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Research Article

EVALUATION OF INHIBITORY EFFECT OF *VERNONIA CINEREA* L. LEAF EXTRACTS ON DIFFERENT FUNGAL SPECIES

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ABSTRACT

Objective: Fungal infection is a very serious cosmetic problem which is of utmost concern globally, one such infection being Dandruff. Finding anti-dandruff molecules from natural sources is the current research focus among cosmetic researchers. Medicinal plants may offer a valuable source for antidandruff molecules. Hence, in the present study, *Vernonia cinerea* L. was studied for its antidandruff activities.

Methods: Initially, *V. cinerea* plant leaves were extracted with different solvents and studied for their antifungal activity against *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis.* Furthermore, the antidandruff activity of ethyl acetate extract was studied and compared with a commercial antidandruff shampoo. In addition, the ethyl acetate extract was subjected to preliminary phytochemical screening and thin layer chromatography to elucidate the bioactive principles present in the extract.

Results: From the data obtained, it was found that the ethyl acetate extract of *V. cinerea* leaves has shown very good antifungal activity at all concentrations tested. Also, the ethyl acetate extract exhibited excellent antidandruff activity against *Pityrosporum ovale* and *Pityrosporum folliculitis*. Ethyl acetate extract of *V. cinerea* has exhibited increased synergistic antidandruff activity with commercial product. Comparatively, the *V. cinerea* extracts alone or in combination with commercial product exhibited more antidandruff activity against *P. folliculitis.* Qualitative phytochemical tests, thin layer chromatography of ethyl acetate extracts demonstrated the presence of common alkaloids, carbohydrates and glycosides, glycosides, saponins, proteins, amino acids. Moreover, phenols were found to be the major active constituent in *V. cinerea* which constitutes as a good source of antidandruff agents.

Conclusion: The results of the study suggest that the leaves of *Vernonia cinerea* can be further exploited for its therapeutic use against several fungal infections.

Keywords: *Vernonia cinerea,* Antifungal activity, Antidandruff activity, Phytochemicals.

INTRODUCTION

Dandruff is a common scalp problem that occur when dead skin is shed, producing irritating white flakes and possibly an itchy scalp. Hair fall is very common in dandruff sufferers [1, 2]. Generally, dandruff is thought to represent the mildest form of seborrheic dermatitis of the scalp. Its pathogenesis remains to be entirely elucidated, although the yeast organism *Malassezia* has been proposed as an etiological factor [3]. The genus *Malassezia* belongs to the basidiomycetous yeasts and is classified in the Malasseziales (Ustilaginomyces, Basidiomycota) [4]. Among the different *Malassezia* species, *M. globosa* and *M. restricta* have been most closely associated with dandruff in humans [5]. *Malassezia* may stimulate cytokine production by keratinocytes (epidermal cells that synthesize keratin), further contributing to the inflammatory component of seborrheic dermatitis and dandruff [6]. It is also stated that, *Malassezia* sp. may produce tryptophan metabolites which are active on the aryl hydrocarbon receptor, thus produce inflammation [7].

At present, many chemical substances are used for treating dandruff by controlling the abundance of yeasts on the scalp. The main active agents used currently for controlling dandruff include imidazole derivatives such as ketoconazole and other compounds. They act by removing the scales, reduce *Malassezia* species adherence to corneocytes and restrain its growth. Though a wide variety of antifungal agents are available for the treatment of dandruff, a complete cure is far from reach. Further, most of the available drugs are either fungistatic or expensive in nature and phytochemicals are less potent [8]. As fungal resistance to synthetic antibiotics is in rise [9] an alternative in the way of herbal medicine in controlling the same is the need of the hour. The medicinal value of plants can be attributed to some chemical substances which produce a definite physiological action on the human body. The most important of such bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds [10]. In the present study, an attempt was made to investigate the potential antidandruff activity of *Vernonia cinerea* (L.) as an alternative strategy.

Vernonia cinerea (Family: Asteraceae) is a terrestrial annual erect herb, widely distributed in India, Bangladesh, Sri Lanka and Malay island [11]. The plant is extensively used in indigenous medicine as stomachic and for cold, asthma and bronchitis [12]. The leaf juice extract is used to treat skin diseases and treating dysentery in children [13], where as the roots of the plant are used for the treatment of eruptive boils, quicker healing of accidental wounds, filariasis and toxic viral fevers. However the antidandruff activity of the same plant has not been explored yet. Hence, to promote the use of medicinal plants as potential sources of antimicrobial compounds it is necessary to thoroughly investigate their composition and activity [14]. Therefore, the present study was taken up to assess the crude leaf extracts of *V. cinerea* for its antidandruff action against *Malassezia* sp.

MATERIALS AND METHODS

Plant material

The fresh plant of *Vernonia cinerea* (Linn.) was collected from fields located in Bharathiar University Campus at Coimbatore, India. The leaves were separated from the steam, washed with tap water, rinsed with distilled water, and air-dried for 1h. Then the leaves were shade dried in room temperature for one week. They were ground into powder and subjected to extraction with different solvents.

Plant extracts preparation

The ground leaves were extracted with different solvents such as, chloroform, ethyl acetate and methanol following the method of

Eloff [15]. The extraction of the leaf powder was done with solvents in the ratio of 1:10 under shaking condition. The extracts were collected in different conical flasks and the same was repeated thrice to attain maximum extraction. Then the solvents were condensed to concentrate the extracts obtained. The concentrated extracts were weighed and re-dissolved in respective solvents to yield 10mg/mL solutions for further analysis.

Antifungal activity

Well diffusion assay [16]

Potato Dextrose agar was prepared, sterilized and poured in the Petri dishes (9 cm). 24 h growing culture of *Candida albicans* (MTCC 183), *Candida parapsilosis* (MTCC 2509) and *Candida tropicalis* (MTCC 184) were swabbed on the solidified plates and wells (10mm dia.) were made onto them using a sterilizes cork borer. The different concentrations (250µg, 500µg, 750µg and 1000µg) of the crude extracts were placed in the wells in triplicates. Dimethyl sulphoxide was used as control in the present study. The plates were then incubated at 37 ºC for 24 h, and the zone of inhibition was measured, if any.

Anti-Dandruff Activity

The Antidandruff activity of the plant extract was carried-out by the method of Kumar *et al*., [17] with small modifications. Broth cultures of the organisms (*Pityrosporum ovale, Pityrosporum folliculitis*) were swabbed over the surface of Potato Dextrose Agar. Wells of 8 mm diameter were cut at the surface of the agar and 100 µl of the above prepared samples (10mg/ml) were loaded on the wells respectively. Plates were incubated at 30 ^{0}C for 2-3 days. The zone of inhibition was measured and recorded.

Thin Layer Chromatography

The ethyl acetate extract of *V. cinerea* was loaded on pre-coated silica plates which were developed using the solvents ethyl acetate and hexane (1:9). The spots were identified both in the UV light, far light and in the iodine chamber. Then R_f value was calculated as the ratio of distance traveled by the solute to the distance traveled by the solvent.

Phytochemical screening of *V. cinerea* crude extract

Qualitative phytochemical tests for the identification of alkaloids, phenols, flavonoids, terpenoids, steroids and saponins were carried out for all the extracts (freshly prepared) by the methods described previously [11, 18].

RESULTS AND DISCUSSION

Dandruff is a universal disorder affecting the scalp and can be a discomforting situation. Presently existing treatment options have certain confines, either due to reduced efficacies or due to acquiescence issues. Furthermore, these drugs are incapable to avoid recurrence, which is familiar bothersome clinical problem. The antifungal activity of *V. cinerea* leaf extracts showed positive results against all the tested fungal pathogens, *C. albicans*, *C. parapsilosis* and *C. tropicalis* (Table 1). Methanol extracts failed to inhibit few fungal pathogens at the lower concentrations tested. Chloroform extracts at all concentrations have exhibited moderate antifungal activity against all the tested pathogens. Among different solvents used, the ethyl acetate extract had shown maximum inhibition as compared to methanol and hexane extract and chosen for further analysis.

Table 1: Antifungal activity of different solvent extracts of *V. cinerea* leaves

S. No.	Concentration (µg/ml)	Zone of inhibition (mm)								
		Methanol extract			chloroform extract			Ethyl acetate extract		
		C. albicans	*C. paropsilosis*	*C. tropicalis*	*C. albicans*	*C. paropsilosis*	*C. tropicalis*	*C. albicans*	*C. paropsilosis*	*C. tropicalis*
1	250	-	-	-	-	-	10	11	12	-
2	500	-	11	11	11	11	10	12	13	13
3	750	13	15	11	12	12	12	15	16	15
4	1000	14	18	12	12	12	12	17	20	18

The '-' sign denotes no visible zone of inhibition was obtained

The ethyl acetate extract of *V. cinerea* recorded MIC value of 250µg/ml (with a zone of inhibition of 11mm) exhibited very good antifungal activity against the tested fungal cultures (Table 2). In general, increasing concentrations of all the extracts including ethyl acetate extracts exhibited increased trend of antifungal activity. Previous reports also suggested the effectiveness of ethyl acetate extracts against a wide range of bacteria and fungi [19, 20, 21].

Medicinal plant extracts are the promising sources of antifungal drugs, even though they have relatively mild effect against human pathogenic fungi when compared with commercial synthetic antifungal drugs [22, 23]. An array of synthetic antifungal agents is available for the treatment of dandruff, though a complete cure is still far from reach. The fungi responsible for dandruff often develops resistance to chemical agents and synthesis of such chemicals remains expensive and posing threat to environment due to effluents and byproducts. Herbal extracts with antifungal activity against many fungal pathogens may offer a very good source for antidandruff compounds. Hence, an attempt was made to find out the synergistic antidandruff activities of the ethyl acetate extract of *V. cinerea* with commercial products. For this, *Pityrosporum ovale* and *Pityrosporum folliculitis* were chosen as tested organisms. The synergistic study was performed by making the mixtures of *V. cinerea* extract and commercial product (shampoo) in equal proportions. Results indicated that their effects (commercial product and *V. cinerea* extract) were similar in its inhibitory potential as shown in Table 2. This part of study seems to have importance in finding effective antidandruff activity from a commonly available plant *V. cinerea.*

Table 2: Antidandruff activity of ethyl acetate extract of *V. cinerea* leaves

S. No.	Sample	Zone of Inhibition (mm)	
		P. ovale	*P. foliculitis*
1	Plant extract	19	20
2	Commercial product	21	24
3	Plant extract + Commercial product	22	25

Plants are found to be rich sources of valuable of bioactive metabolites such as tannins, saponins and phenolics which make them to have marvelous medicinal properties. Phytochemical screening is an important approach in profiling the active ingredients of either whole plant or their solvent extracts of different parts. Phytochemical screening is an indispensable element in herbal technology. In the present study, various qualitative tests were performed with ethyl acetate extracts of *V. cinerea.* The chromatogram developed with 15 % ethyl acetate in hexane revealed the presence of ten major compounds at R_f value of 0.22; 0.34; 0.48; 0.57; 0.68; 0.74; 0.80; 0.88 and 0.97 as visualized under iodine vapor and UV illumination. The broad-spectrum antimicrobial

activities of the plant extract may be, possibly, due to the presence of phenols, tannins, saponins, alkaloids, steroids and flavonoids as shown in table 3. Review of the literature on the phytochemical constituents of the plants tested revealed that steroids, triterpenoids, sesquiterpenes, flavonoids and tannins are the major components of *V. cinerea* [24].

Table 3: Qualitative phytochemical screening of ethyl acetate extract of *V. cinerea* leaves

S. No.	Compound	Result
1	Alkaloids	+
2	Carbohydrates and glycosides	+++
3	Glycosides	-
4	Saponins	+++
5	Proteins and amino acids	+
6	Phenolic compounds	+++

Antimicrobial activities of flavonoids, tannins and sterols have been well documented [25, 26, 27]. During the past few decades, there has been a spectacular increase in the use of natural products in cosmetics. The consciousness and necessitate for cosmetics with herbs is on the rise, primarily because it is believed that these products are generally safe to use without side effects. Plant extracts and essential oils from medicinal plants are popularly used as ingredients in herbal shampoos and other cosmetic products. There are large numbers of plants which are reported to have beneficial effects on hair and are commonly used in shampoos [28]. In this condition, demonstration of antifungal and antidandruff activity of medicinal plant *V. cinerea* has been found a significant improvement in the natural cosmetics research.

CONCLUSION

In the present study, an attempt was made to evaluate the anti-dandruff activity of the leaf extracts of *V. cinerea* plant. Results indicated that, the leaf extract has a powerful antifungal activity against the tested organisms. The phytochemical analysis also revealed the presence of many secondary metabolites which might help in the antimicrobial properties of the leaf extracts. The synergistic activity of *V. cinerea* leaf extract with commercial product (shampoo) as antimicrobial agents had shown significant detrimental effect on the tested organisms which can be applied as a development strategy for targeting dandruff in future.

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