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Research Article

QUANTITATIVE MICROSCOPY AND *IN VITRO* ANTI DANDRUFF ACTIVITY OF *DICHROSTACHYS CINEREA* WIGHT AND ARN

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ABSTRACT
Natural products have been used in traditional medicine for thousands of years and recently have been of increasing interest, since the cost are usually lower and they are considered less toxic by the public. The plant *Dichrostachys cinerea* is profusely branched thorny shrub distributed throughout the dry and warm parts of India belonging to the family Mimosaceae. The standardization of the leaves of *D. cinerea* were performed by Quantitative microscopy such as stomatal no, stomatal index, vein islet no. and vein termination no and foam index were examined. The aim of our study was to assess *in vitro* antidandruff activity of ehanolic extract of leaves of *D. cinerea* by disc diffusion method, the zone of inhibition were measured. It was observed that, no profound activity at lower concentration (50 mg/ml, 100 mg/ml) and showed only moderate activity at higher concentration (250 mg/ml and 500 mg/ml) against the fungi *Mallazessia furfur* or *Pityrosporum ovale.*
Keywords: Anti Dandruff activity, *Mallasezia furfur*, *Pityrosporum ovale*, *D. cinerea.*

INTRODUCTION

Dandruff is a major cosmetic problem that possesses very great public health concern both in developed and developing countries. The problem manifests as profuse white to silvery powdery scales in the scalp region often with moderate to severe itching. Dandruff also referred as (Pityriasis simplex) is a common embarrassing disorder, which affects 5 % of the global population. Dandruff mostly occurs after puberty (between ages 20 – 30 Years), and affects males more than females.[1] *Pityrosporum ovale (Malassezia furfur*), a yeast like lipophilic basidiomycetous fungus, is considered to be the chief cause of the problem. Besides this, *candida* species is also suspected in the disease process of dandruff. These organisms are widely considered to be the commensally flora of the scalp and skin region. *Pityrosporum ovale* on the dermal liquids and proteins and facilitates lipase activity, which releases pro inflammatory free fatty acids (FFAS) causing dermal inflammation and tissue damage. The lipase activity indicates that in addition to hypersensitivity, *Pityrosporum ovale* releases toxic chemicals which contribute to the development of fungi infection.[2] Currently available treatment options for the management of dandruff include therapeutic use of zinc pyrithione, salicylic acid immidazole derivatives, glycolic acid. However these agents have certain limitations either due to poor clinical efficacy or due to compliance issues. Furthermore these drugs are unable to prevent recurrence which is the commonest problem. Moreover the drugs used may affects the eyes during their application. So it is necessary to screen a drug especially of herbal origin to treat dandruff without affecting eye. Review of literature revealed that, the plant belongs to the family Mimosaceae is used for the treatment of ophthalmia. Previously it was reported that, the n-hexane and chloroform extracts of aerial part of *D. cinerea* showed antifungal activity against *Aspergillus niger* and Mucor species at 10 mg/ml concentration but the chloroform extract showed activity only at higher concentration.[3] This study and the ethno medical use prompted us to carry out the Antidandruff screening study.

MATERIALS AND METHODS

The plant *D. cinerea* was collected at dry forests area of Perambalur District in Tamil Nadu, India during the first week of August and it was authenticated by the Taxonomist. The leaves portion was washed thoroughly and dried in shadow. The shadow dried leaves were powdered.

Preparation of Extract

The plant material was powdered sieved, (powder mesh size 60). The powder was defatted with pet ether by continuous hot percolation using soxhlet apparatus then extracted with ethanol (99 %) for six hours. The solvent was removed under reduced pressure. The residue (2.75 %) was semisolid dark green in color, viscous in consistency. For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5 % sodium hydroxide and epidermal peeling by partial maceration employing jeffrey's maceration were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials.

Determination of Vein Islet Number and Vein Termination Number

Leaf lets were cleared in chloral hydrate, stained and mounted on a slide. A camera Lucida is set up and by means of a stage micrometer the paper in divided into squares of 1 mm^2 using a 16 mm objective. The stage micrometer is then replaced by the cleared preparation and the veins are traced in four contiguous squares, either in a square 2 mm x 2 mm (or) rectangle 1 mm x 4 mm. When counting, it is convenient to number each vein-islet on the tracing. Each numbered area must be completely enclosed by veins and those which are incomplete are excluded from the count if cut by the top and left-hand sides of the square (or) rectangle but included if cut by the other two sides.[4,5]

Determination of Stomatal Number

Stomatal number is defined as the number of stoma present in one square mm area of the photosynthetic tissues. Using fresh

leaves, replicas of leaf surface may be made which are satisfactory for the determination of stomatal number and stomatal index. An approximate 50 % gelatin and water gel is liquified on a water – bath and smeared on a hot slide. The fresh leaf is added, the slide inverted and cooled under a tap and after about 15-30 minutes the specimen is stripped off. The imprint on the gelatin gives a clear outline of epidermal cells, stomata and trichomes[4,5].

Determination of Stomatal Index

It is the percentage, which the numbers of stomata from the total number of epidermal cells, each stoma being counted as one cell.

$$I, \text{Stomatal index} = S/S + E \times 10,$$

Where S = Number of stomata per unit area and
E= Number of epidermal cells in the same unit area

The procedure adopted in the determinations of stomatal number was observed under high power (45 x). The epidermal cells and the stomata were counted. From these values the stomatal index were calculated using the above formula.

Determination of Foaming Index

One gram of the coarsely powdered leaf was weighed and transferred to 500 ml conical flask containing 100 ml of boiling water. The flask was maintained at moderate boiling, at 80-90^{o}C for about 30 minutes. Then it was cooled and filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100 ml (V1). Ten stopper test tubes were cleaned (height 16 cm, diameter 1.6 cm) and marked from 1 to 10. Measured and transferred the successive portions of 1, 2, 3 ml up to 10 ml and adjusted the volume of the liquid in each tube with water to 10 ml. Then the tubes were stopper and shaken lengthwise for 15 seconds, uniformly and allowed to stand for 15 minutes and measured the length of the foam in every tube.[6] The length of the foam was less than 1 cm in every tube and hence the Foaming index in less than 100.

Collection and Maintenance of the Culture

Pure culture of *M. furfur* (MTCC: 1374) was obtained from institute of Microbial type of culture collection, Chandigarh, India. The culture was maintained in SDA medium.

Inoculum Preparation

The peptone was added to the liquid SDM in the concentration of 5, 10, 15 and 20 g/lr. Pure culture of *M. furfur* grown in liquid medium was inoculated and incubated at 30 ± 2^{o}C for 7 days.

Preparation of the Medium

2 g of SDA medium and 1 g of Agar was dissolved in 50 ml of distilled water heat to boiling to dissolve the medium completely, sterilize by autoclaving at 15 lbs pressure (121^{o}C for 15 mts p^{H} is adjusted to (5.6 ± 2^{o}C). The medium was poured into the sterile petridishes to get a thickness of 5-6 mm. The medium was allowed to solidify and petridish was inverted and were dried at 37^{o}C just before inoculation.[7]

Antimycotic Assay (Disc – Diffusion Method)

The broth culture of *M. furfur* was swabbed over the sabouraud dextrose agar by using sterile cotton buds. Sterile 5 mm diameter whatman No. 32 filter paper discs were dipped in plant extracts and Clotrimazole (Standard drug10 µg/disc) and control DMSO disc were placed equidistantly (3 cm apart) round the margin of the plates (Plate No-18, 19). Three replicates were maintained. The plates were incubated at 30 ± 2^{o}C and zone inhibition was observed after 3 days. The results were tabulated in Table 3.

RESULTS AND DISCUSSION

The Pharmacognostical standardization of fresh leaves of *Dichrostachys cinerea* was studied by quantitative microscopy, in order to prevent the adulterations. The vein islet and vein termination number of *D. cinerea* were given in (Table 1). The stomatal number and stomatal index were reported in. (Table 2).

Table 1: Vein islet and vein termination number

Parameters	Minimum	Average	Maximum
Vein islet number	10	12.5	15
Vein termination number	6	7.4	9

Table 2: Stomatal number and stomatal index

Parameters	Minimum	Average	Maximum
Stomatal number			
Lower epidermis	75	79.9	84
Stomatal index			
Lower epidermis	23.26	25.04	27.08

The antidandruff activity of ehanolic extract of *D. cinerea* against *Malassezia furfur* were studied by disc diffusion method, in SDA medium (Plate No.- 18) the zone of inhibition was measured. The results were tabulated in Table 3.

Table 3: Antidandruff activity of ethanolic extract of *D. cinerea* against *M. furfur*

S. No.	Drug	Concentration	Zone of inhibition
1.	Ethanolic extract	50 mg/ml	Nil
		100 mg/ml	Nil
		250 mg/ml	8 mm
		500 mg/ml	11 mm
2.	Standard (Clotrimazole)	10 µg/disc	15 mm
3.	Control DMSO	-	-

It was observed that the zone of inhibition was moderate when compared to the standard drug Clotrimazole and Zone of inhibition was slightly concentration dependent and not significant and having moderate inhibition. There was mild increase of inhibition by increasing concentration above 500 mg/ml. The diameter of the zone of inhibition is influenced by a variety of factors such as diffusibility of the drug, disc concentration, the nature and composition of the medium, its thickness and presence of inhibitory or stimulatory substances, pH of the medium and the time of incubation. During incubation, the therapeutic agent diffuses out from the disc in all directions. Agents with lower molecular weights diffuse faster than agents with higher molecular weight might be a powerful inhibitor even though it may diffuse only a small zone of inhibition. Moreover, results obtained *in vitro* often differ from those obtained *in vivo*. Metabolic processes in the body of a living organism may inactivate or inhibit on antimicrobial compound.[8]

CONCLUSION

The standardization of the leaves of *D. cinerea* were studied by Quantitative microscopy such as stomatal number stomatal index vein islet and vein termination number in order to check the purity of the leaves. In this study also concluded that the ethanolic extract of the leaves of *D. cinerea* posses only moderate antidandruff activity against *M. furfur*.

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