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"Evaluation of Antifungal Activity of Pomegranate (Punica Granatum L.) Peel Extract and Formulation of Herbal Shampoo"

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KEYWORDS

Punica granatum peel extract; Herbal shampoo; Antifungal activity; Phytochemicals; Chemical health risk; Scalp infection

ABSTRACT

Introduction: Fungal infections of the scalp and skin are increasing worldwide, and resistance to synthetic antifungal agents has become a major chemical health concern. Natural plant-based alternatives are being explored for safer therapeutic options.

Objectives: The present study aimed to evaluate the antifungal activity of *Punica granatum* peel extract and to formulate and evaluate a herbal shampoo incorporating the extract.

Methods: Pomegranate peels were shade dried, powdered, and extracted using 70% hydroalcoholic solvent by maceration. Preliminary phytochemical screening was performed. Antifungal activity was evaluated against *Candida albicans*, *Aspergillus niger*, and *Fusarium solani* using the agar well diffusion method with Amphotericin B as standard. A herbal shampoo was formulated and evaluated for physicochemical parameters including pH, viscosity, foam stability, wetting time, surface tension, and stability.

Results: The hydroalcoholic extract showed the highest extractive value (12% w/w). Phytochemical screening confirmed the presence of tannins, flavonoids, phenolic compounds, saponins, glycosides, and terpenoids. The extract demonstrated concentration-dependent antifungal activity, with maximum inhibition observed against *Candida albicans* (17.3 ± 0.28 mm at 500 µg/mL). The formulated shampoo showed acceptable pH (6.1 ± 0.2), viscosity (3200 cP), foam stability, and good stability profile.

Conclusions: *Punica granatum* peel extract possesses significant antifungal activity and can be effectively incorporated into herbal shampoo formulations, offering a safe, eco-friendly alternative to synthetic antifungal products.

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1. Introduction

Fungal infections represent a significant and growing public health concern worldwide, affecting millions of individuals each year **[1]**. Superficial and cutaneous fungal infections caused by opportunistic and pathogenic fungi such as *Candida albicans*, *Aspergillus niger*, *Fusarium* species, and dermatophytes are particularly prevalent in tropical and subtropical regions. These infections are commonly associated with scalp disorders, dandruff, seborrheic dermatitis, and other skin-related conditions, which may negatively impact quality of life and, in severe cases, lead to secondary complications. The increasing incidence of fungal infections is further aggravated by factors such as immunosuppression, poor hygiene, environmental pollution, and prolonged use of broad-spectrum antibiotics **[1,2].**

Conventional antifungal agents, including azoles, polyenes, and allylamines, are widely used for the management of fungal infections. However, their long-term use has been associated with several limitations, such as adverse side effects, toxicity, drug–drug interactions, and the emergence of antifungal resistance **[2,3]**. The development of resistant fungal strains poses a serious chemical and health risk, reducing the effectiveness of existing therapies and increasing treatment costs. In addition, frequent exposure to synthetic chemicals present in antifungal shampoos and topical preparations may cause scalp irritation, dryness, and allergic reactions, highlighting the need for safer alternatives **[2,3]**.

In recent years, medicinal plants have gained increasing attention as potential sources of novel antifungal agents due to their chemical diversity, biocompatibility, and reduced risk of resistance development **[4]**. Plant-derived secondary metabolites such as tannins, flavonoids, phenolic acids, alkaloids, and terpenoids exhibit broad-spectrum antimicrobial activity and play a crucial role in traditional and modern healthcare systems. The use of herbal formulations is also aligned with the principles of green chemistry and sustainable healthcare, as they are generally biodegradable and environmentally friendly **[4,15].**

Fig. No 1: Pomegranate

Punica granatum L. (pomegranate), a member of the family Lythraceae, is an ancient medicinal plant widely cultivated for its nutritional and therapeutic value. While the edible arils are commonly consumed, the peel constitutes nearly 30–40% of the fruit and is often discarded as agro-industrial waste. Interestingly, pomegranate peel is a rich source of polyphenols, ellagitannins (punicalagin and punicalin), flavonoids, and phenolic acids, which have been reported to exhibit strong antioxidant, antimicrobial, and antifungal properties **[5,6]**. Previous studies have demonstrated that these compounds can disrupt fungal cell membranes, inhibit ergosterol synthesis, and interfere with essential enzymatic pathways, leading to fungal growth inhibition **[6,14,18]**.

From a chemical health risk perspective, the valorization of pomegranate peel as a bioactive antifungal agent offers dual benefits: reduction of environmental waste and development of safer, plant-based alternatives to synthetic antifungal chemicals. Incorporation of such natural extracts into personal care products, particularly herbal shampoos, may reduce chemical exposure to the scalp while providing effective control of fungal infections and dandruff **[22,24].**

Therefore, the present study was designed to evaluate the antifungal activity of *Punica granatum* peel extract against selected pathogenic fungi and to develop a herbal shampoo formulation incorporating the extract **[4–6,12].** The study aims to scientifically validate the traditional use of pomegranate peel, assess its potential in reducing chemical health risks associated with synthetic antifungal agents, and explore its application in a safe and effective topical formulation.

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2. Materials and Methods

2.1 Materials

Fresh fruits of *Punica granatum* L. were procured from a local market. All chemicals and reagents used in the study, including ethanol, distilled water, and media components, were of analytical grade. Potato Dextrose Agar was used for antifungal studies. Standard antifungal drug Amphotericin B was used as the reference standard. Herbal ingredients such as shikakai (*Acacia concinna*), reetha (*Sapindus mukorossi*), fenugreek (*Trigonella foenum-graecum*), and aloe vera gel were obtained from authenticated herbal suppliers.

2.2 Collection and Authentication of Plant Material

The pomegranate fruits were washed thoroughly with distilled water. The peels were separated manually and shade dried at room temperature for 7–10 days until a constant weight was obtained. The dried peels were pulverized using a mechanical grinder and passed through a 60-mesh sieve to obtain a uniform powder. The plant material was authenticated by a qualified botanist **[16].**

2.3 Preparation of Pomegranate Peel Extract (Maceration Method)

Dried pomegranate peels of *Punica granatum* L. were coarsely powdered using a mechanical grinder. About 100 g of the powdered material was accurately weighed and transferred into a clean, dry glass container. The material was macerated with 70% ethanol in a ratio of 1:10 (w/v). Ethanol–water mixtures are widely employed for the efficient extraction of phenolic compounds and flavonoids from plant materials due to their polarity and extraction efficiency **[7].** The container was tightly closed and kept at room temperature for 72 h with intermittent shaking to enhance solvent penetration and extraction efficiency **[8].**

After completion of the maceration process, the mixture was filtered initially through muslin cloth to remove coarse particles, followed by filtration through Whatman No. 1 filter paper. The filtrate was concentrated by evaporation on a water bath at temperatures below 40 °C to prevent the degradation of heat-sensitive phytoconstituents **[9].** The dried extract was weighed to calculate the percentage yield and stored in an airtight container at 4 °C until further use for phytochemical screening, herbal shampoo formulation, and antifungal activity studies.

2.4 Preliminary Phytochemical Screening

The pomegranate peel extract was subjected to qualitative phytochemical analysis to detect the presence of major secondary metabolites **[7,9,10,13]** such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycosides, steroids, and terpenoids using standard phytochemical tests.

2.5 Test Microorganisms

Fungal strains including *Candida albicans*, *Aspergillus niger*, and *Fusarium solani* were used for the antifungal study. The cultures were maintained on Potato Dextrose Agar slants at 4 °C and subcultured prior to use **[17].**

2.6 Antifungal Activity Study

The antifungal activity of pomegranate peel extract was evaluated using the agar well diffusion method **[1,2,14]**. Sterile Potato Dextrose Agar plates were inoculated with fungal suspensions adjusted to appropriate turbidity. Wells of 6 mm diameter were punched aseptically, and different concentrations of the extract (50, 100, 250, and 500 µg/mL) were introduced into the wells. Amphotericin B was used as the positive control, while solvent served as the negative control. Plates were incubated at 28–30 °C for 48–72 h. Zones of inhibition were measured in millimeters **[18]**.

2.7 Formulation of Herbal Shampoo

The herbal shampoo was formulated using pomegranate peel extract as the primary antifungal active ingredient along with selected herbal excipients known for their cleansing, conditioning, and hair-protective properties. Shikakai (*Acacia concinna*) and reetha (*Sapindus mukorossi*) were used as natural surfactants and foaming agents due to their saponin content, while fenugreek (*Trigonella foenum-graecum*) was incorporated for its conditioning and hair-strengthening properties. Aloe vera gel was added for its soothing, moisturizing, and scalp-protective effects. Guar gum was used as a natural thickening agent, and rose water served as a fragrance and aqueous vehicle.

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Table 1. Composition of Herbal Shampoo

INGREDIENT	QUANTITY(g)	FUNCTION
Pomegranate peel extract (aqueous)	4	Antifungal agent
Shikakai powder	1	Natural cleanser
Reetha liquid extract	2	Foaming agent
Fenugreek seeds	0.6	conditioning and strengthening agent
Fresh Aloe vera gel	1	Moisturizer
Guar gum powder	0.3	Thickening agent
Rose water	2	Fragrance and soothing agent
Purified water	q.s. to 20 g	Vehicle

Method of Preparation:

1. Take shikakai powder (1 g) and fenugreek seeds (0.6 g).

2. Add 10 g of purified water and boil gently for 10–15 minutes.

3. Allow the mixture to cool and filter the extract.

4. Add pomegranate peel extract (4 g) and mix thoroughly.

5. Add reetha extract (2 g) slowly with continuous stirring.

6. Disperse guar gum (0.3 g) gradually to avoid lump formation.

7. Add aloe vera gel (1 g) and mix gently.

8. Add rose water (2 g).

9. Make up the volume to 20 g using purified water and mix until uniform. **[19]**.

2.8 Evaluation of Herbal Shampoo

The formulated herbal shampoo was evaluated for various physicochemical parameters to assess its quality, safety, and performance using standard methods **[11,12,22,24].**

2.8.1 Physical Appearance Evaluation

The formulated shampoo was evaluated visually for color, clarity, consistency, homogeneity, and odor. The formulation was examined for the presence of any particulate matter or phase separation to ensure uniformity and aesthetic acceptability **[20].**

2.8.2 Determination of pH

The pH of the herbal shampoo was determined using a calibrated digital pH meter at room temperature. A 10% (w/v) shampoo solution was prepared in distilled water, and the pH was measured in triplicate. The pH value was maintained within the acceptable range for scalp application to avoid irritation and dryness **[21].2.8.3 Foam Formation Ability**

Foam formation ability was evaluated by the cylinder shake method **[22].** A measured volume of the shampoo solution was transferred into a graduated cylinder and shaken uniformly for a fixed time. The total volume of foam formed was recorded immediately, and foam stability was assessed by measuring the foam volume after a specific time interval

2.8.4 Viscosity Measurement (Ostwald Viscometer)

The viscosity of the herbal shampoo was measured using an Ostwald viscometer at room temperature. The time taken for the shampoo solution to flow between two marked points was recorded and compared with that of distilled water. The viscosity was calculated using standard equations to assess the flow characteristics of the formulation.

2.8.5 Surface Tension Measurement (Stalagmometer Method)

Surface tension of the shampoo solution was determined using a stalagmometer by the drop count method. The number of drops of the shampoo solution and distilled water were counted separately under identical conditions. Surface tension was calculated using the ratio of drop numbers and densities to evaluate the wetting and cleansing efficiency of the formulation **[23].**

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2.9 Determination of Percentage Yield

The percentage yield of the pomegranate peel extract was calculated by dividing the weight of the dried extract obtained after evaporation by the initial weight of the powdered plant material and multiplying by 100.

2.10 Determination of pH of Extract and Shampoo

The pH of the pomegranate peel extract and the formulated herbal shampoo was determined using a calibrated digital pH meter at room temperature. Measurements were carried out in triplicate to ensure accuracy

2.11 Stability Studies

Stability studies of the formulated herbal shampoo were conducted by storing the formulation at different temperature conditions (room temperature and refrigerated conditions) for a specified period. The formulation was periodically evaluated for changes in color, odor, pH, phase separation, and consistency **[24].**

2.12 Skin Irritation Test (Patch Test)

A preliminary skin irritation test was performed on healthy human volunteers after obtaining informed consent **[25].** A small quantity of the formulated shampoo was applied to a marked area of the skin and

2.13 Statistical Analysis

All experiments were carried out in triplicate, and the results were expressed as mean ± standard deviation, with statistical analysis performed to ensure the accuracy, reproducibility, and reliability of the experimental data.

3. Results and Discussion.

3.1 Extractive values

The extractive values varied with the solvent used. The aqueous extract showed 11.12% w/w, ethanolic extract 10% w/w, and hydro-alcoholic extract the highest value at 12% w/w. Benzene and chloroform extracts showed lower extractive values of 4.2% w/w and 6.8% w/w respectively, indicating better extraction with polar solvents.

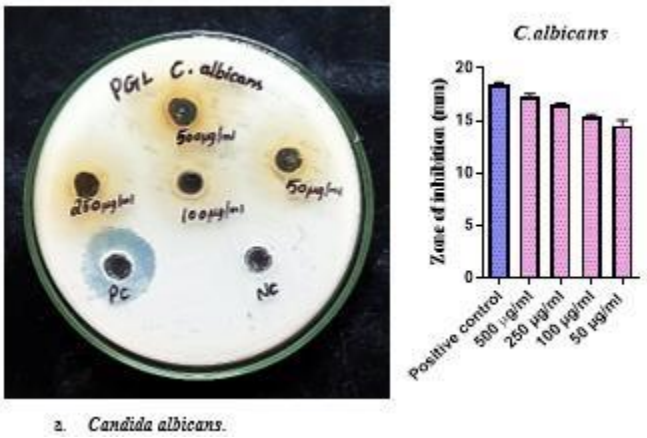
3.2 Phytochemical Screening

Preliminary phytochemical screening of the *Punica granatum* peel extract revealed the presence of tannins (+++), flavonoids (++), phenolic compounds (+++), saponins (++), glycosides (+), and terpenoids (+), while alkaloids and steroids were absent. The abundance of tannins and phenolic compounds supports the strong antifungal potential of the extract.

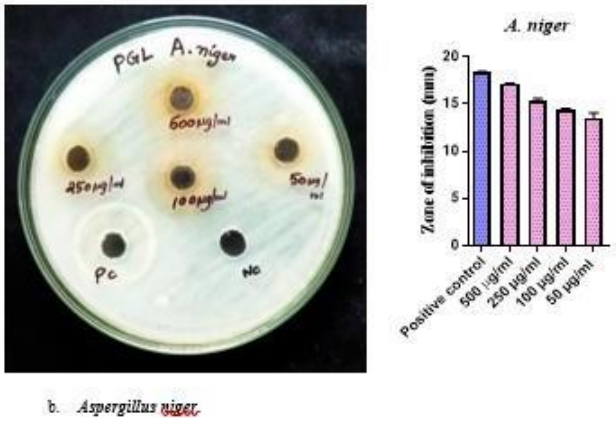
3.3 Antifungal Activity

The antifungal activity of the pomegranate peel extract was evaluated against *Candida albicans*, *Aspergillus niger*, and *Fusarium solani* using the agar well diffusion method. The extract exhibited a concentration-dependent increase in the zone of inhibition against all tested fungal strains .

Fig No 2. Zone of inhibition images *(a) Candida (b) Aspergillus (c) Fusarium*



a. *Candida albicans.*



b. *Aspergillus niger*

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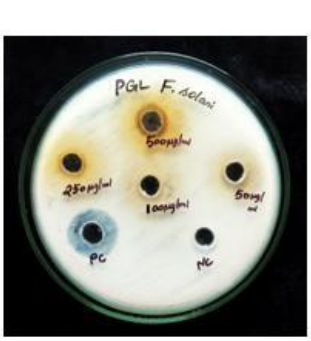
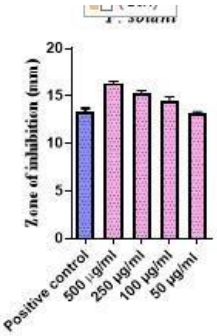




C.: Fusarium solani

Table 2. Antifungal activity of *Punica granatum* peel extract (Zone of inhibition in mm)

S. No	Test Organism	Concentration (µg/ml)	Zone of inhibiton (mm)
1	*Candida albicans*	50	14.5 ± 0.56
		100	15.35 ± 0.21
		250	16.45 ± 0.21
		500	17.3 ± 0.28
		Positive control	18.35 ± 0.21
2	*Aspergillus niger*	50	13.5 ± 0.56
		100	14.35 ± 0.21
		250	15.3 ± 0.28
		500	17.15 ± 0.07
		Positive control	18.35 ± 0.07
3	*Fusarium solani*	50	13.2 ± 0.14
		100	14.5 ± 0.42
		250	15.3 ± 0.28
		500	16.35 ± 0.21
		Positive control	13.35 ± 0.35

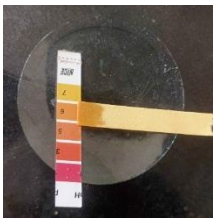
The results indicate that the extract showed maximum activity against *Candida albicans*, followed by *Aspergillus niger* and *Fusarium solani*. The antifungal efficacy is attributed to the presence of tannins and phenolic compounds, which are known to disrupt fungal cell membranes and inhibit essential enzymatic pathways.

3.3 Evaluation of Herbal Shampoo

Evaluation of herbal shampoo is a crucial step to ensure its quality, safety, stability, and consumer acceptability. Each evaluation parameter provides important information regarding the performance of the formulation.

The formulated herbal shampoo was subjected to various physicochemical and performance evaluation tests, and each test is described in detail below. The formulated herbal shampoo was evaluated for various physicochemical parameters, and the results are presented in **(Table 2)**.

Fig No:3 **Evaluations of Herbal Shampoo**



(a) pH determination of herbal shampoo

(b) Foam determination of herbal shampoo

The pH of the formulation was found to be within the acceptable range for scalp application, indicating suitability for topical use. Adequate viscosity and foam formation confirmed good cleansing and handling properties of the shampoo. The reduced surface tension value suggests effective wetting and detergency action. Stability studies showed no significant changes in physicochemical parameters, indicating good formulation stability.

Overall, the results demonstrate that *Punica granatum* peel extract exhibits significant antifungal activity and can be effectively incorporated into a stable and safe herbal shampoo formulation for the management of fungal scalp infections.

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Parameter	Observation /Value	Standard/Acceptable range
Physical appearance	Light brown,Pleasant herbal odor,Smooth texture,Semi-viscous	Uniform,Smooth,Free from phase separation
pH	6**.1 ± 0.2**	4.5 - 7.0
Foam formation ability	10 ml	8 - 15 ml
Wetting time	52 seconds	40 – 60 seconds
Viscosity	3200 cP	2000 – 4000 cP
Dirt dispersion test	Dirt remained in water	Dirt should remain in water
Surface tension	36 dynes/cm	30 – 40 dynes /cm
Stability studies	No changes observed	Nophase separation,Color,Odor, or consistency change

4. Conclusion

The present study confirms that *Punica granatum* peel extract possesses significant antifungal activity against common pathogenic fungi such as *Candida albicans*, *Aspergillus niger*, and *Fusarium solani*. The observed antifungal efficacy can be attributed to the presence of bioactive phytoconstituents, particularly tannins, flavonoids, and phenolic compounds, which are known to disrupt fungal cell membranes and metabolic pathways .

The successful formulation of a herbal antifungal shampoo incorporating the pomegranate peel extract demonstrated acceptable physicochemical properties, good stability, and absence of skin irritation, indicating its suitability for topical application. The retention of antifungal activity in the formulated shampoo highlights the potential of pomegranate peel as an effective natural alternative to synthetic antifungal agents commonly used in hair care products.

Overall, the utilization of pomegranate peel, an agro-industrial by-product, not only provides a sustainable and eco-friendly approach but also reduces chemical health risks associated with synthetic antifungal formulations. The findings support the potential application of *Punica granatum* peel extract in the development of safe, effective, and natural antifungal hair care products. Further studies involving advanced formulation optimization and clinical evaluation are recommended to validate its large-scale applicability.

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