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# **Exploring the Anti-dandruff Potential of Selected Medicinal Plants**

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## ABSTRACT

Dandruff is the most common problem that affects many people leading to an embarrassing condition. It results from three main factors namely: *Malassezia* fungi, sebaceous secretion and individual sensitivity. The liphophillic yeast *Malassezia furfur* is the most common dandruff causing agent in India. In this study dandruff flakes were isolated from patient and cultured in appropriate media like Potato dextrose agar and Sabouraud's dextrose agar. The antidandruff activity of three selected medicinal plants-*Piper cubeba, Cissus quadrangularis* and *Bauhinia vahlii* was studied by well diffusion and broth dilution assay. On screening, methanol extract of *Piper cubeba* showed good activity and further on broth dilution assay gave a Minimum inhibitory concentration (MIC) at 100µg/ml and IC50 at 800µg/ml. On partial purification through TLC and bio-autography an R<sub>f</sub> value of 0.705 was obtained where the active compound had activity against dandruff.

Keywords: Dandruff; Malassezia furfur; Potato dextrose agar; MIC; IC50.

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## INTRODUCTION

Dandruff is a common scalp disorder defined as a slight-to-moderate scaling of the scalp with varying degrees of irritation or erythema. The characteristic flaking and scaling of the scalp suggest impairment in the desquamation process. The etiology of dandruff is multi-factorial, influenced by *Malassezia*, sebum production and individual susceptibility. The commensal yeast *Malassezia* is a strong contributory factor to dandruff formation. The lipophilic yeast *Malassezia* utilizes sebum lipids as a nutrient source, and sebum production is hypothesized to be required to support growth of *Malassezia*. It has been widely theorized that this increase in sebum production and *Malassezia* proliferation triggers the development of dandruff. The exact mechanism of dandruff formation is now believed to be the result of the formation of enzymes called lipases. The *Malassezia* fungus uses these enzymes to break down sebum to oleic acid. The oleic acid then penetrates the top layer of skin and causes increased skin cell turnover in susceptible people. This in turn causes dandruff flakes and sometimes leads to itching and redness.

The current strategy for treating dandruff with antidandruff products and most are chemicals too crude for daily use. Several fungistatic compounds have been shown to improve dandruff condition. Besides the chemical substances, there is a wide range of herbal ingredients like pepper extract, basil extract, neem extract, rosemary oil, basil oil, clove oil, coleus oil, tea tree oil which have been documented to have good antidandruff activity. Herbal medicine, now a days are gaining importance for treating many diseases due to their significant effect and lesser side effects as compared to allopathic medicines. In view of the above said facts, the present study was designed to investigate and determine the antidandruff activity of three different medicinal plants – *Piper cubeba* (Valmilagu, Kavab cini ), *Bauhinia Vahlii* (Mandarai, Malu), *Cissus quadrangularis* (Pirandai, Hadjod).

### MATERIALS AND METHODS

The fresh leaves of *Bauhinia vahlii*, stem of *cissus quadrangularis* and seeds of *Piper cubeba* were collected from local sources and then they were grind into powder and stored in room temperature. Finely grinded plant material was extracted with hexane, ethyl acetate and methanol in the ratio of 1:10 in conical flask in shaking condition for overnight. The extract was filtered through the Whatmann No. 1 filter paper in a separate container. The process was repeated three times and the same plant material was used with fresh solvent. The solvent was removed by placing the extracts in distillation unit and maintained in respective temperature. The extracted residues were weighed and re-dissolved in different solvents to yield 10mg/ml solutions for further analysis.

Dandruff causing agent samples were collected by scraping the lesions of patients and stored in sterile containers in refrigerator until use. Different media formulations (potato dextrose agar, Sabouraud's dextrose agar) were supplemented with olive oil and inoculated with the sample. The plates were incubated at 37°C for 3- 5 days .The culture was stained with



lactophenol cotton blue stain and examined under the high power objective of microscope and the characters were recorded.

Various concentrations (250-1000µg/ml) of the extracts were prepared in DMSO from the resultant extract to determine its antidandruff activity. Control experiments were performed by using DMSO with identical concentration used to test the extract. Isolates from dandruff were inoculated by swabbing on the surface of gelled media plates. Wells of 6 mm in diameter were performed in the PDA media, and each well was filled with 50 µl of certain concentration of extract. The plates were kept in laminar air flow for 30 minutes for proper diffusion of the extract and thereafter incubated at  $37^{\circ}$ C for 3-5days. The radius for the zone of inhibition was in millimeters and recorded against the corresponding concentration.

Broth Dilution assays are standard method used to compare the inhibition efficiency of the antimicrobial agents. 5ml of the potato dextrose broth, 0.1ml of the 24 h growing culture (*M. furfur*) and the different concentration ( $100\mu g$ ,  $200\mu g$ ... $1000\mu g$ ) of the crude extract dissolved in Dimethyl sulphoxide. The tubes were incubated at  $37^{\circ}C$  for 24 h. The optical densities were measured spectrometically at 600 nm (Cos *et al.*, 2006). The percentage of viable cells was calculated by using the following formula.

O.D = Optical density

The organism was biochemically analysed by assays such as Catalase test, Nitrate reduction test and Urea hydrolysis test using standard procedures.

## RESULTS

The extract of three plants *Piper cubeba, Bauhinia vahlii, Cissus quadrangularis* are obtained by direct solvent extraction method. In this method, different solvents were used namely hexane, ethyl acetate and methanol .The flakes from different patients were collected and cultured in potato dextrose broth and sub-cultured in Sabouraud Dextrose Agar (SDA)and Potato Dextrose agar(PDA) by swabbing and streaking method.The significant growth was observed in PDA. Well diffusion was done to determine the anti-fungal activity of three selected plants *Piper cubeba, Bauhinia vahlii, Cissus quadrangularis* with different solvents at different concentrations (250, 500, 750, 1000)  $\mu$ g/ml. The activity was found to be effective in the methanol extract of *Piper cubeba* and it was taken for further assay. Table 1 Broth dilution assay was performed to know the minimum inhibitory concentration of *Piper cubeba*. The MIC was 100 $\mu$ g/mL and IC<sub>50</sub> was found to be 800 $\mu$ g/mL [.Table 2 and Graph 1]



#### Table 1: Well Diffusion

S .No	Conc.	Zone of inhibition (mm)									
5.110	(μg/mL)	Piper cubeba			Bauhinia vahlii			Cissus quadrangularis			
		Methanol	Ethyl acetate	Hexane	Methanol	Ethyl acetate	Hexane	Methanol	Ethyl acetate	Hexane	
1	250	-	-	-	-	-	-	-	-	-	
2	500	12	-	-	-	-	-	-	-	-	
3	750	16	-	-	13	-	-	13	-	-	
4	1000	18	-	-	16	-	-	15	-	-	

#### **Table 2: Broth Dilution Assay**

S .No	Concentration(µg/ml)	% Inhibition
1	100	32.45
2	200	35.81
3	300	37.62
4	400	40.47
5	500	43.26
6	600	45.45
7	700	48.99
8	800	51.65
9	900	55.33
10	1000	60.93





#### DISCUSSION

Currently available treatments for dandruff include therapeutic use of Zinc Pyrithione, salicylic acid, Imidazole derivatives, Glycolic acid, Steroids, Sulfur and Tar derivatives. However, these tools have limitations in the application, or due to poor clinical efficacy or due to poor response to their use. Hence the herbal drugs are prove to be eco-friendly, alternative for synthetic drugs. In order to avoid the harmful effects of synthetic drugs for controlling dandruff, it is suggested to choose herbal medicines for its control. In the present study among the three



medicinal plants, methanol extract of *Piper cubeba* shows significant antidandruff activity against *Malassezia furfur* giving a zone of inhibition of 18mm at 1000µg/ml and an IC<sub>50</sub> value at 800µg/ml. Thus, methanol extract of *Piper cubeba* can be suggested as effective and safe therapeutic agent against dandruff. Hence such type of study will certainly bring out scope for further research to identify the potent bioactive compound having remarkable therapeutic value against dandruff.

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