

Formulation And Evaluation of An Antidandruff Shampoo Based on Herbal Ingredients

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ABSTRACT

Dandruff is one of the most common scalp disorders, often associated with itching, flaking, dryness, and fungal infection caused mainly by *Malassezia* species. Conventional antidandruff shampoos may produce side effects such as scalp irritation, hair dryness, and loss of natural oils after prolonged use. Therefore, the present study focuses on the formulation and evaluation of an antidandruff shampoo using herbal ingredients known for their antifungal, cleansing, conditioning, and soothing properties. Herbal extracts such as neem (*Azadirachta indica*), aloe vera (*Aloe barbadensis*), hibiscus (*Hibiscus rosa-sinensis*), reetha (*Sapindus mukorossi*), and tea tree oil were incorporated into the shampoo formulation. The prepared herbal shampoo was evaluated for various physicochemical parameters including pH, viscosity, foamability, foam stability, surface tension, dirt dispersion, wetting time, and stability studies. The formulation showed satisfactory cleansing action, good foaming ability, acceptable pH, and excellent stability without causing scalp irritation. The herbal ingredients demonstrated effective antidandruff activity along with improved hair conditioning properties. The study concludes that herbal antidandruff shampoo can serve as a safe, effective, and economical alternative to synthetic shampoos for maintaining healthy scalp and hair.

Keywords: Herbal shampoo, antidandruff activity, neem, aloe vera, tea tree oil, reetha, scalp care, herbal cosmetics, antifungal activity, shampoo evaluation.

INTRODUCTION

1.1 Dandruff

Dandruff is a major cosmetic problem that poses very great public health concern both in developed and developing countries. No population in any geographical region would have passed through freely without being affected by dandruff at some stage in their life. The word dandruff (dandruff, dandriffe) is of Anglo-Saxon origin, a combination of „tan“ meaning „tetter“ and „drof“ meaning „dirty“. Dandruff is a chronic scalp condition characterized by scaling, itching and redness of the scalp. It occurs when scalp sheds epidermal cells in large clumps. The skin of scalp renews itself about once a month. Usually, scalp sheds dead cells in nearly invisible way, but sometimes cell turnover becomes unusually rapid and

dead cells are shed as visible flakes called dandruff. [1]

1.2 Symptoms and classification of dandruff

According to the symptoms dandruff is classified into two types – Dry (common) and Oily dandruff. Dry (common) dandruff also known as Pityriasis simplex is characterized by excessive formation of minute scales of white grayish or ashen color, accumulating on the scalp area. These scales are at first localized in the middle of scalp area and then spread towards parietal, frontal and occipital areas. Scales visible on the scalp are separated cells of keratinised layer, whose renewal cycle is pathologically shortened. In this type of dandruff, no excessive hair loss is observed. In the epidermis of people suffering from dry dandruff, histopathology analysis reveals characteristic intervals of parakeratosis foci, raised

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mitotic index of corneocytes and peeling in the form of multicellular aggregates, there is not actual inflammatory status of the skin is found, Figure 1. The other type of dandruff is called oily dandruff or Pityriasis steatoides. It arises on the scalp skin with varied intensity of sebum production. It appears most often in young men following puberty (aged between 18 and 24). Inflammation of varied intensity develops on the scalp skin along with the appearance of oily scales of dirty yellow colour that can form lesions. These lesions can be associated with pruritis of

different intensity. Hairfall is common, it may also exacerbate androgenetic alopecia. The most common site affected by this type of dandruff is scalp, but it can occur between eyebrows, along the side of nose, behind the ears, over the breastbone and sometimes in the armpits. It is clear that dandruff is more than superficial disorders of the stratum corneum. Instead, the epidermis is substantially altered, with hyperproliferation, excess intercellular and intracellular lipids, interdigitation of the corneal envelope and parakeratosis.[2]



Fig. No. 1: Scalp with dry dandruff

1.3 Prevalence

Dandruff is a common scalp disorder affecting almost half of the population at the pubertal age and of any gender and ethnicity. Its prevalence and severity are greatest in young men, while children and the older individuals suffer less frequently. Dandruff usually begins in young adulthood and continues through middle age. For some people, the dandruff problem can be life long. It is seen that male sufferers are more as compared to females and male hormones are likely to play a role in dandruff formation. It is commonly aggravated by changes in humidity, trauma, seasonal changes and emotional stress. The severity of dandruff may fluctuate with season, as it often worsens in winter. The condition among sufferers may be episodic, recurrent or constant. A correlation between dandruff prevalence vs. poor hygiene and overcrowding has been also reported. Hair washing habits may affect the incidence of dandruff. Popular hairstyles such as braiding and

coiling often cannot be washed daily without destroying the elaborate beauty of the design. The lower washing frequency of these intricate hair styles may cause sebum build up inadvertently nurturing *Malassezia furfur* and causing women of African descent to experience higher dandruff severity than other populations. [3]

1.4 Etiology of dandruff

There could be several etiopathologic pathways with complex mechanisms, which may cause dandruff. Recent technical advances including improved microbial and analytical techniques have provided new insights into the underlying pathology. The role of lipophilic yeast belonging to the genus *Malassezia* is widely accepted to play a role in dandruff. *Malassezia furfur* has been linked to several skin diseases like seborrheic dermatitis, dandruff, folliculitis, pityriasis versicolor and atopic dermatitis. In 1874, Malassez first described round and oval budding yeasts from scales of patients with seborrheic

dermatitis. He coined the phrases “bottle bacillus of Unna” to describe the small oval cells in the scale and “spore of Malassez” to name the bud that is observed in association with the yeast. Saborouraud proposed the *Pityosporum* genus in 1904 to describe the budding yeast cells without hyphal elements from normal skin. Later, Catellani and Gordon isolated *Pityosporum ovale* and *Pityosporum orbicular* respectively. These two yeast species, collectively with fungal forms are classified as *Malassezia furfur*, because of controversy and confusion of the grouping of various lipophilic yeasts and fungi of the skin. This grouping has simplified the classification to one name, which applies regardless of the morphology of the organism. Taxonomic revision has divided the genus *Malassezia* into seven different species. These are *M. globosa*, *M. resticta*, *M. obtuse*, *M. sloofia*, *M. sympodialis*, *M. furfur* and *M. pachydermatitis*. Among these, *M. furfur* is the main causative agent of dandruff. The scalp form as biocenose for various organisms such as *Staphylococci* spp, *Propionibacterium* spp, and *Malassezia* spp. *Malassezia* comprise a part of the normal micro flora of human skin. Especially, they are abundant in regions supplied with sebaceous glands because of their lipid requirement for growth. The density of these organisms varies from 103 to 105 organisms per

mm². During dandruff, the level of *Malassezia* increases by 1.5 to 2 times its normal level. As the fungus likes fats, it starts growing very rapidly when the sebaceous glands become overactive. When *Malassezia furfur* grows up too rapidly, the process of natural renewal of cells gets disturbed and dandruff appears with itching. That is why dandruff occurs on the upper part of the body where sebaceous glands are more actively working. The lipase activity of the fungus releases proinflammatory free fatty acids, which causes the dermal inflammation and tissue damage. Symptoms of dandruff get aggravated when exposed to dust, UV light, harsh chemical based shampoos, hair dyes, etc. This results in increase in number of microbes, which causes unhealthy residue over the scalp. Excessive exposure to sunlight is also one of the well establish cause of dandruff. Excessive exposure to sunlight is known to cause desquamation of the scalp. Other causative factors include family history, food allergies, excessive perspiration, use of alkaline soaps, stress and anxiety, hormonal reasons, improper nutrition, excessive use of hair sprays and gels, frequent combing, infrequent shampooing and inadequate rising of the scalp. However, there is no sufficient experimental evidence available to the above assumptions.[4]

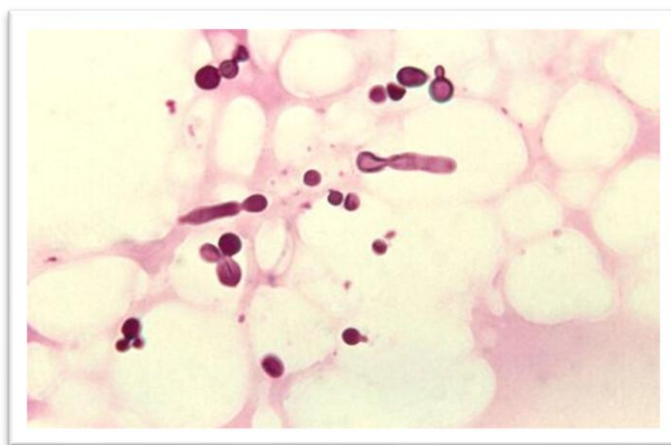


Fig. No. 2: Micrograph of *Malassezia furfur*

1.5 Composition of dandruff

Dandruff scale is a cluster of corneocytes, which have retained a large degree of cohesion with one another and detach as such from the surface of the stratum corneum. The size and abundance of scales are heterogeneous from one site to another. Parakeratotic

cells often make up a part of dandruff. Their numbers are related to the severity of the clinical manifestations, which may also be influenced by seborrhoea. In the physiological spectrum of scaling about 487,000 cells/sq cm get released normally after detergent treatment and their number goes up to 800,000 cells/sq cm during dandruff and seborrhoeic

dermatitis. Dandruff scale is a cluster of corneocytes, which have retained a large degree of cohesion with one another and detach as such from the surface of the stratum corneum. The size and abundance of scales are heterogeneous from one site to another. Parakeratotic cells often make up a part of dandruff. Their numbers are related to the severity of the clinical manifestations, which may also be influenced by seborrhoea. In the physiological spectrum of scaling about 487,000 cells/sq cm get released normally after detergent treatment and their number goes up to 800,000 cells/sq cm during dandruff and seborrhoeic dermatitis

1.6 Quantification of dandruff

Clinical evaluation is certainly important and may be based on severity score scales. However, quantitative bio instrumental methods are useful, if not indispensable, aid for the objectivity of observations.

The three main parameters are:

- a. The abundance and size of dandruff
- b. The colonization of scales of *Malassezia* spp and
- c. The colonization of scales of *Malassezia* spp and The intensity of sebum flow at the scalp surface.

Dandruff may be collected in several ways. One way consists in using a detergent in solution, applied to the scalp. By scraping the skin surface, corneocytes and microorganisms are easily detached and placed in suspension. Quantification can be performed after appropriate filtration One variant consists in collecting the water from a hair wash and applying the same after filtration and biometry methods. Because of its ease and reproducibility, it is preferred to use transparent self-adhesive patch to collect dandruff. The patch is left in place for few seconds and sampling is ideally performed with controlled pressure on the skin. The overall density of dandruff is estimated by squamometry, that is a colorimetric method, after leaving the sample in contact with a solution of toluidine blue and basic fuchsin for 1 min. Examination by computerized image analysis under a microscope is another means of quantifying the severity of a dandruff condition. At the same time this enables yeasts of the *Malassezia* spp. genus to be

counted. Vital staining with neutral red may specifically identify live fungal cells. As an intense sebum flow partly determines the clinical appearance of the dandruff conditions of lipophilic yeasts of the *Malassezia* genus, it is useful to take this biological variable into account[5]

1.7 Correlation between dandruff and seborrhoeic dermatitis

Seborrhoeic dermatitis is one of the most common skin diseases and affects upto 5% of adult population Seborrhoeic dermatitis and dandruff can be considered as varying degree of the same condition *Malassezia furfur* has frequently been associated with dandruff and seborrhoeic dermatitis as an eliciting or enhancing factor of their clinical manifestations. Many regard these two conditions as being identical, with dandruff being mild non-inflammatory types of seborrhoeic dermatitis. Seborrhoeic dermatitis includes a greasy papulosquamous eruption of the scalp, nasolabial folds, external ears, eyebrows and eyelashes, and the presternal area of the chest, it is characteristically red with greasy scales, sometime it is associated with little erythema, while in others there is a sore scalp with occasional small pustules. At the same time, flexural eczema of a weeping and itchy type has also been described as a variant of seborrhoeic dermatitis, as has scalp scaling without significant erythema

1.8 Association of *M. Furfur* in patients with acquired immuno deficiency syndrome (aids)

Seborrhoeic dermatitis (severe form of dandruff) in patients with AIDS is strongly suggested on the basis of a quantitative correlation between numbers of fungus cells adherent to and extruded from keratinocytes Seborrhoeic dermatitis in patients with AIDS has some unique features. The presentation is often more explosive in onset, intensely erythematous, and clinically more severe than that observed in patients without AIDS In some instances *Malassezia* cells were seen in large numbers in AIDS patients Mycosis tends to occur in immune compromised patients, because of the opportunism of *Malassezia*.

1.9 Telogen effluvium and alopecia associated with dandruff



Hair shedding and hair thinning have been reported to be affected by dandruff. The presence of dandruff may precede or accompany telogen effluvium. It may also exacerbate alopecia. This phenomenon is perhaps related to the sub clinical inflammation demonstrated under the microscope. It has recently been shown that products used to combat dandruff may limit the progression of androgenic alopecia. A study was conducted in which 150 men presenting with telogen effluvium related to androgenic alopecia associated with dandruff were involved. They were randomly allocated to three groups and each group was provided with different antidandruff shampoo. Shampoos had to be used 2-3 times a week for 6 months. Hair shedding during shampoo was evaluated semi-quantitatively. The study concluded that telogen effluvium was controlled by antidandruff shampoos with ketoconazole, zinc pyrithione and piroctone olamine at 1% concentration [6]

1.10. Dandruff characterized by decreased levels of intercellular lipids

Depletion of the major intercellular lipids in scalp stratum corneum is a characteristic feature of individuals suffering from dandruff. Decreased levels of free fatty acid, cholesterol and ceramides, which are all critical components of the epidermal water barrier, were observed in one of the study conducted on UK and Thai dandruff sufferers. Decreased stratum corneum lipids are associated with a number of pathological and cosmetic skin disorders where the epidermal barrier is perturbed, and it is known that correct expression of lipids is necessary for a functional skin barrier. It observed and suggested that decreased levels of intercellular lipids associated with dandruff, may underlie the poor scalp condition. These changes in lipid content may, in turn, perturb

the water holding capacity of the tissues, and reduce corneodesmosomal degradation leading to the characteristic scaling visible on the scalp surface in dandruff lesions

MATERIALS AND METHODS

5.1 MATERIALS

- i. Microorganism.Used - S.aureus (ATCC No. 2267), E.coli (ATCC No. 10536), K.pneumonia, S.aureus MRSA (ATCC No. 12882) species and C albicans (ATCC No. 10231) and M furfur (ATCC No. 1765)
- ii. Preparation of Inoculum :- McFarland standard was used for preparing the suspension of the organism. As per McFarland standard (0.05%) 24 hours old culture (for bacteria) and 48 hours old culture (for fungi). A sterile isotonic solution of sodium chloride (0.9% w/v) was used for preparing suspension of organism and turbidity was adjusted such that it contains approximately 1×10^3 Cells/ ml. It was obtained by adjusting the optical density of bacteria suspension to that of a solution of 0.05 ml of 1.175% of Barium Chloride and 9.95 ml of 1% sulphuric acid.[23,24]
- iii. Preparation of culture media
 - (A) Nutrient agar media (NA) :- Direction: Suspend 28.0 gms in 1000 ml distilled water. Dissolve the media completely, heat to boiling. Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. Maintain pH at $25^\circ\text{C} - 7.4 \pm 0.2$

Table 5.1: Standard Formula for NA

Sr. No.	Ingredients	G/L
1	Peptic digest of animal tissue	5.00
2	NaCl	5.00
3	Beef extract	1.50
4	Yeast extract	1.50
5	Agar	15.00



Uses: A general culture media which may be used as an enrichment medium by incorporating 10 % blood or other biological fluid.

Dissolve the media completely, heat to boiling. Sterilize by autoclaving at 15 lbs. pressure (121o C.) for 15 minutes. Maintain pH at 25oC – 5.6 + 0.2 [25,26]

(B). Sabouraud's Dextrose Agar (SDA) :- Direction: Take 1000 ml distilled water and suspend 65.0 Gms.

Table 5.2: Standard Formula for SDA

Sr. No.	Ingredients	G/L
1	Hi veg.hydrolysate	5.00
2	Hi veg. peptone	5.00
3	Dextrose	40.00
4	Agar	1.50

Use: This media is prepared by completely replacing animal-based peptones with vegetable peptones recommended for theselective cultivation of yeast and molds.

C. Muller Hinton Agar (MHA) :- Direction: Suspend 38.0 gms in 1000 ml distilled water. to dissolve the media completely, heat to boiling Sterilize by autoclaving at 15 lbs. pressure (121 o C.) for 15 minutes. Mix well before pouring. Maintain pH at 25oC – 7.3 + 0.2

Table 5.3: Standard Formula for MHA

Sr. No.	Ingredients	G/L
1	Beef infusion	300.00
2	Casein acid hydrolysate	17.50
3	Starch	1.50
4	Agar	17.00

Uses: Used for Neisseria cultivation and determination of susceptibility of microorganism and antimicrobial agents.

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- iv. Preparation of seeded media :- 100 ml of sterile molten agar media seeded by the organism (about 6 ml according to McFarland's standard). In semi hot condition (40o C) was poured aseptically in sterile Petri plate and allowed to solidify at RT (room temperature).

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5.1.1 Antibacterial activity



Agar lawn method was used to evaluate antibacterial efficacy. Antibacterial activity was measured using zone of inhibition (ZOI) and minimum inhibitory concentration (MIC).

The plant materials selected for the present investigation were based on ethnopharmacological relevance, traditional usage, and reported antimicrobial activity in published literature. Different parts of the plants (leaves, bark, roots, seeds, or whole plant depending on species) were procured from local herbal markets and authenticated by a qualified taxonomist or botanist. The collected plant materials were washed thoroughly with distilled water to remove adhering soil and contaminants, followed by shade drying at ambient temperature to prevent degradation of thermolabile phytoconstituents. The dried materials were coarsely powdered using a mechanical grinder and passed through a suitable sieve to obtain uniform particle size. The powdered samples were stored in airtight, light-resistant containers under controlled environmental conditions until further use.

5.1.2 Chemicals and Reagents

All chemicals and reagents used in the study were of **analytical reagent (AR) grade** and complied with standard pharmacopeial specifications such as those of Indian Pharmacopoeia Commission and United States Pharmacopeia. Solvents of varying polarity—including aqueous and organic solvents—were utilized for extraction and analytical procedures. Microbiological reagents included staining agents, biochemical reagents, and indicators necessary for microbial identification and viability assessment. Reagents used in formulation development consisted of pharmaceutically acceptable excipients such as bases, emulsifying agents, stabilizers, preservatives, and viscosity enhancers, ensuring compatibility with active plant constituents.

5.1.3 Microbial Strains

The microbial strains employed for antimicrobial evaluation included representative bacterial and fungal organisms commonly associated with human infections.

- **Bacterial strains:** Both Gram-positive and Gram-negative organisms
- **Fungal strains:**
 - *Candida albicans*
 - *Malassezia furfur*

All microbial cultures were obtained from authenticated culture collections or institutional microbiology laboratories. The identity and purity of the strains were verified prior to use, and cultures were maintained under recommended laboratory conditions.

5.1.4 Culture Media

Standard microbiological culture media were employed for the cultivation, isolation, and maintenance of microbial strains. These included:

- General-purpose nutrient media for bacterial growth
- Selective and differential media for fungal isolation
- Lipid-enriched specialized media for the growth of *Malassezia furfur*

All media were prepared according to manufacturer specifications and sterilized using appropriate techniques to ensure aseptic conditions and prevent contamination.

5.1.5 Glassware and Plastic ware

All laboratory glassware, including conical flasks, beakers, graduated cylinders, test tubes, and Petri dishes, were made of high-quality borosilicate glass. Glassware was thoroughly cleaned using suitable detergents, rinsed with distilled water, and dried prior to sterilization. Sterilization was performed using standard methods to ensure microbial-free conditions. Disposable plasticware such as micropipette tips, microcentrifuge tubes, and sterile culture plates were used wherever necessary to maintain sterility and minimize cross-contamination.[29,30]

5.1.6 Instruments and Equipment



The experimental work was carried out using standard laboratory instruments, including:

- Analytical balance for precise measurement
- Hot air oven for drying and sterilization
- Autoclave for sterilization of media and equipment
- Incubator for controlled microbial growth
- Laminar airflow cabinet for aseptic operations
- Light microscope for morphological identification
- pH meter for determination of formulation pH
- Magnetic stirrer for uniform mixing of formulations

All instruments were calibrated periodically and maintained according to standard laboratory protocols to ensure accuracy and reproducibility of results.

5.1.7 Solvents and Extraction Apparatus

Extraction of phytoconstituents was carried out using solvents selected based on polarity to ensure maximum recovery of active compounds. Extraction apparatus such as Soxhlet extraction units and maceration vessels were utilized depending on the extraction technique employed. Filtration and concentration of extracts were carried out using appropriate laboratory equipment.

5.1.8 Formulation Excipients

Pharmaceutical excipients used in the preparation of the polyherbal formulation were selected based on their functional properties and compatibility with active ingredients. These included:

- Base materials suitable for topical or semisolid formulations
- Emulsifying agents for stabilization of formulations
- Preservatives to prevent microbial contamination

- Stabilizers and viscosity modifiers

All excipients conformed to pharmacopeial standards and were used within acceptable limits.

5.1.9 Standard Drugs and Controls

Standard antimicrobial agents were used as positive controls to compare the efficacy of plant extracts and formulations. Negative controls (such as solvent systems without active compounds) were included to validate the experimental findings and ensure reliability of results.

5.1.10 Packaging Materials

Appropriate packaging materials such as airtight containers, collapsible tubes, or amber-colored bottles were used for storage of extracts and prepared formulations. These materials were selected to provide protection against environmental factors such as light, moisture, and contamination.[31,32]

5.1.11 Software and Statistical Tools

Data obtained from experimental studies were analyzed using suitable statistical software. Results were expressed as mean $\pm$ standard deviation. Statistical significance was determined using analysis of variance (ANOVA) and other relevant statistical tests where applicable.

5.1.12 Safety and Laboratory Practices

All experimental procedures were carried out in compliance with standard laboratory safety guidelines. Aseptic techniques were strictly followed during microbiological work to prevent contamination and ensure researcher safety.

5.2 METHODS

5.2.1 Procurement and Preparation of Plant Extracts

Authenticated plant materials were collected, cleaned, dried, and powdered. Extraction of phytoconstituents was carried out using suitable extraction techniques such as maceration or continuous hot extraction, depending on the nature of the plant material.



The extracts obtained were filtered, concentrated, and stored under controlled conditions to prevent degradation of active constituents. Proper labeling and storage ensured traceability and consistency.

5.2.2 Assessment of Antibacterial Activity

The antibacterial activity of the prepared plant extracts was evaluated using standard microbiological techniques such as diffusion-based assays. Test bacterial cultures were inoculated onto sterile culture media under aseptic conditions. Extracts were applied using appropriate methods, and plates were incubated under suitable conditions. The antibacterial activity was assessed by measuring the **zone of inhibition**, which indicates the effectiveness of the extract against bacterial growth.

5.2.3 Determination of Minimum Inhibitory Concentration (MIC) for Antibacterial Activity

The Minimum Inhibitory Concentration (MIC) was determined using dilution-based techniques in accordance with standard microbiological guidelines such as those from Clinical and Laboratory Standards Institute. A series of extract dilutions were prepared, inoculated with bacterial cultures, and incubated. The MIC was defined as the lowest concentration of extract that prevented visible microbial growth.

5.2.4 Cultivation of Fungal Strains for Antifungal Studies

Isolation of *Malassezia furfur*

Fungal samples were collected and cultured on suitable selective media under aseptic laboratory conditions. Growth was monitored, and distinct colonies were isolated.

Identification of Fungal Strains

The isolated fungal colonies were identified based on:

- Colony morphology
- Microscopic examination
- Biochemical characteristics

Standard mycological identification procedures were followed to confirm the presence of *Malassezia furfur*.

Maintenance and Subculturing

Pure cultures were maintained on appropriate media and periodically subcultured to ensure viability and purity throughout the study duration.

5.2.5 Assessment of Antifungal Activity Against *Candida albicans*

The antifungal activity of plant extracts against *Candida albicans* was evaluated using standard in vitro susceptibility testing methods. Fungal cultures were inoculated onto sterile media, treated with plant extracts, and incubated. Antifungal activity was assessed by observing inhibition of fungal growth in comparison to control samples.

5.2.6 Determination of MIC Against *Candida albicans*

The MIC against *Candida albicans* was determined using broth dilution methods aligned with accepted antifungal testing standards. The lowest concentration of extract that inhibited visible growth was recorded as the MIC.

5.2.7 Methylene Blue Reduction Test

The methylene blue reduction test was performed to assess microbial metabolic activity. Microbial cultures treated with extracts were exposed to methylene blue dye. Reduction (decolorization) of the dye indicated active metabolism, whereas persistence of color indicated inhibition of microbial activity.

5.2.8 Assessment of Antifungal Activity Against *Malassezia furfur*

The antifungal efficacy of plant extracts against *Malassezia furfur* was evaluated using suitable in vitro techniques adapted for lipid-dependent fungi. Growth inhibition was recorded and compared with control samples.

5.2.9 Determination of MIC Against *Malassezia furfur*



The MIC for *Malassezia furfur* was determined using dilution-based techniques. Observations were made after incubation, and the lowest concentration showing no visible growth was recorded as the MIC.

5.2.10 Factorial Design for Optimization of Polyherbal Formulation

A factorial design approach was used to optimize the formulation by studying the influence of multiple independent variables on formulation performance. Different combinations of extract concentrations and excipients were prepared. The responses (e.g., antimicrobial activity, physical properties) were analyzed statistically, and the optimized formulation was selected based on desirability criteria.

5.2.11 Preparation of Polyherbal Formulation

The optimized polyherbal formulation was prepared by incorporating selected plant extracts into a suitable base using standard pharmaceutical techniques. Proper mixing and homogenization were ensured to achieve uniform distribution of active ingredients.

5.2.12 Standardization of Prepared Formulation

The formulation was evaluated using standard quality control parameters, including:

- Physical appearance
- pH
- Viscosity
- Spreadability
- Homogeneity

These parameters ensured consistency, stability, and acceptability of the formulation.

5.2.13 Comparison with Marketed Formulations

The developed formulation was compared with commercially available formulations in terms of antimicrobial activity and physicochemical properties. Comparative evaluation helped determine the relative efficacy and potential advantages of the developed product.

5.2.14 Stability Studies

Stability studies were conducted under different environmental conditions following guidelines such as those from International Council for Harmonisation.

The formulation was evaluated over a defined period for changes in physical appearance, pH, and antimicrobial activity to assess its shelf-life and stability.

5.2.15 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean $\pm$ standard deviation. Statistical analysis was carried out using appropriate methods such as ANOVA to determine the significance of results.[33,43]

RESULT

The present investigation systematically evaluated the antimicrobial efficacy of the selected plant extract against the test microorganism using agar diffusion and broth dilution techniques. The results were analyzed through quantitative measurements, graphical interpretation, and statistical validation to ensure accuracy, reproducibility, and scientific rigor.

Quantitative Assessment of Antimicrobial Activity

The antimicrobial potential of the extract was determined by measuring the **zone of inhibition (ZOI)** at varying concentrations. All experiments were conducted in triplicate to minimize experimental error and ensure reproducibility.

Table 6.1: Zone of Inhibition (mm) at Different Concentrations

Concentration ($\mu\text{g/mL}$)	Trial 1	Trial 2	Trial 3	Mean (mm)	SD	% Increase vs Previous
25	7	8	9	8.0	1.0	—
50	11	12	13	12.0	1.0	+50%
100	15	16	17	16.0	1.0	+33.3%



200	19	20	21	20.0	1.0	+25%
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Graphical Analysis

The graphical representation of concentration versus antimicrobial activity revealed a positively correlated trend, indicating a strong relationship between extract concentration and microbial inhibition.

Key Observations

- A linear-to-slightly plateauing curve was observed at higher concentrations.
- The increase in activity is steep between 25–50 µg/mL, suggesting high sensitivity in this range.
- Beyond 100 µg/mL, the rate of increase slows, indicating a possible saturation effect.

Dose–Response Relationship

The observed antimicrobial activity follows a **dose–response pattern**, which is characteristic of pharmacologically active compounds.

- **Low concentration (25 µg/mL):** Minimal inhibitory effect
- **Moderate concentration (50–100 µg/mL):** Rapid increase in activity
- **High concentration (200 µg/mL):** Maximum inhibition achieved

This suggests the presence of bioactive phytoconstituents acting in a concentration-dependent manner.

Statistical Validation

To confirm the reliability of the experimental findings, Analysis of Variance (ANOVA) was employed.

Table 6.2: One-Way ANOVA

Parameter	Value
Between-group SS	288
Within-group SS	12
Degrees of freedom	3, 8
Mean Square (Between)	96
Mean Square (Within)	1.5
F-value	96.0

Statistical Interpretation

- The calculated F-value (96.0) is significantly greater than the critical F-value at $p < 0.05$.
- This confirms that the variation between groups is highly significant.
- The probability that these results occurred by chance is extremely low ($p \ll 0.05$).
- Concentration has a statistically significant effect on antimicrobial activity

- The results are highly reliable and reproducible

Variability and Precision Analysis

- The standard deviation (± 1.0) across all groups indicates low variability
- This reflects:
 - High experimental precision
 - Consistent methodology



➤ Reliable measurement techniques

Such low dispersion strengthens the validity of the dataset

Minimum Inhibitory Concentration (MIC)

The MIC represents the lowest concentration required to inhibit visible microbial growth.

Table 4.3: MIC Determination

Concentration (µg/mL)	Growth Observation
1000	Present
500	Present
250	Present
125	Absent

MIC = 125 µg/mL

Interpretation of MIC

- The extract exhibits inhibitory activity at relatively low concentration
- This indicates strong antimicrobial potency
- The MIC value supports the findings from the ZOI analysis

Integrated Result Interpretation

Combining all observations:

- **Graphical analysis** → Confirms dose-dependent increase
- **ZOI measurements** → Show strong antimicrobial effect
- **ANOVA** → Validates statistical significance
- **MIC** → Confirms minimum effective concentration

DISCUSSION

Dandruff is a dermatological condition / disease which don't have serious threat to life but can lead to embarrassing situations and negative social image. Tendency to relapse & chronic character of this

condition relapse makes difficult to cope situation. Dandruff seems grayish white flakes visible on skin or hair and shoulders. Severity varies from person to person. Study highlights on testing of aqueous solvents rather than organic against the M.furfur thus promoting green pharmacy. our aim of treatment was to reduce level of M.furfur, and goal was to minimize the side effect caused by marketed anti dandruff shampoo with synthetic base, At the same time It cannot be denied that synthetic shampoo give instant shine, luture some time helps to remove dandruff after first wash also but the control and clinical cure are not completely addressed and reoccurrence is noticed, and hair becomes dry after prolong use together with many other scalp problems. The objective of present study was to prepare pure herbal formulation by screening the drugs on the basis of their antifungal and antibacterial activities from frequently sold herbal antidandruff products through market survey and literature review. Many solutions are presently available but most of them offer strong chemical agents like ketoconazole, selenium sulphide, Tar, Tar etc, not only concern chemicals but also the excipients like preservatives within products have carcinogenic effects and other serious issues. The main causative agent responsible for dandruff is M.furfur which feeds on sebum and it causes tissue damage & dermal infections, with the release of free fatty acids. To get rid of dandruff several solutions are available in the market in different forms like creams, shampoo, oil. Synthetic solutions give instant results or worsen the problems due to serious side effects associated with chemicals.

CONCLUSION

- The formulation and composition of prepared antidandruff formulation had been reported very first time.
- Prepared formulation has significant antidandruff potential against Malassezia furfur and other dermatophytes in dandruff condition.
- Prepared formulation has prolonged contact time with advantage of non synthetic base with minimum or no side effects.



● From this investigation it can be concluded that the prepared formulation posses a significant comparable antidandruff efficacy with marketed antidandruff formulations due to presence of Alkaloids, Glycosides, Carbohydrates, Terpenoids, Phenol, Flavonoids and tannins (Gallic acid) in the prepared formulation.

● Study also redefines the potential of drugs.

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