ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

ANTIDANDRUFF ACTIVITY OF A POLYHERBAL FORMULATION AGAINST *MALASSEZIA* ISOLATES FROM HUMAN SCALP *IN VITRO*

Susan G. Suganya*, Adhithya Subramanian G.

PG and Research Department of Zoology, Bishop Heber College (Autonomous), Tiruchirapalli, Tamil Nadu, India

**Corresponding author: Susangsuganya10677@gmail.com*

ABSTRACT

Dandruff, a most common dermatological disorder, is an unpleasant, chronic and pruritic scalp condition. This study attempted to know the activity of a polyherbal formulation against a dandruff causing organism *Malassezia furfur* which was cultured and identified from human dandruff sample. *M. furfur* was bottle-shaped and was able to form biofilm. Various parts of 3 different plants (leaves of *Phyla nodiflora*, *Azadirachta indica* and ripen fruit seeds of *Piper nigrum*) were made into a Poly herbal Formulation (PHF). The mixture was extracted with water (APHF) and methanol (MPHF), and its anti-malassezial activity was evaluated based on diffusion dependent assays. Both extracts showed effective results comparable to a standard antidandruff shampoo. APHF was qualitatively tested for various classes of phytochemicals and MPHF was analyzed by GC-MS; both revealed the presence of many active metabolites which possibly inhibit *M. furfur* and could be used as a potential anti-dandruff drug.

Keywords: Anti-dandruff, *Malassezia furfur*, Polyherbal, *Phyla nodiflora*, *Azadirachta indica*, *Piper nigrum*.

1. INTRODUCTION

Dandruff is a most common dermatological disorder that affects around 50% of the population worldwide at some time during their lifetime [1]. It is a chronic, pruritic condition restricted to scalp, characterized by scaling and shedding loosely adherent, small white to gray flakes accumulated in patches, often with itching. On the other end, more severe condition of this spectrum is seborrheic dermatitis. Dandruff is a major cosmetic problem in both developed and developing countries, and lead to loss of self-esteem and negative social effects on its presence [2-5].

A Basidiomycetous fungal genus *Malassezia*, previously known as *Pityrosporum*, is considered as a major component of skin pathogen associated with dandruff and many other conditions including atopic dermatitis, seborrheic dermatitis and pityriasisversicolor [6]. First, in this work a dandruff sample was cultured and the organism was identified as one of the lipophilic yeast of this family, *Malassezia furfur* (previously called *Pityrosporum ovale*), which is always seen as a central cause of dandruff. Its pathogenesis is majorly influenced by 3 etiologic factors-individual susceptibility, sebaceous secretions and Malassezial colonization. Other factors responsible for dandruff include hormonal imbalance, weather, poor hygiene, stress and poor diet [7, 8].

There are many antifungal drugs available to treat dandruff. The synthetic active substances include ketoconazole, zinc pyrithione, selenium sulfide and salicylic acid, where many of them are used to control the abundance of fungi in scalp [9]. But problems like development of fungal resistance to antibiotics, reduced efficiency and increased toxicity of synthetic drugs, are now creating the need for an alternative way of treatment. By the way, ethnobotanical research gained more interest and phytoactive constituents of plants are now some effective approach to deal antimicrobial resistance [10]. Herbal drugs are actually known for formulations. A Polyherbal Formulation (PHF)-blend of herbs is more effective than individual herbal extract-highlights “Sarangdhar Samhita”, an Ayurvedic text. PHFs are regarded with comparable efficiency and fewer side effects [11].

To promote the use of medicinal plants as potential sources of antimicrobial compounds, it is necessary to thoroughly investigate their composition and activity. The present study was taken up to assess the anti-dandruff potential of a Polyherbal Formulation (PHF) made of leaves of *Phyla nodiflora* (Frog fruit or Turkey tangle), *Azadirachta indica* (Neem) and ripen fruit seeds of *Piper nigrum* (White pepper) against the isolated fungi *Malassezia furfur*. This study also tries to find the

phytochemical constituents of this herbal mixture when extracted with water (by preliminary phytochemical analysis) and with methanol (by GC-MS).

2. MATERIAL AND METHODS

2.1. Isolation and Identification of Dandruff causing Fungi

2.1.1. Sample Collection and Culture

Flakes shedding from scalp of a volunteer were collected by partitioning with a sterile comb and scrapping using a sterile blunt scalpel. The specimen was then inoculated to Potato Dextrose Broth and observed for growth by incubating at 25°C for 48 hours. Later it was stored at 2-8°C for further use.

The fungi broth culture was introduced to a Potato Dextrose Agar (PDA) plate, incubated at 25°C for 48 hours to observe colony characters. PDA plates were prepared by adding 10% tartaric acid to maintain the pH at 3.5, to inhibit bacterial growth.

For direct microscopy, dandruff specimen was placed in a glass slide with a drop of KOH and methylene blue, and was covered using a coverslip. The fungal morphology was also examined using a smear of the organism grown in PDA plate. In both of the above experiments objective microscope was used for imaging. Fungi broth culture was analyzed by Scanning Electron Microscopy (VEGA 3, TESCAN) at an accelerating voltage of 5kV.

2.2. Preparation of polyherbal extract

2.2.1. Collection of plant materials

The plant materials used in this study were leaves of *Phyla nodiflora* (Verbenaceae), *Azadirachta indica* (Meliaceae) and ripen fruit seeds of *Piper nigrum* (Piperaceae). All these samples were collected from Trichy Local market and N.Govindasamy & Co. Herbals, Trichy (September 2018).

2.2.2. Preparation of polyherbal extracts

Selected plants materials were air-dried at room temperature for 2 weeks and ground into uniform powder of 40 mesh size separately. Equal quantity of ground powder of all the 3 plants was mixed together. To check the activity of the formulation, aqueous and methanolic extracts were taken. For aqueous PHF (APHF), 15g of mixture was extracted with 150ml of distilled water by maceration for 24 hours; then filtered and stored. Another extract was prepared by hot percolation, where 50g of mixture was extracted with 500ml of methanol (MPHF) in a soxhlet extractor, continuously till the solvent flowing through siphon tube became colorless. Later it was filtered and stored.

2.3. Determination of anti-dandruff activity

Anti-dandruff activity was tested by disc diffusion and agar well diffusion methods; the diffusion dependent activity of the PHQ was evaluated based on Zone of Inhibition (ZOI).

APHF was tested by disc diffusion method. Hot air sterilized Whattmann Filter paper discs (No: 1) of 6 mm diameter was loaded with APHF of various diluted concentrations (50µl, 100µl and 150µl). Ketafung Z Plus lotion containing Ketoconazole and Zinc Pyrithione (30µl) was also loaded to disc and used as test standard. Standardized fungal test suspension was inoculated and uniformly spread on a PDA plate by a cotton swab. Using sterile forceps, the discs containing standard and APHF of varied concentrations were laid over the surface of the agar plate; incubated at 25°C for 48 hours and observed.

Activity of MPHF was determined by agar well diffusion method. Wells of 10 mm diameter was punched on a PDA plate which is uniformly inoculated with fungal suspension. Standard and, MPHF of various diluted concentrations (50µl, 100µl and 150µl) were loaded into the wells and incubated. Triplicates were done for both assays.

2.4. Phytochemical analysis

The APHF was qualitatively tested for the presence of preliminary phytochemicals including Tannins, Phlobatannins, Saponins, Flavonoids, Steroids, Terpenoids, Triterpenoids, Alkaloids, Carbohydrate, Protein, Anthroquinone, Polyphenols and Glycosides.

The MPHF extracted using soxhlet was subjected to GC-MS analysis. Experiment was carried out on Shimadzu-QP2020 gas chromatograph–mass spectrometer with an Elite-1(100% Dimethyl poly siloxane), 30m x 0.25 mm ID x 1µm capillary column. Injection volume was 1 ml and the total run time was around 25 minutes. Different constituents were identified comparing the relative retention times and mass spectra with reference to Wiley spectral library database.

3. RESULTS AND DISCUSSION

The fungus causing this chronic cosmetic problem was identified, by analyzing the scalp samples and culturingthe collected dandruff sample. It appeared to grow in white to cream color, smooth and pasty colonies in PDA plate (fig. 1). The direct microscopy of shedding

flakes showed grape like clusters of yeast and hyphae: typical "spaghetti and meatball" appearance (Fig. 2). On microscopic view of culture, the cells appeared bottle shaped (fig. 3) and these observations led to the identification of *Malassezia furfur*. In SEM, *M. furfur* showed its adhesive nature and biofilm formation (fig. 3). The adherence of the fungi to the abiotic container surface could be the reason for the large extracellular matrix to form. Biofilm actually favors the enhancement of virulence and may also be responsible for development of anti-fungal resistance [12].

Fig. 1: Growth in PDA plate

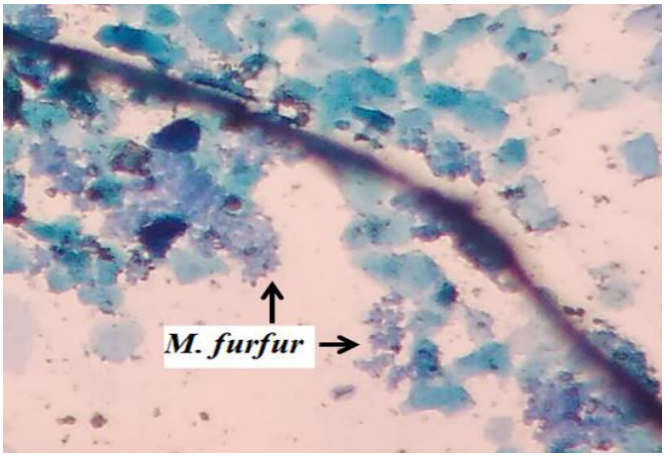

Fig. 2: Direct microscopy of Dandruff flakes

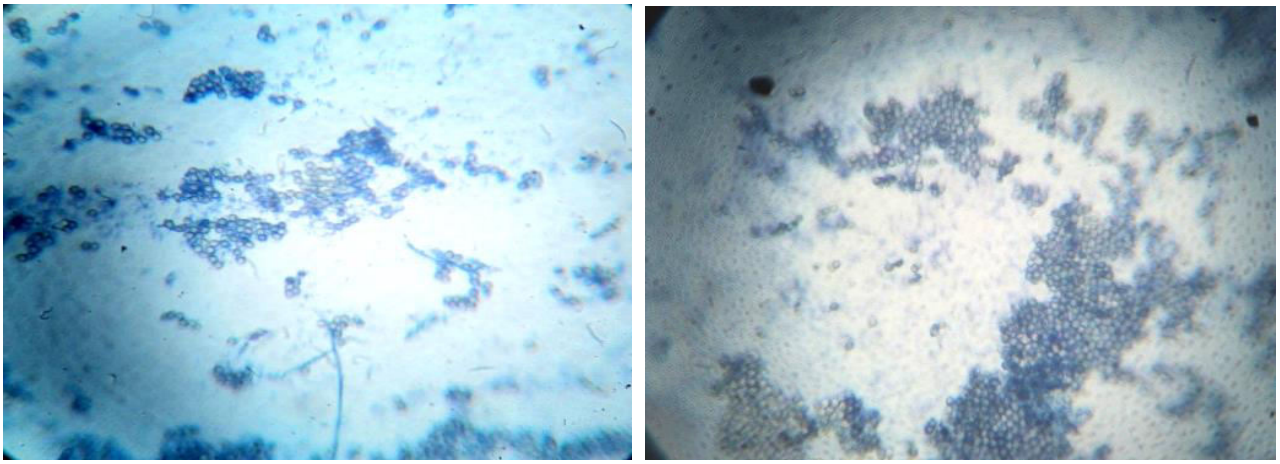

Fig. 3: Microscopic view of *M. furfur*

Both APHF and MPHF exhibited a potential activity against the fungi *M. furfur* in the antibiogram. The ZOI was comparable to that of standard and represented in table 2. The ZOI showed by APHF on disc diffusion was 12.66±0.47, 18.83±0.62 and 25.83±0.23 mm at 50, 100 and 150 µl concentrations, respectively. The MPHF on agar well diffusion exerted a ZOI of 14.33± 1.24, 19.16±0.62 and 24.33±0.47 mm at 50, 100 and 150 µl concentrations respectively. The standard solution at 30 µl concentration exhibited ZOI of 31.16±1.02 and 29.16±0.84 mm in disc and agar well diffusion methods respectively.

Despite differences in the phytochemical constituents the APHF and MPHF, in both disc and agar well diffusion methods the ZOI was similarly expressed. With increased concentration, the ZOI range increased and it was comparable to that of the standard used. Few patients using ketoconazole suffer dryness and itching. Irritant contact dermatitis was also seen in few cases after using ketoconazole and zinc pyrithione [13]. Such problems can be avoided by herbal alternatives.

Preliminary phytochemical analysis of APHF revealed the presence of variety of phytochemicals including tannins, saponins, flavonoids, terpenoids, triterpenoids,

alkaloids, anthroquinone, polyphenols and glycosides (fig. 5). The results are shown in Table 1. APHF had higher presence of flavonoids, polyphenols and triterpenoids. Many classes of phytochemical compounds are reported to function as effective antimicrobials, by acting in multiple ways. Phenols disturb cell homeostasis, dissolve and leak the cytoplasm, and inhibit enzymes working in electron transport and oxidative phosphorylation while flavonoids inhibits nucleic acid synthesis and stops membrane functioning [14]. Terpenes are capable of disrupting cell membrane and can cause cell death because of their low molecular weight and high lipophilic nature [15].

Table 2: Measurements of zone of inhibition

Sample	Concentration (µl)	Zone of Inhibition (mm)	
		APHF-Disc Diffusion	MPHF-Agar Well Diffusion
PHF	50	12.66±0.47	14.33±1.24
PHF	100	18.83±0.62	19.16±0.62
PHF	150	25.83±0.23	24.33±0.47
Standard	30	31.16 ± 1.02	29.16 ± 0.84

Values are expressed in mean ± S.D.

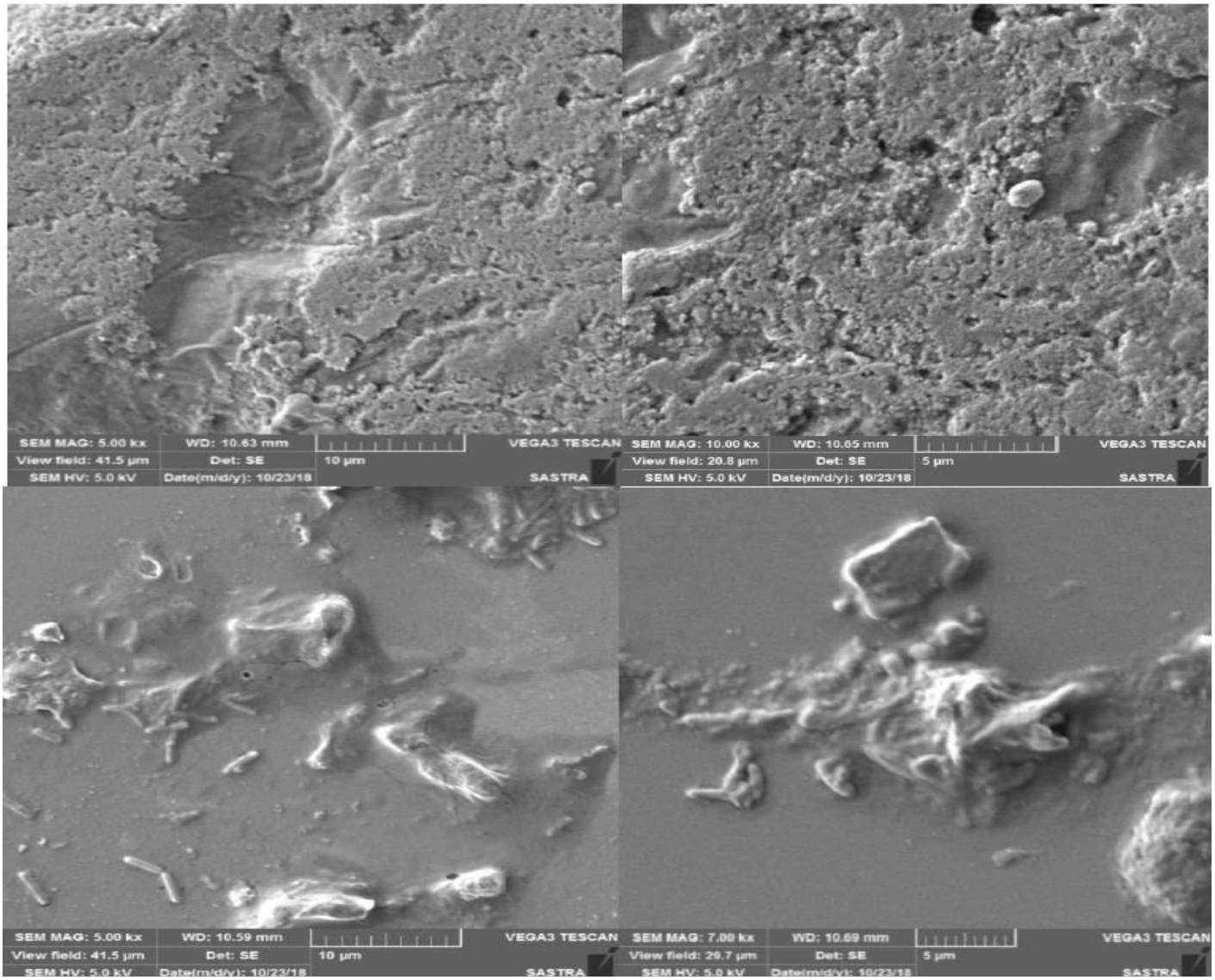

Fig. 4: SEM of *M. furfur*

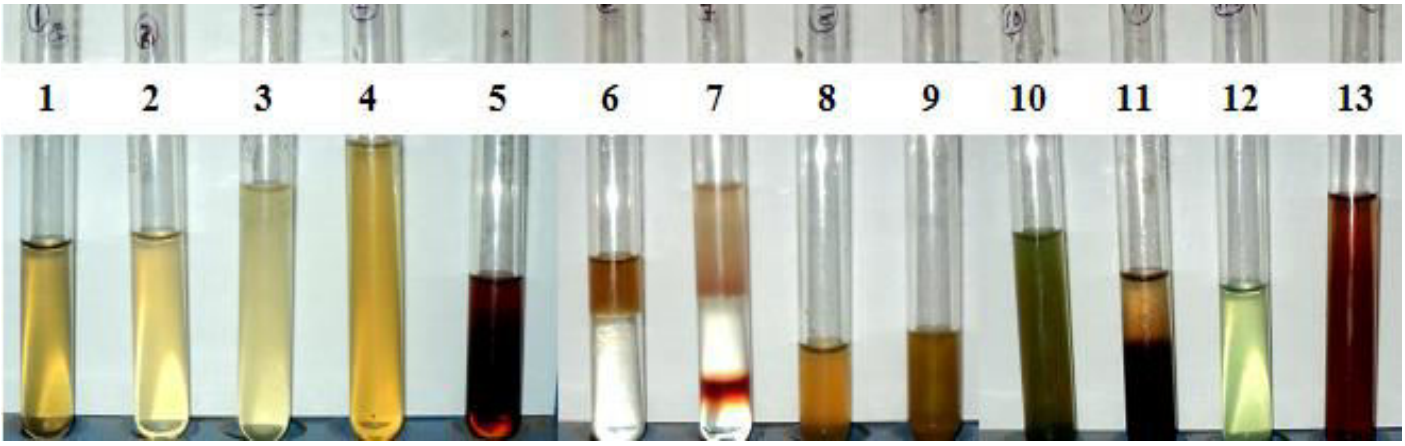

Fig. 5: Qualitative preliminary phytochemical tests of APHF

Table 3: Preliminary phytochemical analysis of APHF

Test analysis	Polyherbal extract
Tannin	+
Phlobatannin	-
Saponin	+
Flavonoids	++
Steroids	-
Terpenoids	+
Triterpenoids	++
Alkaloids	+
Carbohydrate	-
Protein	-
Anthroquinone	+
Polyphenol	++
Glycoside	+

"+" indicates the presence; "++" indicates the presence in higher amount and "-" indicates the negligible amount.

The results of GCMS lead to identification of many compounds from the GC fractions of MPHF extracted using soxhlet extractor. The chromatogram revealed the presence of various phytochemical compounds (Fig. 6) including camphor, Terpinen-4-ol, α-Terpineol, coumaran, 5-Hydroxymethylfurfural, Piperonal, Syringol, Tyrosol, Pellitorine, n-Hexadecanoic acid, Piperanine and Piperine. Previous studies report the strong antifungal activity exhibited by the essential oil containing higher amount of camphor [16, 17]. T erpinen-4-ol and α-Terpineol, also components of tea tree oil (an effective topical dandruff therapeutic agent), has been shown as a potential antifungal [18]. 5-Hydroxy-methylfurfural is reported for its antibiofilm activity against *Pseudomonas aeruginosa* [19]. Compounds like Piperanine and Piperine might have come from the white pepper, where Piperine is known for its anti-inflam-matory property [20].

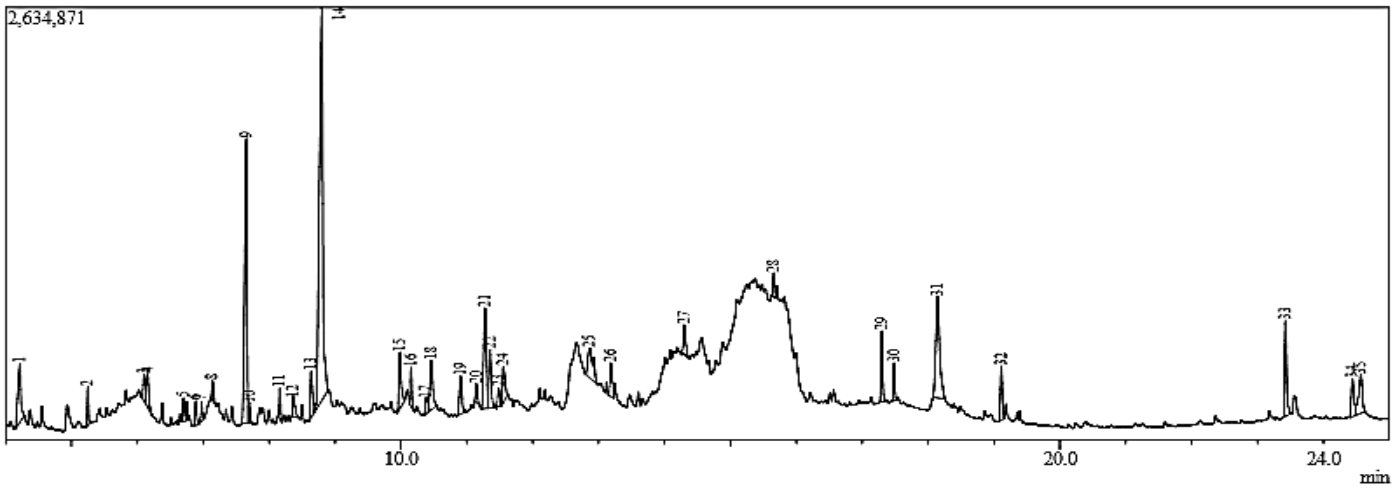

Fig. 6: GC-MS chromatogram of MPHF

4. CONCLUSION

Malassezia furfur cultured from scalp sample was bottle shaped; grown into cream, pasty colonies; was able to form biofilm. This study revealed the effectiveness of PHF for the management of dandruff and this kind of strategy would help to combat problems like antimicrobial resistance. *In vitro* evaluation of aqueous and methanolic PHF proved its appreciable diffusion dependent Zone of Inhibition against *M. furfur*. The preliminary classes of phytochemical components in APHF and the individual GC fraction components of MPHF, both results proved the presence of active metabolites against *M. furfur*, by the way suggesting this formulation as a potential anti-dandruff drug.

5. ACKNOWLEDGEMENT

This study was carried out at PG and Research Department of Zoology, Bishop Heber College (Autonomous), Tiruchirappalli, Tamilnadu, India.

6. REFERENCES

1. Ranganathan S, Mukhopadhyay T. *Indian journal of dermatology*, 2010; **55(2):**130-139.
2. Schwartz JR, DeAngelis YM, Dawson TL. *Practical modern hair science*, 2012; **1:**389-413.
3. Krishnamoorthy JR, Ranganathan S, Shankar SG, Ranjith MS. *African Journal of Biotechnology*, 2006; **5(10):**123-132.
4. Elewski BE. In *Journal of Investigative Dermatology Symposium Proceedings*, 2005; **10(3):**190-193.
5. Borda LJ, Wikramanayake TC. *Journal of clinical and investigative dermatology.*, 2015; **3(2):**221-230.
6. Saunders CW, Scheynius A, Heitman J. *PLoS Pathog.*, 2012; **8(6):** p.e1002701.
7. Hati D, Bhatnagar SP, Sethi KK. *Pharmacognosy Journal*, 2010; **2(10):**328-334.
8. DeAngelis YM, Gemmer CM, Kaczvinsky JR, Kenneally DC, Schwartz JR, Dawson TL. In *Journal of Investigative Dermatology Symposium Proceedings,* 2005; **10(3):**295-297.
9. Sharma S, Upadhyay UM, Upadhyay SU, Patel T, Trivedi P. *International Journal of Phytopharmacy Research*, 2013; **4(1):**23-28.
10. Gupta PD, Birdi TJ. *Journal of Ayurveda and integrative medicine*, 2017; ***8*(4):** 266-275.
11. Parasuraman S, Thing GS, Dhanaraj SA. *Pharmacognosy reviews*, 2014; ***8*(16):**73-81.
12. Angiolella L, Leone C, Rojas F, Mussin J, De Los Angeles Sosa M, Giusiano G. *Medical mycology*, 2018; **56(1):**110-116.
13. Vijayakumar R, Muthukumar C, Kumar T, Saravanamuthu R. *Indian journal of Dermatology*, 2006; **51(2):**145-152.
14. Mehdi N, Roya AB, Mohsen T, Rezvan T, Mohaddese M. *Iranian Journal of Dermatology*, 2015; **18(1):**10-15.
15. Nazzaro F, Fratianni F, Coppola R, Feo VD. *J of Pharmaceuticals*, 2017; **10(4):**86-92.
16. Pragadheesh VS, Saroj A, Yadav A, Chanotiya CS, Alam M, Samad A. *Industrial crops and products*, 2013;**49:**628-633.
17. Mokbel AA, Alharbi AA. *Australian Journal of Crop Science*, 2015; ***9*(6):**532-544.
18. Hammer K, Carson CF, Riley TV. *Journal of applied microbiology*, 2003; **95(4):**853-860.
19. Vijayakumar K, Ramanathan T. *Journal of ethnopharmacology*, 2020; **24(6):**112-124.
20. Tiwari A, Mahadik KR, Gabhe SY. *Medicine in Drug Discovery*, 2020; **9(11):**100-127.