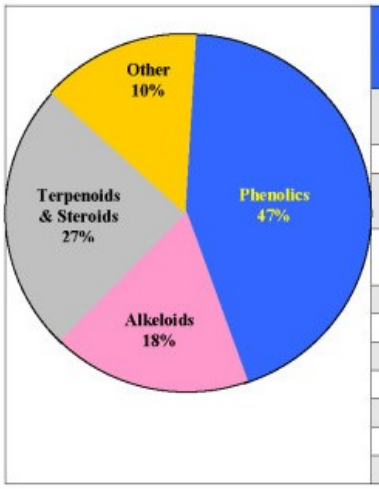
Figure 1. Classification of Phytochemicals

Phytochemists study phytochemicals by extracting and isolating compounds from the plant which is followed by laboratory model studies, such as cell cultures, *in vivo* and *in vitro* experiments in both preclinical and clinical experiments by using experimental laboratory animal models for screening of active biological phytochemical constituent

compounds against particular diseases. Still, there is a challenge for the scientists of ethnopharmacology which is in isolating specific compounds and determining their structural complexity and identifying the particular phytochemical compounds that are primarily responsible for a particular biological activity. This is because there are no studies on these phytochemical compounds regarding biochemical pathways and molecular signaling pathways they follow to induce a response against diseases.

The classification of phytochemicals is still quite controversial. Each phytochemical individually or in combination with others has different biochemical pathways and physiological effects on human health as a result of synergistic effects. Classification of phytochemicals in this chapter is shown in Figure 1.

Many phytochemical constituents which are abundantly found plants and natural products are polyphenols or phenolics. They indicate the emphasizing dietary intake directly either through food or indirectly by bioavailability of the gastric, intestinal and hepatic metabolism processes. Polyphenols are the largest category of phytochemicals among all because of their powerful activities due to the presence of various hydroxyl groups present in them. So, they will be the focus in the chapter in the phytochemistry of Chia seeds and various berries containing them. The contents of major class of basic phenolic compounds are shown in Figures 2 and 3.



No of carbon atoms	Basic skeleton	Class
6	C_6	Simple phenols Benzoquinones
7	C_6-C_1	Phenolic acids
8	C_6-C_2	Acetophenones Tyrosine derivatives
9	C_6-C_3	Hydroxycinnamic acid Coumarins
10	C_6-C_4	Naphthoquinones
13	$C_6-C_1-C_6$	Xanthones
14	$C_6-C_2-C_6$	Stilbenes
15	$C_6-C_3-C_6$	Flavonoids
18	$(C_6-C_3)_2$	Ligands
30	$(C_6-C_3-C_6)_2$	Bioflavonoids
N	$(C_6-C_3-C_6)_N$	Condensed tannins

Figure 2. Pie chart of major polyphenolics compounds in plants

PHENOLIC ACIDS	**Hydroxybenzoic acids**	Gallic acid,
		Ellagic acid,
		Vanillic acid,
		Syringe acid,
		Protocatechuic acid,
		Salicylic acid
	Hydroxycinnamic acids	Caffeic acid,
		Caftaric acid,
		Chlorogenic acid,
		Cinnamic acid,

		Coumaric acid,
		Ferulic acid,
		Curcumin
FLAVANOIDS	**Flavones**	Luteolin,
		Apigenin
	Flavanoids	Quercetin,
		Rutin,
		Isohammetin,
		Myricetin,
		Kaempferol
	Flavon-3-ols	Catechin,
		Gallocatechin,
		Epicatechin
	Isoflavones	Genestein,
		Daidzin,
		Glycitein,
		Equol
	Flavanones	Naringin,
		Hesperidin
	Anthocyanidins and Anthocyanins	Malvidin,
		Cyanidin,
		Delphinidin,
		Peonindin
OTHER PHENOLICS	**Stilbenes**	Resveratrol,
		Piceatannol
	Lignans	Pinoresinol,
		Lariciresinol,
		Matairesinol,
		Secoisolariciresinol,
		Sesamol,
		Enterodiol,
		Enterolacton
	Tannins	Hydrolyzable tannins • Tannic acid, • Galloetannins, • Ellagitannins Nonydrolyzable tannins • Condensed tannins/Proanthocyanidins • Procyanidin B2 • Procyanidin B2
	Xanthones	
	Lignin	
	Chromones	
	Anthraquinones	

Figure 3. Types of polyphenols/phenolics

Phytochemicals act against several diseases such as hypertension, tumor, asthma, arthritis, diabetes, chronic kidney disorder, obesity, inflammation, and bacterial, viral, fungal and pathogenic infections, cancer etc. Unlike pharmaceutical chemicals since these are derived from plants or nature they do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings they are regarded as "human-friendly medicines". So, before the manufacturing of these valuable biological compounds, it is necessary to follow all the steps such as plant material collection, extraction, isolation, qualitative and quantitative estimations.

The various techniques which are commonly used in the field of phytochemistry are extraction, isolation and structural elucidation by MS, 1D NMR and 2D NMR of natural products and herbal ingredients as well as chromatography techniques such as MPLC, HPLC, LC-MS, TLC, CC and analytical methods to find various constituents functional groups by transmittance and absorbance UV, FTIR, IR spectroscopy.

General Methods for Plant Material Collection:

- The general procedure of plant material collection is from herbaria or forests. When the plants are collected from the wild areas, there may be a possibility of incorrect identification but there are fewer chances of having pesticides.
- After collection, they are processed for cleaning to remove any adulteration and deterioration of phytochemicals present in them.
- The cleaning process is done very carefully either by washing, peeling, cleaning, or stripping of leaves from stems and this is better to do manually.
- After cleaning the next step is drying to remove the extra water content and storage. Drying is done either naturally or by an artificial process, otherwise water causes spoilage of the plants.
    1. **Natural drying process:** This includes sun-drying and it takes a few weeks for complete drying and the time duration depends upon temperature, humidity and climatic conditions.
    2. **Artificial drying process:** With the help of artificial dryers, this process reduces the time from several hours to minutes. The commonly used process is warm-air drying.
- After drying the plants can either be powdered, cut, crushed, or used whole and sent for further steps of extraction and isolation of particular phytochemical constituents.

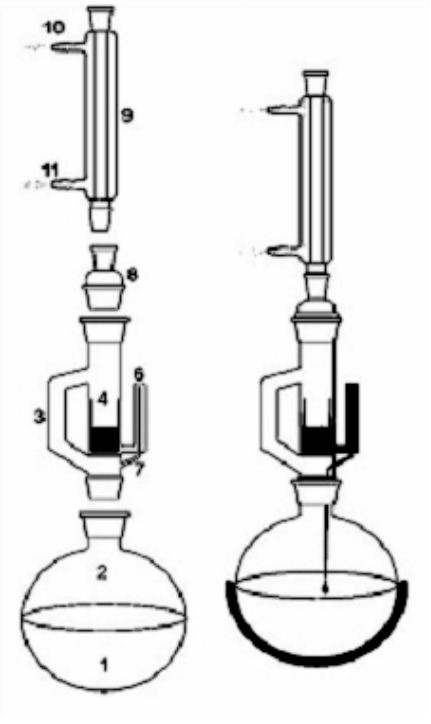
General methods for plant phytochemical extraction:

- **Plant tissue homogenization:** The method of extraction of phytochemical constituents in a solvent medium is widely used by scientists, researchers and phytochemists. The procedure followed is that dried, or wet fresh plant parts, fruits, seeds, leaves, or stems etc are ground in a blender to produce fine particles, and then put in a certain quantity of solvent and shaken vigorously for about 5-10 min or left 24 hours after which the extract is filtered by using vacuum filtration, or by Whatman filter paper. Then the filtrate may be dried under reduced pressure and re-dissolved in the solvent medium to determine the concentration. But some

researchers, however, use a centrifugal technique to produce a clear extract with high potential compounds.

- **Serial exhaustive extraction:** It is a common method of extraction for the analysis of phytochemicals. It involves successive extraction by using solvents of high polarity from non-polar compound hexane to more polar solvent methanol to ensure that a high range and wide polarity of compounds could be extracted. Organic solvents are used in Soxhlet extraction which is used by researchers for dried plant materials. But there is a limitation of this technique that it is not used for thermolabile compounds as prolonged heating or high temperature could lead to deterioration of the active metabolites or bioactive compounds present in the herbs.
- **Soxhlet extraction:** Soxhlet extraction is a laboratory technique with equipment designed for the processing of certain kinds of solids. This technique was invented by scientist Franz von Soxhlet in 1879. He was the first who quantified fat in milk and frequently used it for the extraction of lipids for agricultural chemistry. These devices allow for continuous treatment of a sample with a solvent over hours or days to extract compounds of particular interest. This technique of extraction is only required when the desired compound has limited solubility in a solvent and the impurity is insoluble in that solvent.

  If the desired compound has a high solubility in a sample, then a simple filtration process can be used for the separation of the compound from the insoluble substances. This method is also not used for thermolabile compounds because it causes denaturation of compounds present in extracts. A schematic of Soxhlet extractor equipment is shown in Figure 4.



Components of Soxhelet Extractor in the figure	
1	**Stirrer bar**
2	**Still pot**
3	**Distillation path**
4	**Thimble**
5	**Solid**
6	**Siphon top**
7	**Siphon exit**
8	**Expansion adapter**
9	**Condenser**
10	**Cooling water inlet**
11	**Cooling water outlet**

Figure 4. A schematic representation of a Soxhlet extractor equipment with its components

The procedure of Soxhlet Extraction:

- Solid material containing some of the desired compounds is placed inside a thimble made from thick filter paper which is loaded into the main chamber of the Soxhlet extractor.
- Then the Soxhlet extractor is placed onto a flask containing the extraction solvent and equipped with a condenser.
- The solvent is heated to reflex, the solvent vapor travels up a distillation arm, and floods into the chamber housing the thimble of solids. The condenser ensures that the solvent vapor cools and drips back down into the chamber housing the solid material.
- The chamber then is slowly filled with the warm solvent in which some of the desired compounds dissolve.
- When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon sidearm, with the solvent running back down to the distillation flask. This cycle may be repeated many times, over hours or days.
- During each cycle, a portion of non-volatile compounds dissolves in the solvent.
- After many cycles, the desired compounds are concentrated in the distillation flask.
- After extraction, the solvent is removed and dried using a rotary evaporator, yielding the desired compound. The insoluble compounds remain in the thimble and are usually discarded.
- **Sonication:** The sonication procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz because it increases the permeability of cell walls and produces cavitation. Although the process is used in some cases like extraction of phytochemical constituents from Rauwolfia serpentina, the root part for application is limited due to the high cost. There are some disadvantages of this extraction process. It can produce free radicals due to ultrasound waves more than about 20 kHz on the particular active potential bioactive constituents of medicinal herbs and damage their therapeutic value by causing undesirable changes in the drug molecules and insert hindrances in the pharmacological actions of the compounds.
- **Microwave-Assisted Extraction (MAE):** MAE nowadays attracted attention as a better technique for the extraction of several phytochemical compounds from a wide variety of plants and natural sources. The microwaves at frequencies between 300 MHz and 300 GHz, and wavelengths of between 1 mm and 1 m consist of both electrical and magnetic fields of energy. It is an advanced technique, which leads to conserving the high-quality bioactive compounds without increasing extracting time. It is an alternative technique used for the extraction of antioxidants and phenolics from medicinal plants because of its ability to reduce time and solvent volume. Their activity is measured by following ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and total polyphenolic content (TPC). The extraction can be changed due to some factors which hinder the extraction temperature, solvent composition and extraction time.
- **Extraction of Phenolics Compounds Using Solvents:** There are different types of solvents used by researchers such as methanol, hexane, ethanol, ethyl alcohol, for the extraction of antioxidants from leaves and roots parts of medicinal plants. For the extraction of different phenolics from the compounds with high accuracy,

various polarity solvents are used in varied ratios. As such methanol is found to be best suited polar solvent with high polarity and effectiveness towards antioxidants. The solvents used for the extraction process of different biomolecules from medicinal plants are chosen based on the polarity of the solute of interest.

- **Ultrasonic-Assisted Extraction (UAE):** It is known as one of the easiest extraction techniques because it uses common laboratory equipment such as an ultrasonic bath. In this, the sample is smashed and mixed with a suitable solvent and then placed in the ultrasonic bath, while the temperature and extraction time duration are under control. It is the most effective method for the extraction of antioxidants. The method results in high quality of production of extracts from fruits, seeds, roots, and leaves. It can reduce the degradation of the antioxidant and polyphenolic content of the extracts.

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Biographical sketches

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During her academic sessions, she undertook 90 days of hospital training as an intern from Saral nursing home, Lal Bangla, Kanpur, and 45 days of industrial training as a trainee in Surya Pharmaceuticals, Ramnagar, Chandauli. She received certificates for her academic performance, participation in national and international seminars, conferences, webinars, workshops etc . After completing B. Pharm (Pharmacy Council of India) now, she is a certified Pharmacist under the pharmacy act 1948. She writes various review articles and research articles on the topics which cover the pharmacology aspects related to Hypertension, Role of Berries, Coronavirus SARS-CoV-2 and COVID-19, Chia Seeds (Salvia hispanica) and its role on cardiovascular diseases, cognitive and meditation therapies in depression, postpartum depression and postmenopausal depression in adult women, toxicological study of ethyl acetate polyherbal extract obtained from berries and flaxseed (EAPEG-BF) on male Wistar rats (Research article), phytochemical evaluation and standardization of EAPEG-BF (Research article). The articles were published in journals such Bentham Medical Science-The Open Biology Journal (BMS-TOBJ), Asian Journal of Pharmaceutical and Clinical Research (AJPCR), International Journal of Advanced Research (IJAR), Current Psychopharmacology-Bentham Medical Science (CPSP-BMS), Journal of Biomedical and Pharmaceutical Research (JBPR) and International Journal of Pharmaceutical Sciences and Researches (IJPSR).

Dr. K L Krishna has done his post-graduation in Pharmacology from Delhi University and obtained doctoral degree in Pharmaceutical Sciences from Nirma University, Ahmedabad. He is presently working as Assistant Professor in the Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India. He has more than 20 years of teaching as well as research experience in the research area of Neuropharmacology, Toxicity studies and has more interest in Phytopharmacological research. He has published more than 73 research and review papers in peer reviewed national and international journals with Google citations of more than 1000. He has visited Europe and Malaysia to present a scientific paper, for which he was awarded with UGC and JSSU Travel Grant award respectively. He has guided more than 47 M Pharm students and 1 PhD student, currently guiding 4 PhD Scholars. He is a member of many National professional bodies and technical editor/reviewer for many national and international journals. He has special concern to the societal works and currently looking after National Service Scheme (NSS) affairs of the University as JSS AHER NSS Co-ordinator. He has been recognized as CPCSEA nominee and representing CPCSEA at many institutions IAEC and actively involved in preclinical research. He is recognized as Pharmacy Council of India (PCI) Inspector to conduct the PCI Inspections of Pharmacy Colleges across India.

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same institution where she is presently working. To her credit, she has 8 years of teaching and research experience in area of Neuropsychopharmacology and Nutritional neuroscience, mainly focused on Depression. She is both diligent in her research practice and committed to the profession. She has 25 research and review publications and authored 4 Book chapters in the indexed national and international journals and books respectively. In 2018, she received a travel grant from JSSAHER to attend an international conference at Paris, France, as a speaker in Young research forum. She has guided 6 post graduate (M Pharm) students and serving as an Advisory board member, reviewer and editorial member for many national and international journals and presently, she is also carrying out a research project which is funded by the JSS AHER.