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RESEARCH ARTICLE

ANTIMICROBIAL POTENTIAL OF ROOT, STEM AND LEAF EXTRACT OF *Aristolochia bracteolata* LAM

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ABSTRACT

The present study describes the antimicrobial activity of the acetone, ethanol and petroleum ether extracts of the leaves, stem and root of *Aristolochia bracteolata* Lam. using agar diffusion method against human pathogenic bacteria, such as *Escherichia coli, Pseudomonas aeruginosa, Bacillus aureus* and *Klebsiella pneumoniae.* In the present research, all the extracts were found to be effective against four human bacterial species, *E.coli, P.aeruginosa, K.pneumoniae* and *B.aureus* sensitive to all the plant extracts. The study recommends that the extract of the plant parts possesses novel broad spectrum antibacterial properties. The antimicrobial activity of petroleum ether extracts was found to be highest than that of ethanol extracts. However, the root extract showed more inhibitory effect than the stem and leaf extracts. The findings provide support for the use of the plant in traditional medication.

INTRODUCTION

Plants have been an integral part of life in many local communities for food and medicine both. India has more than 3000 years of medicinal heritage based on medicinal plants. Medicinal plants are widely used by all sections of the population either directly as folk remedies or indirectly in the preparation of modern pharmaceuticals. India is endowed with a rich wealth of medicinal plants, microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. An antimicrobial is substances that destroy or inhibits the growth of microorganisms such as bacteria, fungi or protozoan's as well as destroying viruses (Chan-Bacab and Pefia-Rodriguez, 2001). Plants produce a diverse extent of bioactive molecules, making them wealthy source of various types of medications. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine (Sukanya *et al.,* 2009). The use of plant extracts and phytoconstituents, both with known antimicrobial screening can be of numerous significance in therapeutic treatments. A larger part of the total population in developing countries still uses traditional folk medicine obtained from plant resources (Srivastava *et al.,* 1996). About 80 percentage of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants. In the last few years, a number of reported have been conducted in various countries to manifest such ability. Numerous plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. Therefore, such plants should be examined to better understand their activities, trustworthy and efficiency (Prusti *et al.,* 2008). Antimicrobial compounds of plant source may be found in plant roots, stems, leaves, flowers or fruits, bark (Beuchat *et al.,* 1998). *Aristolochia* is a large plant genus with over 500 species. *Aristolochia bracteolata* Lam commonly called as Worm Killer (due to supposed antihelminthic activity) in English and aadutheendaapaalai in Tamil. *Aristolochia bracteolata* is a herbaceous perennial plant. This plant belongs to the family Aristolochiaceae. The whole plant was used as antipyretic, purgative, anthelmentic and anti-inflammatory agents. The root part was used to treat gonorrhea, syphilis and also used during labors to increase uterine contraction. The plant contain Aristolochic acid has many medicinal properties in various disease condition (Kirtikar and Basu, 1935). Decoction of the whole plant is given in fever, worms, skin disease and snake bite (Alagesaboopathi, 2009). The plant 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), antimicrobial activity (Manikandar *et al.,* 2006) antiarthritis activity (Havagiray *et al.,* 2009), antiallergic activity (Chitme *et al.,* 2010) and antioxident property (Kalpana devi *et al.,* 2011). Therefore the present study has been undertaken to investigate the antimicrobial activity of leaf, stem and root extracts of *Aristolochia bracteolata* by disc diffusion method (Hugo *et al.,* 1987).

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh root, stem and leaves of *Aristolochia bracteolata* were collected in December 2010 from Pethampatti, Salem Taluk,

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Salem district of Tamilnadu, India and is authenticated by botanist of Government Arts College (Autonomous), Salem, Tamilnadu and voucher specimen (No.16/24/12/2010 CA) deposited in the department of Botany, Government Arts College, Salem for the future reference.

Test microorganisms

Human pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Bacillus aureus* and *Klebsiella pneumoniae* were collected from Microbiology Lab, Biomedical Engineering Research Foundation, Salem, Tamilnadu, India. All the test bacterial species were maintained on nutrient agar media.

Preparation of plant extracts

Fresh plant materials (leaves, stem and root) collected were washed individually under running tap water to remove soil particles and other dirt. The leaves were air dried in the laboratory at room temperature (30±1°C) for 10 days. While the root and stem materials were dried at 50±1°C for 60-72 h in an oven. The dried leaves, stem and root samples were ground well into a fine powder with a mixer grinder. The powder was stored in airtight bottles at room temperature before extraction. The method of Alade and Irobi (1993) was adopted for preparation of plant extracts. A fixed weight 25 g of powdered plant material was soaked separately in 150 ml each of acetone, ethanol and petroleum ether for 72 h. Each mixture was stirred at 24 h interval using a sterile glass rod. At the end of the extraction, each extract was passed through Whatman No.1 filter paper (Whatman England), and the filterate obtained was concentrated in vaccum using evaporator. Then the extracts were used for antibacterial assay.

Antimicrobial activity

The media and test bacterial species were poured into dishes (Muller-Hinton agar media). The test strain (0.2 ml) has an inoculum size (108 cells/ml) when the temperature reached 37 to 39°C. Care was taken to assure correct homogenization. The plant extracts were tested for antimicrobial assay in the agar well diffusion activity (Perez *et al.,* 1990) against *Escherichia coli, Pseudomonas aeruginosa, Bacillus aureus* and *Klebsiella pneumoniae.*

Agar well diffusion method

The antimicrobial assay was tested against (acetone, ethanol and petroleum ether) leaves, stem and root of *Aristolochia bracteolata.* The innoculation of microorganisms was prepared from bacterial species (Parihar and Bohar, 2006). About 20-25 ml of Muller-Hinton agar medium was poured in the sterilized petridishes and allowed to solidify. One drop of bacterial strains was spread over the medium by a rod. Wells of 5 mm in diameter and about 2 cm apart were punctured in the culture medium using sterile cork borers. About 75 ml of the plant extracts were added to the wells. The plates were incubated for 24 h at 37°C (Esimone *et al.,* 1998). At the end of incubation, the plates were observed and zones of inhibition were measured. The average zones of inhibition were recorded. 10 µg/ml of streptomycin served as control.

RESULTS AND DISCUSSION

The antimicrobial screening of acetone, ethanol and petroleum ether extracts of different parts of *Aristolochia bracteolata* Lam were observed using Agar well diffusion method by measuring the diameter of the growth inhibition zone. Table 1 show the antimicrobial activity of plant parts extracts of *A. bracteolata*. The acetone, ethanol and petroleum ether extracts of the leaf, stem and root of *Aristolochia bracteolata* showed consiterable antimicrobial activities. The inhibitory zone for acetone extracts of the leaf showed most potent antibacterial activity against *E. coli* (10.12 ±0.11), *P. aeruginosa* (9.40 ± 0.02), *B. aureus* (7.03 ± 0.10) and *K. pneumoniae* (11.12 ± 0.03). The investigation made on ethanol extracts of the leaf maximum activity against *P. aeruginosa* (10.22 ± 0.07), *E. coli* (8.20 ± 0.09 and *B. aureus* (8.12 ± 0.12). It has no inhibition against pathogen like *K. pneumoniae*.

Table 1. Antimicrobial activities of the various leaf, stem and root extracts of *Aristolochia bracteolata*

		Zone of inhibition in mm			
Plant Parts	Extracts	*E.coli*	*P.aeruginosa*	*B.aureus*	*K.pneumoniae*
Leaf	Acetone	10.12±0.11	9.40±0.02	7.03±0.10	11.12±0.03
	Ethanol	8.20±0.09	10.22±0.07	8.13±0.12	-
	Petroleum ether	12.13±0.04	10.08±0.05	12.10±0.02	8.08±0.08
Stem	Acetone	8.07±0.05	10.15±0.01	9.21±0.14	-
	Ethanol	7.11±0.09	-	7.13±0.11	7.03±0.13
	Petroleum ether	10.20±0.06	9.14±0.03	10.09±0.20	11.20±0.09
Root	Acetone	10.13±0.01	9.05±0.20	14.11±0.13	9.13±0.02
	Ethanol	9.10±0.12	10.11±0.08	10.15±0.20	7.11±0.10
	Petroleum ether	11.21±0.07	10.20±0.02	16.21±0.09	10.16±0.11
	Streptomycin 10 µg/ml	19.0±0.15	21.0±0.07	22.0±0.11	20.0±0.10

Data given are mean of triplicates ± Standard error. - indicates no inhibition. Concentration used 50 µg/ml.

The leaf extracts of petroleum ether showed a positive significant antimicrobial activity against *E. coli* (12.13±0.04), *B. aureus* (12.10±0.02) and *P. aeruginosa* (10.08±0.05). While moderate activity against *K. pneumoniae* (8.08±0.08). The petroleum ether extracts of the stem showed significant and highest antimicrobial activity against *K. pneumoniae* (11.20±0.09), *E. coli* (10.20±0.06) and *B. aureus* (10.09±0.20). Whereas moderate degree of activity against *P. aeruginosa* (9.14±0.03). The stem extracts of ethanol showed moderate activity against *B. aureus* (7.13±0.11), *E. coli* (7.11±0.09) and *K. pneumoniae* (7.03±0.13). No antimicrobial activity was recorded against *P. aeruginosa*. The zone of inhibition in stem acetone extracts showed maximum activity against *P. aeruginosa* (10.15±0.01), *B. aureus* (9.21±0.14) and the minimum activity against *E. coli* (8.07±0.05). No antimicrobial activity was found against *K. pneumoniae*. The significant and highest antimicrobial activities of the root petroleum ether extracts against *B. aureus* (16.21±0.09),*E. coli* (11.21±0.07), *P. aeruginosa* (10.20±0.02) and *K. penumoniae* (10.16±0.11). Acetone extracts from root was activity against *B. aureus* (14.11±0.13), *E. coli* (10.13±0.01), *K. pneumonia* (9.13±0.02) and *P. aeruginosa* (9.05±0.20). The antimicrobial activities of the root ethanol extracts showed maximum activity against *B. aureus* (10.15±0.20), *P. aeruginosa* (10.11±0.08) and *E. coli* (9.10±0.12). While moderate activity against *K. pneumoniae* (7.11±0.10). The standard drug streptomycin (10 µg/m1)

showed high degree of inhibition against *E. coli, P. aeruginosa, B. aureus* and *K. pneumoniae*. Previous observations on *A. bracteolata* leaf and root extracts showed considerable antimicrobial activity (Manikandar *et al.,* 2006; Kavitha and Nirmaladevi 2009; Angalaparameswari *et al.,* 2009; 2010). In another investigation, the species of *Aristolochia* like *Aristolochia longa, Aristolochia paucinervis* were reported as worthy antimicrobial drugs (Hinou *et al.,* 1990; Gadhi *et al.,* 1999). In another research, acetone, aqueous and petroleum ether extracts of *Aristolochia indica* was found to be good antimicrobial activity (Alagesaboopathi, 2011). The antimicrobial compounds may be found as flavonoids, tannins, alkaloids, steroids, saponins, triterpenoids and phenolic compounds whose presence may be attributed to the therapeutic properties of plants (Santhi *et al.,* 2006). The results of the present study are found to be directly correlated with the observations of previous workers (Shariff *et al.,* 2006; Jain *et al.,* 2004; Igbinosa *et al.,* 2009). Other details are needed to isolate and characterize the bioactive principles to develop modern antimicrobial medicines. This antimicrobial investigation of the plant extracts described that folk medication can be as efficient as current medicine to combat pathogenic microorganisms.

CONCLUSION

In conclusion, the acetone, ethanol and petroleum ether extracts of *Aristolochia bracteolata* leaf, stem and root possess critical inhibitory result against the tested microorganisms. The results of the study support the traditional declare of this plant.

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