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Anti-dermatophyte activity of methanol and aqueous extracts of *Calycopteries floribunda* (Roxb.) poiret leaves

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ABSTRACT

The present study was undertaken to investigate the phytoconstituents and antifungal activity of solvent extracts of leaves of *C. floribunda*. The powdered plant material was subjected to extraction using methanol and water as solvents. The extracts were screened for antifungal activity against human pathogenic dermatophytes namely *Microsporium gypsium*, *Chrysosporium keratinophilum*, *Chrysosporium indicum* and *Trichophytum rubrum* by Agar well diffusion method. The preliminary phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, saponins and terpenoids. Marked anti-dermatophyte activity was observed. *M. gypsium* and *C. keratinophilum* were more inhibited by the aqueous extract as compared to methanol extract while the methanol extract was more effective against *T. rubrum* and *C. indicum*. The anti-dermatophyte activity of solvent extracts may be due to the presence of various phytoconstituents. Further studies are to be carried to isolate active constituents and reveal the efficacy of extract *in vivo*.

KEYWORDS

Calycopteries floribunda (Roxb.) Poiret;
Dermatophytes;
Soxhlet extraction;
Phytochemical screening;
Antifungal activity;
Agar well diffusion.

INTRODUCTION

Phytomedicines derived from plants have shown great promise in the treatment of various diseases including viral infections. Single and poly herbal preparations have been used throughout history for the treatment of various types of illness[1]. In recent years development of multidrug resistance in the pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases[2]. This and other problems such as toxicity of certain antimicrobial drugs on the host tissue triggered interest in search of new antimicrobial substances/drugs of plant origin[3,4]. Over 50% of all modern clinical drugs are of natural product origin[5]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds[6]. Considering the rich diversity of plants, it is expected that

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screening and scientific evaluation of plant extracts for their antimicrobial activity may provide new anti-microbial substances. Mycotic infections are probably the most common cause of skin disease in developing countries of tropical regions. The drugs used against dermatophytosis exhibit several side effects and have limited efficacy[7]. So that there is a distinct need for the discovery of new safer and more effective antifungal agents. The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice in many parts of the world[8]. This use has been supported by the isolation of active antifungal compounds from plant extracts[9-12]. *Calycopteries floribunda* (Roxb.) Poiret (Combretaceae), locally called Marasuttu balli, is a straggling shrub, leaves opposite, acuminate and round at the base with sub-terminal branched raceme inflorescence, calyx five angled lobes acrescent with ten stamens, fruit ovoid, five angled narrow. Flowering and fruiting occurs in between January to April. The plant commonly grows in Semi-evergreen forests of Western Ghats. The leaves are biter and astringent, anathematic and are used in colic[13]. The present study aims to screen the presence of various phytoconstituents and antibacterial activity of methanol and aqueous extracts of *C. floribunda*.

MATERIALS AND METHODS

Collection and identification

The plant material was collected in Hosanagara, Shimoga district during November 2008 and authenticated to identity in the dept. of Applied Botany, Jnanasahyadri, Kuvempu University, Shankaraghatta, Shimoga. The voucher specimen (KU/AB/KSV/212) was deposited in the department for future reference.

Soxhlet extraction and phytochemical analysis

The plant material was washed thoroughly with running tap water, shade dried, powdered and used for extraction. A known amount of powdered material (500gm) was subjected to soxhlet extraction and exhaustively extracted with methanol for about 48 hours. The extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the desiccator[14]. Methanolic extract was subjected to preliminary phytochemical screening to screen the presence of various secondary metabolites in the solvent extracts[15].

Hot water extraction

Ten gram of plant material was boiled in 100ml of distilled water with constant stirring for about an hour. The solution was allowed to attain room temperature and filtered through three fold muslin cloth followed by Whatman filter paper No. 1. The filtered extract was evaporated condensed and used for antibacterial studies[16].

Antifungal activity of methanol and aqueous extract

The efficacy of methanol and aqueous extracts of *C. floribunda* was tested against human dermatophytes namely *Microsporium gypsium*, *Chrysosporium keratinophilum*, *Chrysosporium indicum* and *Trichophytum rubrum* by Agar well diffusion method[17]. Then, aseptically wells of 6mm diameter were bored in the inoculated plates with the help of gel puncher and the extracts (10mg/ml of DMSO), Standard (Amphotericin B, 1mg/ml) and Control (10% DMSO) were added into the respectively labeled wells. The plates were incubated at 28°C for 72 hours in upright position and the zone of inhibition (ZOI) was recorded. The experiment was carried in triplicates to get average reading.

RESULTS AND DISCUSSION

TABLE 1 : Antifungal activity of methanol and aqueous extracts of *C. floribunda*

Test fungi	Zone of inhibition (ZOI) in mm			
	Methanol extract	Aqueous extract	Standard	Control
M.gypsium	10	12	19	-
C.keratinophilum	12	14	19	-
T.rubrum	14	12	20	-
C. indicum	11	10	21	-

The preliminary phytochemical tests showed the presence of tannins, terpenoids, alkaloids, saponins, steroids and flavonoids in the solvent extract. The result of antifungal activity of methanol extract of *C. floribunda* is given in TABLE 1. Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated

the absence of fungal growth and it as reported as positive and absence of zone as negative[18]. The fungi have exhibited differential sensitivity to the extracts. In case of methanol extract, more susceptibility was shown by *T. rubrum* (ZOI 14mm) followed by *C. keratinophilum* (12mm), *C. indicum* (11mm) and *M. gypsium* (10mm). *C. keratinophilum* was found to be more affected by aqueous extract (ZOI 14mm) followed by *M. gypsium* (12mm), *T. rubrum* (12mm) and *C. indicum* (10mm). Overall, *M. gypsium* and *C. keratinophilum* were more inhibited by the aqueous extract as compared to methanol extract while the methanol extract was more effective against *T. rubrum* and *C. indicum*. Standard drug Amphotericin B exhibited more potent activity than both of extracts. Control (10% DMSO) did not reveal any inhibition of test fungi.

Antibacterial and antioxidant efficacy of Dichloromethane-methanol extract of leaves of *Calycopteris floribunda* and its aqueous 90% methanol soluble fractions was studied. It was shown that significant antibacterial activity against *B.subtilis, S.pyogenes, S.aureus* and *S.typhi* was observed[19]. *Calycopteris floribunda* is a large climbing woody shrub well distributed in south-east Asian countries. Traditionally, *C. floribunda* has been used in colic, as an antihelminthic, astringent and carminative, and for the treatment of diarrhoea, dysentery, jaundice and malaria in many countries[20]. The aqueous 90%, methanol and 1-butanol soluble fractions of the leaves showed significant antioxidant activity. Two pure compounds, 3, 8-di-O-methyl ellagic and 2, 3, 7-tri-O-methyl ellagic acids were isolated from the 1-butanol soluble fraction of the parent extract[19]. Neocalycopterone and its methyl ether, along with two new biflavonoids, calyflorenones A and B from dried leaves of *C. floribunda*[20]. Pachypodol, a flavonol isolated from the leaves of *C. floribunda* inhibited the growth of CaCo 2 colon cancer cell line *in vitro*[21]. Three new biflavonoids calycopterone, isocalycopterone, and 4-demethylcalycopterone and the known flavone 4',5-dihydroxy-3,3',6,7-tetramethoxyflavone was isolated as cytotoxic constituent from the flowers of *C. floribunda* Lamk. Compounds showed a wide range of activity against a panel of solid tumor cell lines. Among the biflavonoids, calycopterone is the major constituent[22]. The structures of five biflavonoids, 6″-demethoxyneocalycopterone, calyflorenone C, 6″-*epi*-calyflorenone B, 6″-*epi*-calyflorenone C and calyflorenone D from the green parts of *C. floribunda* were established by NMR and MS[23].

Fungal infections particularly those involving the skin and mucosal surfaces constitute a serious problem, especially in tropical and subtropical developing countries[24]. Dermatophytes have been reported to be potentially pathogenic[25] and are directly connected with the skin fungal infections. Unlike other fungal infections, cutaneous mycosis has been considered important in which host immune responses are highly evoked resulting in severe pathologic changes, which are extended deeper into epidermis as well as hair and nails. Such fungal infections are mainly caused by *Microsporum* species[26]. Although several antimycotic drugs are available at present, its use is becoming limited by a number of factors, such as low potency, poor solubility, development of resistant strains and drug toxicity. Therefore, there is a distinct need for the discovery of new, safer and more effective antifungal agents. Recently, extracts and their biological active compounds isolated from plant species has been the centre of interest[27,28]. It is estimated that there are 250000 to 500000 species of plants on earth[29]. Relatively small percentages (1 to 10%) of these are used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes[30]. In developing countries like India where poverty and malnutrition is rampant, knowledge of plant derived metabolites could reduce the cost of health care. India has a rich history of using various herbs and herbal components for treating various diseases[20]. Antimicrobial activity of tannins[31,32] flavonoids[33,34], saponins[35,36], terpenoids[37], alkaloids[38,39] have been documented. The extract was found to possess most of the phytoconstituents. The antifungal activity of solvent extracts could be chiefly due to the presence of various phytoconstituents.

CONCLUSION

Dermatophytes are the major cause of superficial mycoses of man and remain a public health problem

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especially in tropical countries such as India. The results of this study showed that the extracts possess broad spectrum antifungal activity and are promising against the fungi tested. The extracts could be used to treat skin infections caused by these pathogenic fungi.

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