

International Journal of Pharmacy and Pharmaceutical Science

ISSN Print: 2664-7222
ISSN Online: 2664-7230
IJPPS 2026; 8(1): 01-06
www.pharmacyjournal.org
Received: 01-10-2025
Accepted: 06-11-2025

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Optimizing extraction and enhancing stability of flaxseed oil: Toward functional herbal haircare formulations with antimicrobial properties

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DOI: <https://www.doi.org/10.33545/26647222.2026.v8.i1a.284>

Abstract

Flaxseed oil is a rich source of omega-3 fatty acids, lignans, and bioactive compounds with potential applications in hair care. This study evaluated flaxseed oil extracted via Soxhlet and cold-press methods, its stabilization by blending with olive oil, and formulation into herbal hair products including hair oil, serum, and mask. Cold-pressed oil exhibited superior oxidative stability and antioxidant activity compared to Soxhlet-extracted oil. Blending with olive oil further enhanced stability and bioactive retention. Phytochemical screening revealed the presence of alkaloids, carbohydrates, proteins, tannins, steroids, and glycosides. Antioxidant activity of the blended oil was highest (29.23% inhibition). Herbal hair formulations displayed desirable sensory properties and pH compatible with scalp physiology. Antioxidant evaluation showed highest radical scavenging activity in hair serum (52.86%). All formulations exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and antifungal activity against *Malassezia* spp., with the hair mask and hair oil showing strongest effects, respectively. These findings demonstrate that flaxseed-olive oil-based herbal formulations are multifunctional, safe, and effective for scalp protection and hair health.

Keywords: Flaxseed oil, cold-pressed, herbal hair products, antioxidant, antimicrobial

Introduction

Natural oils have gained increasing importance in hair care due to their bioactive properties, safety, and sustainability [8]. Flaxseed (*Linum usitatissimum* L.) oil is rich in alpha-linolenic acid, lignans, and antioxidants, making it a potential ingredient for hair protection, scalp health, and hair growth promotion [1, 15, 19]. Olive oil is commonly used as a stabilizer and emollient, providing polyphenols and vitamin E for enhanced oxidative protection [20, 28, 38]. Previous studies demonstrated that cold-press extraction preserves sensitive bioactives better than solvent-based methods, which may degrade nutrients due to heat [16, 26, 36]. The present study aimed to extract flaxseed oil via Soxhlet and cold-press methods, stabilize it through blending with olive oil, and formulate herbal hair products, while evaluating their antioxidant, antibacterial, and antifungal properties.

Materials and Methods

Materials

Flaxseed (*Linum usitatissimum*) seeds were procured from a local market. Olive oil (extra virgin) was used for blending. Medicinal plant extracts with known antioxidant and antimicrobial properties were incorporated into formulations [4-6, 11]. All chemicals and reagents were analytical grade.

Methods

Extraction of Flaxseed Oil

1. Soxhlet Extraction: Ground seeds were extracted with petroleum ether for 6 hours [31, 36].
2. Cold-Press Extraction: Seeds were mechanically pressed without heat or solvent [16, 26].

Oil Blending

Cold-pressed flaxseed oil was blended with olive oil in a 1:2 ratio to enhance oxidative stability and bioactive retention [20, 28].

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Phytochemical Screening

Qualitative phytochemical analysis of Soxhlet-extracted, cold-pressed, and flaxseed-olive oil blended samples was performed using standard chemical tests to identify major bioactive constituents [7]. Alkaloids, carbohydrates, amino acids, proteins, tannins, steroids, glycosides, saponins, and flavonoids were detected using established assays including Hager's, Benedict's, Ninhydrin, Biuret, gelatin, Liebermann-Burchard, Keller-Killiani, foam, and ferric chloride tests. The identified phytochemicals contribute to antimicrobial and antifungal activity (alkaloids, tannins), antioxidant and anti-inflammatory effects (flavonoids), mild cleansing action (saponins), moisturizing and conditioning properties (carbohydrates), follicular stimulation (glycosides), keratin synthesis and hair strength (amino acids), and scalp barrier protection (steroids), thereby enhancing the overall efficacy of the herbal hair formulations [4, 5, 8, 11, 13, 25, 29, 33, 38].

Analytical Characterization

Acid value, peroxide value, *p*-anisidine value, iodine value, and saponification value were determined using standard protocols [3, 14, 17].

Formulation of Herbal Hair Products

Hair oil, serum, and mask were formulated using blended oil and medicinal plant extracts. Physical properties such as color, appearance, texture, odor, and pH were evaluated [11, 33, 37].

Antioxidant Activity

DPPH radical scavenging assay assessed antioxidant potential of oils and formulations [2].

Antimicrobial Activity

- Antibacterial Activity:** Disc diffusion method against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [2, 11].
- Antifungal Activity:** Broth microdilution against *Malassezia* spp., measuring optical density at 600 nm [9, 10, 30].

Results and Discussion

Oil Extraction, Selection, and Phytochemical Analysis

Flaxseed oil obtained by Soxhlet extraction appeared golden yellow and yielded the highest oil recovery (25.7%). However, this oil exhibited comparatively lower oxidative stability, which was attributed to prolonged exposure to heat and organic solvent during extraction. Cold-pressed flaxseed oil appeared light yellow in color and yielded 18.5% oil, while demonstrating superior antioxidant potential, indicating better preservation of bioactive compounds. The flaxseed-olive oil blend produced a light-golden, clear oil and exhibited the highest antioxidant activity among all samples, confirming a synergistic stabilizing effect of olive oil on flaxseed oil (Fig. 1).

Qualitative phytochemical screening revealed the presence of alkaloids, carbohydrates, proteins, tannins, steroids, and glycosides in cold-pressed and blended oils. Amino acids were detected only in Soxhlet-extracted oil, whereas saponins and flavonoids were absent in all samples (Table 1).

Analytical quality assessment further supported these observations. The blended oil showed the lowest acid value

and peroxide value, indicating enhanced oxidative stability, while cold-pressed oil exhibited the highest iodine value, reflecting a greater degree of unsaturation and nutritional quality. Soxhlet oil showed comparatively higher degradation indices, confirming the negative impact of thermal extraction. The detailed analytical characteristics were summarized in Table 2.

Based on antioxidant activity, phytochemical composition, and superior oxidative stability, the flaxseed-olive oil blend was identified as the most suitable oil base for the development of herbal hair care formulations. This selection ensured improved product stability, enhanced bioactivity, and better compatibility for cosmetic application.

Physical Evaluation of Herbal Hair Products

Herbal hair formulations prepared using the selected flaxseed-olive oil base exhibited acceptable and uniform physical characteristics. The hair oil appeared golden yellow with a clear, oily appearance, while the hair serum exhibited a reddish-orange color and translucent liquid nature. The hair mask formed a light brownish to peach-colored opaque gel. All formulations possessed a smooth texture and pleasant odor, indicating good aesthetic acceptability.

The pH values of the formulations ranged from 4.52 to 5.5, which falls within the normal physiological range of the scalp. Hair oil showed a pH of 5.5, hair serum ranged between pH 4.52-5.0, and hair mask exhibited a pH of 5.3, suggesting that all products were non-irritating and suitable for regular scalp application.

Qualitative phytochemical screening of the herbal hair formulations revealed the presence of carbohydrates and steroids in all three products (hair oil, serum, and mask), indicating their role in moisturizing, conditioning, and scalp barrier support. Alkaloids and flavonoids were detected only in the hair serum and hair mask, suggesting enhanced antimicrobial and antioxidant potential in these formulations. Amino acids were present in the hair serum and hair mask, contributing to hair strength and keratin support. Tannins and glycosides were observed variably among the formulations, with the hair oil and hair mask showing tannins, while glycosides were present in the hair oil and serum. Saponins were absent in all formulations, indicating that the products provide bioactivity without surfactant-related irritation.

Antioxidant Activity of Herbal Hair Products

The antioxidant potential of the herbal hair formulations was evaluated using the DPPH radical scavenging assay. Among the formulations, hair serum exhibited the highest radical scavenging activity, followed by hair oil and hair mask. These results demonstrated that incorporation of the flaxseed-olive oil blend significantly enhanced the antioxidant capacity of the herbal hair formulations. Comparative antioxidant activity was represented graphically in Fig. 2.

Antimicrobial Activity of Herbal Hair Products

Antibacterial Activity

The antibacterial efficacy of the herbal hair formulations was evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The hair mask exhibited the strongest antibacterial activity against *S. aureus*, showing the largest zone of inhibition. Hair serum demonstrated moderate antibacterial activity against both test organisms,

while hair oil showed comparatively lower inhibition. The positive control exhibited significant antibacterial activity, whereas no inhibition was observed with the negative control. The antibacterial results were graphically in Figure 3.

Antifungal Activity

Antifungal activity against *Malassezia* spp. showed a concentration-dependent decrease in optical density for all formulations. Hair oil exhibited the strongest antifungal effect at 100% concentration, followed by hair serum and

hair mask. The dose-response antifungal activity of the formulations was represented graphically in Fig. 4, demonstrating effective fungal growth inhibition with increasing concentration.

Overall Interpretation

Overall, the results demonstrated that selecting a stabilized flaxseed-olive oil blend as the formulation base was critical in achieving herbal hair products with desirable physical characteristics and significant antioxidant, antibacterial, and antifungal activity.

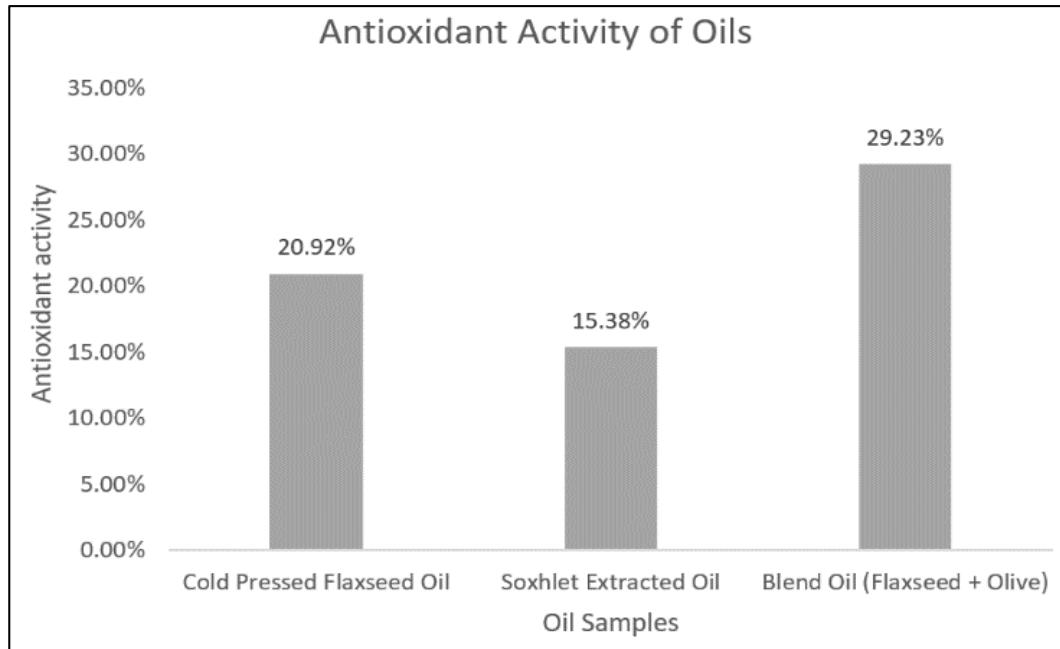


Fig 1: Antioxidant activity of Soxhlet, cold-pressed, and blended flaxseed oils.

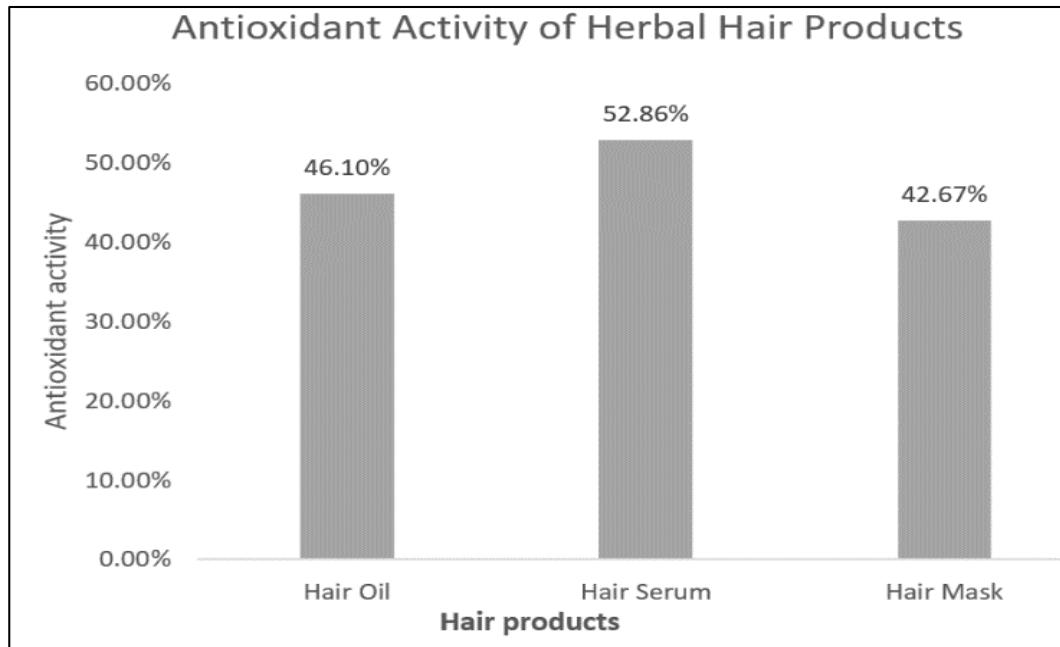
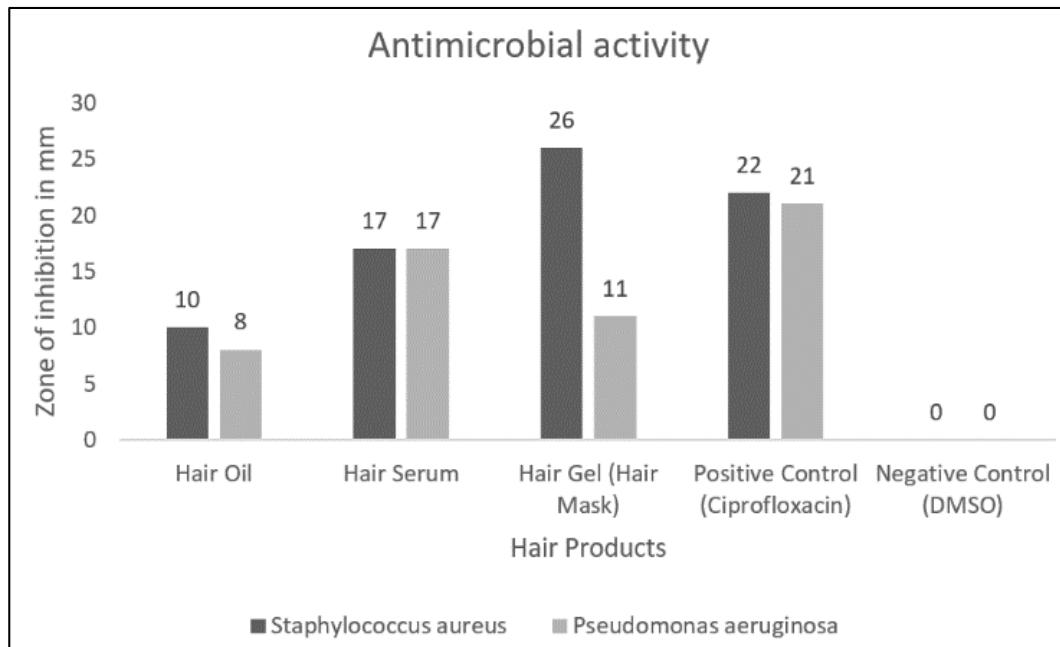
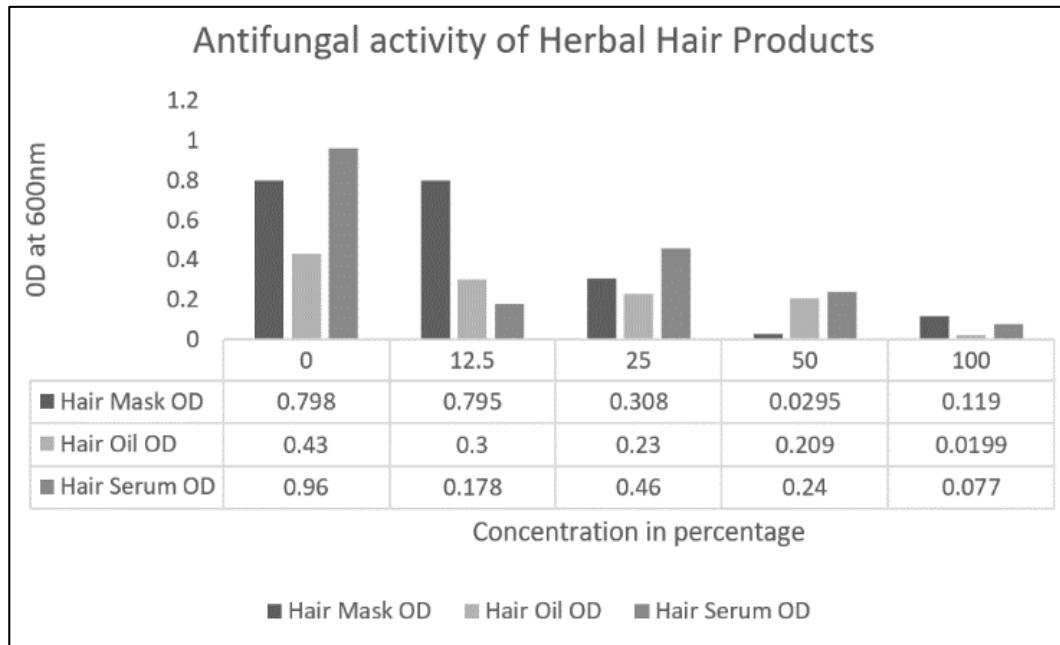


Fig 2: Antioxidant activity of Herbal Hair Products

**Fig 3:** Antimicrobial activity of Herbal Hair Products**Fig 4:** Antifungal activity of Herbal Hair Products**Table 1:** Phytochemical Screening of Flaxseed Oils

Phytochemical	Cold-Pressed Oil	Soxhlet Oil	Blend Oil
Alkaloids	+	+	+
Carbohydrates	+	+	+
Amino Acids	-	+	-
Proteins	+	+	+
Tannins	+	+	+
Steroids	+	-	+
Glycosides	+	+	+
Saponins	-	-	-
Flavonoids	-	-	-

Table 2: Analytical Characteristics of Oils

Parameter	Soxhlet Oil	Cold Press Oil	Blend Oil
Acid Value (mg KOH/g)	1.726	1.683	0.224
Peroxide Value (meq O ₂ /kg)	4.2	3.6	3.0
p-Anisidine Value	0	0.164	0.056
Iodine Value (g I ₂ /100g)	139.59	187.18	131.98
Saponification Value (mg KOH/g)	172.4	196.35	201.46

Conclusion

Cold-pressed flaxseed oil stabilized with olive oil provides a bioactive-rich, oxidative-stable base suitable for herbal hair formulations. Hair products demonstrated significant antioxidant, antibacterial, and antifungal activity, desirable sensory properties, and pH compatibility with the scalp. These findings support the potential use of flaxseed-olive oil-based formulations as multifunctional hair care products.

Acknowledgments

The authors acknowledge the support of Rapture Biotech Laboratory facilities and thank mentor for experimental assistance. No conflicts of interest were declared.

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