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Short Communication

Assessment of *Aristolochia bracteolata* leaf extracts for its biotherapeutic potential

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The present study focuses on the antibacterial and antifungal activity of the medicinal plant *Aristolochia bracteolata*. Aqueous, methanol and chloroform extracts of this plant were evaluated against the bacterial strains *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, *Shigella flexneri*, *Proteus vulgaris* and the fungal strains like *Aspergillus niger, Aspergillus terreus, Penicillium notatum* and *Rhizopus stolonifer*. Among the three extracts assessed, methanol extract was found to have the significant activity followed by the chloroform extract against certain bacteria. Water extract did not have any activity against bacteria. Antifungal activity assessment indicated that the tested fungal strains are more susceptible to aqueous extract followed by methanol extract and chloroform extract.

Key words: Antibacterial, antifungal, *Aristolochia bracteata,* minimum inhibitory concentration.

INTRODUCTION

Worldwide infectious disease is the number one cause of death accounting for approximately one half of all death in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries like United States. Death from infectious disease ranked 5th in 1981, has become the 3rd leading cause of deaths occurring in US (Pinner et al., 1996).

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents (Cohen, 1992). The problem of microbial resistance is growing and outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore actions must be taken to reduce this problem including control of the use of antibiotic, more research to understand the genetic mechanisms of resistance and development of new drugs. The ultimate goal is to offer appropriate and efficient antimicrobial drugs for the human well-being (Gislene, 2000).

Medicinal plants occupy a distinct place in the life of human, right from the primitive till today (Latha and Pari, 2003). Use of plants as a source of medicine has been inherited and is an important component of health care system in India (Seth et al*.,* 2004). *Aristolochia bracteolata* is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites (Negi et al., 2003). This plant belongs to the family Aristolochiaceae and known as kidmar. It has insecticidal properties. Its roots and leaves are bitter and antihelmintic, and are medicinally important. Almost every part of the plant have medicinal usage. Identifying bioactive compounds and establishing their health effects are active areas of scientific enquiry (Etherton et al., 2004). The present study is to determine *A. bracteolata* antimicrobial property.

MATERIALS AND METHODS

Collection of plant material

A. bracteata plants were obtained from places near Coimbatore. The plant specimens were identified, certified and the voucher specimen number (M3) were deposited at the herbarium of the

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Table 1. Antibacterial activity of the leaf extracts of *Aristolochia bracteolata* by agar well diffusion method and Minimum inhibitory concentration.

Microorganisms	Aqueous		Methanol		Chloroform		Control
	ZOI (mm)	MIC (µg/ml)	ZOI (mm)	MIC (µg/ml)	ZOI (mm)	MIC (µg/ml)	ZOI (mm)
Bacillus subtilis	8.33±0.06	5000	13.7±0.15	39.06	11.0±0.1	2500	27±0.05
Escherichia coli	-	-	9±0.1	5000	11.33±0.32	312.5	22±0.30
Shigella flexneri	9.0±0.1	5000	-	-	9.0±0.1	5000	27±0.10
Salmonella typhimurium	-	-	-	-	-	-	30±0.05
Klebsiella pneumoniae	9.33±0.06	5000	-	-	9.0±0.1	5000	26±0.01
Staphylococcus aureus	10.3±0.15	5000	11.33±0.06	2500	-	-	25±0.05
Pseudomonas aeruginosa	-	-	12.7±0.06	1250	10.6±0.12	2500	27±0.15
Salmonella typhi	-	-	13.0±0.1	625	11.33±0.32	2500	22±0.05
Proteus vulgaris	-	-	-	-	-	-	15±0

ZOI = Zone of inhibition (Mean±S.D.).
MIC = Minimal inhibitory concentration.

Botanical Survey of India, Southern Circle, Coimbatore.

Preparation of the extract

The solvents used were chloroform, methanol and water. 10 g of leaf and bark powder was taken and the extract was prepared with Soxhlet using 100 ml of each solvent. The extract was filtered through membrane filter (0.45 µm pore size) with the aid of suction pump. The obtained filtrate was evaporated to dryness at 40°C. The extract was then weighed and dissolved in minimal volume of dimethyl sulphoxide (Silva et al., 1997).

Test microorganisms

The bacterial strains used for the experiments include *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 9144), *Klebsiella pneumoniae* (ATCC 33495), *Pseudomonas fluorescens* (ATCC 13525), *Shigella flexneri* (ATCC 29508) and *Proteus vulgaris* (ATCC 13315). The fungal strains used were *Aspergillus niger* (ATCC6275), *Aspergillus terreus* (ATCC11877), *Penicillium notatum* (ATCC9479) and *Rhizopus stolonifer* (MTCC553).

Antibacterial assay

The agar well diffusion method was employed for the determination of antibacterial activity of the extracts (NCCLS, 1999). Petri plates containing 20 ml of Mueller Hinton Agar medium were seeded with 24 h culture of the bacterial strain. The wells (6 mm in diameter) were cut from the agar and extract solution at a concentration of 5 mg/ml was delivered into them. The plates were incubated at 37°C for 24 h. The activity was assayed by the diameter of the inhibition zone formed around the well (NCCLS, 1993).

Determination of minimum inhibitory concentration

Plant extracts that are active against the tested microorganisms (with zone of inhibition above 6 mm) were taken for the determination of minimal inhibitory concentrations (MIC). Two fold dilutions of each extract to be tested were prepared in 250 µl volume of sterile nutrient broth to give a range of concentrations from 5000 to 4.9 µg/ml in a 96 well microtitre plates. One drop of suspensions of test microorganism was added to the extract/dilutions (Sokmen et al., 1999). These were incubated for 18 h at 37°C and then the plates were observed for the growth. MIC of each extract was taken as the lowest concentration that showed no growth.

Antifungal assay

Antifungal activity of Aristolochia was demonstrated in a radial growth inhibition assay (Schlumbam et al., 1986). A fungal plug was placed in the center of the Potato Dextrose Agar plate. Extracts of 30 mg/ml concentrate was pipetted into the wells. The petriplates were incubated in the dark at 23°C. Antifungal activity was observed as a crescent shaped zone of inhibition at the mycelial form. The effect on fungal growth was expressed qualitatively. Nystatin was use as positive control.

RESULTS AND DISCUSSION

In vitro antibacterial studies of the three different leaf extracts of this plant revealed that the methanol extract had significant activity against most of the organism, while the chloroform extract possessed moderate activity (Table 1). Methanol extract exhibited the maximum inhibitory effect against *B. subtilis*, *P. aeruginosa, Sal. typhi* and considerable inhibitory activity against *E. coli* and *Staph. aureus*. Chloroform extract had significant inhibitory activity against *E. coli* and moderate activity against *B. subtilis, Shig. flexneri, K. pneumoniae, P. aeruginosa* and *Sal. typhi*. No zone of inhibition was found against *Sal. typhimurium* and *P. vulgaris* using the aqueous and methanolic extracts.

B. subtilis was found to be the most susceptible bacterium with minimal inhibitory concentration of 39.06 µg/ml for the methanol extract. But chloroform extract and aqueous extract inhibited the same organism at a higher concentration.

Table 2. Antifungal activity of the leaf extracts of *Aristolochia bracteolate*

Fungal strain	Aqueous	Methanol	Chloroform
Aspergillus niger	+	-	-
Aspergillus terreus	+++	-	+
Penicillium notatum	+	+	-
Rhizopus	-	+++	-

+++ = Highly susceptible, ++ = moderately susceptible, + = mildly susceptible, - = resistant.

Antifungal activity indicated that the tested fungal strains are more susceptible to aqueous extract followed by methanol extract and chloroform extract (Table 2). *Rhizopus* was inhibited onle by the methanol extract. The chloroform extract had activity only against *A. terreus*. Thus the investigations revealed that the plant has pronounced antibacterial activity and antifungal activities.

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