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Research Article

Physico-Chemical & Phytochemical Investigation of Linseed *(Linum usitatissimum L.)* oil grown in Maharashtra (India) and Analytical Study by HPLC

Shobha S. Borhade

Department of Drug Chemistry, S.M.B.S.T.College Arts,Science and Commerce Sangamner Affiliated to University of Pune, Sangamner Dist. Ahmednagar, Maharashtra , India 422605.

Abstract

Linseed *(Linum usitatissimum L.)* is a multi-purpose crop and its consumption is beneficial for human health. The nutritional components of flaxseed are oil, protein, lignans, fiber and vitamin. Linseed is a good source of plant omega-3 fat, dietary fibre and other nutrients. The purpose of this study was investigating physico-chemical & phytochemical analysis of Linseed *(Linum usitatissimum L)* oil & Analytical study by HPLC. The seed of Linseed (*Linum usitatissimum L.)* from Maharashtra was analysed for its proximate composition and physico-chemical characterization of the oil. Physico-chemical parameters such as Moisture content, Percentage ash, Colour, taste, Odour, Refractive index, Specific gravity, Acid value, Iodine value, Saponification number, Unsaponifible mater content and Peroxide number were determined. Preliminary phytochemical analysis of Linseed *(Linum usitatissimum L.)* oil showed presence of flavonoids, steroids and terpenoids which may be active compounds. The result justifies the use of the plant in folk medicines. The high performance liquid chromatography (HPLC) is a suitable analytical method for seed oil of Linseed. It was detected by HPLC chromatogram.

Keywords : Linseed *(Linum usitatissimum L)* , Proximate composition, Physico-chemical Characterisation and Instrumental analytical techniques, HPLC.

Introduction

Herbal medicine has produced number of distinguished researchers and due to its accessibility to traditions it is stillpracticed even by lay practitioners. Ayurveda, the ancient healing system of India, flourished in the Vedic era in India. According to historical facts, the classical texts of Ayurveda, Charaka Samhita and Sushruta Samhita were written around 1000 B.C. The Ayurvedic Materia Medica includes 600 medicinal plants along with therapeutics. The formulations incorporate single herb or more than two herbs (polyherbal formulations). Linseed *(Linum usitatissimum L)* is a cool temperature annual herb with erect stems, is an angiosperm belonging to the Linaceae family and Linum genus. ,60-80 cm tall. It has little branching except at the apex. Leaves are alternate, lanceolated and greyish-green with 3 veins. Flowers are 5 petalled in a cluster, bright blue or white in some forms. The sepals are lanceolated and nearly as long as the pointed fruit. The fruit are spherical capsules , the seed capsule is round, 5-9 mm diameter and contains several seeds. The seeds are oval, somewhat flattened, 4-6mm long and are pale to dark brown and shiny have a crisp and chewy texture . Flax seeds range in colour from a deep brown to a light yellow .Seed colour is determined by the amount of pigment in the outer seed coat – the more pigment, the darker the seed. Dried seed contains from 33 to 45 percent oil. flax seed contains both the soluble and insoluble types of fiber. In fact, flax seed often acts as a laxative because of its fiber content. Flax seed is made up of 22% high-quality protein and contains all 9 essential amino acids. Most of the oil is alpha linolenic acid (or ALA), a type of omega-3 that is a precursor to the fatty acids (EPA and DHA) . The essential fatty acids in flaxseed oil are vital components of the phospholipids that are a major part of the architectural structure of cellular membranes throughout the body. [1] The essential fatty acids composition of linseed oil is dominated by C18 fatty acids C18:2 (16 % of oil) C18:3 (50% of oil) essential for humans . Linseed oil is extracted from seed by n-hexane for industrial uses.[2] and is Insoluble in water and less dense than water. Hence floats on water. Contains principally glycerides of linolenic, linoleic, oleic and palmitic acid.[3] Llinseed oil is cures various ailments like arthritis, coronary heart diseases and stroke, hypertension and inflammatory and autoimmune disorders, asthma. The omega-3 fatty acids make linseed oil a great health booster and caretaker of heart . The essential fatty acids are precursors in the production of prostaglandins, which are hormone-like regulatory chemicals that control a tremendous amount of biochemical activity in all cells of the body .[4] The essential fatty acids are important regulators of cholesterol metabolism in the body .[5] The essential fatty acids are precursors in the production of prostaglandins, which are hormone-like regulatory chemicals that control a tremendous amount of biochemical activity in all cells of the body . The essential fatty acids are precursors in the production of prostaglandins, which are hormone-like regulatory chemicals that control a tremendous amount of biochemical activity in all cells of the body.Omega-3 fatty acids lower plasma triglycerides, reduce platelet aggregation, relax blood vessels, and lower blood pressure . High intake of flaxseed dietary fiber significantly reduced blood levels of triacylglycerols which could moderate several risk factors for cardiovascular disease .[6] Omega -3 resulted in a decrease

in asthma attacks and a reduction in the use of medications .[7] Low levels of omega-3 fatty acids are associated with increased incidence of breast cancer ,[8] prostate cancer ,9 and colon cancer, [10] Dietary alpha-linolenic acid is reported to lower cholesterol levels in the blood and in liver tissues. [11-13] Flaxseed oil supplementation in healthy humans reduces the production of inflammatory cytokines, which results in increased calcium absorption and increased bone density . [14] Flaxseed supplementation reduced the frequency and intensity of hot flashes in postmenopausal women .[15] Linseed oil lowers high blood pressure in Hypertension sufferers. It improve the mental function of many old age pensioners. It can help in the treatment of Multiple Sclerosis. It can relieve some cases of Premenstrual Syndrome (PMS) in females .[16] Presence of flavonoids in linseed oxidative cell damage suggesting antiseptic, anticancer, anti-inflammatory effect and mild hypersensitive properties.[17-20]

MATERIALS AND METHODS

Collection of seeds

The seeds of. Linseed *(Linum usitatissimum L.)* were purchased from village Pathardi . This place is situated in Ahmednagar, Maharashtra, India, its geographical coordinates are 19° 10' 0" North, 75° 11' 0" East and its original name (with diacritics) is Pāthardi. The average annual rainfall in the area is 218cm. and means monthly temperature is 25-30 ^{0} C .Seeds were obtained by breaking the fruit capsule. These Seed were cleaned and dried at room temperature. The dried seeds were then ground to a powder using high speed blender . The powder was thoroughly mixed and dried in an air circulating oven at 50 ^{0} C for 1 hr.stored at 5ºC, and used as the stock seed samples for further extraction of oil. All reagents used in quantitative and chemical investigation were of analytical grade.

Extraction of seed oil

Powder material of Linseed *(Linum usitatissimum L.)* was used for extraction . The oil was extracted with n-hexane (1:4 w/v) by continous extraction in a Soxhlet apparatus for 10 hours. The solvent was evaporated at 40 ^{0} C to dryness. The extracted oil was stored in sealed and dark bottles. Physico-chemical studies, Phytochemical studies and HPLC of extracted oil was done. The physical state, colour & odour were evaluated by means of sensory organs.

Physico-chemical parameters

Linseed *(Linum usitatissimum L.)* oil was a liquid at room temperature with yellow colour. Paint like odour and pleasant nutty taste. Oil density was determined picnometrically, whereas refractive index was determined at 40°C with Abbey Refractometer equipped with a thermostated circulator. Specific gravity was determined at 25 ^{0} C using a 25 ml capacity specific gravity bottles. The oil viscosity was determined by Ostwald method (Standard Base, 2010). The seed oil was assessed for various chemical properties. Standard methods described by Association of Official Analytical Chemists (AOAC, 1990) were used for the determination of moisture Physical and chemical analyses of the extracted oil were carried out by using AOAC methods (AOAC, 1990). Iodine value was determined using Wij's method as reported in AOAC methods (AOAC, 1990). The procedures of Egan et al. (1981) were adopted for the estimation of S aponification values, unsaponifiable matter content and acid value of the oil sample. Protein was determined using micro-Kjeldhal method as described by Allen and Quarmby (1989). A factor of 6.25 was adopted for protein content estimation.Carbohydrate content was determined by colorimetric method (Allen and Quarmby 1989).

Qualitative Analysis of Phytochemicals

The analysis of phytochemicals from Linseed *(Linum usitatissimum L.)* oil was individually carried out using various qualitative tests for Alkaloids (Harborne 1973), Carbohydrates and glycosides, Flavonoids ,Steroids , Terterpenoids , Saponin , Tannin , Protein and Volatile or essential oil

Preparation of Reagents

Preparation of Maeyer's Reagent: 0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

Preparation of Dragendorff's Reagent: Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in 1:1 ratio.

Test for Alkaloids : Alkaloid determination using Harborne (1973) method

The sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. Beaker was covered and allowed to stand for 4 h. Then it was filtered and the extract was concentrated on a water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to stand till its settlement. The precipitate was easily collected from the solution and was washed with dilute ammonium hydroxide and filtered. The residue was the alkaloid which was weighed after complete dryness and the percentage was calculated.

Test for Carbohydrates and Glycosides

Small quantity of extract was dissolved separately in 5 ml of distilled water and filtered. The filtrate may be subjected to Molisch's test to defect the absence of carbohydrates. Another small portion of extract was hydrolysed with dilute hydrochloric acid for few hours in water bath and was subjected to Liebermann-burchar's

test to defect absence of different glycosides. (pink to red colour indicate presence of glycosides).

Test for flavonoids : Flavonoids determination using Sofowara, 1993; Harborne, 1973.

0.5 g of the methanolic extract was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests:

• 3 ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube and the colour was observed. A dark yellow colour indicated the presence of flavonoids

•5 ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H_2SO4 . The appearance of the yellow colouration indicated the presence of flavonoids.

Test for steroids

0.5 g of the methanolic extract fraction was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

Test for terpenoids (Salkowski test)

5 ml of each plant extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H2SO4). A layer of the reddish brown colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

Test for Saponin : determination by (Obadoni and Ochuko, 2001)

20 g of each grounded sample was put into a conical flask and 100 Cm3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h. with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel, added 20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage.

Test for tannins

0.25 g of the methanolic plant extract was dissolved in 10 ml distilled water and filtered. 1% aqueous Ferric chloride (FeCl3) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples.

Test for protein : Mellon's reaction

Million's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating.

Test for volatile oil or essential oil

Place a thick section of drug on glass slide. Add a drop of sudan red 3rd reagent and after two minutes wash with 50 % alcohol mount in glycerin.

HPLC analysis of Extract

Extract prepared by Soxhlet Apparatus was subjected to HPLC for the separation and identification of constituents present in the oil. Different compounds are detected by HPLC.

RESULTS AND DISCUSSION

The physical constants evaluation is an important parameter in detecting adulteration or improper handling of drug. The Linseed *(Linum usitatissimum L)* oil was liquid at room temperature and has a clear and yellow colour. The odour perceived was paint like while the taste was pleasant nutty. The odour and taste is consequences of its composition, specific method of production and state of rancidity in the fat. The oil was observe to freeze within the range of 12-75 ^{0}C. It indicate high content of polysaturated fatty acids. The seed oil has refractive index 1.4525 and specific gravity 0.8325. The Linseed *(Linum usitatissimum L.)* oil has acid value of 1.05 (mg KOH / g of oil) . This value measures of the amount of free fatty acids present. Acid value is an indicator for edibility of oil and suitability for industrial use. The low free fatty acid value suggested that linseed oil is stable. The saponification number is 191.2 (mg KOH / g of oil) Iodine value 163.5 value (g / 100 g of oil) indicate a high level of unsaturated fatty acid is an assest in nutrition as high content of saturated fatty acids is implicated in cardiovascular diseases.The peroxide value was 0.98 number (Meg / Kg) . It indicate that the oil has lower degree of rancidity. The ash value is 3.6 % is important to determine purity of drug i.e. the presence or absence of foreign inorganic matter. Since the Linseed is useful in traditional medicine for the treatment of various ailments. The content of moisture, protein,ash,fibre and carbohydrates were 4.5 % ,19.8 %, 3.6 %, 5.2 %, 27.6 % respectively. The whole seed moisture contents were quite low. The linseed seed is good source of nutrition. The high oil and protein content makes the seed oil a potential source of commercial vegetable oil and protein.The result suggest that the oil is edible & can also be used in the manufacture of paint and varnishes. Phytochemical screening revealed that Linseed *(Linum usitatissimum L.)* oil contain flavonoids,steroids and terpenoids which could make the plant useful for the synthesis of various drugs of human use. It lowers high blood pressure.

CONCLUSION

Experimentally is found that Linseed *(Linum usitatissimum L.)* oil is used a medicinal important . Present study deals with Physico-chemical, Phytochemical investigation of dried seed .The physico-chemical ivnvestigation of the certain medicinal plant will be helpful for evaluation of nutritive value and preparation of modern drugs and medicines. Phytochemical screening helps to reveal the chemical nature of the constituents of Linseed *(Linum usitatissimum L.)* extract. Phytochemical analysis of extract showed that it contain flavonoids,sterols and triterpenes. Flavonoids were found in the extract and are potent water soluble antioxidants.The major fatty acids shows 19.00 % Linolenic acid, 6.8 % Linoleic acid, 5.6 % Palmitic acid 3.2 % Oleic acid, 2.2 % Stearic acid respectively. HPLC study revealed presence of number of constituents were detected and further investigations are in progress in the laboratory.

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Figures : HPLC

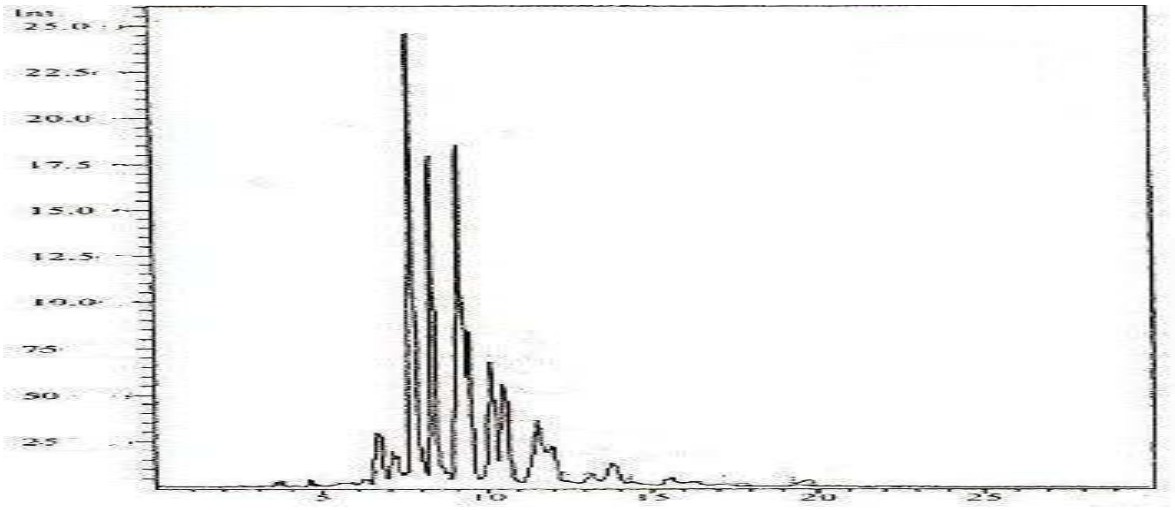

Fig 1 . HPLC of Linseed (Linum usitatissimum) oil

Table 1 . Proximate Analysis of Linseed (Linum usitatissimum) oil

Evaluation parameters	Values (%) Seed oil
Moisture content	4.5 %
Oil	32 %
Protein	19.8 %
Total ash value	3.6 %
Fibres	5.2 %
Carbohydrates	27.6 %

Table 2 . Physical properties of Linseed (Linum usitatissimum) oil

Evaluation parameters	Values (%) Seed oil
Physical state	Liquid
Colour	Yellow
Taste	Pleasant nutty
Odour	Paint like
Congealing point	12-75 ^{0} C
Specific gravity (25 ^{0} C)	0 .8325
Refractive index (40 ^{0} C)	1.4525

Table 3 . Chemical properties of Linseed (Linum usitatissimum) oil

Parameters	Value
Acid value (mg KOH / g of oil)	1.05
Iodine value (g / 100 g of oil)	163.5
Saponification number (mg KOH / g of oil)	191.2
Unsaponifible matter (% of oil)	0.26
Peroxide number (Meg / Kg)	0.98

Table 4. Phytochemical Investigation of extract of Linseed (Linum usitatissimum)

Test for active constituents	Results
Alkaloids	-
Carbohydrates	+
Glycosides	-
Flavonoids	+
Steroids	+
Terpenoids	+
Saponin	-
Tannin	-
Protein	+
Volatile oil / Essential oil	+

(+) Positive Result (-) Negative Result