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Research Article

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Studies on antibacterial, antifungal activity and phytochemical analysis of *Aristolochia bracteata* Retz.

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ABSTRACT

The phytochemical analysis of both the aqueous and methanolic extracts of the medicinal plant Aristolochia bracteata Retz. and their antibacterial and antifungal activities against six pathogenic bacteria such as Staphylococcus aureus, Streptococcus pyogenes, Leuconostoc lactis, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi as well as four fungus namely Aspergillus niger, Aspergillus flavus, Rhizopus indicus and Mucor indicus were investigated. The phytochemical analysis revealed the presence of carbohydrates, alkaloids, steroids, saponins, tannins, phenols, fixed oils & fats, proteins and flavonoids in varying concentration. Antibacterial potentiality of aqueous and methanol solvent extract of mature leaves of A. bracteata was evaluated against the bacteria, highest antibacterial activity was observed against Streptococcus pyogenes (42 mm) and followed by Pseudomonas aeruginosa (38 mm) in methanol extract. But in the case of antifungal activity the maximum inhibition zone found against Rhizopus indicus (18) in methanol extract. There is no activity antibacterial and antifungal activity found in the aqueous extract.

Keywords: *Aristolochia*, antibacterial, antifungal, aqueous extract, methanol extract.

INTRODUCTION

Mainstream medicine is increasingly receptive of the use of antimicrobial and other drugs derived from plants, as traditional antibiotics become ineffective and because of the rapid rate of plant species extinction. There is a feeling among natural-products chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably [1].

In India thousands of species are known to have medicinal values and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times [2]. Medicinal plants are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity also play an important role in the modern medicine [3]. It is well known that even the most synthetic drugs have their origin from plant products [4]. Recently scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. The efficacy of current antimicrobial agents has been reduced due to the continuing emergence of drug resistant organisms and the adaptations by microbial pathogens to commonly used antimicrobials. Therefore the search for new drugs from plants continues to be a major source of commercial drugs. Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease hence, further exploration of plant antimicrobials need to occur [5]. The screening

of plant extracts and their products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes [6]. The selection of crude plant extracts for screening programs is potentially more successful in initial steps than the pure compounds [7]. Such screening of various plant extracts has been previously studied by many workers [8]. Even though hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not yet been evaluated [9].

EXPERIMENTAL SECTION

Preparation of plant extracts

Fresh Plant leaf of *Aristolochia bracteata* Retz. was collected from Kattampoondi Village, Thiruvannamalai district, Tamil Nadu, India; they were identified with the help of Gamble's flora.

Preparation of powder

The leaves of plants were collected and dried under shade. These dried materials were mechanically powdered sheaved using 80 meshes and stored in an airtight container. These powdered materials were used for further physiochemical, phytochemical and fluorescent analysis [10].

Extraction of plant material

Various extracts of the study plant was prepared according to the methodology of Indian Pharmacopoeia [11]. The leaves were dried in shade and the dried leaves were subjected to pulverization to get coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). Both the extracts were stored in a refrigerator in air tight containers. Both the extracts were analyzed for phytochemical screening of compounds, antimicrobial and pharmacological activity.

Phytochemical studies

Qualitative phytochemical analyses were done by using the procedures of Kokate *et al.* [12] Alkaloids, carbohydrates, tannins, phenols, flavonoids, gums and mucilages, phytosterol, proteins and amino acids, fixed oils, fats, volatile oil and saponins were qualitatively analyzed.

Table 1: Analysis of fluorescence characters of leaf powder of *Aristolochia bracteata* in different chemical reagents

Sl. No	Chemical reagent	Appearance
1.	Powder colour	Pale green
2.	5% NaOH	Light brown
3.	10% NaOH	Brown
4.	Con. H_2SO_4	Black
5.	Acetic Acid	Brown
6.	1N NaOH in H_2O	Brown
7.	5% KOH	Brown
8.	50% HNO_3	Dark brown
9.	5% $FeCl_2$	Green
10.	1N HCl	Light brown
11.	Con.HNO_3	Brown
12.	1N NaOH in Ethanol	Brown
13.	50% H_2SO_4	Light brown
14.	50% HCl	Light brown
15.	Con. HCl	Brown

Test organisms

The stored culture of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Leuconostoc lactis* and *Salmonella typhi* were collected from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology. Sector 39-4, Chandigarh, India.

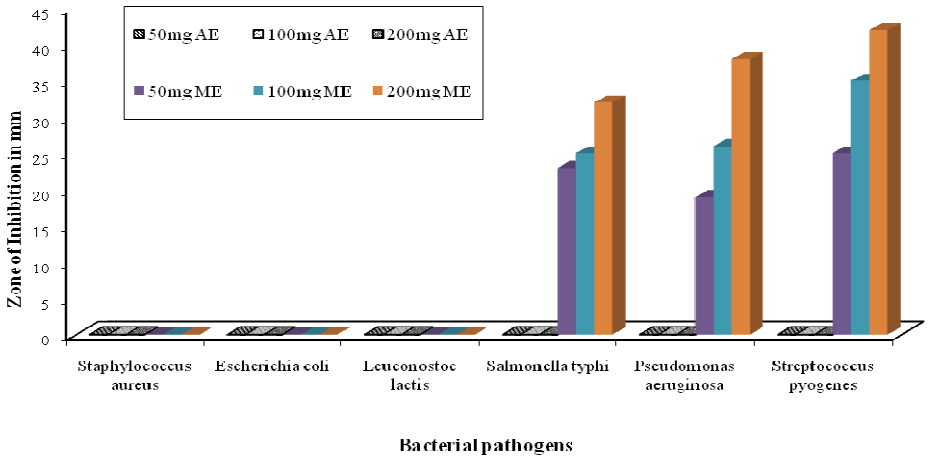
The pathogenic fungal strains *Aspergillus niger, Aspergillus flavus, Rhizopus indicus* and *Mucor indicus* were collected from the Microbiological Lab, Christian Medical College, Vellore, Tamil Nadu, India.

Table 2: Results of phytochemical screening of aqueous leaf extracts of *Aristolochia bracteata*

S. No.	Name of the compounds	Name of the test	Status of the substances	
			Aqueous extract	Methanolic extract
1.	Carbohydrates	Fehling's	++	+++
		Benedict's	++	+++
2.	Alkaloids	Mayer's	+	+++
		Hager's	+	+++
		Wagner's	-	++
		Dragen Dorfff's	++	++++
3.	Steroids	Chloroform + Acetic acid + H_2SO_4	+	+
4.	Tannins & Phenols	10% Lead Acetate	-	+
		5% Ferric Chloride	++	++
		1% Gelatin	+	+
5.	Saponins	Foam test	+	+++
6.	Fixed oils & Fats	Spot test	+	++
7.	Gums & Mucilage	Alcoholic Precipitation	-	-
8.	Proteins	Biuret test	+	++
9.	Flavonoids	NaOH / HCl	+	++
10.	Volatile oils	Hydro distillation method	-	-

++++ = *High rich amount; +++ = Rich amount; ++ = Moderate amount; + = Minimum amount; - = absent*

Fig.1 Graphical representation of antibacterial activity of *Aristolochia bracteata* leaf extract



AE - Aqueous Extract ME - Methanol Extract

Antibacterial Studies

Bacterial Media (Muller Hindon Media)

Thirty Six grams of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were bored with 6mm dia cork porer. The plates with wells were used for the antibacterial studies.

Antifungal studies

Fungal media (PDA)

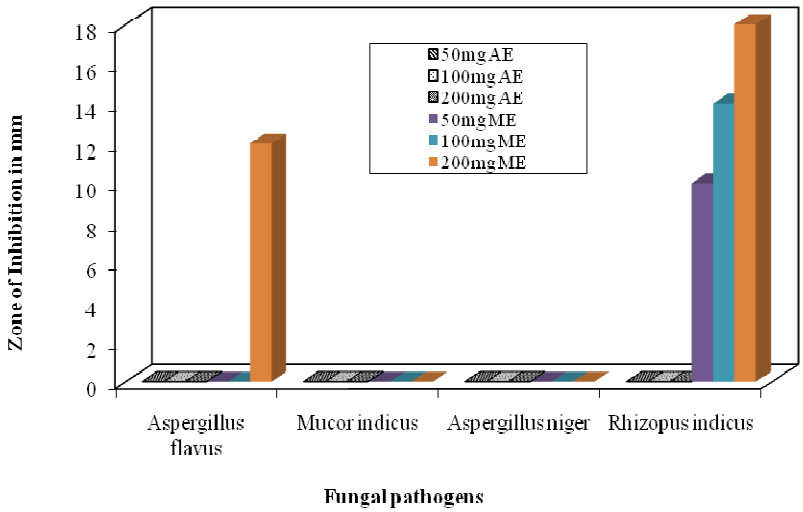
Two Hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm dia cork porer.

Well diffusion method

Antibacterial and Antifungal activity of the plant extract was tested using well diffusion method [13]. The prepared culture plates were inoculated with different bacteria and fungus by using plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2^{o}C for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed for the zone formation around the wells.

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

Fig.2 Graphical representation of antifungal activity of *Aristolochia bracteata* leaf extract



AE - Aqueous Extract ME - Methanol Extract

RESULTS AND DISCUSSION

Fluorescence analysis of leaf powder

The powder of root is examined in daylight, to detect the fluorescent compounds and the observations are given in Table 1. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples [14].

Preliminary phytochemical screening

The results of phytochemical examination of both the extracts were given in Table 2. The phytochemical screening carried out on the *Aristolochia bracteata* leaf extracts. Phytochemical compounds present were found to be carbohydrates, flavonoids, saponins, alkaloids, steroids and proteins in both the extracts, fixed oils, fats, gums, mucilage and volatile oils were not detected in both the extracts. The variation in type of phytochemicals present in

different solvents as shown in the result of phytochemical screening might be attributed to the ability of the solvents to dissolve into solution specific type of phytochemicals as reported by Yusha'u *et al*. [15]. Tannins bind to proline rich proteins and interfere with the protein synthesis [16]. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [17].

The results of antibacterial activity were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and absence of zone as negative [18]. The zone of inhibition against bacterial pathogens ranged between 42 – 19mm in methanolic extract and there is no activity found in aqueous extract. The maximum activity (42mm) was recorded from 200mg of methanolic extract of *A. bracteata* against *Streptococcus pyogenes* followed by 38mm against *Pseudomonas aeruginosa* and minimum (19mm) against *P. aeruginosa* at 50mg level (Fig. 1).

The antifungal activity of both the extract of *A. bracteata* leaf extracts were determined against four fungal strains and recorded in Fig. 2. The zone of inhibition against fungal pathogens ranged between 18 - 10mm in methanolic extract and there is no inhibition in aqueous extract. The maximum activity (18mm) was recorded from 200mg of methanolic extract against *Rhizopus indicus* and minimum (10mm) by the above fungus at 50 mg level. There was no activity observed in aqueous extract against the tested fungus. Our findings are in accordance with the observations of Ravindra *et al*., [19] who proved that highest antifungal activity was observed with methanolic extract of *Capparis pepiaria* against the tested fungal strains.

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