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PHYTOCHEMICAL CHARACTERIZATION AND IN-VITRO ANTIFUNGAL ACTIVITY OF FLAXSEED (*LINUM USITATISSIMUM*): A COMPREHENSIVE REVIEW FOR PHARMACEUTICAL APPLICATIONS

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ABSTRACT

Flaxseed (*Linum usitatissimum*), traditionally known as linseed, is a nutrient-dense functional food widely recognized for its diverse therapeutic properties. This review highlights the botanical profile, ethnomedicinal relevance, phytochemical composition, and bioactivity of flaxseed and flaxseed oil, with particular emphasis on their antifungal potential. Flaxseed contains a rich array of bioactive compounds, including lignans, omega-3 fatty acids, fixed oils, alkaloids, tannins, proteins, carbohydrates, and phenolic constituents, which collectively contribute to its antioxidant, anti-inflammatory, antidiabetic, antihyperlipidemic, and anticancer effects. The study further evaluated the pharmacognostic characteristics and performed preliminary phytochemical screening, confirming the presence of multiple secondary metabolites. Antifungal activity was assessed using Sabouraud Dextrose Agar against *Aspergillus* species, where both flaxseed extract (F1) and flaxseed oil (F2) demonstrated measurable zones of inhibition, with the aqueous seed extract exhibiting superior activity (4.3 cm) compared to the oil (1.8 cm). These findings affirm flaxseed's potential as a natural antifungal agent in addition to its established health benefits. Overall, flaxseed represents a valuable source of therapeutic compounds and may serve as a promising candidate for the development of functional foods and plant-based antifungal formulations.

KEY WORDS: Antifungal activity, *Aspergillus* species, Dextrose Agar, *Linum usitatissimum*, Sabouraud.

INTRODUCTION

Flax-seed (Linum usitatissimmum) is also known as linseed, and these terms are used interchangeably. Flax-seeds are obtained from the blue flowering annual herb and belong to the Linaceae family. Flax plants grow from 12 to 40 inches high. The plant has a slender and fibrous stem, and its bright blue flowers are up to 3 cm in diameter. The flowers have five petals and form a five-celled ball that can contain up to 10 seeds. Flowering continues until plant growth stops. Almost all parts of the linseed plant are utilized for various purposes. Flax-seeds and flax-seed oil are considered to have potential health benefits due to the presence of different biologically active compounds and elements.

In this modern period, one of the major obstacles that current living faces are poor health, as individuals are suffering from a variety of degenerative lifestyle disorders. Foods that can act like medicine have sparked attention as people become aware of the importance of diet and their desire for wellness. Flax-seeds possess a wide range of nutritional properties that encourage health professionals and nutritionists to incorporate them into the development of functional meals and the selection of a healthy diet. Flax-seeds are obtainable in two forms: whole or ground. Both kinds have their own set of advantages.

Flaxseed has anti-oxidant properties due to the presence of SDG (Secoisolariciresinol Di glucoside) lignin. Moreover, eicosapentaenoic and docosahexaenoic acids inhibit neutrophilic inflammatory responses in humans, leading to anti-inflammatory responses. Flaxseed has many potential therapeutic benefits. The seeds of this plant have been used in the treatment of diseases, such as upper respiratory infections, constipation, abdominal pain, urinary tract infections, and skin infections.

Flaxseed has been used since ancient civilizations and cultures. According to archaeological evidence, flax seeds have been used from the time of Nefertiti in Egypt. Flax, also known in India as “Tisi” or “Alsi”, was utilized to supplement the food of the hardworking villagers. Flax seeds have been described as having a “nice nutty fragrance and aroma”, making it potentially suitable for use in a variety of meals.

Fig. 1: Brown flaxseeds

Fig. 2: Yellow flaxseeds.

Fig. 3: Flaxseed plant.

Plant Profile

Plant name	Linum Usitatissimum
Common name	Linseed, Linum
Synonyms	Linseed, Flax, and Alsi
Family	Linaceae
Biogeography	Linseed is found in Russia, Canada, the USA, Argentina. In Egypt, Algeria, Greece, Italy only flax fiber is cultivated. In India, it is cultivated for fibers as well as oil.
Vernacular names	In English - Linseed, Flax Hindi - Alsi Telugu - Aviselu Tamil - Aali Vidhai Urdu- Katan Marathi: Jawas, Atasi Oriya: Pesi Punjabi: Teesi

Taxonomical History

Kingdom	Plantae
Class	Magnoliopsida [Dicotyledons]
Order	Malpighiales
Family	Linaceae
Genus	Linum

Differences between flax-seed and flax-seed oil

S.no	Flax-seed	Flax-seed oil
1	Brown and yellow colour	Pale yellow colour clear liquid.
2	Unpleasant and sour odour	Characteristic odour
3	Slightly bitter in taste	Pleasant taste
4	Soluble in water	Soluble in chloroform, ether, and petroleum ether

Health benefits for Flax- Seeds

❖ **Flax seeds reduce LDL and Improves HDL levels**

Flax seeds reduce LDL cholesterol levels and Improves HDL cholesterol levels because of presence of omega-3 fatty acids (ALA), lignans and soluble fibres.

❖ **Flax seeds reduce blood sugars**

Flax seeds can help reduce blood sugar levels, especially in people with type 2 diabetes or insulin resistance and they cause sharp increases in blood sugar.

❖ **Flax seeds reduce insulin resistance and Improves insulin sensitivity**

Flax seeds Improves insulin sensitivity. Regular intake may help the body use insulin more effectively. They also Supports weight and blood sugar control, which indirectly improves insulin function.

❖ **Flax seeds reduce the chances of renal diseases**

Flax seeds may reduce the risk of renal disease by lowering inflammation, blood pressure, and blood sugar — key contributors to kidney damage, especially in high-risk individuals.

❖ **Flax seeds reduce the chances of breast, prostate and colon cancer**

Flax seeds reduce the risk of colon cancer. They also slow the growth of prostate cancer cells. Flaxseed may be associated with decreased risk of breast cancer and shows "antiproliferative" effects in breast tissue.

❖ **Flax seeds show anti-fungal activity**

Flaxseed and its derivatives (oil, protein extracts, and flour) have demonstrated antifungal activity in laboratory and food preservation studies, showing inhibition against certain fungal strains like Aspergillus, Penicillium, and Fusarium.

❖ **Flax seeds show antiproliferative properties**

Flaxseeds (and their derivatives) have shown antiproliferative activity in laboratory and animal studies, they can inhibit cell growth/proliferation under certain conditions.

❖ **Flax seeds exhibit anti-inflammatory effects**

Flaxseed exhibits significant anti-inflammatory effects, primarily due to its content of ALA, lignans, and fiber. These effects have been demonstrated in both laboratory and human studies, particularly in individuals with chronic inflammation or metabolic conditions. While not a substitute for medication, flaxseed can be a valuable part of an anti-inflammatory diet.

❖ **Flax seeds show anti atherogenic and anti-arrhythmic properties**

Flax seeds demonstrate promising anti-atherogenic and anti-arrhythmic properties in animal studies and some early human trials: they may reduce atherosclerotic plaque development, improve lipid/inflammatory profiles, and lower the incidence of arrhythmias in experimental settings.

❖ **Flax seeds reduce skin sensitivity and improve skin health**

Regular intake of flaxseed or flaxseed oil has been shown to improve skin hydration, reduce trans epidermal water loss, and strengthen the skin's natural barrier. Flaxseed oil supplementation may help decrease skin roughness, scaling, redness, and sensitivity—particularly in people with dry or sensitive skin types.

MATERIALS AND METHODS

Commercially available flax seeds and flax seed oil were purchased from a local distributor in Nellore, Andhra Pradesh, India. Peptone, glucose and agar were purchased from New Himalaya scientific co., Nellore, AP, India.

Pharmacognostic study

Physical characteristics study

It refers to use of five sense organs of humans namely, eyes, ears, nose, tongue, touch to evaluate the characteristic features of crude drugs, especially the crude drugs of plant origin. Flax seeds are observed, and physical characters are performed.

Phytochemical screening

Preliminary Plant chemical screening of various solvents was performed in order to determine the presence or absence of secondary metabolites such as alkaloids, steroids, tannin's, triglycerides, proteins, and amino acids. The following standard procedures were used:

S. NO	TEST	OBSERVATION	INFERENCE
1	**Test for alkaloids:** To 1 ml of the sample +1ml of Mayer's reagent.	Cream coloured precipitate was observed	Presence of alkaloids.
2	**Test for steroids:** To 0.5 g of the sample + 2ml of acetic anhydrous + 2ml of concentrated sulfuric acid were added.	Change colour from violet to blue or green.	Presence of steroids.
3	**Test for Terpenoid:** To 0.5 g of the sample +2ml of chloroform + 2ml of concentrated sulfuric acid were added.	A reddish-brown colour was observed.	Presence of terpenoids.
4	**Test for Flavonoid:** Few drops of ammonia solution were added to the sample and further, concentrated hydrochloride acid was layered.	Yellow colour was observed.	Presence of flavonoids.
5	**Test for Saponins:** About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath + 10ml of the filtrate was mixed with 5ml of the distilled water and shake vigorously for froth formation.	Foam was observed	Presence of saponins.
6	**Test for phenol compounds:** To 1ml of extract + few millilitres of gelatin solution.	White precipitate was observed.	Presence of Phenol compounds and tannin
7	**Test for tannin:** 1ml of extract + few drops of lead acetate solution	White precipitate was observed	Presence of tannin
8	**Test for Cardiac triglycerides:** 2ml of the sample + 2ml of glacial acetic acid + few drops of ferric chloride solution + 1ml of concentrated sulfuric acid were gently layered.	Brown ring or green ring was obtained.	Presence of Cardiac triglycerides.
9	**Test for proteins:** 1ml of extract + 1ml of concentrated nitric acid + boiled and cooled for few minutes + 20% of sodium hydroxide	Orange colour was observed	Presence of proteins
10	**Test for amino acids:** Few ml of the sample + three drops of ninhydrin solution + boil in water bath for 10 minutes.	Purplish blue colour was observed.	Presence of amino acids
11	**Test for carbohydrates:** 1ml of the extract + 2ml of Molish's reagent	Reddish brown ring was observed	Presence of carbohydrates
12	**Test for fixed oil:** **Spot test:** crush a small piece of the flax seed tissue + place on a clean piece of filter paper.	If there is the appearance of greasy	Presence of translucent spot of liquid

Anti-fungal activity of flax seeds and flax seed oil

Anti-fungal activity: Anti-fungal activity means to kill or inhibit the growth of fungi in a substance.

Sample preparation

- **Grinding-** Flax seeds were obtained from the local Market. The seeds were washed, cleaned of Extraneous matter and shade dried completely. The Seeds were dried at room temperature under shade for A period of one week. The dried seeds were grinded Using a laboratory grinder.
- **Extraction-** mix the powdered flax seed (10gm) with sterile distilled water
- **Soaking–** incubate the mixture for 24-48 hours at room temperature
- **Filtration –** filter the mixture using Whatman filter paper to remove seed particles and store the extract

Preparation of Sabouraud Dextrose Agar (SDA) Medium

- Weigh the required amount of dehydrated SDA powder and add it to the distilled water in a flask. Heat the mixture gently while continuously stirring. Bring it to a brief boil to ensure all the powder, especially the agar, is completely dissolved.
- The medium should look clear or slightly amber. Cap the flask loosely (to allow steam to escape) and sterilize it in an autoclave. Standard sterilization is 121º for 15 minutes (15 lbs pressure).
- Petri plates – pour about 20ml of sterile, molten agar medium into sterile petri plates and allow it to solidify under aseptic conditions
- Prepare the Fungus inoculum (Plates 1and 2):
  - Prepare a solution of Aspergillus spores (mix spores in sterile water/saline).
  - Dip a sterile cotton swab into the spore solution.
  - Evenly spread the fungus across the entire surface of the two SDA plates.
- Add flax seed extract to plate 1 and flax seed oil to plate 2
- Incubate. Place the plates in the incubator for 3 to 7 days at approximately 28ºC.
- Observe and compare clear ring (Zone of Inhibition) around the oil disc or the flax seed extract. Use a ruler to measure the diameter of any clear ring.

RESULTS AND DISCUSSION

Pharmacognostical evaluation

Organoleptic evaluation

SEED

Colour: Brown

Odour: Nutty scent

Texture: smooth and glossy or shiny

Taste: Nutty flavour

OIL

Colour: Clear, uniform and golden yellow

Odour: Crisp, mildly nutty

Taste: Mildly nutty

Texture: Liquid oil

Phytochemical screening

S. No	Chemical constituents	Name of the test	Observation	Results
1	Test for fixed oil	Hagar's test	Formation of yellow precipitate	Indicates presence of alkaloids.
2	Test for tannins	Gelatin test	Appearance of white precipitate	Indicates that tannins are present
3	Test for proteins	Biuret test	Violet or red colour formation	Indicates that proteins are present
4	Test for carbohydrates	Benedict's test	Appearance of a blue colour	Indicates the presences of starch
5	Test for starch	Iodine test	Appearance of a blue colour	Indicates the presence of starch
6	Test for fixed oils	Spot test	An oily, translucent spot is formed	Indicates presence of fixed oil

Fig. 4: Phytochemical screening.

Fig. 5: Spot test.

Assessment of Anti-fungal activity

A clear ring with zone of inhibition is observed in plate 1 (F1- ground flax seed extract) and plate 2 (F2 - flax seed oil) and it indicates that flax seed and flax seed oil has anti-fungal activity against Aspergillosis.

For F1, the zone of inhibition is 4.3 cm

F2, the zone of inhibition is 1.8 cm

Fig. 6: Flax seed and Flax seed oil.

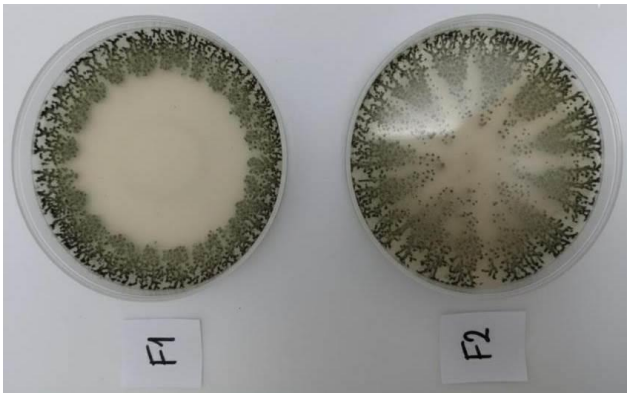

Fig. 7: Anti-fungal activity (Zone of inhibition).

CONCLUSION

The present study successfully evaluated for antifungal activity of flax seeds and flax seed oil using Sabouraud Dextrose Agar (SDA) Medium.

- Flax seeds possess high medicinal properties and are used in traditional medicine to treat variety of conditions. In conclusion, the study demonstrates the phytochemistry and anti-fungal properties of flax seeds and flax seed oil.
- The results indicated that there are various bio-active compounds present in flax seeds such as alkaloids, fixed oils, tannins, proteins and carbohydrates.

The anti-fungal activity of flax seed extract is more when compared to flax seed oil in the present study. Both flax seed oil and flax seeds should be considered as useful source of material for human health.

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