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Antibacterial Activity of *Aristolochia Bracteolata* Lam

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Abstract:

The present study was subjected to evaluate the antibacterial activity of the aqueous leaf and aqueous root extract of the medicinal plant *Aristolochia bracteolata* using the standard disc diffusion method against four bacterial species, viz., *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas flavus* strains. The antibacterial activity revealed that the root extract has more effective than the leaf extracts. *Staphylococcus aureus* was the most sensitive organism among the tested organism. A preliminary phytochemical screening was conducted on the plant extracts using standard qualitative procedures that revealed the presence of the secondary metabolites, alkaloids, saponins, phenols, flavonoid and tannin in rich status.

Keywords: *Aristolochia bracteolata*, phytochemical screening, antibacterial activity, disc diffusion method

1. Introduction

The limited life span of antibiotics has rendered and necessities to search for have antimicrobial substances from various sources such as medicinal plant. Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. Plants and plant derived agents have long history to clinical relevance source of potential chemotherapeutic agents¹. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of anti infective agents with possibly novel mechanism of action²⁻⁵.

Aristolochia bracteolata is a climbing perennial medicinal plant with cordate leaves and dark-purple colour tubular flowers, belonging to the family Aristolochiaceae. It is mainly indicated for skin diseases, snake bites, arthritis and diabetes in the literature of Indian system of medicine. Root powder is combined with honey and given internally in the case of gonorrhoea, boils, ulcers and other skin infections sank 2010.⁶ The whole plant was used as anthelmintic, anti pyretic and anti inflammatory agents. The plant contain Aristolochic acid has many medicinal properties in various disease conditions.⁷ Root extract was reported to have antibacterial activity.⁸ The selection of the medicinal plant is based on its uses in Siddha medicine for the treatment of microbial infections.

The present study was conducted to investigate the antibacterial activity of aqueous extract of root and leaves of the medicinal plant *Aristolochia bracteolata* against some clinical associated pathogens. A preliminary phyto chemical screening was studied to identify the biochemical compounds present in the extract.

2. Material and Methods

2.1. Collection of Plants

The leaves and roots of *Aristolochia bracteolata* have collected from Kangaikondan near Tirunelveli in Tamil Nadu and identified by consulting taxonomists and the voucher specimen of the plant was kept in Department Herbarium, Department of Siddha Medicine, The Tamil University, Thanjavur. The plant parts leaf and root have washed with water and shade dried, ground into coarse powder and stored in airtight container.

2.2 Bacterial Culture

Four bacterial strains, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas flavus* were used to evaluate the antibacterial study. All bacterial cultures were maintained on Nutrient Agar slants stored at 4 °C.

2.3 Preparation of Extracts

The leaves and root of the shade dried powder of *Aristolochia bracteolata* were soaked separately in distilled water (200ml) for 72 hours. At the end of the extraction, the extract was filtered using Whatman No. 1 filter paper. The filtrates were concentrated in vacuum at 30 °C. After complete evaporation, the extracts were preserved aseptically. The graded concentrations of the extract were prepared and then subjected to screen the phyto chemicals and antibacterial assays.

2.4 Preparation of Susceptibility Test Disc

5 mm diameter disc were prepared by using Whatman No.1 filter paper, sterilized and put it in extract of different concentrations.

2.5 Antibacterial Screening

The extracts were screened for antibacterial activity using the disc diffusion technique.⁹ The paper disc impregnated with leaf extract of 10, 20 and 30mg/10ml and root extract of 10, 20 and 30mg/10ml were placed a nutrient agar medium containing petriplates which is previously uniformly seeded with the test organism of 48 hrs cultures viz., *Klepsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas flavus* and *Escherichia coli*. Streptomycin containing disc was used as standard control. After the placement of the discs the petriplates were kept in an incubator at 35°C for better growth of the organism. After 18 hrs the inhibition zones were recorded in mm.

3. Results and Discussion

The phyto chemical screening indicated that the presence of alkaloids, saponins, flavonoid, phenol and tannin in rich status (Table-I). Previous studies on *Aristolochia bracteolata*, the aqueous leaf extract showed the presence of alkaloids, tannins, phenol, flavonoids, glycosides, lignin and saponin¹⁰⁻¹¹. The *in vitro* antibacterial activity of the aqueous extract of *A. bracteolata* leaves and root against the micro organisms *Klepsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas flavus* and *E. coli* were assessed by the presence of inhibition zones. In this study, the antibacterial activity of the leaf and root extracts were compared with Streptomycin used as positive control. The results were shown in table II. The antibacterial activity revealed that the root extract has more effective than the leaf extracts. This findings supported by the previous investigations on *Aristolochia bracteolata* that different extracts showed positive antimicrobial activity and maximum antibacterial activity found in root extracts followed by leaf and finally in the stem¹². The results indicated that *Klepsiella pneumoniae*, *Staphylococcus aureus* and *E. coli* were more sensitive to the extract. The study report is in agreement with previous work which show that the antibacterial activity of the plant *Aristolochia bracteolata* aqueous leaf extract had significant inhibiting activity against *Klepsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus* and *E. coli*¹³. *Staphylococcus aureus* was the most sensitive organism among the tested organism with the inhibition zone of 17mm in 30mg/10ml of the leaf extract and 14mm in 10mg/10ml of the root extract. Previous studies on *Aristolochia bracteolata* leaf and root extract showed considerable antimicrobial activity¹⁴. The extracts (500 g/ disc) of *A. bracteolata* showed moderate antibacterial activity with the average inhibition zone of 7-18mm by disc diffusion method¹⁵. The antibacterial activity of dried extracts of *A. bracteolata* against few gram positive and gram negative bacteria.¹⁶

The presence of secondary metabolites in plants, produce some biological activity in man and animals. Tannins and phenols are known to possess antimicrobial properties.¹⁷ Alkaloids and saponins have been implicated as bioactive antibacterial and antifungal agents of plants.¹⁸⁻²⁰ Hence, the presence of the phyto chemical such as alkaloid, saponin and tannin may be responsible for its antibacterial activity.

Sl.No.	Phytochemicals	Leaf	Root
01.	Alkaloid	+	++
02.	Flavonoid	++	++
03.	Saponin	++	++
04.	Phenol	+	++
05.	Tannin	+	+
06.	Glycosides	-	-

Table 1: Phytochemical Screening of *Aristolochia bracteolata*

Key: + Present, - Absent.

Sl. No.	Name of the test organism	Zone of diameter in mm						
		Drug concentration mg/10ml						
		L			R			S
1.	<i>Klepsiella pneumonia</i>	8	9.5	10	10	11	12	19
2.	<i>Staphylococcus aureus</i>	8	10.5	12	12	12.5	14	17
3.	<i>Escherichia coli</i>	7	8	8.5	9	10	12	18
4.	<i>Pseudomonas flavus</i>	6.8	6.5.	6.5	7	7.5	8	18

Table 2: Antibacterial activity of the aqueous extracts of the plant *Aristolochia bracteolata*

S - Streptomycin, L - Leaf, R - Root.

4. Conclusion

The previous studies on the plant and the present results concluded that the plant *Aristolochia bracteolata* was an effective antibacterial drug. The extracts are more sensitive on higher concentrations in leaf extracts and lower concentrations in root extract. The preliminary phytochemical screening revealed that the presence of alkaloids, phenol, saponin, flavonoid and tannin. Among the four tested bacterial organisms, *Staphylococcus aureus* was more sensitive to the plant *Aristolochia bracteolata*.

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