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PREPARATION OF ANTI-DANDRUFF SHAMPOO FROM POLY HERBAL FORMULATION AND ITS ANTIOXIDANT, ANTIMICROBIAL AND ANTIINFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF DIFFERENT PARTS OF *AZADIRACHTA INDICA, OCIMUM TENUIFLORUM, ECLIPTA PROSTRATA, PHYLLANTHUS EMBLICA, HIBISCUS ROSA-SINENSIS, AND SAPINDUS MUKOROSSI*- A NOVEL APPROACH

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ABSTRACT

The aim of the present study to evaluate the phytochemical screening, preparation of Anti-Dandruff shampoo from poly herbal formulation and its antioxidant, antimicrobial and anti-inflammatory activity of Aqueous extract of different parts of medicinal plants. The aim of the recent work was to develop the polyherbal antidandruff shampoo and evaluate the formulated shampoos for physical parameters. Shampoos are the products which are used for the removal of the surface grease and dirt from the hair scalp and shaft. The present study focused on the preparation of a polyherbal anti-dandruff shampoo using selected medicinal plants known for their beneficial effects on hair and scalp health. The plants used in this formulation *included Azadirachta indica, Ocimum tenuiflorum, Eclipta prostrata, Phyllanthus emblica, Hibiscus rosa-sinensis, and Sapindus mukorossi*. These plants were selected based on their traditional use in herbal medicine and their reported biological activities such as antimicrobial, antioxidant, and anti-inflammatory properties. Several evaluation tests were performed to assess the quality and effectiveness of the prepared herbal shampoo. The preliminary photochemical screening showed that the most of the secondary metabolites mainly found in aqueous extract of polyherbal formulation. Especially bioactive compounds such as Alkaloids, Flavonoids, Phenols, Saponin and Tannin were highly present in the aqueous extract when compared to other solvents like Chloroform, Ethyl acetate, Petroleum ether and Aqueous extract based on the solubility. The physicochemical properties of the shampoo, such as pH and foam stability, were determined. The pH of the shampoo was found to be within a range that is compatible with the natural pH of the scalp. Maintaining an appropriate pH is important for preventing scalp irritation and maintaining hair health. The results indicated that the polyherbal formulation possessed significant antioxidant activity due to the higher free radical scavenging activity which may help protect scalp tissues from oxidative stress and support healthy hair growth. Antimicrobial activity was another important parameter evaluated in the study. The antimicrobial assay study revealed that the aqueous extract of different part of polyherbal shampoo showed potential antibacterial activity against *Staphylococcus, Streptococcus pyogens* species were selected viz., bacterial cultures in a maximum concentration based on concentration dependent manner and also antifungal activity against *candida albicans*. The plant extracts used in the formulation are known to possess natural antimicrobial compounds that inhibit the growth of microorganisms responsible for scalp infections and dandruff. The results suggested that the polyherbal shampoo formulation exhibited antimicrobial activity, which contributes to its anti-dandruff effect. The outcome of the present studies revealed that polyherbal shampoos can be prepared easily from the natural plant sources having antimicrobial properties and herbal antidandruff shampoos are better alternatives than synthetic shampoos.

KEYWORDS: Polyherbal Shampoo, Anti-oxidant Assay, DPPH, Anti-microbial assay, Anti-Inflammatory activity.

INTRODUCTION

Medicinal plants are admired in the traditional medicine systems of many countries because of the huge variety of bioactive compounds and therapeutic efficacy.[1] Flavonoids, phenolic compounds, glycoside, and tannin are antimicrobial, antioxidant, and anti-inflammatory active compounds found in plants and could be used in the treatment of scalp infections and dandruff. Especially, flavonoids have been reported to have very high biological activity due to their capacity to engage cellular activities and stop the growth of microbes.[2] Scalp and hair disorders, particularly dandruff, pose a significant challenge worldwide, affecting individual' hygiene and selfesteem. While synthetic shampoos are generally used for treatment, concerns about their side effects have increased interest in herbal formulations.[3] Natural medicinal plants are known for their bioactive compounds, offer promising alternatives due to their antimicrobial and therapeutic properties.

Numerous plant-derived active compounds have been traditionally used in overcoming dandruff and hair disorders. Bioactive constituents like saponins, flavonoids, tannins, terpenoids, alkaloids, and essential oils that contribute to their various treatment. Acacia concinna (Shikakai), Sapindus mukorossi (Reetha), and Phyllanthus emblica (Amla) have been thoroughly documented for their cleansing, antifungal, and scalp-nourishing properties.[4-6] Likewise, Reetha exhibits natural foaming and antimicrobial properties, offering a non-toxic alternative to synthetic surfactants. Its phytochemical profile includes triterpenoids and saponins that ensure gentle cleansing and inhibit microbial growth on the scalp.[7]

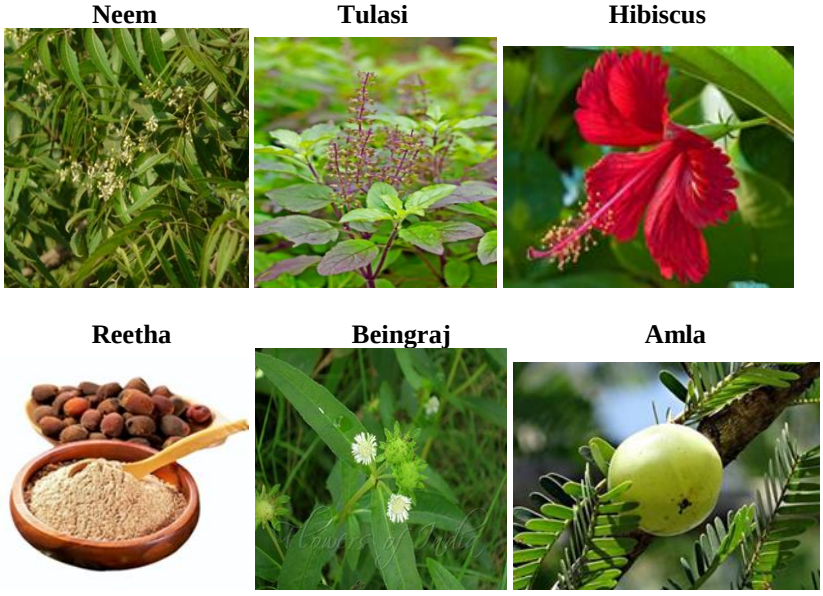
Amla, a potent antioxidant and anti-inflammatory agent, is admired in Ayurveda for its hair-rejuvenating effects. Its high vitamin C content enhances the collagen production, strengthens hair follicles, and prevents hair loss and premature greying. It also aids in decreasing oxidative stress, which is a contributing factor in scalp inflammation and consecutive dandruff formation [8] Thus, this research was conducted to prepare a natural poly herbal shampoo based on the preference of herbal plants and to determine its physicochemical characteristics and antifungal effects against microorganisms that cause dandruff.[9]

METHODS AND MATERIALS

Collection of Plant Materials

The plant materials used in this study were Neem, Tulsi, Bhringraj, Amla, Hibiscus, and Reetha. The plants include *Azadirachta indica*, *Ocimum tenuiflorum*, *Eclipta prostrata*, *Phyllanthus emblica*, *Hibiscus rosa-sinensis*, and *Sapindus mukorossi*.

These plant materials were collected from local herbal markets and gardens. The collected samples were washed with clean water to remove dust and impurities and then dried under shade at room temperature.



Preparation of Plant Powder

The dried plant materials were ground separately using a grinder to obtain fine powders. The powdered materials were passed through a fine sieve to ensure uniform particle size. The powders were stored in airtight containers until further us.

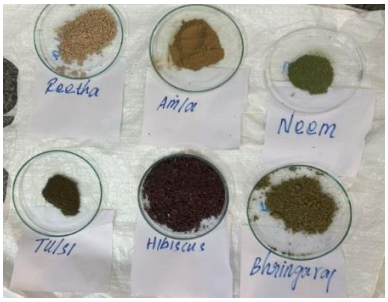


Figure 1: Plant powder.

Preparation of Plant Extract

About 10–20 g of each powdered plant material was mixed with distilled water and heated gently for 30–40 minutes to prepare the aqueous extract. The mixture was allowed to cool and then filtered using filter paper to remove solid residues. The filtrate obtained was used as the plant extract.

Figure 2: Preparation of Polyherbal Shampoo.

The polyherbal shampoo was prepared by mixing the extracts of all selected medicinal plants in suitable proportions. Reetha extract was used as the natural cleansing agent because it contains saponins that produce foam. Neem, tulsi, bhringraj, amla, and hibiscus extracts were added to provide therapeutic properties such as antimicrobial, antioxidant, and anti-inflammatory effects. The mixture was stirred continuously until a uniform solution was obtained.

Filtration

The boiled mixture was cooled and filtered using muslin cloth or filter paper to remove solid particles. The clear filtrate obtained contained the herbal extracts.

Preparation of Shampoo Base

Reetha extract was used as the natural surfactant because it contains saponins that produce foam and cleansing action.

Mixing of Herbal Extracts

The extracts of neem, tulsi, bhringraj, amla, and hibiscus were added to the reetha extract in suitable proportions and stirred continuously to obtain a uniform mixture.

Final Preparation

The mixture was allowed to cool and stored in a clean bottle. The prepared formulation was labeled as polyherbal anti-dandruff shampoo and used for further evaluation tests.

Phytochemical analysis

Phytochemical studies were performed for observing the secondary metabolites present in the different extracts using procedures described by Trease and Evans.[10]

The preliminary qualitative phytochemical studies were performed for testing the different active compounds present in different extracts.

Detection of Alkaloids

Extracts were dissolved individually dilute Hydrochloric acid and filtered.

Mayer's test

Filtrates were treated with Mayer's reagent (Potassium mercuric iodide). Yellow coloured precipitate indicates presence of alkaloids.

Wagner's test

Filtrates were treated with Wagner's reagents . (Iodine and Potassium iodide) for identification of alkaloids.

Detection of Flavonoids

Alkaline reagent test

Extracted were treated with few of drops of sodium hydroxide solution. Formation of intense yellow color, which became colorless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate test

Extracts were treated with few drops of lead acetate

solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Phenols
Ferric chloride test
Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols

Detection of Glycosides
Extracts were hydrolyzed with dil. HCL, and then subjected to test for glycosides.

Modified Borntrager's test
Extracts were treated with ferric chloride and immersed in boiling water bath for about 5 minutes. The mixture was cooled and extracted equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonia layer indicates the presence of anthranilic glycosides.

Legal's test
Extract were treated with sodium nitro pruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

Detection of Saponins
Foam test
0.5 gm of extract was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of Tannins
3-5 ml test solution with few drops of 1ml lead acetate and observed red precipitate was observed and indicates the presence of tannin.

Detection of Steroids
3 ml of test solution and minimum quality of chloroform was added with 1-4 drops of acetic anhydride and one drop of concentrated nitric acid.

Detection of Proteins
Xanthoproteic test
The extracted were treated with few drops of Conc. Nitric acid. Formation of yellow colour indicates the presence of protein.

Detection of Diterpenes
Copper acetate test
Extracts were dissolved in water and trail willy 3-4 days drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

ANTIOXIDANT ACTIVITY
DPPH radical scavenging activity
The free radical scavenging activity of plant extract was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) The scavenging activity for DPPH free radicals was measured according to the procedure described by (Braca *et al.*, 2001). An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.5 to 2.5 µl of plant extract/ascorbic acid at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of DPPH radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes.[11]

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)}}{\text{A518 (control}} \times 100$$

ANTIMICROBIAL ACTIVITY
To perform antimicrobial activity using *Staphylococcus*, *Streptococcus pyogens* species were selected viz., bacterial cultures.

Preparation of the Bacterial Inoculam
Stock cultures were maintained at 4^{o}C on slopes of nutrient agar and potato dextrose agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes 50 ml nutrient broth bacterial cultures were incubated with agitation for 24 hours and 37^{o}C on shaking incubator and fungal cultures were incubated at 27^{o}C for 3-5 days. Each suspension of test organism was subsequently stroke out on nutrient agar media and potato dextrose agar. Bacterial cultures then incubated at 37^{o}C for 24 hours and fungal incubated at 27^{o}C for 3-5 days. These stock cultures were kept at 4^{o}C. For use in experiments, a loop of each test organism was transferred into 50 ml nutrient broth and incubated separately at 37^{o}C for 18.

Well Diffusion method
The antibacterial activity and antifungal activity of crude extract extracts was determined by Well Diffusion method (Bauer *et al*., 1996) ... The 25-100 µl of Plant extract was poured into the wells. After that, the plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. The tested cell suspension was spread on muller hintonagar plate and potato dextrose agar. well were put into the agar medium using sterile forceps. plant extract were poured on to wells. Then plates were incubated at 37°c for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm.[12]

ANTI-INFLAMMATORY ACTIVITY
Principle
The albumin denaturation method for analyzing anti-inflammatory activity is based on the principle that inflammatory agents cause the denaturation (unfolding) of proteins, like albumin, which can be prevented or

reduced by anti-inflammatory substances. In this method, albumin is exposed to heat or chemicals that induce denaturation, and the test sample (suspected to have anti-inflammatory properties) is added. If the sample prevents or reduces the denaturation, it demonstrates anti-inflammatory activity. The extent of inhibition is measured spectrophotometric ally, with a higher percentage of inhibition indicating stronger anti-inflammatory potential.

Procedure

The albumin denaturation method was performed to analyse the anti-inflammatory property of plant extracts. 1ml of herbal formulations extracts were taken in test tubes. To the tubes, 3 mL of PBS and 1mL of egg albumin are added and mixed and incubated at 37°C for 20min. Denaturation was induced by increasing the temperature to 90°C for 2-3 min. Absorbance was taken at 660nm after cooling down the mixture. As a positive control, diclofenac was utilized, while distilled water serves as the negative control.[13] The percentage of denaturation inhibition is calculated by.

Ac-As/ Ac× 100

Evaluation parameters of Shampoo

a. Determination of pH

The pH of the cream can be measured on a standard digital pH meter at room temperature by taking adequate amount of the formulation diluted with a suitable solvent in a suitable beaker.[14]

b. Physical appearance and consistency

Separability the physical appearance of the cream can be observed by its colour, roughness and graded. The color and odor of the newly formulated shampoo were assessed through visual inspection. The consistency of the formulated shampoo was evaluated manually by taking a small amount and rubbing it between the fingers to determine its texture and uniformity.[15]

c. Determines the Percentage of Solid Content. A 4 g shampoo was placed to a clean, dry evaporating dish, and the combined weight of the dish and shampoo was measured. The dish was then cooked on a warm-plate until the liquid components had fully evaporated. After drying, the residual solid residue was weighed, and the percentage of solid material was determined.[16]

d. Viscosity

Viscosity of formulated creams can be determined by using Brookfield Viscometer e. Homogeneity: The formulation was tested for the homogeneity by visual appearance and by touch.

e. Skin irritation test

Mark an area of 1sq.cm on the left-hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, enema was checked, if any, for regular intervals upto 24hrs and reported. Evaluation Parameters and Observation.

RESULTS AND DISCUSSION

PHYTOCHEMICAL ANALYSIS: Table -1

PHYTOCHEMICALS	Herbal extract
Alkaloid	+++
Flavanoid	+++
Saponin	+++
Tannin	+++
Reducing sugar	+++
Phenol	+++
Steroid	++
Phytosterol	++
Protein	++

ANTI-OXIDANT ACTIVITY

DPPH radical scavenging activity: **Table – 2**

Concentration	Standard (Ascorbic acid)	*Sample*
10 µl	15	17.47 %
20 µl	38	46.11 %
30 µl	56	52.00 %
40 µl	73	65.65 %
50 µl	88	86.00 %

ANTI- MICROBIAL ASSAY

Well diffusion method: Table -3

Organisms Concentration	*E. Coli*	*S. aureus*	*Candida albicans*
25 µl	1.5 mm	1.0 mm	0.5 mm
50 µl	2.0 mm	2.0 mm	1.0 mm
75 µl	3.0 mm	2.5 mm	2.0 mm
100 µl	5.0 mm	4.0 mm	3.0 mm
Standard	5.0 mm	5.0 mm	5.0 mm

ANTIMICROBIAL ASSY OF POLYHERBAL SHAMPOO AGAINST BACTERIA & FUNGI

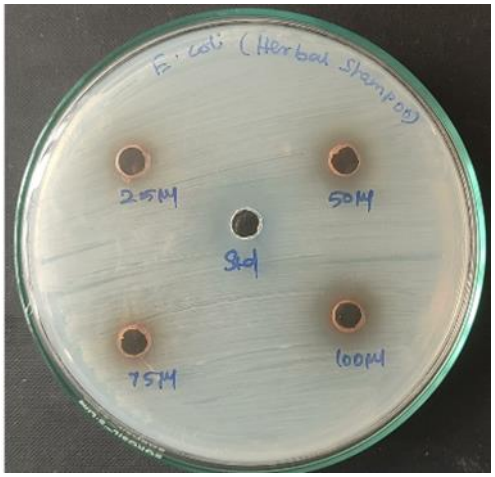


Figure-3 *E. coli* – Bacteria.

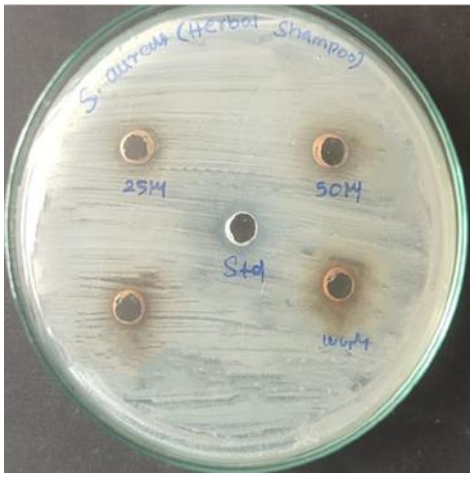


Figure-4 *S. aureus*- Bacteria.

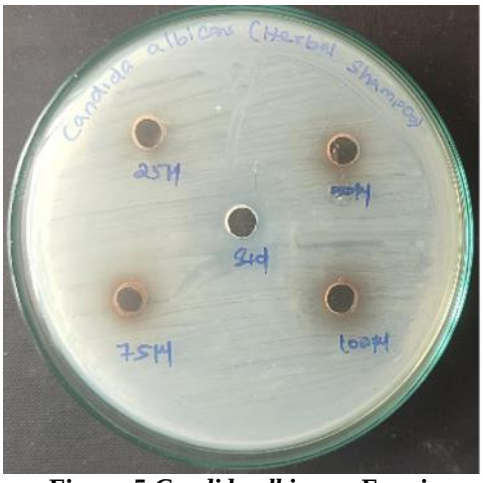


Figure-5 *Candida albicans*- Fungi.

ANTI- INFLAMMATORY ACTIVITY

Inhibition of protein denaturation: Table -4

CONCENTRATION	% INHIBITION OF STANDARD(Aspirin)	% INHIBITION OF Sample
10 µl	33	15.00 %
20 µl	43	29.00 %
30 µl	58	41.00 %
40 µl	79	58.80 %
50 µl	88	63.50 %

SENSORY EVALUTION OF SHAMPOO: Table -5

S.no	Evaluation Parameters	Observations
1	Physical Apperance	Smooth in texture
2	Colour	Dark Brown
3	Odor	Good smell
4	Washability	Easily washable
5	PH test	6.8
6	Consistency	Semi Liquid
7	Smoothness	After applying skin becoming smooth

PATCH TEST: Table -6

1.	Swelling	Nil
2.	Redness	Nil
3.	Irritation	Nil

DISCUSSION

The present study focused on the preparation of a polyherbal anti-dandruff shampoo using selected medicinal plants known for their beneficial effects on hair and scalp health. The plants used in this formulation included *Azadirachta indica, Ocimum tenuiflorum, Eclipta prostrata, Phyllanthus emblica, Hibiscus rosa-sinensis, and Sapindus mukorossi*. These plants were selected based on their traditional use in herbal medicine and their reported biological activities such as antimicrobial, antioxidant, and anti-inflammatory properties.

Dandruff is one of the most common scalp disorders affecting a large number of individuals worldwide. It is characterized by the excessive shedding of dead skin cells from the scalp and is often associated with itching and irritation. The condition is commonly linked with the growth of certain microorganisms on the scalp, particularly the yeast Malassezia. These microorganisms break down scalp oils and produce irritating substances that lead to inflammation and flaking of the scalp. Therefore, the development of formulations with antimicrobial and anti-inflammatory properties is important for the effective management of dandruff.

The use of herbal ingredients in cosmetic formulations has gained significant attention in recent years due to their safety and therapeutic benefits. Unlike synthetic chemicals, herbal ingredients are generally considered mild and less harmful to the scalp and hair. In the present study, a polyherbal approach was adopted in which several plant extracts were combined in a single formulation. The concept of polyherbal formulation is widely used in traditional medicine systems, where the combination of multiple herbs produces a synergistic therapeutic effect.

The physicochemical evaluation of the prepared herbal shampoo indicated that the formulation possessed suitable properties for hair cleansing. The pH of the shampoo was found to be within a range that is compatible with the natural pH of the scalp. Maintaining an appropriate pH is important for preventing scalp irritation and maintaining hair health.

Foam formation and foam stability are important characteristics of shampoo formulations because consumers often associate foam with cleansing efficiency. The presence of natural saponins from reetha contributed to good foam formation in the prepared herbal shampoo. The foam produced was stable and persisted for a reasonable period of time, indicating effective cleansing properties.

The antioxidant activity of the polyherbal formulation was also evaluated. The results indicated that the plant extracts exhibited significant free radical scavenging activity. This antioxidant activity can be attributed to the presence of phenolic compounds and flavonoids in the plant extracts. These compounds help protect scalp tissues from oxidative stress and support healthy hair growth.

The antimicrobial activity of the herbal shampoo was another important aspect evaluated in the study. The plant extracts used in the formulation possess natural antimicrobial properties that help inhibit the growth of pathogenic microorganisms. This antimicrobial effect plays a crucial role in controlling dandruff and preventing scalp infections.

The anti-inflammatory activity of the herbal formulation also contributes to its effectiveness in managing dandruff. Inflammation of the scalp is one of the major factors responsible for itching and irritation associated with dandruff. Plant extracts such as neem and tulsi contain bioactive compounds that help reduce inflammation and soothe the scalp.

Safety evaluation of the prepared herbal shampoo was performed through patch testing. The results indicated that the formulation did not produce any signs of irritation, redness, or swelling on the skin. This suggests that the herbal shampoo is safe for topical application and suitable for regular us the results of the present study indicate that the polyherbal shampoo formulation possesses several beneficial properties including cleansing ability and antimicrobial activity.

CONCLUSION

Several evaluation tests were performed to assess the quality and effectiveness of the prepared herbal shampoo. The physicochemical properties of the shampoo, such as pH and foam stability, were determined. The pH of the shampoo was found to be within a range that is compatible with the natural pH of the scalp. Maintaining an appropriate pH is important for preventing scalp irritation and maintaining hair health.

Foam formation is an important property of shampoo formulations because it indicates the cleansing ability of the product. The herbal shampoo prepared in this study showed good foam formation due to the presence of natural saponins in reetha. The foam produced was stable and effective in removing impurities from the hair.

The antioxidant activity of the plant extracts was also evaluated. Antioxidants are important because they help neutralize free radicals that can damage scalp cells and hair follicles. The results indicated that the polyherbal formulation possessed significant antioxidant activity, which may help protect scalp tissues from oxidative stress and support healthy hair growth.

Antimicrobial activity was another important parameter evaluated in the study. The plant extracts used in the formulation are known to possess natural antimicrobial compounds that inhibit the growth of microorganisms responsible for scalp infections and dandruff. The results suggested that the polyherbal shampoo formulation exhibited antimicrobial activity, which contributes to its anti-dandruff effect. Overall, the study demonstrated that the prepared polyherbal shampoo possesses beneficial properties such as cleansing ability, antioxidant activity, antimicrobial activity, and anti-inflammatory effects. These properties make the formulation suitable for controlling dandruff and maintaining scalp health.

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CONFLICT OF INTEREST

None declared.

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