Pharmacogn. Res., 2025; 17(3):909-913.
https://www.phcogres.com

Original Article

GC-MS Analysis and *in vitro* Anti-Dandruff Activity of *Phyla nodiflora* against *Malassezia furfur*

Priyanka Manmode[1], Dighe Santosh Bhausaheb[2,*]

[1]Department of Pharmacy, Bhagwant University, Sikar Road, Ajmer, Rajasthan, INDIA.
[2]Department of Pharmacology, Pravara Rural College of Pharmacy, Loni, Maharashtra, INDIA.

ABSTRACT

Background: The common skin ailment known as dandruff mostly affects the scalp. It will impact nearly half of the prepubescent population, regardless of gender or race. The fungus *Malassezia globosa*, which is unique to the scalp, was found to be the causative culprit. The balloon vine, or Karnasphotha (*Cardiospermum helicacabum* Linn.), belongs to the Sapindaceae family and is traditionally used to treat dandruff. *In vitro* Antidandruff activity of *Karnasphota Mula* and *Beeja* by Agar cup method and Biocidal activity. **Materials and Methods:** *Phyla nodiflora in vitro* antidandruff activity using the Agar cup method and biocidal activity. **Results:** *Phyla nodiflora* demonstrated effective antidandruff properties against *Malassezia furfur* at varying concentrations. **Conclusion:** The *Phyla nodiflora* is having significant Antidandruff properties.

Keywords: Dandruff, Ketoconazole, Anti-microbial, Cup method, *Malassezia furfur*.

Correspondence:
Dr. Dighe Santosh Bhausaheb
Associate Professor, Department of Pharmacology, Pravara Rural College of Pharmacy, Loni, Maharashtra, INDIA.
Email: priyankamanmode2015@gmail.com

Received: 24-02-2025;
Revised: 07-04-2025;
Accepted: 16-06-2025.

INTRODUCTION

An integral part of the universe are plants. Numerous medicinal plants have been found as sources of significant medication following a variety of observations and experiments.[1] In order to survive, people also employ plant-based medications that are grown close to their settlements. Their experiences, whether positive or negative, led them to identify the medicinal properties of various plants in relation to specific illnesses. Offspring inherit these experiences from their parents.[2] Searching for active chemicals in medicinal plants is crucial. Many nations' health care systems, including China, India, and others, have relied heavily on plant-based traditional medicine. Approximately 70-80% of the world's population still uses herbal medicine as their primary source.[3] *Phyla nodiflora* is the important member of the family verbenaceae showing a variety of medicinal uses. It can be the source of the indigenous medicine. It is distributed in India, Sri Lanka, Ceylon, Baluchistan, South and Central America and Tropical Africa. It is native of California. In India, it is found in the warmer parts including A.P., Karnataka, Kerala, and Maharashtra, some parts of Rajasthan, Tamil Nadu, U.P. and W.B. It is common in wet places along bunds or irrigation canal edges and sliver banks. The most prevalent condition affecting humans that harms skin and hair is dandruff. The existence of dandruff, which is produced by Malassezia species and is thought to include a number of hereditary and environmental factors, has drawn a lot of attention lately because it can result in hair loss, low self-esteem, and a negative social image.[4] Dandruff is said to be caused by the fungus *Malassezia globosa*, according to older literature. Although the skin surface of both healthy individuals and those with dandruff naturally contains this species, it was found in 2007 that *M. globosa*, a fungus that is exclusive to the scalp, is the causative culprit. *M. globosa* and *Malassezia restricta* are the species that are currently most closely linked to severe dandruff or dandruff. But other authors also included *Malassezia obtusa, Malassezia sloofia, Malassezia furfur,* and *Malassezia sympodialis*.[5,6] Different kinds of abnormalities of the scalp exist. These include psoriasis (raised reddish patches), dandruff, tinea capitis, alopecia (hair loss), seborrhoeic dermatitis (inflammation of the scalp skin characterised by scaly, itchy, and flaky skin), and scalp folliculitis (inflammation of the hair follicle).[7] Thus, the goal of this study is to test *Phyla nodiflora* phytochemical content for dandruff. Therefore, the goal of this study is to assess the phytochemical analysis and anti-dandruff properties of neem organic bark extracts against *Phyla nodiflora* species.

OPEN ACCESS

Manuscript TECHNOMEDIA

DOI: 10.5530/pres.20252156

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

MATERIALS AND METHODS

Plant Collection and Authentication

Phyla nodiflora, a medicinal herb, was gathered from the Bhima River's edge.

Herbal Drug and Extraction Process

Drying Method

After being gathered and well cleaned, the entire *phylum nodiflora* plant was allowed to shade dry for 15 days at room temperature.

Grinding process

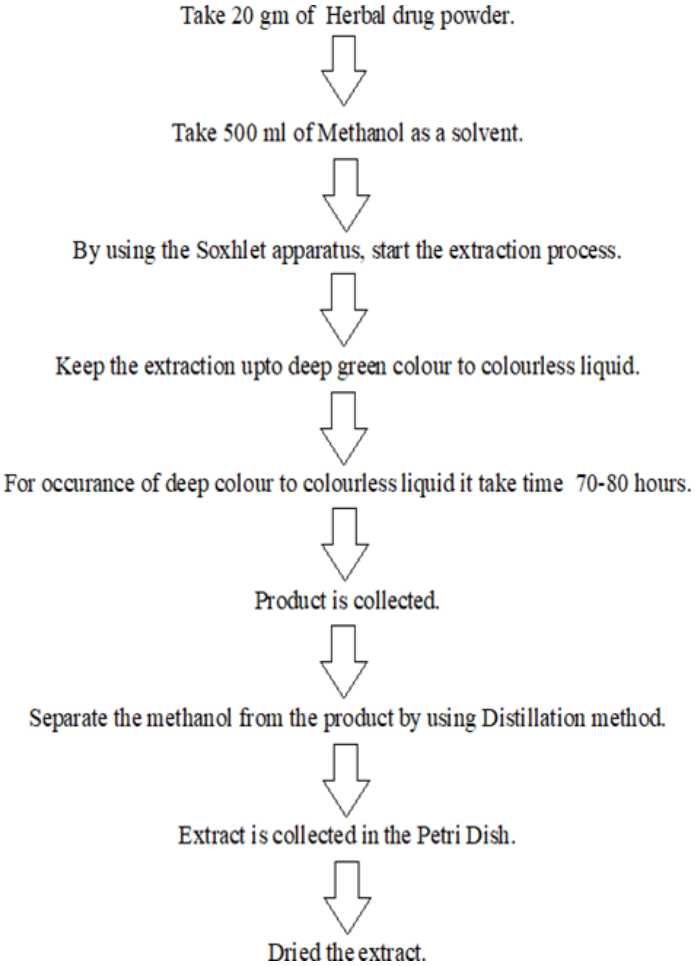
A mixer grinder was used to thoroughly crush the plant material into a fine powder.

Sieving Process

The herbal medication is sieved using Sieve No. 10, then sealed in airtight polythene bags and maintained at room temperature.

Extraction Process

The above flow chart represents the extraction process of powder of *Phyla nodiflora* plant using Methanol in soxhlet apparatus for 3 days.



GC–MS analysis

Using a nonpolar capillary column (BP5MS, 5% phenylpolysilphenylene siloxane) with dimensions of 30 m×0.25 mm, i.d. 0.25 µm, 2 µL of each sample was injected into a SHIMADZU GC-MS instrument, model GCMS-17A/QP5050, at a flow rate of 1 mL/min. The carrier gas was helium with a 99.9999% purity. Samples were injected using chloroform as the solvent. The thermal program began at 80°C for 4 min and then rose by 10°C every minute until it reached 200°C. This temperature was held for 5 min, and then it was raised from 200°C to 300°C at a rate of 7°C per minute, and then it was held for 15 min. It took 50.3 min to run in total. In the 70 eV ionization energy mode, mass spectra were obtained between 42 and 600 m/z, with the ionization source temperature set at 280°C.[8]

Identification of the components

By comparing the mass spectrum of the analysed compound with the spectrum of known compounds stored in the libraries accessible on the instrument's computer, the chemical compounds contained in each extract were determined by interpreting the GC-MS mass spectrum using the NIST and WILEY databases.

In vitro Anti-dandruff Activity

Collection of Dandruff

Samples of dandruff were collected from volunteers by scraping the scalp with sterile scalpel and stored in sterile container.

Isolation of Fungi in Pure culture

On a sterile Sabouraud dextrose agar plate, the collected dandruff was inoculated. For 3-5 days, it was incubated at 32-37°C. The presence of distinctive white growths surrounding the flakes was suggestive of the organism responsible for dandruff.

Growth and Identification

The creature was identified using biochemical, microscopic, and cultural techniques. By using pure culture in Sabouraud dextrose agar medium with chloramphenicol added, the colonies were determined to be *Malassezia furfur* fungal species. Agar offers a selective medium for the growth of fungi of medical significance, while chloramphenicol, an antibiotic, prevents the establishment of undesirable bacterial floras.

Preparation of test sample

Phyla nodiflora was prepared at 100%, 75%, 50%, and 25% in distilled water for conducting zone of inhibition.

Evaluation of Antidandruff Activity against *M. furfur* By Zone of Inhibition by Agar Cup Plate Method

A sterile borer was used to create wells on sterile SDA plates. *M. furfur* was used as the inoculum on the plates. 100%, 75%, 50%, and 25% of the test material (*Phyla nodiflora*) was put using a micropipette into each plate. For 72 hr, all plates were incubated at 30±2°C. Following incubation, antimicrobial activity was assessed and the zone of inhibition surrounding the well was identified.[9] Using the agar well diffusion method, the extracts' anti-dandruff properties were ascertained. The fungal strains that had been cultured on PDA for 48 hr at 32°C were suspended in olive oil

and mixed with 0.5% cycloheximide and 0.05% chloramphenicol. A sterile inoculated loop was used to apply the inoculum of the tested *Malassezia* species to the surface of the hardened PDA. Sterile disc agar was used to create agar wells with a diameter of 3 mm. To ensure that the plant extracts diffused evenly into the agar, the plates were pre-incubated for 2 hr at 32°C. The plates were incubated for two days at 32°C following pre-incubation. Ketoconazole (20, 40, 60, 80, and 100 µg) was also employed as a positive control. The inhibition zone diameter was measured in millimetres following a seven-day incubation period to assess the anti-dandruff activity. The experiments were carried out in triplicate, and the mean±standard deviation was used to represent the results. The simplest estimated effectiveness of control and sample inhibition was determined using the formula IC_{50}.[10]

$$IC_{50}=50\ (I)/I+1$$

Where, IC_{50}=Half of inhibitory concentration and I=Minimum inhibitory.

Determination of Minimum Inhibitory Concentration (MIC)

Using the micro broth dilution procedure, the extract's MIC was ascertained.[11] The fungus (*Malassezia species*) was serially diluted (10, 20, 40, 60, 80, and 100 µg/mL) in a plate and incubated at 32°C for 48 hr in order to determine the MIC fold. Wells were checked for any obvious growth after incubation. The lowest concentration of the extract at which no discernible growth occurred was considered as the MIC.[12]

RESULTS

Phytochemicals such alkaloids, flavanoids, phenolics, terpenoids, saponins, and glycosides have been found in the ethanolic leaf extracts of *Phyla nodiflora*, according to phytochemical screening assays (Table 1). The results showed that *Phyla nodiflora* had alkaloids, flavanoids, terpenoids, and glycosides, whereas phenolics and saponins were absent. The Gas Chromatography-Mass Spectrometry analysis carried out in ethanolic leaf extracts of *Phyla nodiflora* was shown in Figure 1.

In vitro Anti-dandruff Activity

The antidandruff properties of *Phyla nodiflora* against *Malassezia furfur* were good. There was a concentration-dependent anti-dandruff effect. The greatest inhibition zone against *Malassezia furfur* was seen at a clear zone with a diameter of 17 mm at 100% concentration. When it came to *Malassezia furfur, Phyla nodiflora* exhibited an inhibitory zone of 16 mm at 75% concentration, 14 mm at 50% concentration, and 13 mm at 25% concentration (Figure 2).

In vitro Anti-dandruff Activity of *Phyla nodiflora* Extract

The l extract has the largest inhibitory zone (14 mm), while positive control ketoconazole had the maximum zone of inhibition growth, measuring 17 mm and 16 mm. For all extract concentrations employed, the study demonstrates the anti-dandruff properties of *Phyla nodiflora* extracts. The inhibitory zones in the ethanol extract measured 7.57±3.4 mm, or 7-14 mm. Comparing the current study's results to the control group (ketoconazole), the inhibition zone is smaller (9.85±3.5 mm and 9.57±3.7 mm). The 25 mm inhibitory zone and chemical characterisation of *Phyla nodiflora* extracts utilising ethanol extract on dandruff-causing *Malassezia species* were obtained from the anti-dandruff properties of cured neem extract.

Minimal Inhibitory Concentration (MIC)

Diethyl ether extract yielded an inhibition zone of 5.85±2.0 mm while ketoconazole yielded a 9.42±4.2 mm anti-dandruff activity range at 100 µg/mL. The extract's effectiveness was 31.5%, while ketoconazole's was 38%. At 100 µg/mL, the extract's anti-dandruff activity range was 8.57±3.4 mm, while ketoconazole's was 9.57±3.7 mm. The extract's effectiveness was 39.4%, while ketoconazole's was 40.5% (Table 2).

Table 1: Phytocomponents identified in the ethanolic leaf extract of *Phyla nodiflora* by GC-MS.

Sl. No.	Compound	Molecular Weight	Molecular Formula	Retention Time	Quantity (mg)
1	Trans, Trans-Muconic Acid	142	$C_6H_6O_4$	14.478	0.003
2	Cyclohexanone, 2-(1-Methyl-2-Oxopropyl)-	168	$C_{10}H_{16}O_2$	14.698	0.016
3	1H-Imidazole, 2-(Diethoxymethyl)-	170	$C_8H_{14}O_2N_2$	15.453	0.001
4	Trans,Cis-1,7-Dimethylspiro[4.5]Decane	166	$C_{12}H_{22}$	16.404	0.002
5	1,6-Anhydro-.Alpha.-D-Galactofuranose	162	$C_6H_{10}O_5$	18.365	0.007
6	Emylcamate	145	$C_7H_{15}O_2N$	18.710	0.044
7	Benzene, 1,1'-[1,4-Butanediylbis(Oxymethyl ene)]Bis-	270	$C_{18}H_{22}O_2$	19.200	0.011
8	Oxalic Acid, CyclobutylPentadecyl Ester	354	$C_{21}H_{38}O_4$	19.540	0.001

Table 2: **Antidandruff activity of ethanol extract of *Phyla nodiflora*.**

Conc. (µg/mL)	*Phyla nodiflora* (diameter/mm)	Ketoconazole (diameter/mm)	IC_{50} (µg/mL)
10	3.61±1.6	4.26±2.2	*Phyla nodiflora* 39.2±1.6 Ketoconozole 40.7±1.7
40	4.54±1.7	5.27±1.6	
60	5.40±1.9	7.70±2.1	
80	6.86±2.9	8.56±2.9	
100	8.55±3.4	9.58±3.7	

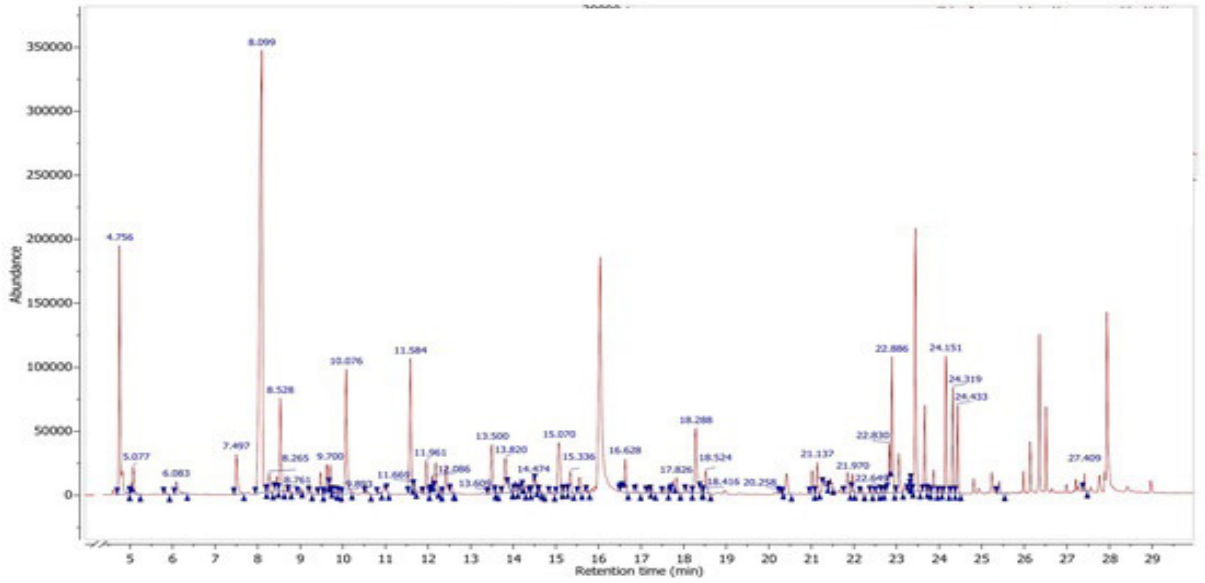


Figure 1: GC-MS chromatogram of *Phyla nodiflora* ethanolic leaf extract.

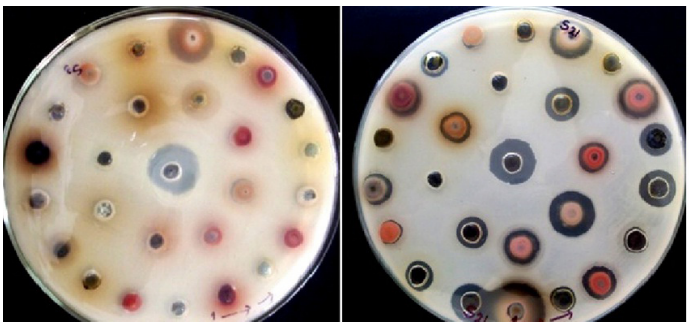


Figure 2: Antidandruff activity of *Phyla nodiflora* by zone of inhibition using agar cup plate method.

DISCUSSION

The phytochemical evaluation revealed the presence of alkaloids, flavanoids, terpenoids, and glycosides in *Phyla nodiflora* whereas phenolics and saponins were absent. The Gas Chromatography-Mass Spectrometry analysis carried out in ethanolic leaf extracts of *Phyla nodiflora.* The antidandruff properties of *Phyla nodiflora* against *Malassezia furfur* was significant. There was a concentration-dependent antidandruff effect was observed. *Phyla nodiflora* has significantly controlled the Minimal Inhibitory Concentration.

CONCLUSION

The ethanolic extract of *Phyla nodiflora* had strong anti-dandruff properties against two species of *Malassezia*, namely *M. globosa* and *M. restricta*. As a result, *Phyla nodiflora* was found to have good anti-dandruff properties. The background information for using *Phyla nodiflora* as a possible therapeutic anti-dandruff medication and to treat fungal-related illnesses will be provided by this work.

ACKNOWLEDGEMENT

The authors thankful to the Department of Pharmacy, Bhagwant University, Sikar Road, Ajmer (Raj.), India.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectrometry; **IC_{50}:** Half of Inhibitory Concentration; **MIC:** Minimum Inhibitory Concentration; **SDA:** Sabouraud Dextrose Agar; **PDA:** Potato Dextrose Agar; **U.P.:** Uttar Pradesh; **W.B.:** West Bengal; **A.P.:**

Andhra Pradesh; ***M. globosa*:** *Malassezia globosa*; ***M. restricta*:** Malassezia restricta; ***M. furfur*:** *Malassezia furfur*; ***M. obtuse*:** *Malassezia obtusa*; ***M. sloofia*:** *Malassezia sloofia*; ***M. sympodialis*:** *Malassezia sympodialis*; **NIST:** National Institute of Standards and Technology; **BP5MS:** (5% phenylpolysilphenylene siloxane); **eV:** Electron Volt; **m/z:** Mass-to-charge ratio; **µg:** Microgram; **mL:** Milliliter; **mm:** Millimeter; **hr:** Hour; **µm:** Micrometer.

SUMMARY

Dandruff is a common skin condition that mainly affects the scalp. Almost half of the population in the pre-pubertal age and of any gender and ethnicity will be affected. No population in any geographical region would have passed through freely without being affected by dandruff at some stage in their life. Thus, the goal of this study is to test *Phyla nodiflora* phytochemical content for dandruff. Therefore, the goal of this study is to assess the phytochemical analysis and anti-dandruff properties of neem organic bark extracts against *Phyla nodiflora* species. The background information for using *Phyla nodiflora* as a possible therapeutic anti-dandruff medication and to treat fungal-related illnesses will be provided by this work.

REFERENCES

1. Malik HM. Treatment through herbs. In: Medicinal plants of Pakistan, (Eds.). 3rd ed. Pakistan; 2001. p. 21-3.
2. Qureshi R. Floristic and ethnobotanical study of Desert- Nara Region, Sindh. Shah Abdul Latif University, Pakistan research repository. Pakistan; 2004. p. 454.
3. Sudershan C. Shoot brid regeneration from leaf explants of a medicinal plant. *Enicosternma axillare*. Curr Sci. 1998; 74: 1099-100.
4. Borda LJ, Wikramanayake TC. Seborrheic dermatitis and dandruff: A comprehensive review. J Clin Investig Dermatol. 2015; 3(2): 1-5. doi: 10.13188/2373-1044.1000019 , PMID 27148560.
5. Gupta AK, Batra R, Bluhm R, Boekhout T, Dawson TL. Skin diseases associated with *Malassezia* species. J Am Acad Dermatol. 2004; 51(5): 785-98. doi: 10.1016/j.jaad.2003.12.034, PMID 15523360.
6. Ranganathan S, Mukhopadhyay T. Dandruff: the most commercially exploited skin disease. Indian J Dermatol. 2010; 55(2): 130-4. doi: 10.4103/0019-5154.62734, PMID 20606879.
7. Narshana M, Ravikumar P. An overview of dandruff and novel formulations as a treatment strategy. Int J Pharm Sci Res. 2018; 9: 417-31.
8. Khdera HA, Saad SY. Chemical composition of organic extracts of *Phyla nodiflora* L. in Syria by GC-MS. Heliyon. 2024; 10(14): e34686. doi: 10.1016/j.heliyon.2024.e34686, PMID 39149040.
9. Kochari A, Byadigi SD. *In vitro* AntiDandruff Activity of Karnasphotha (*Cardiospermum helicacabum* Linn.) against *Malassezia furfur*. J Ayurveda Integr Med Sci. 2024; 6: 61-6.
10. Esterly JS, Wagner J, Mclaughlin MM, Postelnick MJ, Qi C, Scheetz MH. Evaluation of clinical outcomes in patients with bloodstream infections due to gram-negative bacteria according to carbapenem MIC stratification. Antimicrob Agents Chemother. 2012; 56(9): 4885-90. doi: 10.1128/AAC.06365-11, PMID 22777044.
11. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001; 48 Suppl 1: 5-16. doi: 10.1093/jac/48.suppl_1.5, PMID 11420333.
12. Zia M. Detection and identification of *Malassezia* species in domestic animals and aquatic birds by PCR-RFLP. Iran J Vet Res. 2014; 16: 136-41.

Cite this article: Manmode P, Bhausaheb DS. GC-MS Analysis and *in vitro* Anti-Dandruff Activity of *Phyla nodiflora* against *Malassezia furfur*. Pharmacog Res. 2025;17(3):909-13.