International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

A review of phytochemicals and uses of flaxseed

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Abstract

Essential oils are concentrated liquids of complex mixtures of volatile compounds and can be extracted from several plant organs. Aromatic plants and oils have been used for thousands of years, as perfumes, cosmetics and culinary applications. Now-a-days, essential oils are used as a good source of several bioactive compounds, which possess antioxidative and antimicrobial properties. In addition, some essential oils have been used as medicine. Flaxseed is valued for its oil content, which traditionally was used for paints and coatings, printing inks, soap, core oils, brake linings, and herbicide adjuvant. This review article is designed to compile recent extraction methods and latest fractionation techniques for isolation of bioactive compounds of essential oils obtained from flaxseeds. Solvent free microwave extraction, supercritical fluid extraction, direct steam distillation, hydro distillation and simple steam distillation are commonly used methods for extraction of oil while the isolation of bioactive components can be carried out by using fractional distillation or vacuum distillation and high speed counter current chromatography. Flaxseed showed high antioxidant activity. Alpha-linolenic acid (ALA), lignans, n-3 fatty acids are a major group of compounds that act as primary antioxidants or free radical scavengers.

Key words: Flaxseed, Phytochemicals, Essential oils, Biological activities, Isolation techniques

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1. Introduction

Essential oils are the mixture of volatile lipophilic (fat loving, i.e., soluble in fat) components, usually obtained from wood pulp, leaf, twig and bark tissues of higher plants, but also broadly found in bryophytes, such as liverworts. However essential oils are just slightly soluble in water, with respect to the polarity (magnetic activity) the aqueous solubility of individual constituents of essential oil varies. Usually, components which have more polar functional groups are assume to be highly soluble in water as compared to the other components [1]. Essential oils (EOs), which are complex mixtures of biologically active substances, are categorized as natural products possesses pharmacological potential that can be of therapeutic benefit in the management of human diseases [2-4]. Plant essential oils are generally extracted by steam distillation because they are hydrophobic liquids, they occur as concentrates. The concentrates contains a mixtures of numerous volatile compounds such as ketones, aromatic phenols, alcohols, esters, lactones and aldehydes, as well as monoterpenes and sesquiterpenes [5]. But composition vary with different seasons [6]. Essential oils are complex mixtures of volatile substances generally present in low concentrations [5-7]. They have to be extracted from the matrix, before such substances can be analyzed. Various methods can be used for the extraction of EOs such as steam distillation, soxhlet extraction, hydro-distillation (HD) and simultaneous distillation-extraction. However, these compounds present in EOs are reactive and thermally sensitive. Low extraction efficiency, losses of some volatile compounds, degradation of unsaturated or ester compounds through thermal or hydrolytic effects and toxic solvent residue in the extract may be encountered using these extraction methods. These limitations have directed to the consideration of the use of new “green” technique in essential oil extraction, which typically use less solvent and energy, such as supercritical fluids, ultrasound and microwave [8]. Essential have wide applications in food flavoring and preservatives industries. Furthermore EOs have wide range applications in medicine due to the presence of bioactive components and also in food preservation. Essential oils are present in aromatic plants which are volatile, natural, and complex compounds. However, EOs are responsible for protecting the plants via their antibacterial, antiviral, antifungal [9], and insecticidal activities. Essential oils are secondary metabolites of plants, which are by their antimicrobial and antifungal activities increase durability in horticulture crops. Various components of EOs can be effective and useful in

controlling microbial growth. The chemical components of the essential oil can be analyzed by gas chromatography-mass spectrometry (GC-MS) [6-10-11]. In gas chromatography, the molecules being separated must be gaseous because its operation relies on gases. Most compounds of plants are not volatile such as sugars and pigments. Their boiling point is so high so before becoming gases they degrade because of high temperature. Essential oils are composed of volatile molecules so this type of chromatography is best for essential oils because it readily shift volatile molecules to the gas phase. GC instrument gives no information on how much of each compound is present and what they are. It only separate molecules and then release them [12].

Flax seed is annual herbaceous plant. Botanical name of flax seed is Linum usitatissimum. In social and economic development of humans flaxseed shows its importance and versatility. First domesticated plant by humans was flaxseed about 8000-10000 years ago. Classical civilization esteemed flaxseed oil in cosmetics and also in cooking and its fiber used to make sail cloth and linen. Ancient people also used flaxseed because of its remedial properties [13]. Earlier, flaxseed oils are used for industrial purposes e.g. production of paints, cosmetics, linoleum, inks and varnishes. Due to its nutritional and pharmaceutical values flaxseed is widely used in functional foods. Protein, lignan, oil, vitamin, soluble fiber, mineral etc. are nutritional components of flaxseed [14].

Flaxseed is an important worldwide seed crop. Flaxseed contains large amount of α-linolenic acid so as a functional food flaxseed is the most studies oilseeds. ALA is also known as omega-3-fatty acid made up of 50-55% of the total flaxseed oil. Different studies show that the risk of mental disorder, cardiovascular disease can be reduced by ingestion of flaxseed oil. The reason of this is, during metabolization in intestine ALA can be converted into docosahexaenoic acid (DHA) and eicosapentaenoic acid. Flax seed oil shows excessive sensitivity towards immiscibility in aqueous food system and oxidation so it has limited utilization in food industry although it has many disease prevention and health benefits properties. Usually, in flax seed 30% diet fiber, 6% moisture, 20% protein, 4% ash and 40% oil is present. Flaxseed also contains potent antioxidants lignans and dietary fiber which contain high amount of shot chain omega-3 fatty acid. It also has approximately 20% monounsaturated fatty acids, 70% ALA fatty acid and 9-10% saturated fatty acids. The percentage of proteins varies from 20-30% in flaxseeds [15].

There are different phytochemicals which are present in flaxseed such as triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, proteins, free amino acids, carbohydrate and vitamin C.

2. Extraction methods

Different volatile chemical compounds are present in many plant species. These compounds can be separated as an essential oil. As an essential oil these compounds can be extracted. From various plant materials these oils are separated by using different methods. Despite the fact that it appears to be generally easy to separate such oils, composition of oil may fluctuate to a large extent relying upon the extraction strategy utilized. For hydrodistillation extraction method Clevenger apparatus is commonly used. Heat was provided to the heating mantle (50 ℃) and process continued for 3 h. Immediately essential oil was collected and analyzed. Obtained flaxseed oil yield was 0.31% [16]. Method which is used as a reference for the quantification of essential oils is hydrodistillation method [17]. Hydrodistillation is environmentally friendly, provides good quality of essential oils and operate in a safe and simple manner. Hydrodistillation also have economic viability so it is used on the industrial level for the extraction of essential oil under atmospheric pressure. The volatile components are evaporated into water and steam dislodge atmospheric oxygen secured the volatiles from oxidation, this is also the advantage of hydrodistillation [18]. Soxhlet extraction (SE) is a helpful apparatus for preparative purposes in which the analyte is concentrated from the lattice in general or isolated from specific interfering substances. In a previous study, 50 g flaxseed was extracting using soxhlet apparatus using hexane as a solvent. Extraction continued for 10 h. Then filtered the solution and in a rotatory evaporator solvent was removed under vacuum at 40 °C. The obtained yield of the oil was 19.4 ± 0.4 g. Ultrasound-assisted extraction (UAE) is also used for the extraction of EOs from plant material. An experiment was conducted by some researchers to find out the yield of flaxseed oil via UAE method. Flaxseed powder was mixed in a solvent 10 mL n-hexane and ultrasonic probe directly inserted into the mixture. At different levels of power output under continuous ultrasonic waves samples were extracted. Temperature was controlled at desired level during extraction. The yield of flaxseed oil obtained by this method was 42.0%. Microwave-assisted oil extraction is a simple technique that involves extraction of oil from plant material with the help of microwave energy. This technique is applicable in both liquid phase (liquid as a solvent) and solid phase (gas as extractant) extraction. Approximately 2.5 g sample of flaxseed powder with 4%, 8%, 12% moisture contents, in a ratio of 1:3 g/mL was mixed with solvent ethanol by some researchers. Microwave oven was used to treat the mixture thermally, with an output microwave power 1025 W and frequency 2450 MHz and operated at 50% power level. In order to avoid heating the sample at high rate, thermal treatment was used. The heating time of microwave treatment was 10 s. After each 10 s, it quickly followed with stirring by cooling time of 5 min. With the help of infrared thermometer, temperature was observed after every 10 s of microwave treatment, which ranging from 55 to 60 °C. By adding ethanol, level of

solvent was maintained in a flask after each treatment. Per sample about 10mL of ethanol was consumed. For each sample total time of treatment was 3 min. After the heat treatment, for 12 h sample was allowed to settle. The supernatant layer was pipetted which containing oil. Evaporated the oil in water bath and then recover the oil and weighed. The obtained yield of flaxseed oil was 10.0% (Ali and Watson, 2014).

3. Isolation of phytochemicals present in flaxseed

Various techniques have broadly been utilized for fractionation of essential oil including (i) high speed counter current chromatography (ii) fractionation by vacuum distillation (iii) fractional distillation also called simple distillation.

3.1. High speed counter current chromatography

High speed counter current chromatography is an ongoing partition system in which two immiscible solvents are utilized in type of two layers for the isolation of EOs components from the EOs where lower layer act as mobile phase and upper layer works as stationary phase. At first, stationary stage is filled in helical section and after that mobile phase is pumped in the coiled column through inlet that is normally found on chromatographic column. Flow rate of mobile phase is nearly 7 milliliter for every minutes utilizing head to tail mode. At the point, when balance is built up, sample of essential oil is introduced which undergo fractionation and continuously detected by detector. High speed counter current chromatography is most generally utilized for purification or sanitization of various functional compounds from standard established Chinese fragrant plants and furthermore different other common characteristic items. In high speed counter current chromatography, exact selection of both extracting solvents is of extreme significance for efficient isolation, effective separation, proper fractionation and complete purification. Selection of proper solvent system ought to accord following guidelines (i) retention time of stationary phase ought to be more noteworthy than 50 percent (ii) solvent's settling time ought to be under 29 seconds (iii) target material should be stable (iv) required components must be soluble in twofold layer solvent system and (v) solvent system ought to be stratified [19].

3.2. Fractionation by Vacuum Distillation

Volatile essential oil can be fractioned through vacuum distillation apparatus in which known volume of sample is kept in round bottom flask having three outputs one for capillary tube to control and build exuberance in flask and second for connection of temperature control sensors to manage temperature of fractioned essential oils all through the distillation and third one made to attach with packed column. Length of fractionating column is 1.5 meters with titanium alloy filled as packing material and expected to give protection against erosion. Thermocouple is used to quantify the temperature of top of fractionating column with precision of about ±1.5°C. In condenser, thermostatic shower is commonly used to control temperature and vacuum pump is used to keep up required pressure. Chemical compounds having low volatility are fractioned earlier in vacuum distillation and slight modification in chemical composition significantly changes acaricidal activities [19].

3.3. Fractional Distillation

Boiling point of any liquid is the most critical physical property that shows important role in fractionation of essential oil, as various fractions are known to have distinctive chemical constituents which are separated on the basis of temperature. In this way, fractions of essential oils are typically gotten at variable temperatures under cautiously controlled conditions and decreased pressure. Each part comprises altogether extraordinary aromas because of various chemical compounds [19].

4. Biological activities of flaxseeds

Flaxseed (*Linum usitatissimum*) is famous for its nutritious and protective properties which are attributed to the presence of good quality omega-3 unsaturated fatty acids, alpha-linolenic acid (ALA), antioxidants such as phenolics, lignin, carotenoids and tocopherols [20-23]. Flaxseed is becoming famous day by day due to its health related benefits such as it is associated with reduced risk of cardiovascular diseases and cancer (especially of prostate and mammary glands). It has anti-inflammatory properties and laxative effect. It is also seemed to be effective in osteoporosis and mitigating the menopausal symptoms [24].

4.1. Biological activities of lignans

Whole or ground flaxseed is a rich source of lignans (frequently named phytoestrogens), including secoisolariciresinol and matairesinol. These lignans are accepted to apply cell reinforcement and phytoestrogenic impacts. They are changed over by microscopic organisms in the colon to the dynamic metabolites enterodiol and enterolactone. These metabolites are accounted for to have more prominent cancer prevention agent action (antioxidant activity), antiplatelet impacts, than the parent lignan secoisolarciresinol diglucoside and furthermore apply either powerless estrogenic or antiestrogenic impact, contingent upon natural dimensions of estradiol [25].

4.2. Biological activities of ALA

Flaxseed contains α-linolenic acid (ALA) that is responsible for the treatment of wide range diseases such as multiple sclerosis (MS), renal disease, Crohn’s disease, diabetes, lupus and ulcerative colitis. Other conditions such as migraine headache, depression, chronic obstructive pulmonary disease (COPD), skin cancer, allergies, psoriasis and eczema are also treated with the use of ALA [26].

4.3. Biological activities of peptides

Bioactive peptides (for example cyclolinopeptide A) are present in flaxseed and they are found to inhibit the Plasmodium falciparum (a malarial human parasite in

culture). A peptide mixture consisting of high levels of Branched chain aminoacids (BCAAs) and aromatic amino acids (AAAs) at low level are observed to show the antioxidant properties by scavenging 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and its antihypertensive properties are shown by inhibition of the angiotensin I–converting enzyme [27].

4.4. Biological activities of n-3 fatty acids

Flaxseeds contains soluble fibers, vitamin E, n-3 fatty acids, lignans, other phenolic and peptide compounds and these components of flaxseeds possess great health beneficial properties such as vessel relaxation, anti-inflammation, antioxidant and anticarcinogenic. Risk of a wide range of chronic diseases such as diabetes, cardiovascular, cancer and obesity is found to be reduced by these bioactive compounds [28].

5. Economic value

A case study in 2009/2010 showed that worldwide about 2.3 million metric tons of flaxseed are produced and in 2014 recorded production of flaxseed is about 1.173 million tons. China is enlisted in the largest flaxseed producing countries with a history of about 2000 years [29]. Flax grown in china are classified into two types; linseed is one and fiber flax is the other type of flax. This classification is on the basis of difference in environmental requirements, harvesting times, botany and cultivation. Difference in these parameters will result in overall difference of seeds thus their utilization also differs. Fiber flax has been utilized in industries like textile and cloth manufacturing industries. Due to nutritional value of flax its utilization as food additives is gaining interest day by day.

6. Applications of flaxseed

6.1. Medicinal applications

The therapeutic uses of flaxseed/linseed are referenced in progress of Avicenna, Hippocrates, Qantes, and Dioscorides, just as in medieval books on restorative herbs in both Asia and Europe. Linseed was suggested as an antitumoral pain, cough relieving and anti-inflammatory remedy. It was additionally utilized for the treatment of spots and nail issue. In the Middle Ages, linseed oil was manage as a diuretic for the treatment of kidney issue. Russian society drug utilized linseed preparations and linseed oil too. In recent years, enthusiasm for utilizing flax preparations for therapeutic purposes extraordinarily emerged once more. Linseed is referenced in the pharmacopeias of different countries. In Russia, commercial "linseed" preparation is managed perorally as a spongy and softening agent (emollient) to lessen irritation brought about by inflammatory and ulcerous procedures in the mucous films of the gastrointestinal tract. This preparation is additionally utilized as a demulcent and applied externally in the form of compresses or poultices for the treatment of various local inflammatory disorders [30].

6.2. Flaxseed as a nutritional additive

Flaxseed is utilized as a healthful added substance in bakery and in the preparation of some dietary items. Specifically, experts of the State Institute of the Baking Industry created innovation for another mix rye–wheat bread with linseed additives. It was prescribed that linseed-containing bakery products be incorporated into the eating regimen, particularly of patients with gastrointestinal tract issue and cardiovascular diseases, specifically, atherosclerosis. One Russian bakery (Rybinsk) produces "an old Russian bread" of developed wheat grain with flaxseed for medicinal prophylactic purposes [31].

6.3. Flaxseed Oil in the Production of Industrial and Home Chemicals

This oil is used in both form either pure or in the form of components or derivatives (acids, hydro peroxides, oxides, etc.), in various products: linoleum; modified (epoxidized) resins; plasticizing agents for obtaining plastics with increased elasticity; additives for asphalt, bitumen's, lacquers, paints, and anticorrosion film coatings for metals; wood impregnates; lubricant compositions; magnetic recording media; polishing compositions with abrasive particles; sulfided surfactants; granulated pesticides; copolymers for various containers (including plastic bottles for water); adhesive and sealing compounds; foam-extinguishing compositions for fermenters; various inks (including printing ink); paper and cardboard; photosensitive compositions; nutritional additives; cosmetics and dermatological compositions; and medicinal [32].

7. Future directions

Flaxseed is certifiably not a significant food plant. Due to cyanogenic glucosides substances and laxative properties, its utilization as food is limited. Flaxseed may even use as health promoting ingredient to foods, because it contain biologically active lignans. Flaxseed also contains polyunsaturated fatty acids (PUFA) which is also very useful for health benefits. Possibility of getting these unsaturated fats from higher plants in large quantity is very attractive. To combine these unsaturated fats hereditarily building would be required so such items could not be created normally by any oil-seed species. Flaxseed may be a choice platform species because it already contained high amount of ALA (alpha-linolenic acid) and precursor to PUFA. One of the future applications of flaxseed is that, the gene and molecular expression experiments are not studied widely in flaxseed. Other than knowing about properties of flaxseed, health benefits of flaxseed can understand by determination of compounds of flaxseed which include prevention of cancer diseases, reduction of coronary heart disease risk factors, improvement in inflammatory and immune responses. Although, excellent progress has already been made, it is expected that the use of different methodologies of potent techniques coupled with rapid,

reliable and sophisticated detectors will become more common in the near future.

8. Conclusion

The most researched biological activities of flaxseed have been related to ALA, lignans, peptides and, to a lesser extent, soluble polysaccharides (gum), since flaxseed is the most abundant prominent source of these components. However, there are many products of flaxseed which shows beneficial effects in commerce. Hence strategies for the economic extraction, modification and isolation of phytochemicals from flaxseed have to be developed for flaxseed to be a truly natural antioxidant. Alpha-linolenic acid has anti-allergic and anti-inflammatory activities, lignans are believed to exert antioxidant and phytoestrogenic effects and peptides have strong immunosuppressive and antimalarial activities.

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