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RADICAL SCAVENGING POTENTIAL OF LINUM USITATISSIMUM

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ABSTRACT
Linum usitatissimum, commonly known as Flax seed. Flaxseed is appeared as an important functional food ingredient because of its rich contents of α- linolenic acid (ALA, omega-3 fatty acid), lignans, and fiber. Flaxseed oil, fibers and flax lignans have potential health benefits such as in reduction of cardiovascular disease, atherosclerosis, diabetes, cancer, arthritis, osteoporosis, autoimmune and neurological disorders. Flax protein helps in the prevention and treatment of heart disease and in supporting the immune system. The antioxidant activity of flax seed oil was measured using free radical scavenging activity with 2-2-diphenyl, 1-picrylhydrazyl (DPPH) and reducing power assay. Due to rich phyto-constituents in flax seed oil the attempt has been made to investigate the radical scavenging potential.

KEYWORDS: Linum usitatissimum, DPPH & reducing power.

INTRODUCTION

Flaxseed is one of the oldest crops, having been cultivated since the beginning of civilization. The Latin name of the flaxseed is Linum usitatissimum, which means "very useful". Flax was first introduced in United States by colonists, primarily to produce fiber for clothing[1]. Every part of the flaxseed plant is utilized commercially, either directly or after processing. The stem yields good quality fibers having high strength and durability[2]. Flax has been used until 1990s principally for the fabrication of cloths (linen) and papers, while flaxseed oil and its sub-products are used in animal feed formulation. There is a small difference in using the terms flaxseed and linseed. Flaxseed is used to describe flax when consumed as food by humans while linseed is used to describe flax when it is used in the industry and feed purpose. In the last two decades, flaxseed has been the focus of increased interest in the field of diet and disease research due to the potential health benefits associated with some of its biologically active components. Flaxseeds have nutritional characteristics and are rich source of ω-3 fatty acid: α-linolenic acid (ALA), short chain polyunsaturated fatty acids (PUFA), soluble and insoluble fibers, phytoestrogenic lignans (secoisolariciresinol diglycoside-SDG), proteins and an array of antioxidants.[3] Its growing popularity is due to health imparting benefits in reducing cardiovascular diseases, decreased risk of cancer, particularly of the mammary and prostate gland, anti-inflammatory activity, laxative effect, and alleviation of menopausal symptoms and osteoporosis.

Fig 1: Flax Seeds and oil.

Health benefits of flax oils are as following.[4]

Various abiotic stresses lead to the overproduction of reactive oxygen species (ROS) in plants and animals which are highly reactive and toxic causing damage to proteins, lipids, carbohydrates and DNA thus leads to oxidative stress. This oxidative stress causes damage to

tissues and results in large number of diseases. Antioxidants neutralize the effects of ROS and thus help in preventing diseases. Antioxidants can be natural or synthetic. Natural antioxidants can be taken up through diet as they are present in fruits, vegetables and spices.[5]

Free radicals are atoms or groups of atoms that have at least one unpaired electron, which make them highly unstable and reactive. Living organisms accumulate free radicals through both normal metabolic processes and exogenous sources. Although radicals have beneficial effect during energy production and as antibacterial, excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death.[6] Antioxidant compounds in food play an important role as a health protecting factor. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro- peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.[7] A imbalance between free radical generating and free radical scavenging systems results in oxidative stress, a condition that has been associated with the cell injury seen in many pathologic conditions. Free radical mediated damage contributes to such varied processes as chemical and radiation injury, ischemia reperfusion injury, cellular ageing and microbial killing by phagocytes. Natural products are becoming the cynosure to inhibit and scavenge these reactive oxygen species.[8]

In the present works an attempt had been done to evaluated antioxidant activity of flax seed oil. The antioxidant was measured using free radical scavenging activity with 2-2-diphenyl, 1-picrylhydrazyl (DPPH) and reducing power assay.

MATERIAL AND METHOD

Plant material collection

The seed was collected from local market Bhopal (M.P.) and authenticated.

Preparation of oil- Oil was prepared by the seed through the oil machine.

Identification of oil

(a)Oil is completely miscible with half volume of light petroleum ether.
(b) Mix oil and alcohol in equal volume. Liquid is obtained which is cooled at 0^0C.Stand the above mixture for 3 hrs. Clear liquid was observed.

Physico-chemical Characterization

The saponification value, peroxide, iodine, and acidity values of the flaxseed oils were determined according to the AOCS official methods (Cd 8-53, Cd 1-25, and Cd 3d-63, respectively).[9] The refractive index of flaxseed oil was determined using an Abbe refractometer. The density of the oils was measured using a densimeter.

Antioxidant Activity

DPPH Radical Scavenging Activity

Preparation of standard solution: Required quantity of Ascorbic acid was dissolved in methanol to give the concentration of 5, 10, 20, 30, 40 and 50µg/ml.
Preparation of test sample: Stock solutions of samples were prepared by dissolving 10 ml of flax seed oil in 10 ml of methanol to give concentration of 1mg/ml.

Preparation of DPPH solution: 4.3mg of DPPH was dissolved in 3.3 ml methanol: it was protected from light by covering the test tubes with aluminum foil.

Protocol for estimation of DPPH scavenging activity

DPPH solution was added to 3 ml methanol and absorbance was taken immediately at 516 nm for control reading. Different concentration of test sample (5,10,20,30,40 & 50) were prepared. Diluted with methanol with up to 3 ml.150 µl DPPH solution was added to each test tube. Absorbance was taken at 516 nm in UV-visible spectrophotometer (Shimadzu, UV-1700Japan) after 15 min using methanol as a blank.[10]

The % reduction and IC50 were calculated as follows
The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

$$\% \text{ antiradical activity} = \frac{\text{Control absorbance} - \text{sample absorbance} \times 100}{\text{Control absorbance}}$$

FRAP method

The ferric reducing antioxidant power (FRAP) was determined using a modified version of the FRAP assay (Szydlowska-Czerniak etal 2008). A freshly prepared FRAP reagent (2.5 mL of 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.1 M acetate buffer, pH 3.6) was incubated at 37°C for 10 min. Next, 0.5 mL of the seed oil and 2 mL of the FRAP reagent were transferred into a 10 mL volumetric flask containing redistilled methanol. The obtained blue solution was maintained at room temperature for 20 min. The absorbance was measured against a reagent blank at 593 nm.[11]

Table 1: Physio-chemical characteristics of oil.

S. No.	Parameters	Results
1.	Acid value(mg/KOH/g)	1.18
2.	Saponification value	188
3.	Iodine value(g of I_2/100g of oil)	178.9
4.	Peroxide value(mcq/kg)	1.98
5.	Refractive index(at 20^0C)	1.481
6.	Density(g/cm^3)	0.9180

Table 2: 50% inhibition (IC_{50}) of seed oil by DPPH method.

S. No.	Sample	IC_{50} (µg/ml)
1.	Ascorbic acid	2.41
2.	Flax seed oil	7.46

Table 3: Total reducing power of Ascorbic acid & flax seed oils.

Concentration (µg/ml)	Absorbance	
	Ascorbic acid (standard)	Seed oil
20	0.130	0.205
40	0.220	0.218
50	0.301	0.514
80	0.744	0.602
100	0.850	0.708

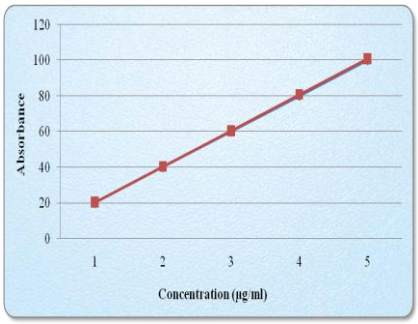

Fig. 3: Reducing Power of Ascorbic acid.

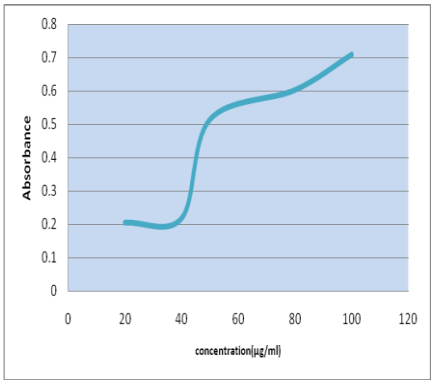

Fig. 4: Reducing Power of seed.

RESULT AND DISCUSSION

Flaxseed is mainly considered as oilseed crop. Moreover, the other nutritional parameters than its oil content, make it more favorable choice for food technologist to develop functional foods. Flaxseed contains good amount of α-Linolenic Acid (ALA), a omega-3 fatty acid, protein, dietary fiber, lignan, specifically Secoisolariciresinol diglucoside (SDG). Several studies reveal that these components work well for nutritional benefit in human being. ALA is beneficial for infant brain development, reducing blood lipids and cardiovascular diseases.[12] Intially physiochemical characteristics was carried out and results was tabulated in table 1.The Antioxidant activity seed oil was carried out by DPPH & reducing power assay by using ascorbic acid as standard. DPPH is a purple-coloured stable radical of organic nitrogen with a maximum absorbance at 517 nm and it is widely used to study radical scavenging activities of extracts and pure compounds. When the odd electron becomes paired off in the presence of a free radical scavenger to form hydrazine, the absorption reduces and the DPPH solution is decolourised from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. IC_{50} (concentration required to obtain a 50% antioxidant capacity or is the concentration of substrate that brings about 50% loss of the DPPH) is typically employed to express the antioxidant activity and to compare the antioxidant capacity of various samples.[13] In the present study, the IC_{50} value of flax seed oil was found to be 7.46µg/ml as compared with standard ascorbic acid having IC_{50} 2.41 µg/ml. The results were tabulated in table 2. The result of Ferric reducing power assay was showed in fig.3-4.The absorbance of the sample and standard were tabulated in table 3.

CONCLUSION

Initially, the physiochemical analysis of flax seed oil was carried out to check their purity. The antioxidant activity of seed oil was assessed by DPPH protocol and ferric reducing antioxidant power assay. A result of these methods confirms the antioxidant efficacy of seed oil.

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