

Polyherbal Formulations of Antidandruff Shampoo and Their Antifungal Activity

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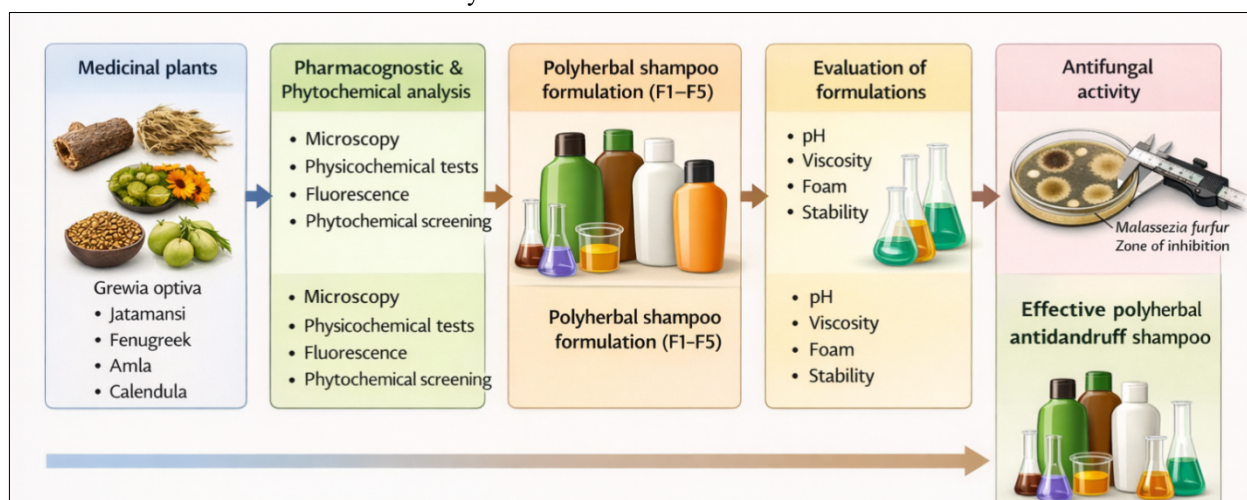
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ABSTRACT

The purpose of this research was to prepare a polyherbal antidandruff shampoo using identified medicinal plants and to determine the antifungal action of the product on the microorganisms that cause dandruff. Dandruff is widely linked to any fungal growth on the head and the use of synthetic shampoos over extended periods of time can lead to irritation and other side effects. Thus, bioactive phytoconstituent herbal formulations are viewed as safer. Some of the possible reasons were the traditional application of *Grewia optiva*, *Nardostachys jatamansi*, *Trigonella foenum-graecum*, *Emblica officinalis*, and *Calendula officinalis* in hair care and antimicrobial effects. Plant samples were verified through macroscopic/microscopic analysis and *Grewia optiva* bark was analyzed on physicochemical/fluorescence and phytochemical. Different proportions of plant extracts were made into five shampoo formulations (F1-F5) and tested on the basis of physicochemical properties and antifungal activity. The bark was found to contain total ash 4.34%, acid-insoluble ash 3.6, water-soluble ash 2.6 and extractive values in acceptable ranges. The formulations of all the shampoos were appropriate with respect to pH (6.2-6.6), viscosity (3100-3400 cP), excellent foamability, and stability. Antifungal assays on *Malassezia furfur* demonstrated an inhibitory range of 11-18 mm as formulation F3 was the most active. The findings prove that the polyherbal formulation has acceptable quality parameters and powerful antifungal action. The synergistic effect of medicinal vegetation increased the antidandruff properties, which means that it is possible that the created polyherbal shampoo will be a safe and efficient alternative to a synthetic one.



Keywords: Polyherbal Shampoo, Antidandruff Formulation, Antifungal Activity, *Malassezia furfur*, Medicinal Plants, Phytoconstituents

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INTRODUCTION

Dandruff is a widely-popular scalp condition located on a significant percentage of the world population and it is often linked to fungal infection, especially by members of the *Malassezia* genus. It is described by the presence of an itch, a scaly surface, and irritability of the head, which also can cause suffering and decreased quality of life. Traditional antidandruff shampoo usually includes artificial antifungal remedies, which can lead to undesirable results like dryness, irritability or resistance with the protracted utilization. Due to these shortcomings, more attention has been given to the development of herbal preparations, which are safer and more sustainable as scalp care. Medicinal plants are popular in the traditional medicine systems of many countries because of the large variety of bioactive compounds and therapeutic effects (Taheri et al., 2021; Soni and Verma, 2025).

Flavonoids, phenolic compounds, glycoside, and tannin are antimicrobial, antioxidant, and anti-inflammatory secondary metabolites found in plants and could be used in the treatment of scalp infections and dandruff. Specifically, flavonoids have been reported to have very high biological activity due to their capacity to engage cellular activities and prevent the growth of microbes (Abotaleb et al., 2018). Phytochemical compositions of several medicinal plants (that are representatives of other genera) and their potential as topical agents have also demonstrated promising anti-microbial and antioxidant effects, which support the use of these plants as topical agents (Lee et al., 2008; Taheri et al., 2021).

The species of the genus *Grewia* have been interesting because of their abundant phytochemical profiles and a wide range of biological functions. Phenolic compounds, flavonoids, and other bioactive constituents have been present in various species of *Grewia* in previous studies, which means that they have a potential to be used in medicine (Jariwala and Parmar, 2024; Suguna and Umesh, 2023). Research of the related species has also shown antimicrobial and antioxidant effects, indicating that these plants can be utilized in formulations to be applied to the skin and scalp (Khangarot et al., 2024; Coin, 2023). Moreover, the bioactive compounds extracted in *Grewia optiva* have also been found to be pharmacologically active, which further confirms its usage as a traditional herbal extract in homeopathic preparations (Ul Bari et al., 2019).

Nardostachys jatamansi, *Trigonella foenum-graecum*, *Emblica officinalis*, and *Calendula officinalis* are used as other traditional medicinal plants in the treatment of hair and scalp. Phytochemicals found in these plants have been known to have anti-microbial, anti-inflammatory and antioxidant effects that can help keep the scalp healthy and avoid fungal infections. Polyherbal recipes are a frequent practice in herbal medicine because it is assumed that combining various plant extracts can increase the therapeutic effect by a synergistic effect of various bioactive compounds (Puri et al., 2024).

The increasing interest in the recent years is in the development of herbal cosmetic and pharmaceutical formulations by applying scientifically proven methods. This is done to identify the authenticity and quality management of crude plant materials by performing pharmacognostic analysis, physicochemical analysis, and phytochemical screening prior to the use of these raw materials in the formulation process (Soni and Verma, 2025). This kind of standardization provides the safety, stability, and reproducibility of the herbal products that are supposed to be used topically.

Although, many medicinal plants have been traditionally used in hair care, the research data on the chemistry and appraisal of polyherbal antidandruff shampoos comprising of *Grewia optiva* and other medicinal plants is limited. Thus, this research was conducted to prepare a poly herbal shampoo based on the choice of medicinal plants and to determine its physicochemical characteristics and antifungal effects against microorganisms that cause dandruff.

MATERIALS AND METHODS

1. Plant Material Collection and Authentication.

The samples of *Grewia optiva* bark, *Nardostachys jatamansi* rhizome, *Trigonella foenum-graecum* seeds, *Emblica officinalis* fruits and *Calendula officinalis* flowers were harvested in the Uttarakhand area, India, in the right time. The vegetal samples were rinsed with sterile distilled water to eliminate soil and debris attached to them, dried at room temperature (25 ± 2 °C), and put in airtight containers and shielded against moisture and light until next use. Identification of the plant materials was done using the basic macroscopic characteristics (colour, odour, size, shape and surface texture) under the general pharmacognostic criteria. A mechanical grinder (Remi Laboratory Instruments, Mumbai, India) was used

to dry the materials and sieve No. 60 was used to fine the powder so that the particle size is homogenous.

2. Pharmacognostic Analysis of *Grewia optiva* Bark.

Grewia optiva bark was cut into thin transversal sections using sharp stainless steel blade and cleared in chloral hydrate solution. The mounts were placed in glycerine using clean glass slides and viewed using a compound microscope (Olympus CX23, Olympus Corporation, Japan). Powder microscopy was done by applying a small sample of powdered bark to a slide, treating with phloroglucinol and concentrated hydrochloric acid in order to lignified tissues, and mounting in glycerin. The features that were used to make a diagnosis (cork cells, fibers, epidermal cells and multicellular trichomes) were observed and photographed with the help of a digital microscope camera attachment.

3. Physicochemical Analysis

The physicochemical parameters of the *Grewia optiva* powdered barks were identified as per the standard procedures as outlined in pharmacognostic manuals. Muffle furnace was used to determine total ash, acid-insoluble ash, and water-soluble ash at 450-600 °C (Tempo Instruments, India). Maceration and subsequent evaporation to dryness of the water bath were used to determine extractive values that were water-soluble and ethanol-soluble. The loss on drying was also done in a hot air oven (Labline Instruments, India) at 105 °C until the weight is constant. The visual separation and weighing of the organic matter were done using the analytical balance (Shimadzu AY220, Japan) to determine the foreign organic matter.

Table 1. Physicochemical parameters of *Grewia optiva* bark powder

S. No.	Parameter	Value (% w/w)
1	Total ash	4.34
2	Acid insoluble ash	3.6
3	Water soluble ash	2.6
4	Water soluble extractive value	3.6
5	Ethanol soluble extractive value	1.2
6	Foreign organic matter	0.2 ± 0.01
7	Loss on drying	1.2

The table illustrates that the ash values, extractive values, moisture content and foreign matter in the powder of the bark were found to be within acceptable limits. These findings are used to signify the purity and stability of the crude drug and serve as reference standards to quality control in formulation research.

4. Fluorescence Analysis

Detection of the *Grewia optiva* bark powder was performed using the fluorescence technique whereby the powder was individually put into chloroform, methanol, ethanol, and distilled water. The samples were placed on glass slides and viewed under a UV chamber at 254 nm and under a microscope using visible light (Early Interactive, 2012). Different conditions were used to ensure that the colour changes were recorded.

Table 2: Fluorescence examination of different Extract of *Grewia Optiva* Bark powder

S.No.	Extracts	Visible Light	Short UV (254 nm)
1	Chloroform	Light Green	Brownish Green
2	Methanol	Light Brown	Light Brown
3	Ethanol	Light Brown	Light Brown
4.	Water	Dark Brown	Dark Brown

The table illustrates the typical colour effects that the powder of the bark undergoes, when subjected to various solvents, and viewed through the naked eye and under

ultraviolet light. These fluorescence characteristics are another identification parameter and are also used to detect adulteration of the crude drug.

5. Phytochemical Screening

The ethanol, aqueous and chloroform *Grewia optiva* bark extracts were done by macerating overnight and then filtrated using the Whatman No. 1 filter paper. Standard chemical tests on carbohydrates (Molisch test), proteins (Biuret test, Millon test), flavonoids (Shinoda test, ferric

chloride test), tannins and phenolic compounds (ferric chloride test, lead acetate test), steroids (Salkowski test), alkaloids (Dragendorff test, Mayer test, Wagner test), glycosides (Borntrager test, Baljet test), and saponins (foam test) were used on the extracts. Reagents were of an analytical grade (Merck, India).

Table 3. Qualitative phytochemical screening of *Grewia optiva* bark extracts

S. No.	Phytochemical Group	Test	Chloroform	Methanol	Water	Ethanol
1	Carbohydrates	Molisch's test	+	+	+	-
		Fehling's test	+	+	+	-
		Benedict reagent	-	-	+	-
2	Proteins and amino acids	Biuret test	-	-	+	-
		Test with Millon's reagent	-	-	+	-
3	Flavonoids	Shinoda test	+	+	+	+
		Ferric chloride test	+	+	+	+
4	Tannins and phenolic compounds	Dilute ferric chloride	+	+	+	+
		Lead acetate solution	+	+	-	-
		Gelatin solution	-	-	+	-
5	Steroids	Salkowski test	+	+	-	-
6	Alkaloids	Dragendorff test	+	-	-	-
		Mayer test	+	-	-	-
		Wagner test	-	-	-	-
		Hager test	-	-	-	-
7	Glycosides and saponins	Anthraquinone glycoside (Borntrager's test)	-	+	-	-
		Cardiac glycosides (Baljet test)	+	+	+	+
		Saponins (Foam test)	+	+	+	+

The table indicates that different solvent extracts contain a number of phytochemical groups that include flavonoids, tannins, phenolics, glycosides, steroids, and saponins. The findings show that *Grewia optiva* does have several bioactive components which could possibly lead to antimicrobial and antifungal effects.

6. Polyherbal Antidandruff Shampoo Preparation.

Powdered extracts of *Grewia optiva*, *Nardostachys jatamansi*, *Trigonella foenum-graecum*, *Emblica officinalis* and *Calendula officinalis* were used to prepare five polyherbal shampoo formulations (F1-F5) in varying ratios. The surfactant, preservatives (methyl paraben and

sodium benzoate), viscosity-adjusting agent (sodium chloride), and vehicle (distilled water) were sodium lauryl sulphate (SLS), methyl paraben, sodium benzoate, sodium chloride, and distilled water, respectively. Extracts of the required quantities of the plant extracts were dispersed in distilled water, which was then mixed with the help of the magnetic stirrer (Remi 2MLH, India). SLS was dissolved individually and added in a gradual manner through constant stirring. The addition of preservatives and sodium chloride was done slowly to acquire a homogenous and uniform shampoo base. The ready formulations were kept in clean, dry and amber-coloured bottles and kept at room temperature.

Table 4. Polyherbal shampoo formulation composition (F1-F5).

Ingredient	F1	F2	F3	F4	F5
<i>Grewia optiva</i>	30	25	28	26	30
<i>Jatamansi</i> (Rhizome)	8	10	10	8	8

Trigonella foenum-graecum	8	8	10	10	8
Emblica officinalis	20	20	20	16	16
Calendula officinalis	20	25	20	20	18
Distilled water	q.s	q.s	q.s	q.s	q.s
SLS (Surfactant)	6	6	6	6	6
Methyl paraben	2	4	4	4	4
Sodium benzoate	2	4	4	4	4
NaCl (pH neutralizer / thickener)	2	4	4	4	4

The table illustrates the various percentages of the plant extracts and excipients in manufacturing the five formulations of shampoos. Composition variation was done in order to achieve an optimized formulation with adequate physicochemical characteristics and antifungal efficacy.

7. Comparison of Shampoo Formulations.

The shampoo solutions that were ready were analyzed on the basis of physicochemical parameters.

The pH was determined by use of digital pH meter (Elico LI-120, India) at room temperature.

Viscosity was measured with the help of Brookfield Viscometer (Model DV-II +, Brookfield Engineering, USA).

Foaming was determined through cylinder shake technique by using a graduated measuring cylinder.

Spreadability was measured using glass slide method where the time of separation of two slides under a given weight was measured.

The formulations were tested in terms of stability by keeping them at room temperature (25 ± 2 °C) and monitoring the colour change, phase separation, and consistency over 30 days.

Table 5. Evaluation parameters of polyherbal shampoo formulations

Formulation	pH	Viscosity (cP)	Foam height (cm)	Spreadability (g·cm/sec)	Stability
F1	6.5	3200	6.2	12.4	Stable
F2	6.3	3350	6.5	12.8	Stable
F3	6.4	3400	7.1	13.2	Stable
F4	6.2	3100	6.0	11.9	Stable
F5	6.6	3250	6.4	12.6	Stable

The table illustrates the physicochemical analysis of all the formulations prepared such as the pH, viscosity, foamability, spreadability, and stability. The findings show that none of the formulations exceeded acceptable limits in areas of topical application, which confirmed them as being suitable as shampoo preparations.

8. Antifungal Activity

Against *Malassezia furfur*, agar well diffusion method was used to determine the antifungal activity of the prepared shampoo formulations (F15). The culture of the

fungi was kept in Sabouraud dextrose agar medium (HiMedia Laboratories, India). It was transferred to sterile Petri plates in an autoclave (Equitron, India) at 121 °C at 15 min. The sterile cork borer was used to make the wells of uniform diameter, and the same amount of each formulation was added to the wells. Bacteriological incubator was used to incubate the plates at 37 °C and 24- 48 h. The diameter of the zone of inhibition measured in millimeters was used to determine the antifungal activity with the help of a digital vernier caliper.

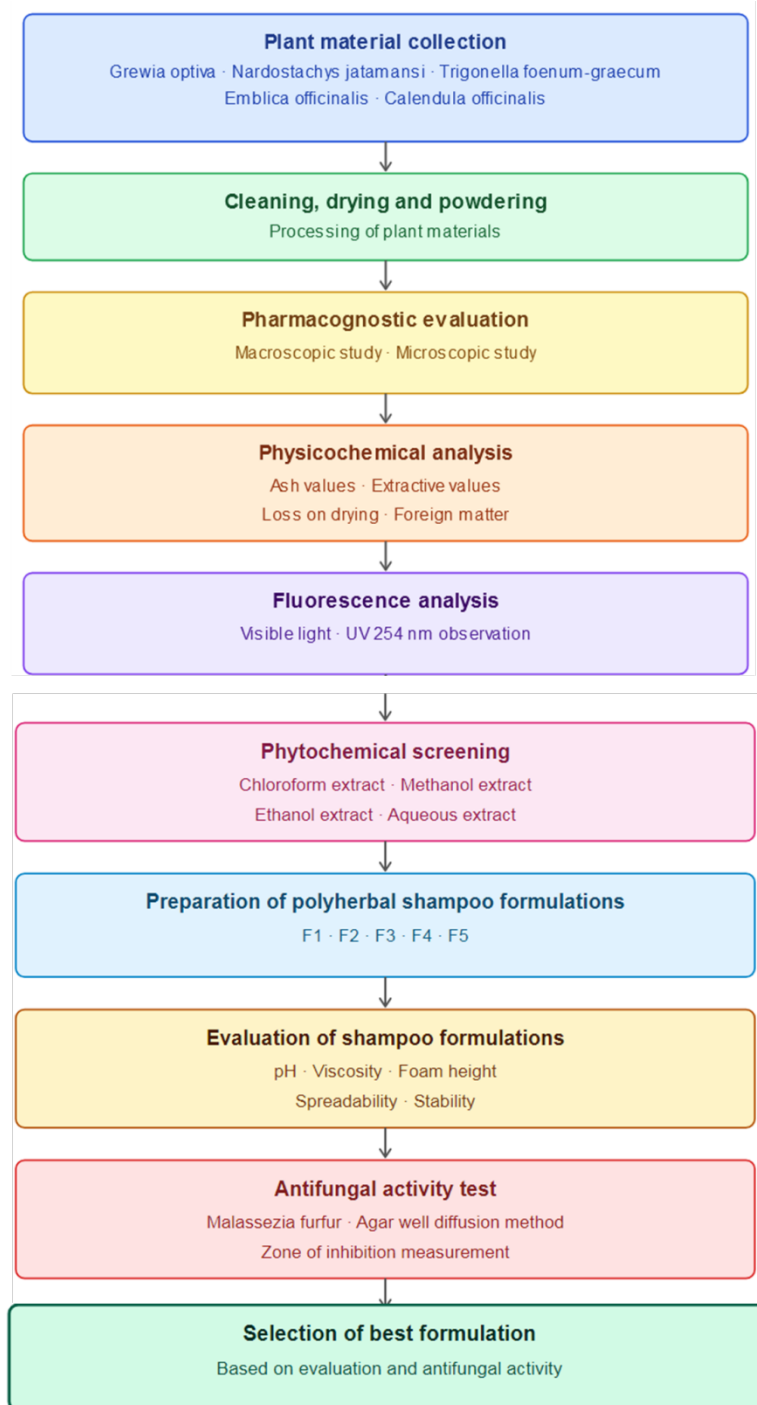


Figure 1. Flowchart indicating procedure of preparation and analysis of polyherbal antidandruff shampoo.

The figure also illustrates the sequential procedure used during the study beginning with the collection of the plants, pharmacognostic and phytochemical analysis, the preparation of polyherbal shampoo formulations, and their evaluation in relation to their physicochemical and antifungal properties. This flowchart shows the

methodical nature of the protocol followed in order to properly authenticate raw materials and scientifically validate the developed formulation.

RESULTS AND DISCUSSION

1. Plant materials Macroscopic characteristics.

The crude drugs that were used in the formulation were analysed based on external morphological features such as colour, odour, texture, and surface appearance. The macroscopic appearance of all the plant materials was typical of their usual descriptions, hence their authenticity and appropriateness to be used in the polyherbal preparation. The correct and accurate identification of

raw materials is a necessary process in the development of a herbal formulation to prevent adulteration, and to achieve reproducibility of the pharmacological activity. The characters observed were congruent with those that are found in the normal pharmacognostic descriptions of plants that are utilized in traditional hair care formulations.

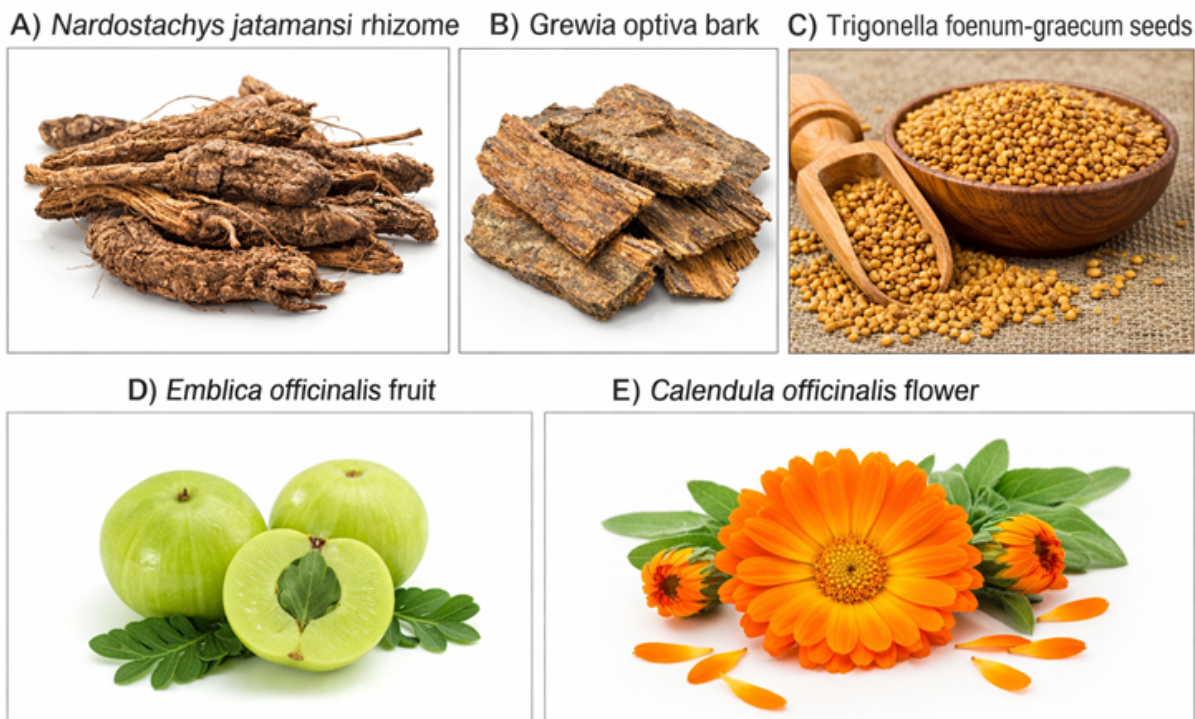


Figure 2. Macroscopic features of vegetal matters utilized in the research.

The figure illustrates the outer morphological appearance of the medicinal plants chosen in the formulation such as the bark, rhizome, seeds, fruits and flowers. The macroscopic characters witnessed affirm the authenticity of the crude drugs and they will show that the right plant materials were used to prepare the polyherbal shampoo.

2. Microscopic characteristics of *Grewia optiva* bark

The microscopic analysis of *Grewia optiva* bark showed clear anatomical features such as cork cells, cortical tissues, fibers, epidermal cells and multicellular

trichomes. These diagnostics characteristics are regarded as significant pharmacognostic indicators that can be used to authenticate the crude drug. A typical occurrence of characteristic fibers and trichomes identifies the plant as the bark, and justifies the use of the plant in herbal preparations. In order to ensure quality, safety, and consistency of polyherbal preparations, there should be pharmacognostic standardization of raw materials, since the variability in the plant material may affect the therapeutic outcome of the finished product.

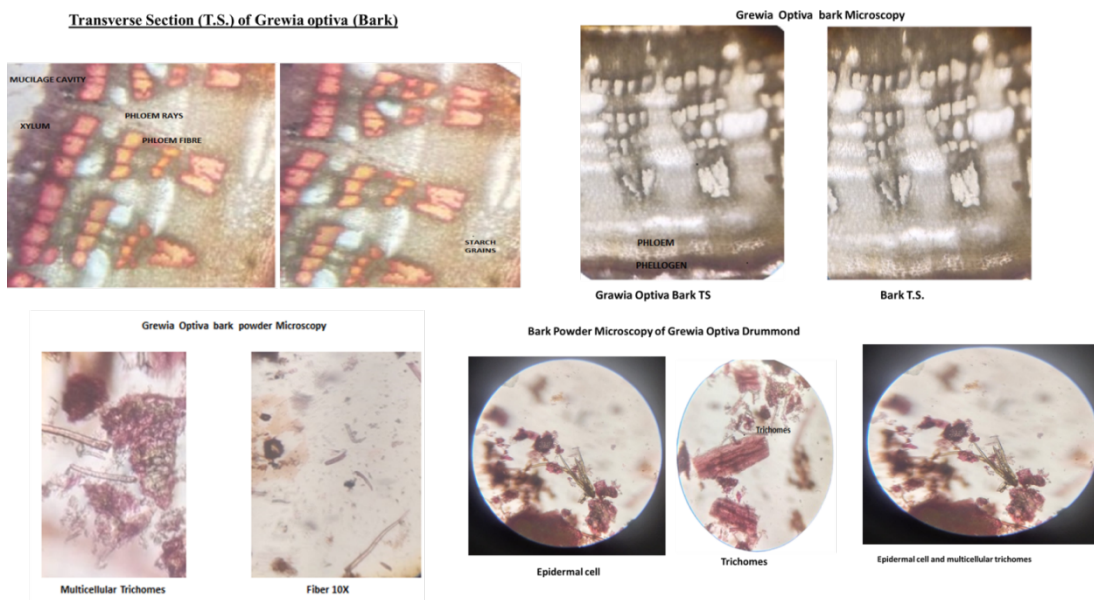


Figure 3. Grewia optiva Grewia optiva Bark microscopic characters.

The figure illustrates the anatomical structures of the Grewia optiva bark in terms of cork cells, fibers, epidermal cells and multicellular trichomes that were examined under the microscope. These diagnostics characteristics are useful pharmacognostic markers of identification and quality control of the used bark in the formulation.

3. Grewia optiva bark physicochemical parameters.

The physicochemical parameters derived on Grewia optiva bark powder were within acceptable values

suggesting that the crude drug was not contaminated with too much inorganic impurities. The values of the ash suggested that there were low content amounts of extraneous matter and the extractive values suggested the presence of both polar and moderately polar constituents. The presence of low moisture content indicates that there is good stability of the powdered drug during storage. It is significant to establish these parameters to have quality control of the herb raw material since physicochemical constants are the reference standards to be used in formulation studies regarding identification and purity.

Table 6. Physicochemical parameters of Grewia optiva bark powder

Parameter	Value (% w/w)
Total ash	4.34
Acid insoluble ash	3.6
Water soluble ash	2.6
Water soluble extractive	3.6
Ethanol soluble extractive	1.2
Loss on drying	1.2
Foreign organic matter	0.2 ± 0.01

The table illustrates the typical physicochemical constants of the bark utilized in the research, which show that there are no excessively impurities and that the quality of the raw material applied in the formulation is guaranteed.

4. Fluorescence characteristics

The treatment of the bark powder with various solvents by fluorescence analysis in the visible and ultraviolet light led to the ability to observe various colour changes. These variations show that there is evidently a variety of classes of chemical constituents which react differently to particular reagent and conditions of light. The

fluorescence behaviour is a common qualitative technique of identification of crude drugs and adulteration detection that is a quick technique. The patterns of observed fluorescence are, thus, further evidence supporting the validity of *Grewia optiva* bark that is used in the current formulation.

5. Phytochemical profile

A qualitative phytochemical screening of various solvent extracts showed that flavonoids, tannins, phenolic compounds, glycosides, steroids, and saponins were

present. These constituents were also exhibited differently when solvents were used, which shows that the extraction efficiency is affected by the solvent polarity. All these types of phytochemicals are reported to have antimicrobial, antioxidant, and anti-inflammatory effects, and these could help to add therapeutic value to the formulation. The existence of various bioactive components justifies the choice of *Grewia optiva* as part of the important ingredients to be included in the polyherbal shampoo.

Table 7. Phytochemical screening results

Phytochemical	Chloroform	Methanol	Ethanol	Water
Carbohydrates	+	+	+	+
Proteins	-	-	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Phenolics	+	+	+	+
Steroids	+	+	-	-
Alkaloids	+	-	-	-
Glycosides	-	+	+	+
Saponins	-	+	+	+

The table illustrates the fluorescence behaviour of various extracts at the visible and ultraviolet light, which indicate the existence of various chemical constituents. These

observations are conducive to the identification and standardization of the crude drug.

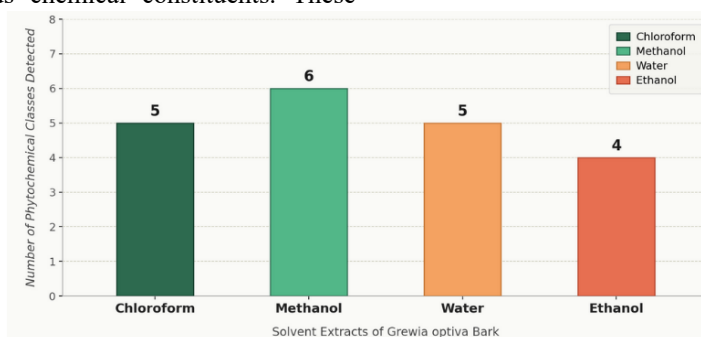


Figure 4. Distribution of phytochemical constituents detected in chloroform, methanol, ethanol, and aqueous extracts of *Grewia optiva* Bark on qualitative phytochemical screening results.

The figure illustrates the difference in the concentration of various phytochemical groups in the various solvent extracts, and this shows that polarity of the solvent affects extraction of bioactive constituents. This distribution justifies the existence of several active compounds that can be involved in the antifungal action of the polyherbal formulation.

Different ratios of *Grewia optiva*, *Nardostachys jatamansi*, *Trigonella foenum-graecum*, *Emblica officinalis* and *Calendula officinalis* were successfully prepared as five polyherbal formulations of shampoo using appropriate excipients. In all the formulations, there was a homogenous and smooth appearance, and no separation of phases, which means that the ingredients have been mixed and are compatible. A combination of

medicinal plants in one formulation is also aimed at the benefit of increased therapeutic effect of different phytoconstituents due to a synergistic effect, and this principle is a standard in polyherbal preparations.

6. Assessment of shampoo formulations.

The topical use of these prepared formulations was tested with regard to their physicochemical properties to ascertain their appropriateness. The pH of each of the formulations fell within the proper acceptable range of

scalp preparations meaning that the shampoos will not be irritating. Shampoo formulations were found to be suitable in terms of viscosity and foamability, implying that the sample was easy to apply and clean the hair. Stability data revealed that there was no significant change in colour, consistency or phase separation over the period of study and this reflected that the formulations were physically stable. These findings prove the ratios of the chosen excipients and plant extracts to be appropriate to prepare a stable polyherbal shampoo.

Table 8. Evaluation parameters of polyherbal shampoo formulations

Formulation	Colour	Odour	Consistency	Foam retention (min)	pH
F1	Brown	Herbal	Smooth	5.2	6.5
F2	Dark brown	Herbal	Smooth	5.5	6.3
F3	Brown	Pleasant	Smooth	6.1	6.4
F4	Brown	Herbal	Slightly thick	5.0	6.2
F5	Light brown	Pleasant	Smooth	5.4	6.6

The table shows the relative analysis of all the formulations of the shampoos that were made using various ratios of plant extracts. The findings have shown that the formulations were physically stable and could be applied in the scalp with some slight variations because of the differences in the composition.

7. Antifungal action of formulations.

Antifungal activity was observed on all the polyherbal shampoo formulations against *Malassezia furfur*, which is the microorganism that is often related to dandruff. The variation in the zone of inhibition in formulations shows that the proportion of plant extracts determine antifungal activity.

Table 9. Antifungal activity of polyherbal shampoo formulations against *Malassezia furfur*

Formulation	Zone of inhibition (mm)
F1	12
F2	14
F3	18
F4	11
F5	15

The table indicates the zone of inhibition of various formulations on *Malassezia furfur*, and antifungal activity varies with the content of plant extracts. The findings indicate that polyherbal formulation of phytochemicals increases antidandruff potential.

The preparations with more significant concentrations of extracts with more phenolic and flavonoid compounds were more active, indicating a potential synergistic action of the joint phytochemicals. The antifungal effect witnessed justifies the conventional role of the chosen medicinal plants in the management of the hair and the scalp and implies that the designed polyherbal shampoo can be an effective antidandruff preparation.

CONCLUSION

The current research has been able to set up the pharmacognostic, physicochemical, and phytochemical characteristics of *Grewia optiva* bark and has applied the authenticated plant materials to design a polyherbal antidandruff shampoo comprising of *Grewia optiva*, *Nardostachy jatamansi*, *Trigonella foenum-graecum*, *Embllica officinalis*, and *Calendula officinalis*. The identity of the crude drugs was determined by means of macroscopics and microscopics, whereas parameters of physicochemical and fluorescence analysis were used as the reference parameters that aid in the quality control. The qualitative phytochemical screening revealed the

occurrence of flavonoid, tannin, phenolic compounds, glycosides, steroid, and saponins implying that selected plants have bioactive ingredients that could involve antimicrobial and antifungal activity.

Five polyherbal shampoo formulations were made with varying content of plant extracts and all the formulations possessed acceptable physicochemical characteristics such as appropriate pH, viscosity, foamability, spreadability, and stability to be used topically. The antifungal research found that all formulations had an inhibitory effect on *Malassezia furfur* and the research proved that the selected medicinal plants have the potential in the treatment of dandruff. It has been established that the antifungal activity between formulations is varying, which implies that the percentage of plant extracts used is significant in the determination of the final product.

The results of the current study contribute to the consideration of the polyherbal combinations as a promising method to designing the safe and effective antidandruff preparations. The laid down pharmacognostic and physicochemical parameters may be used as a reference standard in the future researches about *Grewia optiva* and herbal preparations. It is suggested that further studies using quantitative phytochemical and active constituents isolation, stability studies over time, and clinical testing should be conducted to warrant the therapeutic effectiveness and safety of the polyherbal shampoo developed.

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