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Preliminary Phytochemical Screening and Antidermatophytic activity of *Aristolochia bracteata*. L

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A B S T R A C T

The incidence of fungal infections has been increasingly reported in the recent years. This is mainly due to the advent of newer antifungal agents, increased use of immunosuppressive drugs, climatic factors and poor hygienic conditions. Dermatophyte refers to the infection caused by a group of fungi namely *Trichophyton*, *Epidermophyton*, and *Microsporum*. Topical and systemic therapies are commonly used in the treatment of these infections. However due to the indiscriminate usage of antifungal agents has resulted in the development of drug resistant strains. Ethanomedical plants have been used traditionally since ancient times in the treatment of these infections. In this study the phytochemical and the antidermatophytic activity of *Aristolochia bracteata* has been studied. The plant powder was purchased from the local Siddha medical shop was extracted with two different solvent systems and subjected to Preliminary phytochemical screening. The leaf extracts were subjected to TLC, HPTLC, MS and NMR spectra. The antidermatophytic activity of the plant extract was determined by disc diffusion method. The photochemical analysis of the plant showed phenols, flavanoids, saponin and alkaloids class of compounds. The methanol solvent was found to be the effective extraction of the bioactive fractions of the leaf. The HPTLC fractions of the leaf extracts showed Acetylenic acid derivatives. The structural identification of acetylenic acid analog was readily achieved by H1 NMR spectra and the molecular formula of the acetylenic acid was C₁₆H₂₈O₂ was predicted. The methanol extract exhibited higher activity at 2gm/disc concentration when compared to the other concentration tested by disc diffusion method. The results of the study suggest that the antidermatophytic activity of *Aristolochia bracteata* could be attributed to the presence of acetylenic acid in the leaf fractions contributing to the antidermatophytic activity. However further studies are still required to know the exact concentration at which these fractions are effective for the development of herbal preparation that will be useful in the treatment of dermatophytic infections in the future.

Introduction

Traditional medicinal plants contain a wide range of phytochemicals that are used in the treatment of various infections. The medicinal value of these plants consists of many chemical substances producing a variety of pharmacological properties. In the past few years; the incidence of dermatophytosis has been increasing over the past few years. Dermatophytes refer to the infections involving skin, hair and nails to the superficial layers. They comprise a closely related group of phylogenetic genera namely *Trichophyton*, *Epidermophyton* and *Microsporum* with numerous species. These agents affect the keratinized tissues and spread by direct contact from infected human beings (anthropophilic organisms), animals (zoophilic organisms) and soil (geophilic organisms) and by indirect way from fomites. The clinical signs of dermatophytosis vary depending on the affected region of the body. Due to the indiscriminate and prolonged use of some of the antifungal drugs, there are various drawbacks such as lack of fungicidal efficacy of the drug, emergence of resistant strains, and certain side effects. Hence, there is a need for a novel antifungal agent that is more effective and less toxic than those already existing.

Ethno medicinal plant preparations have been used to treat various infections in the past. Agar-based disk diffusion susceptibility method for dermatophytes is simple, inexpensive, and does not require specialized equipment (Singh *et al.*, 2007). The disk diffusion method has a good correlation with the reference dilution assay. However data on disk diffusion methods for dermatophytes are scarce.

Aristolochia bracteata L is a shrub distributed throughout India, belonging to the Aristolochiaceae family commonly

called as "Worm killer (*Aadutheenda apaalaiin* Tamil). It is used in traditional medicines as a gastric stimulant and also in the lung inflammation, dysentery and snake bites. The Phytochemical analysis of this plant has revealed the presence of alkaloids, triterpenoids, steroids, sterols, flavonoids, tannins, phenolic compounds and cardio glycosides (Devesh *et al.*, 2014; Kokate *et al.*, 1995). In this present study, an attempt has been made for phytochemical screening and evaluation of antidermatophytic activity of this plant *Aristolochia bracteata* against dermatophytes by disc diffusion method (Deepa *et al.*, 2012).

Materials and Methods

Preparation of Plant Extracts

The *Aristolochia bracteata* L was procured from the local siddha shop in Salem in the form of readymade powder. The entire content of the packet (10 gm) was used for this study. This plant powder was subjected to extraction process using Soxhlet apparatus with methanol (Alagesaboopathi, 2011). This extract was evaporated to dryness at reduced temperature and pressure in a rotary evaporator. The water extract of the powder was prepared by stirring the powder continuously in 20 ml distilled water for 6 hours at room temperature, filtered and the filtrate was subjected to reduced temperature and pressure in a rotary evaporator. Both the extracts were subjected to phytochemical screening for compounds followed screening for antimicrobial activity (Kavitha *et al.*, 2009).

Preliminary Phytochemical Analysis

The Phytochemical analysis of the Methanol and Aqueous extracts were carried out using standard procedure following the method of Kokate *et al.*, 1995 (Devesh *et al.*, 2014).

Preparation of the Fungal Inoculum

The dermatophytic cultures were procured from the Medical microbiology Laboratory, Periyar University, Salem. The isolates used in this study were mainly the subcultures of the primary cultures maintained on to the Sabouraud dextrose agar (SDA) slants. The isolates included in this study were *T.rubrum*, *T.tonsurans*, *T.mentagrophytes* and *T. verucosum*. The fungal inoculum was prepared by harvesting the spores by using sterile saline and adjusting the concentration by using Haemocytometer. The viability of the spores was checked by plating on to Sabouraud dextrose agar (SDA).

Antifungal activity of Plant Extract

Different concentrations of the plant extracts was prepared, dipped on to the Whatmann filter paper discs and air dried. The Sabouraud Dextrose agar (SDA) plates were prepared and the fungal inoculums were uniformly spread onto the plates and the disc containing the various concentration of the plant extracts were placed uniformly on to the plate. The plates were incubated at 28°C to 30°C for 5 days (Nweze *et al.*, 2010). Ketoconazole drug was used as the positive control. The diameters of the zone of inhibition were measured and interpreted using standard chart.

Thin Layer Chromatography (TLC) & HPTLC Analysis of the Plant Extract

TLC glassplates were prepared using silica gel G60 and 0.25 mm slurry of silica gel G was applied in a glass plate by means of spreader preciously cleaned with methanol. Plates were activated by applying heat at 100-110°C for an hour. Then Chloroform / methanol/ ethyl acetate (60: 40: 10 v/v) was used as a solvent system for the separation of bioactive compounds. The plates were developed up to 80-100 mm in development

chamber saturated with selected solvent system.

Fresh samples were applied on precoated silica gel G60 aluminum sheets (5X10 cm) with the help of linom at applicator attached to HPTLC system programed through WIN CATS Software. After the sample was loaded, the migration distance was maintained up to 80mm from the lower edge of 10 mm which was performed at 200C with the suitable solvent system n-Hexane: ethyl acetate (60: 40 v/v) in a chamber. Then the plate was dried at 600C in an oven for 5mins. Densitometry scanning was performed with TLC scanner equipped with WIN CATS software. The dried plate was viewed under UV radiation and scanned by densitometer at 368nm.

Mass Spectra and H1 NMR Studies of the Plant Extract

The Mass analysis was performed using the Finnigan LTQ mass spectrometer (Thermo Electron, San Jose, CA) was used in the positive ion mode with an ESI source at SRM Institution, Chennai. The data processing was performed on the spectrometer. Chemical shifts were expressed by parts per million (ppm) from the residual solvent peak of 7.26 ppm for CDCl₃ for ¹H NMR.

Results & Discussion

The preliminary phytochemical analysis of *Aristolochia bracteata* was found to contain phenols, flavonoids, saponin and alkaloids. Sterol were absent in both the extracts. The methanol extract exhibited higher activity at 2gm/disc concentration when compared to the other concentration tested. The methanol extract exhibited strong antidermatophytic activity against *T.mentagrophytes*, *T.tonsurans*, and *T.Verrucosum* indicating that methanol is a good solvent and can be used for the extraction process(Table- 1)(8).

Table.1 Antidermatophytic Activity of *Aristolochia bracteata* by Disc diffusion

S.No	Name of the solvent	Concentration of Plant Extract	<i>T.rubrum</i>	<i>T.tonsurans</i>	<i>T.mentagrophytes</i>	<i>T.verucosum</i>
1	Methanol	250 mg	NZ	NZ	NZ	NZ
		500 mg	NZ	NZ	6mm	6mm
		1gm	15	18	21	18
		2gm	18	26	28	23
2	Water	250mg	NZ	NZ	NZ	NZ
		500mg	NZ	NZ	NZ	NZ
		1gm	12	13	15	14
		2gm	10	15	18	12
3	Fluconazole	Positive Control	16	19	19	18
4	Solvent Control		NZ	NZ	NZ	NZ

NZ- No zone of Inhibition

Table.2 The HPTLC analysis of the methanol extract of *Aristolochia bracteolata* at 368 nm with the area percentage

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	75.6 AU	0.09 Rf	268.4 AU	18.54 %	0.15 Rf	282.6 AU	7369.2 AU	29.45 %
2	0.31 Rf	12.4 AU	0.35 Rf	55.3 AU	4.36 %	0.38 Rf	71.3 AU	1253.4AU	4.12 %
3	0.38 Rf	6.3 AU	0.43 Rf	69.1 AU	4.85 %	0.49 Rf	112.8 AU	3217.9 AU	9.65 %
4	0.86 Rf	112.9 AU	0.88 Rf	236.7 AU	15.96 %	0.81 Rf	253.4 AU	3986.8 AU	12.55 %

Figure.1 HPTLC analysis of the methanol extract of *Aristolochia bracteolata* showing 4 peaks from the developed chromatogram at 368 nm

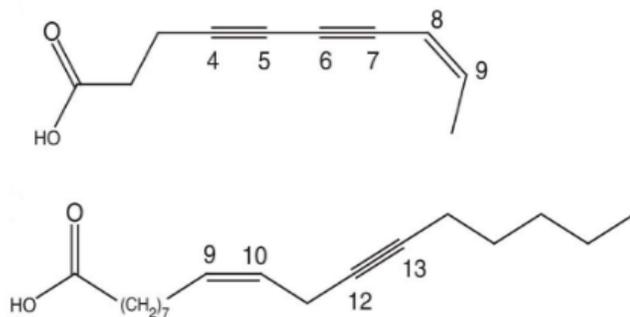
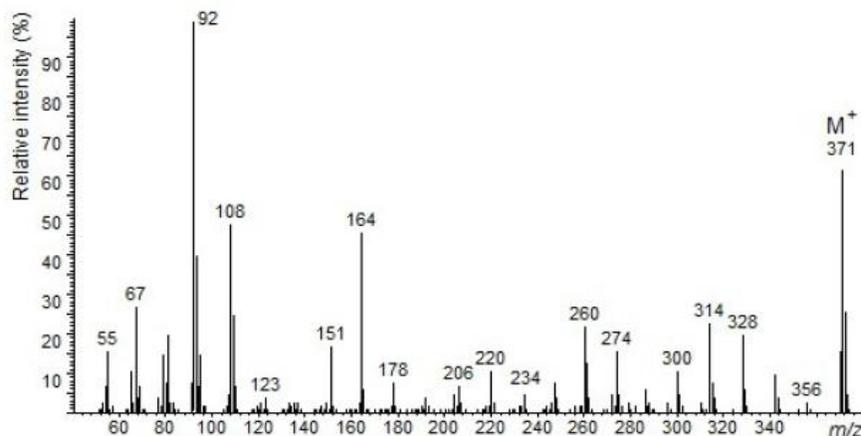


Figure.2 Molecular structure of acetynelic acid as predicted by H1 NMR



The HPTLC analysis showed 4 peaks from the developed chromatogram of *Aristolochia bracteolata* at 368 nm (Fig 1). The four different components present in the methanol extract with the corresponding R_f value with the area percentage are tabulated (Table-2).

The mass studies of the methanol extract showed 17 compounds based on the m/z parent peaks. The graph for ¹H NMR showed that the chemical shift values at 2.2 ppm, 3.6 ppm, 6.7 ppm and 7.2 ppm. Based on this interpretation, the structural identification of acetynelic acid analog was readily achieved by careful comparison of ¹H NMR spectra and the molecular formula of acetynelic acid C₁₆H₂₈O₂ was predicted by comparing NIST Chem web library. The molecular structure of acetynelic acid is shown in (Fig 2)

Conclusion

HPTLC fingerprinting method was found to be more precise and accurate method for compound identification. The results of the present study suggest that the antidermatophytic activity of the leaf of *Aristolochia bracteolata* is attributed due to the presence of acetynelic acid which was

evident by the mass spectra. However further studies are required to assess the exact concentration in which these extract should be given for the effective treatment of dermatophytosis in the future.

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