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Evaluation of Anti-Dandruff Activity of Polyherbal Serum Against the Fungus Malasseiza Fungus from Human Scalp

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Abstract
Dandruff is one of the most common problems faced by people all around the world. It is a dermatological disorder that can be extremely frustrating and embarrassing. Fortunately, a recent study has shown promising results in the fight against dandruff. The study aimed to investigate the effectiveness of a polyherbal formulation against Malassezia furfur, a fungus that is often responsible for causing dandruff. The polyherbal formulation used in the study was composed of various leaves, including Piper betal, Tictona grandis, and Eclipta prostrata, as well as flowers such as Tenner cassia. Diffusion-dependent assays were used to determine the mixture's anti-malassezial activity after it had been extracted with both water (APHF) and methanol (MPHF).The results of the study were very encouraging. Both MPHF and APHF, which underwent GC-MS analysis and qualitative testing for various kinds of phytochemicals, showed the existence of numerous active metabolites that may inhibit M. furfur and have potential for use as anti-dandruff treatments.

1. Introduction:

Chronic scalp infection known as dandruff is manifested by scaling, itching, and redness of the scalp. It develops when the scalp sheds epidermal cells in large clumps. A month or so, the scalp's skin goes through a renewal process. Dead cells usually shed from the scalp in a practically undetectable manner, although. Dead cells can be shed as noticeable flakes known as dandruff when cell turnover becomes exceptionally high. In both developed and developing nations, dandruff is a significant cosmetic issue that is extremely concerning for public health (Zoya, *et al.,* 2016). The fungus genus Malassezia, earlier referred to as Pityrosporum, is thought to be a significant part of the skin pathogen responsible for dandruff and numerous other disorders like, seborrheic dermatitis, atopic dermatitis, and pityriasis versicolor (Saunders, *et al.,* 2012). The majority of individuals use anti-dandruff solutions today that contain chemicals to treat the dandruff infection. Instead of curing, it will cause further negative effects like dryness of the scalp and hair, inflammation of the scalp, discolouration of the hair, and hair loss. Due to their considerable effects and less side effects as compared to allopathic pharmaceuticals, herbal therapy is currently becoming more and more important for treating various disorders. Numerous herbs have been shown to have effective anti-dandruff properties. A poly herbal formulation is a branch of traditional and alternative medicine that is used widely in both developing and developed countries. It is the combination of more than two to three plants at a specific ratio. Plants are known to have a variety of phytoconstituents. (i.e. phytochemicals) that are responsible for the various curable properties that are attributed to them. (Kumar, *et al.,* 2019). The purposes of the current study were to determine the phytochemical components of a polyherbal serum made of *Piper betal, Tictona grandis, Eclipta prostrata,* and *Tenner cassia* flowers and their antifungal and antidandruff effects against the isolated fungus *Malassezia furfur.* This was done through primary phytochemical analysis and GC-MS.

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2. Materials and Methods:

Isolation and Identification of Dandruff causing Fungi

The dandruff-causing agent was removed from the patient's scalp using a scraper, then it was put into sterilized containers and refrigerated until it was needed. In potato dextrose agar (PDA) media that had been enhanced with coconut oil, the causative organism was inoculated. For three to five days, the inoculation plates were incubated at 37 °C. The fungus was identified by staining the fungal culture with lactophenol cotton blue stain and looking at it under a high-power objective microscope. (Kumar, *et al.,* 2019).

Preparation of polyherbal extract

Collection of plant Sample

The leaves of the selected species were collected, washed, and cleaned to remove the dust particles and subsequently they were dried under shade. The polyherbal formulation made up with equal volume (25g of each plant and 250 ml of D.H_2O) grind and take extracts were taken for aqueous PHF (APHF), then later it was filtered and stored (Kumar, *et al.,* 2019).

Sample Collection and Culture

The patient's scalp flake collection process involved dividing using a sterile comb and scraping with a sterile blunt scalpel. The specimen was then injected into Potato Dextrose Agar (PDA) and incubated for 48 hours at 25 C to check for growth. further, it was kept at 2 to 8 C for further use. In order to observe the characteristics of the colony, fungi broth culture was added to a Potato Dextrose Agar (PDA) plate and incubated at 25 c for 48 hours. Using a coverslip, the dandruff sample was placed on a glass slide along with a drop of potassium hydroxide and methylene blue. Imaging was done using the experiment-grade objective microscope. By using a microscope, fungi broth culture was examined (Susan et al., 2021).

Determination of anti-dandruff activity

The diffusion dependent activity of PHQ was evaluated using the Zone of Inhibition (ZOI). Standardised fungal test suspension was inoculated and uniformly spread on a PDA plate by a cotton swab. Using gel puncture, the well containing standard and APHF of varied concentrations were laid over the surface of the agar plate. Incubation at 25 °C for 48 hours and observation were performed.

Phytochemical analysis

Preliminary phytochemicals like tannins, saponins, flavonoids, steroids, and alkaloids were qualitatively examined in the APHF. The MPHF that was extracted using Soxhlet was examined using GC-MS.

3. Results and Discussion

The causal organism *M. furfur* was identified based on morphological characters. The macroscopic nature of colony for *M. furfur* is dull, smooth, or slightly folded, broad base bud as shown in plate 1. According to Susan , *et al.,* 2021 the fungus causing this by analyzing the scalp were identified as *Malassezia furfur.* Similarly Rakkimuthu , *et al.,* 2021 reported as *M. furfur* species can be identified based on their macro/microscopic and Biochemical features - Acclimatization of glycine is positive in *M. furfur* only.

Plate 1-Growth in PDA Plate Fig 1-Microscopy of *M.furfur*

Zone of Inhibition

APHF exhibited a probable activity against the fungi *M. furfur* in the antibiogram. The ZOI was similar to that of standard and signified in table 1. The ZOI exhibited by APHF on agar well diffusion 1 exerted a ZOI of 1.9 for standard as penicillin and 0.8mm, 0.9mm at 25, 50µl concentrations respectively and on agar well diffusion 2 applied a ZOI of 2mm for standard as penicillin and 0.9mm, 0.10mm at 50, 75µl concentrations, agar well diffusion 2 exerted a ZOI of 2mm for standard as penicillin and 0.9mm, 1.0mm at 50, 75µl concentrations, agar well diffusion 3 exerted a ZOI of 2mm for standard as penicillin and 1.0mm, 1.2mm at 75, 100µl concentrations respectively.

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Table 1: Measurements of zone of inhibition

Well diffusion plate	Standard	25 µl	50 µl	75 µl	100 µl
Plate 1	1.9mm	0.8mm	0.9mm	-	-
Plate 2	2.0mm	-	0.9 mm	1.0 mm	-
Plate 3	2.0mm	-	-	1.0 mm	1.2 mm

Figure 2- Zone Of Inhibition

Phytochemical properties

Despite variations in the APHF's phytochemical components, the ZOI was expressed similarly in all three agar well diffusion methods. Concentration increased and so did the ZOI range. Initial phytochemical testing on APHF found a range of phytochemicals, including tannins, saponin, flavonoids, protein, phenol, alkaloids, and terpenoids. The presence of terpenoids and flavonoids was greater in APHF. According to reports, many kinds of phytochemical substances operate in a variety of ways to be efficient antimicrobials. Protein is effective in promoting hair growth. Flavonoids impair membrane function and prevent the production of nucleic acids. Terpenes' low molecular weight and strong lipophilicity make them capable of rupturing cell membranes and resulting in cell death.

Phytochemical	Result
Tannins	+
Saponins	+
Flavonoids	+
Protein	+
alkaloid	+
Phenol	-
terpenoids	+

Table :2 Phytochemical Analysis of APHF

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GC-MS ANALYSIS

Numerous chemicals found in the GC fractions of MPHF that were extracted using a Soxhlet extractor can be identified thanks to the results of GCMS. Numerous phytochemical substances were present, as shown by the chromatogram. (Fig.2) including 4H-Pyran-4-one, 2,3- dihydro-3,5-dihydroxy-6- methyl, Benzofuran, 2,3-dihydro, Hydroquinone, Phenol, 2-methoxy-3-(2- propenyl), Eugenol, Caryophyllene, Humulene, Hydroxychavicol, (1S,4aR,8aS)-1-Isopropyl-7-methyl-4- methylene-1,2,3,4,4a,5, Germacrene D, Phenol, 2-methoxy-4-(2- propenyl)-, acetate, 3-O-Methyl-d-glucose, n-Hexadecanoic acid, Phytol, cis,cis,cis-7,10,13- Hexadecatrienal, cis,cis,cis-7,10,13- Hexadecatrienal.

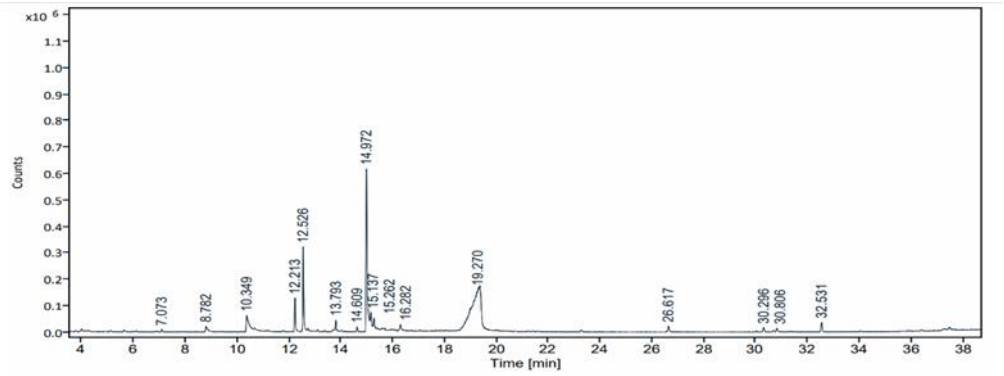

Figure-3: GC-MS Analysis of MPHF

4. Conclusion

However, the effectiveness of these plant extracts against dandruff has not been fully explored. Research is needed to evaluate the efficacy, safety, and formulation of these plants to develop them as potential treatments for dandruff. Additionally, further studies need to be conducted to determine long-term benefits and potential side effects of these natural remedies. *Piper betel, Tictona grandis, Eclipta prostrata,* and *Tenner cassia* exhibit promising anti-dandruff activities through their antifungal mechanisms. Therefore, these natural remedies hold great potential as alternative treatments for dandruff caused by the Malassezia fungus.

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