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Screening and characterization of *Aristolochia bracteolata* plant extract against antibacterial activity of selected microbes

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Abstract

A bioassay-guided assay of acetone extract of *Aristolochia bracteolata* plant leaf was carried out in order to evaluate its antibacterial activity and to identify the active compounds in this extract. Antibacterial activities of acetone extract against gram-positive and gram-negative bacteria strains were investigated by the Kirby-Bauer agar disk diffusion method. Among the strains tested *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Showed the highest sensitivity towards the acetone extracts and hence they are used as test organisms for the bioassay-guided fractionation. From this extract, aristolochic acid has been isolated and has shown the greatest antibacterial activity against both standard strain and clinical isolates of *Escherichia coli* with equal minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 30 and 50 µg/mL. The plant leaf having bioactive compound of aristolochic acid and acetylenic acid and flavonoids. Bioactive components and functional groups present in the plant leaf essential for aristolochic acid antibacterial activity. This investigation it can conclude the *Aristolochia bracteolata* for alternative of treatment for bacterial infection in traditional and current medicinal uses.

Keywords: *Aristolochia bracteolata*, clinical pathogens, bioactive compound and agar disk diffusion method

Introduction

India has different parts of several medicinal plants or their extracts are used treatment for various diseases [1]. *Aristolochia bracteolata* is known as “worm killer” due to supposed antihelminthic activity and trypanocidal effect [2]. Medicinal plants many have been used to centuries as remedies for human diseases because they contain components of therapeutic value [3]. *Aristolochia* is an important genus, both numerically and medicinally, in the family Aristolochiaceae. The genus *Aristolochia* consists of about 400 species of herbaceous perennials, under shrubs or shrubs bearing essential oils and is widespread across tropical Asia, Africa and South America but their effect upon [4]. Various plants are known to consume fungicidal substances and much work has been ratified out on the effects of medicinal and aromatic plant extracts against numerous fungi [5]. This has led to the search for new and effective therapeutic alternatives among natural compounds. Plants remain an important source of diverse chemical entities, which is used as drugs or provide scaffolds from which new drugs have been derived [6]. It is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery, and snakebites [7]. *Aristolochiaceae* has been used by the Sudanese people as analgesic, antiscorpion and it is antsnake. It is also used in the treatment of tumors and malaria and for fevers [8] but its usage as an antimalarial is not recommended in its crude form. *Aristolochia bracteolata* showed a definite positive effect on wound healing, with significant increase in the level of powerful antioxidant enzymes. Its root and leaves were bitter and anthelmintic and are medicinally important. Almost every part of the plant has medicinal usage [9]. The whole plant was used as a purgative antipyretic, and anti-inflammatory. It also possesses a potent antiallergic activity.

Asia has abundant species of medicinal and aromatic plants and traditional medicines have practiced in Asia since ancient times. India has made use of medicinal plants to cure ailments of thousands of years. According to World Health Organization the goal of health for all can't be achieved without herbal medicines, while the demand for herbal medicine is growing in developing countries, there are indications that consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives in traditional medicine [10-12].

Although *Aristolochia* is being known in many countries that is containing a toxic compound AA, but this has not stopped it from being a popular herbal remedy for thousands of years. It is still extensively used in India and in traditional Chinese medicine for slimming, menstrual symptoms, and rheumatism. It is also widespread used in Sudan and other African countries as one of the most effective herbal remedies for infectious diseases. Therefore, it was our objective to assess the potential antimicrobial activity of *Aristolochia bracteolata* using a bioassay-guided fractionation, in order to produce pure compound that can act as the lead compound in developing new, safe, and effective drug to replace the use of the harmful crude plant material [5].

Materials and Methods

Collection of the plant materials

The plant material used in this present study was the plant *Aristolochia bracteolata* collected from Tamil Nadu, in India. The leaves collected and they dried in room temperature. Then they crushed in to small pieces and these dried small pieces are finely powdered [13].

Preparation of plant extracts

The plant powder (15g) of *Aristolochia bracteolata* was Soxhlet - extracted with 300 ml of acetone in the round bottom flask using the Soxhlet extraction method as per the standard procedure at their 70 °C boiling point [14]. The extract recovered from the solvent using rotary evaporator apparatus and stored in a freezer (-4 °C) for further use. The sticky black substances were obtained and stored in refrigerator and these are suspended/ dissolved in DMSO (Dimethyl Sulphoxide) prior to use [15].

Preliminary phytochemical analysis plant extracts of *Aristolochia bracteolata*

Preliminary phytochemical analyses of acetone extract was carried out using standard procedure to identify the phytochemical constituents, alkaloids, flavonoids, glycosides, phytosterol, fixed oils and fats, saponins, tannins and phenolic compounds. Proteins and free amino acids, gums, mucilage, lignin [11].

Preparation of the Bacterial Inoculums

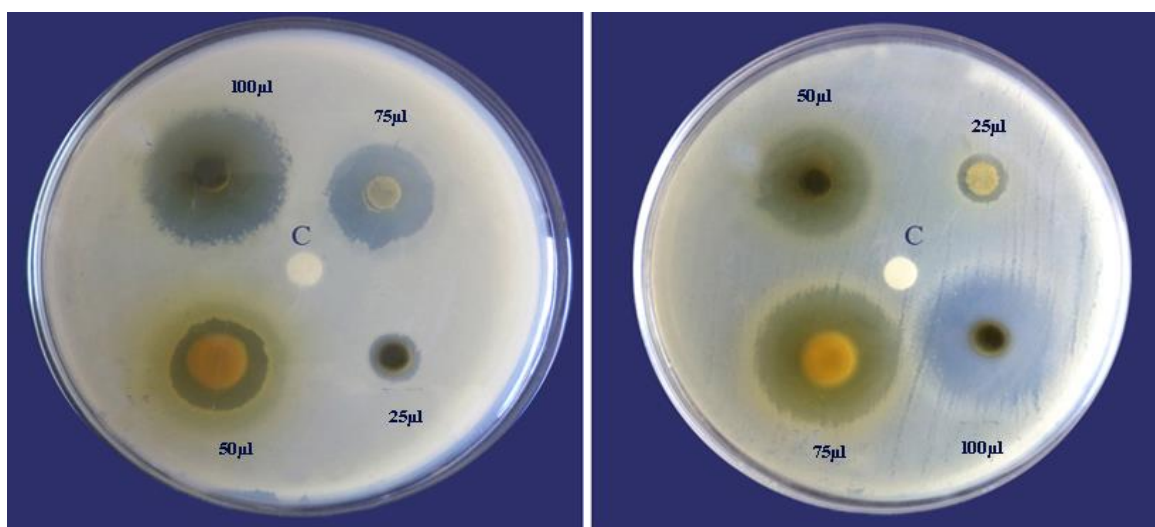
The young microbial inoculums / culture were prepared 24 hours at 37 °C, afterwards the turbidity of culture is adjusted to match that of a 0.5 McFarland standard (10^8 CFU/ml). Absorbance of 0.600 at 450nm determined spectroscopically then it was used as inoculums [1].

Screening for antibacterial assay

The antibacterial activities of the plant were tested against the selected bacterial strains. The sterilized Mueller Hinton agar medium was poured in to each sterile petriplate and allowed to solidify [16]. Using sterile cotton swabs the test bacterial cultures were we evenly spread over the appropriate media. The sterile discs were individually loaded with different concentrations of organic solvents extract acetone of the plant. These discs were we kept in undisturbed place for the evaporation of the solvents. Then the discs were we placed on the top layer of the Petri dishes pertaining to the test cultures. A 100µL suspension of test microorganism added to individual tubes and incubated at 37 °C for 24 h. After the incubation period, the results were we observed and measured the diameter to demarcate inhibition zone around each disc/organisms. The MIC of the compound was defined as the lowest concentration that inhibited the visible bacterial growth and the MBC was defined as the lowest concentration that prevented the growth of the organism after subculture onto antibiotic-free plates. After results were, confirm by two trained laboratory personnel [17]. Microbial strains used for antibacterial assay: Ten different bacterial strains are used for the antibacterial assay they are, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

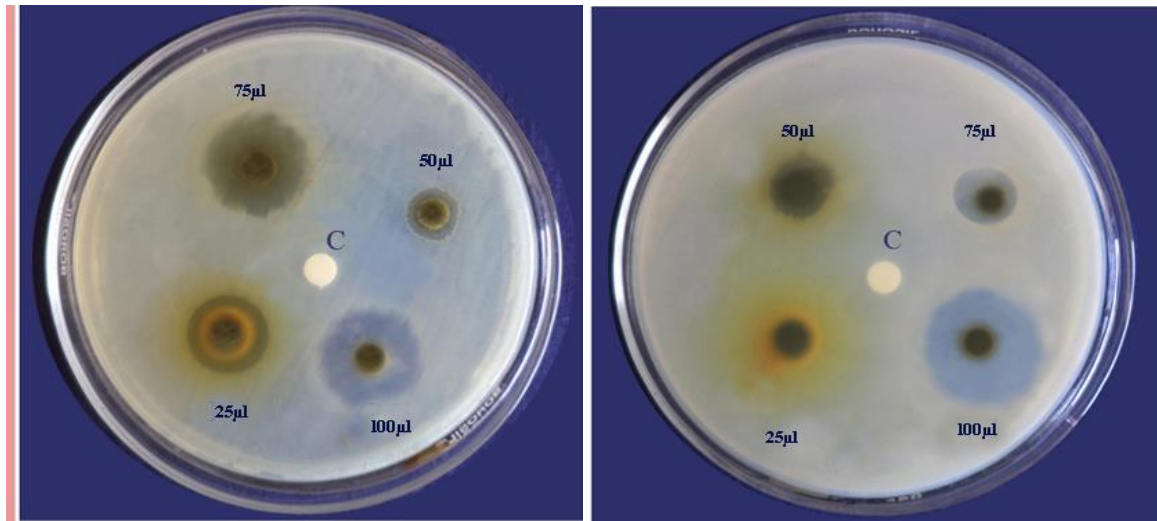
Results and Discussion

Antibacterial Activity Initial steps in new drug discovery involve identification of new chemical entities, which either can be sourced through chemical synthesis or can be isolated from natural products through biological activity guided fractionation. Bioassay-guided fractionation of the identified plant may lead to standardized extract or isolated bioactive lead compounds as the new drug.



Bacillus subtilis

Escherichia coli

*Pseudomonas aeruginosa**Staphylococcus aureus***Plate 1-4:** Antibacterial activity of *Aristolochia bracteolata* leaf extract**Table 1:** Phytochemical investigation of *Aristolochia bracteolata* leaf extract

S. No	Phytochemicals	E. Acetate
1.	Alkaloids	++
2.	Flavanoids	+
3.	Saponin	+
4.	Phenol	+
5.	Phytosterol	+++
6.	Tannins	+
7.	Carbohydrates	+
8.	Glycosides	++

+ Low, ++ Moderate, +++ High

Table 2: Antibacterial activity of *Aristolochia bracteolata* leaf extract

S. No	Test Organisms	Diameter of zone of inhibition (mm)				
		Standard	Acetone			
		100µl	25µl	50µl	75µl	100µl
1.	<i>Staphylococcus aureus</i>	23 ±1.74	07 ±0.8	07 ±1.2	08 ±1.8	11 ±2.6
2.	<i>Bacillus subtilis</i>	21 ±1.55	08. ±1.0	10±1.24	11 ±1.8	13 ±2.1
3.	<i>Escherichia coli</i>	18 ±0.29	10 ±0.9	12 ±0.9	15 ±1.5	17 ±2.5
4.	<i>Pseudomonas aeruginosa</i>	16±0.45	08 ±0.3	09 ±0.8	11 ±1.1	12 ±1.8

Standard – Chloramphenicol

SD - Standard Deviation

The plant materials of *Aristolochia bracteolata* selected for the study to evaluate the formulation containing the extracts of these plants for their antimicrobial activity. Methanol and Ethyl acetate extracts of these plants made by soxhlet extraction method [18] and the obtained extracts concentrated by evaporation and preliminary phytochemical screening made for the obtained extracts by standard procedure [19]. The phytochemical screening indicated that the presence of alkaloids, saponins, flavonoid, phenol and tannin in rich status (Table-1) and Plate 1- 4. Previous studies on *Aristolochia bracteolata*, the aqueous leaf extract showed the presence of alkaloids, flavonoids, saponins, tannins, phenol, carbohydrates, and glycoside.

The plant leaf of *Aristolochia bracteolata* extracted successively with acetone. The resulting fractions tested for antibacterial activity. The crude extract and acetone fraction were significantly active against *Bacillus subtilis* and were moderately active against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The Highest active fraction identified against *Escherichia coli*. (Table-2). The presence of secondary metabolites in plants, produce some biological

activity in man and animals. Tannins and phenols known us possess antibacterial properties. *Aristolochia* is used in traditional medicine for the treatment of various diseases [19], including those associated with bacteria. This study showed clearly that the excellent effect of *Aristolochia* in treating such diseases is due to the toxic compound Aristolochic acids. Although Aristolochic acid is highly effective in killing *Escherichia coli*, it is ineffective against the other microorganisms tested. The widespread use of *Aristolochia* is not sufficient to ensure that it is effective or even that it is safe. Therefore, hit-to-lead exploration is necessary to identify related compounds with low toxicity, low cost, and improved potency that can replace the use of the harmful crude plant material [20].

Conclusion

Using bioassay-guided assay technique, the present study directly linked the antibacterial activity of *Aristolochia bracteolata* to the Aristolochic acid and Acetylenic acid. Although Aristolochic acid had strong activity against *Escherichia coli*, it had a narrow spectrum of activity than

expected based on the activity of the crude extract from which it was isolated or from its traditional usage. This may be the result of synergism between different compounds in the complex extracts or may be due to placebo effect.

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References

- Muthu C *et al.* Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. *Journal of Ethnobiology and ethnomedicine.* 2006; 2(1):43.
- Abdelgadir AA, Ahmed EM, Eltohami MS. Isolation, characterization and quantity determination of aristolochic acids, toxic compounds in *Aristolochia bracteolata* L. *Environmental health insights.* 2011; 5:EHI. S6292.
- Manikandan P *et al.* Antibacterial Efficacy of *Moringa oleifera* Leaf against Medically Important Clinical Pathogens. *International Journal of Current Microbiology and Applied Sciences.* 2016; 5(4):109-116.
- Wu TS *et al.* Terpenoids of *Aristolochia* and their biological activities. *Natural Product Reports,* 2004; 21(5):594-624.
- Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine,* 2006; 27(1):1-93.
- Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. *Journal of natural products.* 2007; 70(3):461-477.
- Thirumal M *et al.* *Aristolochia bracteolata*: an overview on pharmacognostical, phytochemical and pharmacological properties. *Critical Review in Pharmaceutical Sciences.* 2012; 1(1):70-82.
- El Tahir A, Satti GM, Khalid SA. Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Maytenus senegalensis* (Lam.) Exell. *Journal of ethnopharmacology.* 1999; 64(3):227-233.
- Gupta K *et al.*, International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clinical infectious diseases.* 2011; 52(5):e103-e120.
- Tamizhazhagan V, Pugazhendy K. Ethnobotanical and Phytopharmacological review of *Pisonia alba* Span. *Asian J Pharm Clin Res.* 2017; 10(5):69-71.
- Tamizhazhagan V *et al.* Preliminary screening of phytochemical evaluation selected plant of *Pisonia alba*. *IJ Biol. Research.* 2017; 2(4):63-66.
- Tamizhazhagan V *et al.* Antioxidant properties of *Pisonia alba* plant leaf extract. *International Journal of Zoology and Applied Biosciences.* 2017; 2(6):311-314.
- Murugan K *et al.* *Aristolochia indica* green-synthesized silver nanoparticles: a sustainable control tool against the malaria vector *Anopheles stephensi*? *Research in veterinary science.* 2015; 102:127-135.
- Chen C.-N.R., A phytochemical investigation of the bark of *Doryphora sassafras* Endlicher, The Ohio State University, 1972.
- Fritzen-Freire CB *et al.* Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. *Food Research International.* 2012; 45(1):306-312.
- Gunalan G, Saraswathy A, Krishnamurthy V. Antimicrobial activity of medicinal plant *Bauhinia variegata* Linn. *Int J Pharm Biol Sci.* 2011; 1(4):400-8.
- Suliman Mohamed M. *et al.* Activity of *Aristolochia bracteolata* against *Moraxella catarrhalis*. *International journal of bacteriology,* 2014.
- Julkunen-Tiitto R. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *Journal of agricultural and food chemistry.* 1985; 33(2):213-217.
- Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. *Evidence-Based Complementary and Alternative Medicine,* 2011.
- Njuguna NM, Masimirembwa C, Chibale K. Identification and characterization of reactive metabolites in natural products-driven drug discovery. *Journal of natural products.* 2012; 75(3):507-513.