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Antimicrobial activity reported in a weed *Aristolochia Bracteata* Retz

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ABSTRACT

Aristolochia bracteata Retz. family Aristolochiaceae is common annual weed distributed throughout India and widely used indigenously as a medicine. In Ayurveda it is reported that the leaves, seeds and the roots are used for treatment of skin diseases, ulcers, snakebite, treatment of cancer, lung inflammation etc. Attempt has been made to investigate the antimicrobial activity of root, stem and leaf extracts of *Aristolochia bracteata* Retz. by disc diffusion method. The mentioned plant parts of *Aristolochia bracteata* Retz. were extracted with methanol, butanol, petroleum ether and aqueous extracts and tested against bacterial isolates isolated from clinical samples viz. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*. The crude extracts showed significant broad spectrum antibacterial activity. Among four extracts assessed, methanol extract was found to have highest antibacterial activity followed by butanol extracts against certain bacterial isolates. Present study reveals that bioactive compound could be present in the extract as it shows inhibitory activity against isolates from clinical samples. The bio characterization of those compounds is in process.

Key words: *Aristolochia bracteata*, Leaf, Extracts, Antibacterial activity, Medicinal value.

INTRODUCTION

Human beings and plants having medicinal value have unique relationship since time immortal (1). Traditional system of medicine shows that plants have provided mankind a large variety of drugs to cure various diseases. Use of plants as a source of medicine has been inherited and is an important component of health care system. (11).

Aristolochia bracteata Retz, is commonly known as worm killer and is a common dominant weed of Maharashtra (7). This plant is one of the worst weed of black cotton soil. It has also been found to occur in dry and irrigated loamy and clayey soils, though hot to a great extent. It is particularly an account of this habit that the extermination of this weeds has become a matter of grave concern (12). The whole plant was used as purgative and anthelmintic, antipyretic and anti-inflammatory agents. *Aristolochia bracteata* Retz, is used in traditional medicine as gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites (8). Root powder is combined with honey and given internally in the case of gonorrhoea, boils, ulcers and other skin diseases

(Sankarnarayanan *et.al.* 2010).In indigenous system of medicine, it is reported that leaves were used for skin diseases, rheumatism and as analgesic(Manikandar *et.al.*, 2006).Alongwith its use against health disorder, it was felt worthwhile to screen this weed for its antimicrobial activities so that its further role in antibiotics is explored. With this perspective this study has been undertaken.

MATERIALS AND METHODS

Plant Material

Mature root stem and leafs of *Aristolochia bracteata* Retz, were collected from the agricultural and open fields around Baramati during may2011.The plants were identified and confirmed from BSI, Pune. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and ground to powder.

Preparation of Extracts

Five gram of plant material was dissolved in 50 ml of solvent (Aqueous, Petroleum ether, Butanol, Methanol) and kept in centrifuge machine for 10 minutes at10000 rpm. Obtained supernatant were filtered with Whatman Filter paper No.41 and evaporated to dryness. Finally dissolved in minimal volume of Dimethyl Sulphoxide and stored at 4⁰C until used in the assay.

Test Organisms

One strain of Gram positive bacteria- *Staphylococcus aureus* and two strains of Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* were used to evaluate the antibacterial activity. All these bacteria were isolated from clinical samples like urine and pus by streak plate method by using Nutrient Agar Plate. All bacterial cultures were maintained on Nutrient Agar slants stored at 4⁰C and periodically subcultured.

Antibacterial Assay

In vitro antibacterial activity of the different extracts of *Aristolochia bracteata* Retz. was studied by disc diffusion method (Ronald *etal* 1989)using different bacterial strains such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These different extracts were loaded on a sterile Whatman filter paper No. 41 filter paper disc with 6 mm diameter and dried aseptically. The solvent loaded without extracts were served as control. A standard disc of ampicillin was used as control. 24 h old bacterial suspension (0.1ml) was poured on sterile nutrient agar plate. After 4 h, the extract loaded and control loaded disc were placed on the solidified nutrient agar media. These inoculated plates were kept in refrigerator for half an hour for prediffusion and finally incubated at 37±1° in incubator for 24 to 48 hour to allow growth of organisms. After incubation the growth and zone of inhibition plates were observed and the diameter of the zone of inhibition were measured and recorded. Clear zone of inhibition around disc indicated the presence of antibacterial activity. All data on antibacterial activity was average of triplicates. Activity Index (A.I.) of every plant extract was calculated by using following formula:

$$\text{Activity Index(A.I.)} = \frac{\text{Inhibition zone of the sample (mm)}}{\text{Inhibition zone of the standard (Ampicillin) (mm)}}$$

RESULTS

Root, stem and leaf extracts of *Aristolochia bracteata* Retz. Screened showed growth inhibitory activity against test bacteria. Antibacterial studies of the four different extracts viz. aqueous, methanol, butanol and petroleum ether of root, stem, leaves of *Aristolochia bracteata* Retz. revealed that growth inhibition of test organisms. The inhibition zone of various extracts showed moderate antibacterial activity with the average zone of inhibition by 7-19 mm by disc diffusion method (Table 1).

Root extracts showed considerable maximum antibacterial activity in all extracts. Methanolic extract showed maximum zone of inhibition (18 mm) by *E.coli*. Following this, high antibacterial activity was shown by leaf extracts, here also butanolic extracts showed higher activity than others. Minimum antibacterial activity was observed in stem extracts.

Zone Of Inhibition Shown By *E.Coli* in methanolic leaf extracts.Zone Of Inhibition Shown By *S.aureus* in Butanolic stem extracts.**Table 1- Antibacterial activity of Root, Stem and Leaf Extracts of *Aristolochia bracteata* Retz.**

Plant part	Extract	<i>Escherichia coli</i>		<i>Pseudomonas aeruginosa</i>		<i>Staphylococcus aureus</i>	
		ZOI	A.I.	ZOI	A.I.	ZOI	A.I.
Negative Control	Aqueous	8	0.17	-	-	13 ^{ns}	0.65
	Pet.Ether	-	-	-	-	19 ^{ns}	0.95
	Methanol	14*	0.31	9 ^{ns}	0.81	9 ^{ns}	0.45
	Butanol	14*	0.31	9 ^{ns}	0.81	11 ^{ns}	0.55
Root	Aqueous	-	-	9	0.63	11 ^{ns}	0.55
	Pet.Ether	-	-	9	0.81	8 ^{ns}	0.40
	Methanol	18*	0.40	7 ^{ns}	0.63	13 ^{ns}	0.65
	Butanol	12*	0.26	7 ^{ns}	0.63	11 ^{ns}	0.55
Stem	Aqueous	-	-	-	-	-	-
	Pet.Ether	-	-	7	0.63	-	-
	Methanol	12*	0.33	7 ^{ns}	0.63	-	-
	Butanol	10*	0.22	10 ^{ns}	0.90	13 ^{ns}	0.65
Leaf	Aqueous	-	-	7	0.63	-	-
	Pet.Ether	7	0.15	7	0.63	9 ^{ns}	0.45
	Methanol	9*	0.20	8 ^{ns}	0.72	-	-
	Butanol	14*	0.30	8 ^{ns}	0.72	8 ^{ns}	0.40
Positive Control	Ampicillin	45		11		20	

ZOI- Zone of Inhibition

A.I.- Activity index

All the values of ZOI are mean of nine readings.

* - values are significant at 5% level. Where $P < 0.05$.ns- values are non-significant at 5% level. Where $P < 0.05$.

DISCUSSION

The screening of secondary metabolites had shown that higher plants represent a potential source of new anti-infective agents (Kelmanson *et.al.*,2001). In previous studies, Negi *et.al* (2003) had reported the antibacterial activity of dried extracts of *Aristolochia bracteata* Retz against few gram positive and gram negative bacteria. Hinou *et.al* (1990) reported cytotoxic and antimicrobial principles from the roots of *Aristolochia longa*.

The present study supports that some of medicinal plants containing secondary metabolites has antibacterial activities and this study supports the previous work of and role of antimicrobial activities. The presence of bioactive compounds in this study of *Aristolochia bracteata* Retz. might have possible role of antibacterial activity.

Wormkiller is traditional medicinal plant in India, is the rich source of bioactive compounds with diverse chemical structure making them a rich source of different types of medicine. In the present investigation different extracts of *Aristolochia bracteata* Retz. showed positive antimicrobial activity. Maximum antibacterial activity found in root extracts followed by leaf and finally in the stem. Present study reveals that bioactive compound could be present in the extract as it shows inhibitory activity against isolates from clinical samples. The bio characterization of those compounds is in process. It may be taken up for further isolation of pure bioactive compounds for better management of microbial infection and multiple resistance in bacteria.

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