



Original Research Article

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Antidandruff and Anti-Inflammatory Activity of *Piper nigrum* Extract against Dandruff Causing Pathogens

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ABSTRACT

Pepper is known as king of spices belongs to the family Piperaceae. Nearly 55 phytochemical compounds were present in pepper. *Piper nigrum* was extracted with ethyl acetate and chloroform solvents and find its efficacy against dandruff causing fungus. Dandruff samples were collected and fungus were isolated from dandruff and identified as *Microsporum* sp. and *Trichophyton* sp. Antifungal evaluation of different extracts of *Piper nigrum* were tested against dandruff causing isolates. Among the extracts ethyl acetate showed higher antifungal activity against selected fungus. Antidandruff and anti-inflammatory activity of ethyl acetate extract of *Piper nigrum* were carried out and showed better activity on *Microsporum* sp. The results were compared with commercially available shampoos. On comparison, ethyl acetate extract of *Piper nigrum* showed excellent inhibitory activity against anti dandruff and anti-inflammatory.

Introduction

Dandruff was a common scalp disorder (epidermal growth) and the shedding of dead skin cells from scalp. It was associated with seborrheic dermatitis. Keratinocytes play a role in the expression and generation of immunological reactions during dandruff formation. It causes nearly half of the world's population. Probably affects males more than the females. Dandruff was contagious and caused by fungal infection. Dermatophytes were

fungi that require keratin for growth. The most common dermatophytes were *Microsporum* sp. and *Trichophyton* sp. *Microsporum* was a genus of fungi that causes tinea capitis, tinea corporis, ringworm, and other dermatophytoses. The most common *Microsporum* species were *M. gypseum* and *M. canis*. The fungus genus *Trichophyton* was characterized by the development of both smooth-walled macro- and micro-conidia. The most important significant member of genus were *T. tonsurans* and *T. mentagrophytes*.

Most of the traditionally used medicinal plants has got the therapeutic properties and has been used for improving immune system as well as to cure various ailments (Ravichandran et al., 2004). There were many natural and artificial methods were regulated for the control of dandruff. People mostly depend on commercial shampoos, ointments, creams for the control of dandruff. Components present in those products include Zinc Pyrithione, Ketoconazole, and antifungal agents like Clotrimazole, Amphotericin B, Miconazole, Nystatin, etc. (Chandrani et al., 2012). Plant contains natural bio active components like alkaloids, flavonoids, tannins, terpenoids, etc. We choose white pepper (*Piper nigrum*) for the present study because of its antifungal and antidandruff properties and had better effectiveness to treat dandruff.

Materials and methods

Collection of plant material and preparation of plant extract

The fresh white pepper was collected from local market of Mambalam, Chennai. White pepper was crushed by mixer grinder. Cold extraction method was followed using different solvents (Ethyl acetate and Chloroform) and finally the extracts were filtered through Whatman No.1 filter paper. The crude extract obtained was evaporated and stored at 4°C in refrigerator until use.

Isolation of dandruff causing fungus

Samples were collected from scalp of 6 persons suffering from dandruff. Collected samples were inoculated into sterile Sabouraud Dextrose Agar (SDA) media and incubated at 32°C for 3-5 days.

Identification of dandruff causing fungus

Microscopic and macroscopic identification were done to specifically identify fungus. Microscopic identification was done by Lactophenol Cotton Blue (LPCB) test. Macroscopic identification was done by morphological, cultural characteristics on SDA.

Dilution of samples

The commercially available shampoos (ketaconazole and loreal paris), antibiotic, and *Piper nigrum* were diluted using dimethyl sulfoxide (Naga Padma et al., 2015).

Antifungal assay (Surabhi et al., 2015)

To check the Minimum Inhibitory Concentration (MIC) of *Piper nigrum* extracts were made in the range of 1000µg, 750µg and 500µg samples and their antifungal activity were checked using disc diffusion method. Agar disc diffusion method was done to determine the zone of inhibition of extracts against dandruff causing *Trichophyton* sp. and *Microsporum* sp.

Antidandruff assay (Sibi et al., 2012)

Various concentrations of the samples (ethylacetate and chloroform extracts), Loreal Paris and Ketaconazole were diluted in DMSO from the resultant extract to determine its antidandruff activity. Samples were loaded on the sterile discs and placed on the media plates. The plates were incubated at 37°C for 3 to 5 days. The zone of inhibition was measured in two directions at right angles to each other by measuring in mm and recorded against the corresponding concentration.

Anti-inflammatory assay (Gandhisan et al., 1991)

Fresh blood was collected and mixed with EDTA which acts as an anticoagulant. Samples were centrifuged at 10,000 rpm for 15 min at room temperature. The supernatants were carefully removed while the packed red blood cell was washed in fresh normal saline (0.85% NaCl). The process of washing and centrifugation were repeated five times until the supernatants were clear. This RBC suspension was used for the estimation of anti-inflammatory property.

Different concentration of sample and control were separately mixed with 2ml of phosphate buffer, 4ml

of hyposaline and 0.9ml of RBC suspension. All the assay mixtures were incubated at 37°C for 30 minutes. The supernatant liquid was decanted and the hemoglobin content was estimated by spectrophotometer at 620nm.

Results

For the present study was carried out for the period of March 2018- May 2018. *Piper nigrum* was collected and extracted with two different solvents (ethyl acetate and chloroform) and collect crude extract for further studies. Collected dandruff samples were inoculated on sterile SDA (Sabouraud dextrose agar) plates.

Typical white grayish growth around the plate was indicative of dandruff causing fungus. Dandruff

scrapings gave white colonies around dandruff flakes. Isolated fungus was further used for microbiological analysis.

Isolated fungus were stained using Lactophenol cotton blue stain and observed under 45X objective (Fig. 1 and Fig. 2). Fig. 1 showed that the typical formation of macroconidia with hyaline, multiseptate, variable in form, spindle shaped to ovovate ranging from 20 to 300µm in size. It indicates that the most common *Microsporum* sp. was isolated and identified.

Fig. 2 showed that the typical formation of macroconidia that borne laterally directly on the hyphae with short pedicels and thin walled ranged from 8 to 50µm in size. It indicates that the most common *Trichophyton* sp. was isolated and identified.



Fig. 1: *Microsporum*

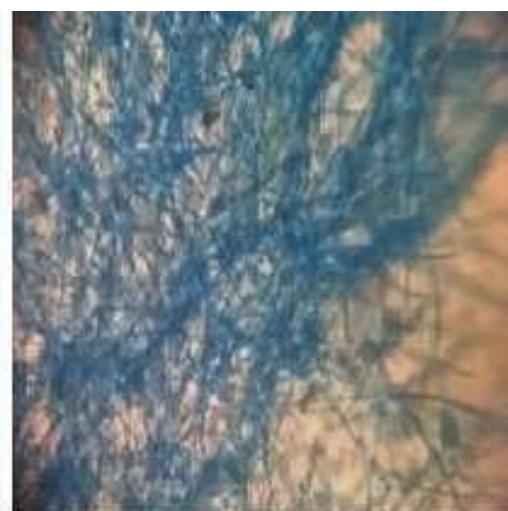
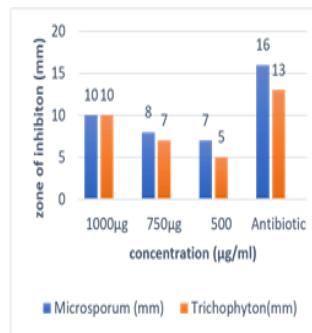
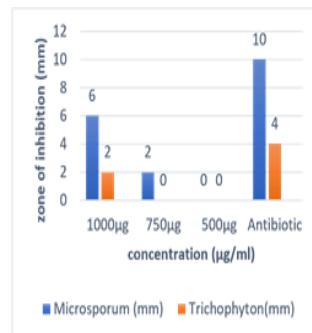


Fig. 2: *Trichophyton*

Total of two solvents (ethyl acetate, chloroform) were used to extract *Piper nigrum*, commercially available shampoos like Loreal Paris, Ketacanozole and standard Antibiotic (Fluconazole) were screened out for the study of antifungal and antidandruff activity against two different fungal sp. namely *Microsporum* sp. and *Trichophyton* sp. isolated from the dandruff samples. Antifungal activity of ethyl acetate and chloroform extracts were represented by concentration vs zone of inhibition in graph 1, 2 and Figs. 3, 4.



Graph 1



Graph 2

Fig. 3 shows the zone of inhibition in *Microsporum* sp. (A) and *Trichophyton* sp. (B) of ethyl acetate extract as antifungal activity.



Fig.3 A

Fig.3 B

Fig. 4 shows the zone of inhibition in *Microsporum* sp. (A) and *Trichophyton* sp. (B) of chloroform extract as antifungal activity. Note: 1-1000 µg, 2-750 µg, 3-500 µg, A-Antibiotic.

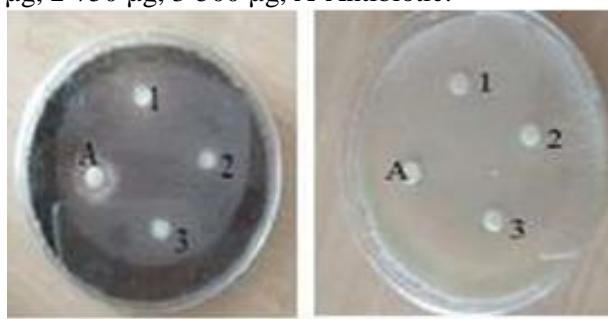
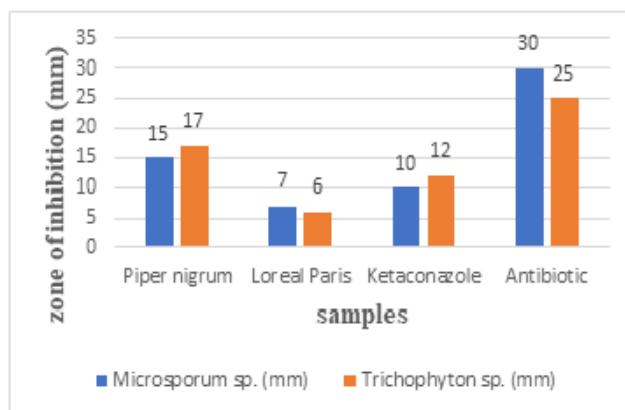


Fig.4 A

Fig.4 B

From the graph 1 & 2 and Figs. 3 & 4 we infer that ethyl acetate shows better results for both *Microsporum* sp. and *Trichophyton* sp. for antifungal activity. Antidandruff activity of samples was represented by concentration vs zone of inhibition in graph 3.



Graph 3

Fig. 5 shows the zone of inhibition in *Microsporum* sp. (A) and *Trichophyton* sp. (B) of samples as anti-dandruff activity. Note : 1-Piper nigrum, 2-Loreal paris, 3-Ketaconazole, A-Antibiotic (Fluconazole).

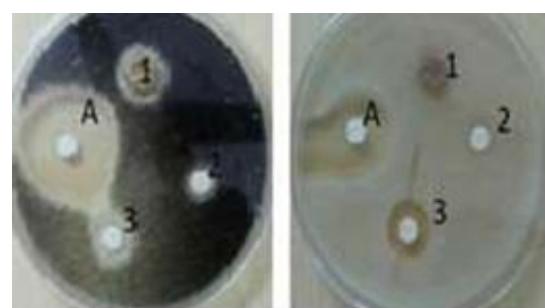
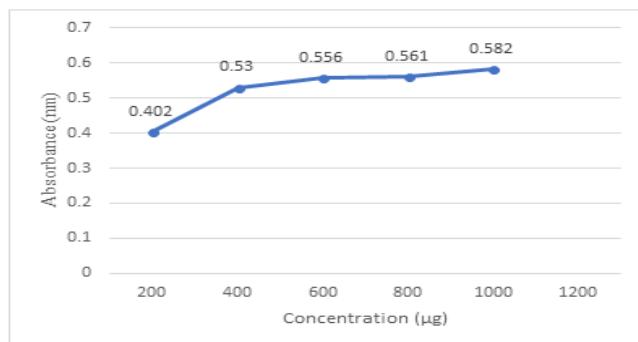


Fig.5 A

Fig.5 B

From the graph 3 and Fig. 5 we infer that ethyl acetate extract shows better results for *Microsporum* and *Trichophyton* as antidandruff activity.

Anti-inflammatory activity of *Piper nigrum* represented by concentration vs absorbance in graph 4. From graph 4 we infer that *Piper nigrum* has better anti-inflammatory activity on ethyl acetate extract.



Graph 4

Discussion

A person's entire body surface continuously sheds dead skin cells and the skin itself sheds every twenty-four days. Dandruff, the shedding of dead skin cells from the scalp at an excessive rate, is the result of the normal growing process of the skin cells of the scalp. The identification of the isolates is done normally by comparing morphological and physiological characteristics (Rasika et al., 2016).

Dandruff may be caused by several different factors, but the exact underlying cause of dandruff is unknown. Dandruff is the visible desquamation of scalp, is the mildest manifestation of seborrheic dermatitis. Common ways to control dandruff is to use agents like imidazoles, selenium sulphide, coal tar and salicylic acid. These shampoos work by slowing down cell turnover and may reduce the number of *Malassezia* (Ravichandran et al., 2004).

During the past few decades, there has been a dramatic increase in the use of natural products in cosmetics. All the antidandruff shampoos had good antifungal activity but there is considerable variation in the potency of their antifungal activity depending on the active compound and

its concentration. In the present study the best antidandruff shampoo was L'Oréal Paris as it contains Zinc pyrithione and arginine. This was followed by Ketoconazole as it contains antifungal compounds like coconut fatty acid diethanolamide and disodium monolauryl ether sulfosuccinate.

The most common cause of dandruff is probably the fungus *Malassezia furfur*. The plants were selected based upon their usage as traditional medicine for treating dandruff (Mamatha et al., 2016). Most of the plant extracts were showing good antifungal activity almost equivalent to that of commercially available shampoos. *Piper nigrum* had more antifungal activity and this could be because of their active compound Piperine and also it has antitumour and anticancer activity. Use of natural products is not only cost effective but also negligible side effects. The present study was significant as not only efficient known plant products but also their better efficacies at minimum concentrations could be identified. Herbal drugs or their formulations are viable alternative to synthetic drugs. During the past few decades, there has been a dramatic increase in the use of natural products in cosmetics (Chandrani et al., 2012). The chemicals used for the treatment of dandruff have certain limitations; they are unable to prevent occurrence, which is a common troublesome clinical problem. Most products which are designed to fight dandruff contain zinc pyrithione. Ketoconazole and ZnPTO based shampoos are used more by the consumers for common dandruff problems. Herbal drugs or their formulations are viable alternative to synthetic drugs.

Further this research work can help make a polyherbal mixture that could be incorporated in hair oil or shampoos for better anti-dandruff activity. The excellent antidandruff action of *Piper nigrum* might have been due to the synergistic antifungal, anti-inflammatory and local immunostimulatory actions of its ingredients, which has been well documented by various research workers (Ravichandran et al., 2004).

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

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References

- Chandrani, D., Lubaina, S. Z., Soosama, M., 2012. A review of antifungal effect of plant extract vs chemical substances against *Malassezia* sp. Int. J. Pharma BioSci. 3(3), 773-780.
- Gandhisan, R., Thamaraiichelvan, A., Baburaj, S., 1991. Antiinflammatory action of *Lannea coromandelica* HRBC membrane stabilization. Fitoterapia. 62, 82-83.
- Mamatha, P., Sridhar, V., Ramakrishna, R., 2016. Antifungal activity of plant extracts against dandruff causing organism *Malassezia furfur*. Int. J. Bioassays. 5(11), 5047-5049.
- Naga Padma, P., Anuradha, K., Divya, K., 2015. Comparison of potency of antifungal action of dandruff shampoos and different plant extracts. Int. J. Med. Res. Health Sci. 4(2), 327-331.
- Nikam, S. R., Khanvilkar, V. V., Jagdale, D. M., Jadhav, A. P., More, S. H., Kadam, V. J., 2013. Evaluation of antibacterial and antifungal activities of marketed anti dandruff shampoos. Indo-Amer. J. Pharmaceut. Res. 3(10), 8097-8100.
- Rasika, M., Parameshwari, S., Sivagurunathan, P., Uma, C., Bhuvaneshwari, M., 2016. Antifungal activity of amla extracts against dandruff causing pathogens. Int. J. Adv. Res. Biol. Sci. 3(1), 209-214.
- Ravichandran, G., Shivram Bharadwaj, V., Kolhapure, S. A., 2004. Evaluation of the clinical efficacy and safety of "Antidandruff Shampoo" in the treatment of dandruff. The Antiseptic. 201(1), 5-8.
- Sibi, G., Gurmeetkaur, Geeta Devi, Dhananjaya, K., Ravikumar, K. R., Mallesha, H., 2012. Antidandruff activity of *Ricinus communis* L. leaf extracts. Int. J. Curr. Pharmaceut. Res. 4(3), 74-76.
- Surabhi, P., Vaishali, M., 2015. Studies on antifungal activities of certain plant extracts against dandruff causing fungus, *Malassezia*. Int. J. Curr. Res. Biosci. Plant Biol. 2(7), 206-211.

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