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Screening and characterization of compound for antidermatophytic activity of *Aristolochia bracteolata*

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Abstract
Antidermatophytic activity *Aristolochia bracteolata* plant extracts using and isolated bioactive compound from plant extract. The using two different solvents plant extract of *Aristolochia bracteolata* leaf completely prevented growth of tested dermatophyte species with agar well diffusion method. The preliminary phytochemical screening indicated the presence of alkaloids, flavonoids, saponins, phenol, phytosterol, tannin, carbohydrate and glycosides. Thin Layer Chromatography (TLC) and HPTLC High Performance Thin Layer Chromatography studies indicate the bioactive compound of the plant. The plant Leaf include the Aristolochic acid and Acetylenic acid and flavonoids. Bioactive components and functional groups present in the plant leaf of *Aristolochia bracteolata*. FT-IR study revealed the presence of various functional groups related to phenols, alcohols, alkenes and Alkyl halides. The twenty-two components identified by GC-MS analysis. The plant extract showed inhibitory activity against dermatophytes genera *Trichophyton* and *Microsporum*. This investigation it can conclude the *Aristolochia bracteolata* for alternative of treatment for dermatophytic infection in traditional and current medicinal uses.

Keywords: *trichophyton, microsporum, aristolochia bracteolata,* bioactive compound, agar well diffusion method

Introduction
India has different parts of several medicinal plants or their extracts are used treatment for various diseases [1]. *Aristolochia bracteolata* is a known as "worm killer" due to supposed antihelminthic activity and trypanocidal effect [2]. Aristolochia is an important genus, both numerically and medicinally, in the family Aristolochiaceae. The genus Aristolochia consists of about 400 species of herbaceous perennials, under shrubs or shrubs bearing essential oils and is widespread across tropical Asia, Africa and South America but their effect upon [2,3]. Various plants are known to consume fungicidal substances and much work has been ratified out on the effects of medicinal and aromatic plant extracts against numerous fungi [4]. Dermatophytes are unexplored dermatophytes are known to cause superficial skin infections like scabies [5]. This plant belongs to the family Aristolochiaceae. It has insecticidal properties. Its roots and leaves are bitter and antihelmintic, and are medicinally important. Virtually every part of the plant has medicinal convention. Isolating bioactive compounds and establishing their health effects are active areas of scientific analysis [6]. The plant materials derivatives as an emmenagogue, anthelmintic, purgative, mosquito repellent, antidote, anodyne and insecticide [7].

There are many antifungal agents are used to treat dermatophytosis. However, not all species of dermatophytes have the same susceptibility pattern and relative or absolute resistance may occur [8]. In Nigeria, many plants are used against infectious diseases, which today are frequent due to very poor hygienic conditions, cost and microbial resistance to the time- honoured antibiotics [9]. The continuing increase in the incidence of fungal infections together with the gradual rise in resistance of bacterial and fungal pathogens for antibiotics and antifungal highlights the need to find alternative sources from medicinal plants [10, 11]. This work was displyed to isolate and identify dermatophytes from infected individuals with physical skin lesions and to screen five medicinal plants for potential antidermatophytic activity. The selection of these plants for evaluation was based on ethanomedical information obtained from traditional healers in who used the plants for treatment of dermatophytic infection. The present study aims to determine the dermatophytic activity of *Aristolochia bracteolata.*

Materials and Methods
Collection of the plant materials
The plant material used in this present study was the plant *Aristolochia bracteolata* collected from Simmakal, Madurai District, Tamil Nadu, in India. The leaves collected and they dried in room temperature. Then they crushed in to small pieces and these dried small pieces are finely powdered.

Preparation of plant extracts
The plant powder (20g) of *Aristolochia bracteolata* was Soxhlet - extracted with 300 ml of Ethyl acetate in the round bottom flask using the Soxhlet extraction method as per the standard procedure at their 77ºC boiling point. The extract recovered from the solvent using rotary evaporator apparatus and stored in a freezer (-4ºC) for further use.

Preliminary phytochemical analysis plant extracts of *Aristolochia bracteolata*
Preliminary phytochemical analyses of Ethyl acetate and Methanol extracts were carried out using standard procedure to identify the phytochemical constituents, alkaloids, glycosides, phytosterol, fixed oils and fats, saponins, tannins

and phenolic compounds. Proteins and free amino acids, gums, mucilage, flavonoids, lignin.

Thin layer chromatography of the plant extract:
TLC glass plates were prepared using silica gel G type 60. A layer of 0.25 mm slurry of silica gel G was applied in a glass plate by means of spreader preciously cleaned with methanol. A layer 0.25 mm thick was used routinely. Plates were activated by heating at 100-1100C for an hour. Different mobile phases Chloroform / methanol/ ethyl acetate (60: 40: 10 v/v) can be used as a solvent system for the separation of bioactive compounds.

HPTLC analysis of the plant extract
A 0.25mm layer of silica gel G60F254 was coated in aluminium packed TLC plate. Samples were applied on precoated silica gel G60F254 aluminium sheets (2X10 cm) with the help of CAMAG LINOMAT applicator attached to HPTLC system programed through winCATS 1.3.4 Software. After spotting the sample, Maintained the migration distance up to 80mm from the lower edge of 10 mm which was performed at 20ºC with the suitable solvent system Ethyl acetate -Butanone-Formic Acid-Water (5:3:1:1v/v) in a chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 366nm. The Peak table, Peak display and Peak densitogram were noted. The software used was win CATS 1.3.4 version.

FT IR Analysis of plant extract:
EXI- Spectrum One Model at St. Joseph College, in Thiruchirapalli, determined the infrared (IR) spectrum of the Aristolochia *bracteolata* extract. The spectrum was obtained using potassium bromide (KBr) pellet techniques in the range of 4000 to 400 cm-1 at a resolution of 1.0 cm-1. Potassium bromide (AR grade) was dried under vacuum at 100ºC and 100 mg of KBr pellet. The spectrum was plotted between intensity and wave number.

GC MS Analysis of plant extract
The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) then components be presentthe separated using Helium as carrier gas at a constant flow of 1 ml/minutes. The mass detector conditions were: transfer line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Preparation of the Fungal Inoculums
Twenty-one days old grown culture of dermatophytes was scraped with sterile needle and harvested in sterile saline & conidial suspension adjusted to absorbance of 0.600 at 450nm determined spectroscopically then it was used as inoculums [12].

Agar well diffusion method
Antifungal screening carried out using the agar well diffusion assay [13]. Muller Hinton agar plates were prepared. After solidification 4wells of 6mm were made using well cutter. The test culture was swabbed on the surface of solid media using sterile cotton swabs and allowed to dry 15mins. The crude extract dissolved in 5% DMSO (100mg/ml). From the plant extract *Aristolochia bracteolata* 25µl, 50µl, 75µl, 100µl were taken and loaded on the respective well. Itraconozole (10µl) was used as positive control. Negative control was prepared using DMSO solvent. The plates were incubated at 6days at 30ºC. Results were determined based on size of the inhibitory zone surrounding the wells. The diameter of zones of inhibition (mm) was measured in mm using measurement scale and compared with the standard antifungal drugs [14].

Results and Discussion
The plant materials of *Aristolochia bracteolata* were selected for the study to evaluate the formulation containing the extracts of these plants for their antimicrobial activity. Methanol and Ethyl acetate extracts of these plants were made by soxhlet extraction method and the obtained extracts concentrated by evaporation and preliminary phytochemical screening were made for the obtained extracts by standard procedure [15].
The phyto chemical screening indicated that the presence of alkaloids, saponins, flavonoid, phenol and tannin in rich status (Table-1). Previous studies on *Aristolochia bracteolata*, the aqueous leaf extract showed the presence of alkaloids, flavonoids, saponins, tannins, phenol, carbohydrates, and glycosides [23-24].
The in vitro antifungal activity of the ethyl acetate and methanol extract of *Aristolochia bracteolata* leaves against the microorganisms *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans, Trichophyton verrucosum, Microsporum canis, Microsporum gypseum, Microsporum furruginum,* and *Microsporum audonii.* The *Trichophyton rubrum* and *Trichophyton mentagrophytes* were assessed by the presence of inhibition zones. In this study, the antifungal activity of the leaf extracts was compared with Itrconozole used as positive control. The results were shown in (table - 6). The antifungal activity revealed that the leaf extract has more effective. This finding supported by the previous investigations on *Aristolochia bracteolata* that different extracts showed positive antimicrobial activity [25].
The results indicated that *Trichophyton rubrum, Trichophyton mentagrophytes* and *Trichophyton tonsurans* were more sensitive to the extract. The study report is in aggrement with previous work which show that the antifungal activity of the plant *Aristolochia bracteolata* Ethyl acetate leaf extract had significant inhibiting activity against *Trichophyton rubrum, Trichophyton mentagrophytes* and *Trichophyton tonsurans*[26].
The presence of secondary metabolites in plants, produce some biological activity in man and animals. Tannins and phenols are known to possess antimicrobial properties.
GC-MS analysis of the ethanol extract of leaf of Aristolochia bracteolata. showed the presence leven bioactive components. Their retention time (RT), molecular formula and concentration (%) were tabulated (table 3 -4). Among the leven identified component few of their activities were listed out in the table 3. Aristolochic acid and Acetylenic acid is found to be antifungal, antimicrobial, anti-inflammatory and anticancer properties [27].
It has been proved that these components have several

applications like antioxidant, anticancer and anti-inflammatory properties [28]. The result obtained through FTIR analysis of leaf extract of Aristolochia bracteolata. Clearly indicates the presence of alcohol, alkyl halides, alkenes and phenols (table 5 and fig 1). Antimicrobial activity of various extracts of medicinal plants is due to bioactive components had been already shown [29]. The presence of OH group in methanolic extract of medicinal plants is responsible for microbicidal activity.

Many investigations were discover plant products that inhibit the fungi like Trichophyton rubrum and Trichophyton mentagrophytes. These two species cause common infections in humans which are difficult to control effectively, and the pharmaceutical arsenal currently available against them is rather limited [30-31].

Table 1: Phytochemical investigation of *Aristolochia bracteolata* extracts

S. No	Phytochemicals	E. Acetate	Methanol	Method
1.	Alkaloids	+	++	Mayor's reagent
2.	Flavanoids	+++	++	NaoH
3.	Saponin	++	+	Foam test
4.	Phenol	+	+	Chloroform/FeCl3
5.	Phytosterol	++	++	Liebermann burchard's test
6.	Tannins	+++	-	FeCl3
7.	Carbohydrates	-	-	
8.	Glycosides	+	+	

Table 2: HPTLC Peak table with Assigned substance name

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.57	525	20399.8	Aristolochic acid standard
Sample	1	0.01	21.5	234.4	Unknown
Sample	2	0.08	615.8	25903.5	Aristolochic acid
Sample	3	0.25	112.7	4178	Unknown
Sample	4	0.31	93.9	3955.2	Acetylenic acid
Sample	5	0.37	103.1	4397.3	Unknown
Sample	6	0.50	99.7	4801.9	Acetylenic acid
Sample	7	0.51	101.1	5266.5	Unknown
Sample	8	0.71	151.3	8343.2	Unknown
Sample	9	0.81	205.2	8774.3	Unknown
Sample	10	0.89	26.6	582.2	Flavonoids
Sample	11	0.96	26.9	720.2	Unknown

Table 3: GCMS molecular formula with molecular weight

S. No.	RT	Name of the Compound	Molecular formula	MW	Peak area %
1	10.26	A-d-glucose	$C_6H_{12}O_6$	180	27.88
2	10.73	3-o-methyl-d-glucose	$C_7H_{14}O_6$	194	24.54
3	11.43	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.00
4	13.23	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.27
5	13.83	Hexadecanoic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$	328	9.46
6	14.72	Phytol	$C_{20}H_{40}O$	296	1.27
7	15.45	Acetylenic acid	C2H2	292	1.08
8	15.99	Linolenic acid, trimethylsilyl ester	$C_{21}H_{38}O_2Si$	350	17.81
9	25.88	Aristolochic acid	$C_{17}H_{11}NO_7$	302	8.96
10	28.61	Flavonoids	$C_6C_3C_6$	430	2.00
11	31.80	ß-Sitosterol	$C_{29}H_{50}O$	414	3.73

Table 4: MASS presented compound name

S. No	Relative intensity %m/z	Compound name referred by NIST Chem web library
1	76.9	Fluoroacetic acid
2	105.0	Benzoyl peroxide
3.	121.0	octadec-6-ynoic acid (Acetylenic resemblance)
4.	133.0	N-methyl-3,4-pyridinedicarboximide
5.	165.2	Acetylenic acid
6.	183.1	Phosphocholine (Lipid derivative)
7.	193.2	Isoxazoline
8.	211.0	Salutaridine
9.	220.8	Methyl esters
10.	239.1	n-propyl 9,12-octadecadienoate
11.	256.0	i-propyl 9,12-octadecadienoate (linoleate)

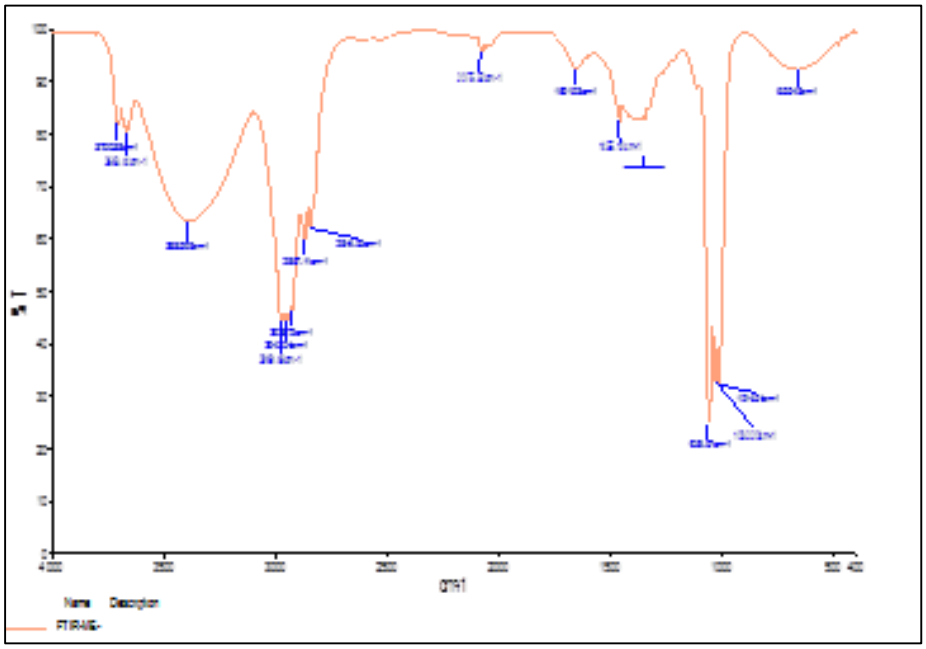

Fig 1*: FTIR Peak Values of Ethyl Acetate Extract of Aristolochia bracteateolata*

Table 5: FTIR Peak Values of Ethyl Acetate Extract of *Aristolochia bracteateolata*

S. No	Peak value	Functional group	Functional group name
1. 1	655.40	C-Br stretch	Alkyl halides
2. 2	1032.2	C-N stretch	Aliphatic amines
3. 3	1054.97	C-N stretch	Aliphatic amines
4. 4	1455.10	C-H bent	Alkanes
5. 5	1651.3	-C=C- stretch	Alkanes
6. 6	2075.52	-C=C- stretch	Alkanes
7. 7	2844.00	C-H stretch	Alkanes
8. 8	2867.11	C-H stretch	Alkanes
9. 9	2923.72	C-H stretch	Alkanes
10. 10	2943.04	C-H stretch	Alkanes
11. 11	2969.48	O-H stretch	Alcohols
12. 12	3385.9	OH-stretch, H – bonded	Phenol and Alcohols
13. 13	3663.4	O-H stretch, free hydroxyl	Alcohols, phenols
14. 14	3700.38	O-H stretch, free hydroxyl	Alcohols, phenols

Conclusion

We conclude that Ethyl acetate extracts of *Aristolochia bracteolata* have remarkable antimicrobial activity. Methanol extracts did not show significant antimicrobial activity against the microorganism under study. Plant are fundamental source for all other organisms. During the evolution process plant represent the first stage and they produce the most important materials such as nutrients, fuel, oxygen, etc. Higher plants also play a dominant role in the maintenance of human health by producing many bio active compounds. To develop the compound with anti dermatophytic activity, so that it can be used in the treatment any dermatophytosis.

The conventional treatment of fungal disease is limited, and part of the reason is due to the limited spectrum of the currently antifungal drugs, and the expensive treatment, particularly due to the need of prolonged Therapy. The ultimate conclusion of this study supports the traditional medicine use of different plant extracts in treating different infections caused by pathogenic fungi in Saudi Arabia either by using a single or combined extract. It also recommends that a great consideration should be paid to medicinal plants which are found to have plenty of pharmacological properties that could be sufficiently better when considering a natural food and feed additives to improve human and animal health.

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