International Journal of Pharmacy and Natural Medicines
ISSN: 2321-6743 | https://doi.org/10.30904/j.ijpnm.2026.4941

Comparative *In-vitro* Antifungal Activity and Phytochemical Profiling of Flaxseeds (*Linum usitatissimum*) and Pumpkin Seeds (*Cucurbita pepo*)

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ABSTRACT

Fungal infections have become a growing public health concern due to increasing resistance to conventional antifungal drugs and their associated toxicity. This study aimed to investigate the phytochemical composition and comparatively evaluate the in vitro antifungal activity of flax seeds (Linum usitatissimum) and pumpkin seeds (Cucurbita spp.), two widely consumed functional foods with potential medicinal properties. Seeds and their oils were procured from a local distributor in Nellore, India, and subjected to pharmacognostical evaluation including organoleptic analysis and preliminary phytochemical screening using standard qualitative tests. Antifungal activity was assessed against Aspergillus species using Sabouraud Dextrose Agar (SDA) medium by measuring the zone of inhibition after incubation. Phytochemical screening confirmed the presence of several bioactive constituents such as alkaloids, flavonoids, tannins, carbohydrates, proteins, sterols, and fixed oils in both seed samples. The antifungal assay revealed measurable inhibition of fungal growth in all tested samples. Ground flax seed extract produced the highest zone of inhibition (4.3 cm), followed by pumpkin seed extract (3.7 cm), pumpkin seed oil (3.0 cm), and flax seed oil (1.8 cm). These findings indicate that both seeds possess notable antifungal potential, likely due to their rich phytochemical composition. In conclusion, flax seeds and pumpkin seeds demonstrate promising natural antifungal activity and may serve as potential sources for the development of plant-based antifungal agents, although further detailed studies are required to confirm their mechanisms and clinical applicability.

Keywords
Flax seeds,
Pumpkin seeds,
Phytochemical screening,
Antifungal activity,
Aspergillus

Received 19-01-2026
Revised 14-02-2026
Accepted 28-02-2026
Published 10-03-2026

Introduction

Medicinal plants have been used for centuries as an important source of therapeutic agents. Even in the modern era, plant‐derived compounds continue to play a crucial role in drug discovery and development. Natural products obtained from plants are known to contain a wide range of bioactive molecules that exhibit pharmacological activities such as antimicrobial, antioxidant, anti‐inflammatory, and anticancer properties [1]. Edible seeds are particularly valuable because they serve as concentrated reservoirs of nutrients and phytochemicals required for plant growth and protection. In recent years, functional foods such as flax seeds and pumpkin seeds have attracted significant attention due to their high nutritional value and potential health benefits [2].

Flax seeds (Linum usitatissimum) are among the oldest cultivated crops and are widely consumed for their nutritional and medicinal properties. They are rich in omega‐3 fatty acids, lignans, proteins, dietary fiber, vitamins, and minerals. These constituents contribute to various health benefits including cardiovascular protection, antioxidant activity, and antimicrobial effects [3]. Pumpkin seeds (Cucurbita spp.) are also widely recognized as nutrient‐dense foods containing unsaturated fatty acids, proteins, phenolic compounds, phytosterols, and essential minerals such as magnesium and zinc. These compounds contribute to antioxidant, anti‐inflammatory, and antimicrobial properties [4].

Fungal infections represent a major global health problem, particularly among immunocompromised individuals. Opportunistic fungi such as Aspergillus species can cause severe infections affecting the respiratory system and other organs. Conventional antifungal drugs are often associated with limitations including toxicity, high cost, and the emergence of drug‐resistant strains [5]. Because of these limitations, researchers are increasingly exploring plant‐derived natural products as potential alternatives to synthetic antifungal drugs. Previous studies have reported antifungal activity of flax seed extracts and pumpkin seed extracts individually against several fungal pathogens [6–8]. However, comparative studies evaluating the phytochemical composition and antifungal potential of these two seeds under similar laboratory conditions

are limited. Understanding their relative efficacy may help identify promising plant‑based antifungal agents for pharmaceutical, nutraceutical applications.

Figure 1: Flax seeds

Figure 2. Flaxseed plant

Figure 3. Pumpkin seeds

Materials and Methods

Commercially available flax seeds, flax seed oil, pumpkin seeds, and pumpkin seed oil were purchased from a local distributor in Nellore, Andhra Pradesh, India. Sabouraud Dextrose Agar (SDA), peptone, glucose, and agar were obtained from a scientific supplier. Organoleptic evaluation was performed to assess color, odor, taste, and texture of the seeds and oils. These characteristics help identify crude drugs and provide preliminary information regarding their quality. Preliminary phytochemical screening was conducted using standard qualitative tests to detect alkaloids, flavonoids, tannins, proteins, carbohydrates, sterols, and fixed oils. For extract preparation, seeds were washed, shade dried for one week, and ground into powder. Ten grams of powdered sample was soaked in sterile distilled water for 24–48 hours and filtered using Whatman filter paper.

Sabouraud Dextrose Agar medium was prepared by dissolving SDA powder in distilled water and sterilizing it in an autoclave at 121°C for 15 minutes. Sterile molten agar was poured into petri plates under aseptic conditions. Antifungal activity was evaluated using the agar diffusion method. Aspergillus spores were spread over the surface of SDA plates and extracts were applied to the plates. After incubation at 28°C for 3–7 days, the zone of inhibition was measured.

Anti-fungal activity of flax seeds and flax seed oil

Sample preparation

Grinding-Flax seeds and pumpkin seeds were obtained from the local Market. The seeds were separately 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) and pumpkin seeds (10gm) with sterile distilled water separately
- Soaking– incubate the mixtures for 24-48hr at room temperature
- Filtration – filter the mixtures 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 121^{0} 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 1 ,2, 3 and 4):

- 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
- Add pumpkin seed extract to plate 3 and pumpkin seed oil to plate 4
- Incubate. Place the plates in the incubator for 3 to 7 days at approximately 28^{0}C.
- Observe and compare clear ring[zone of inhibition] around the disc for flaxseed extract & oil and pumpkin seed extract & oil. Use a ruler to measure the diameter of any clear ring.

Results and Discussion

Table 1: Organoleptic characteristics of seeds

Property	Flax Seeds	Pumpkin Seeds
Color	Brown	Brown‑Green
Odor	Nutty	Characteristic
Texture	Smooth	Firm
Taste	Nutty	Mild

Table 2: Antifungal activity results

Sample	Zone of Inhibition
Flax seed extract	4.3 cm
Flax seed oil	1.8 cm
Pumpkin seed extract	3.7 cm
Pumpkin seed oil	3.0 cm

Figure 4: Flaxseed powder and oil

Figure 5: Pumpkin seed powder and oil

Figure 6: Phytochemical screening of flax seeds

Figure 7: Phytochemical screening of pumpkin seeds

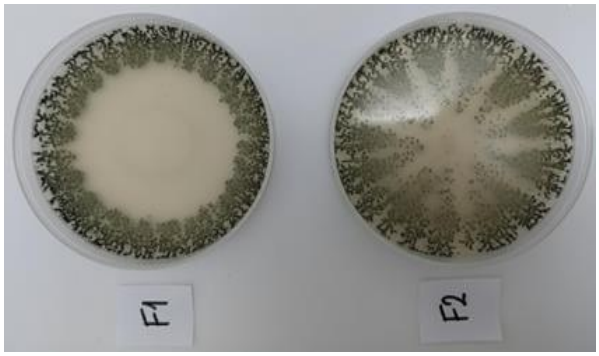

Figure 8: Anti-fungal activity [Zone of inhibition]

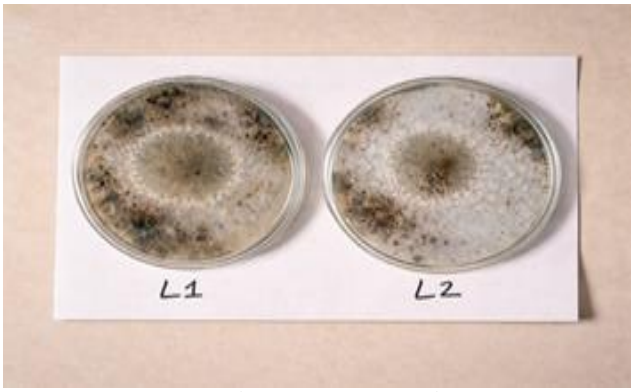

Figure 9: Anti-fungal activity [Zone of inhibition]

Discussion

The present study demonstrates that both flax seeds and pumpkin seeds contain several phytochemicals responsible for antifungal activity. The presence of phenolic compounds and flavonoids may contribute to disruption of fungal cell membranes and inhibition of fungal enzymes. Flax seeds exhibited stronger antifungal activity compared to pumpkin seeds. This may be attributed to the higher concentration of lignans and phenolic compounds present in flax seeds. Pumpkin seeds also showed notable antifungal activity due to the presence of phytosterols and tocopherols which have been reported to possess antimicrobial properties. These findings are consistent with previous research demonstrating antimicrobial activity of flax seed and pumpkin seed extracts. However, the study has certain limitations including evaluation of only one fungal species. Future research should include additional fungal strains, different extraction methods, and quantitative analysis of phytochemicals to better understand the antifungal mechanisms.

Conclusion

The present study confirms that flax seeds and pumpkin seeds possess significant phytochemical constituents and exhibit measurable antifungal activity against Aspergillus species. Among the tested samples, flax seed extract showed the highest antifungal activity followed by pumpkin seed extract. These results suggest that edible seeds may serve as promising natural sources of antifungal compounds. Further studies are required to explore their therapeutic potential and mechanisms of action.

Acknowledgment

The authors thank the, Swathi College of Pharmacy, Nellore, A.P, India, for technical assistance and support.

Conflict of Interests

The authors declare no conflict of interest

Ethics Approval: Not applicable

Funding: This study received no specific funding from public, commercial, or not for profit funding agencies.

AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

Data Availability

Data will be available on request

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