# Evaluation of the Antimicrobial Efficacy of Aristo lochia Tagala Leaf Extract against Selected Human Pathogenic Bacteria and Fungi

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***Abstract*- The aim of this study was to examine the antimicrobial activity of Aristolochia tagala leaf extract with different solvents namely Acetone, Chloroform, Ethanol, Methanol and aqueous against few pathogenic bacteria and fungi. For the antibacterial activity of leaf extract of the target plant, the disc diffusion assay was used against two gram-positive bacterial strains, viz., Staphylococcus lentus and Bacillus cereus and one gram-negative bacterial species Serratia marcescens and three fungal species such as Candida albicans, Candida dubliniesis and Cryptococcus neoformans. The patterns of inhibition varied with the plant extracts, the solvent used for extraction and the organisms tested.**

***Index Terms*- Aristolochia tagala, antimicrobial screening, Human pathogens, Medicinal plant.**

## INTRODUCTION

Medicinal plants have been an integral part of life in various regional communities for food and drug. The wide range of medicinal usages, the present day entails new drugs with more potent and desired activity with less or no side effects against particular disease (Roy et al., 2009). Medicinal plants are largely used by all divisions of the population either directly as folk medications or indirectly in the preparation of recent pharmaceuticals (Pushpangadan, 1995). Numerous plants synthesize substances that are useful in the maintenance of health in humans (Sawarkaret al., 2011). Tamil Nadu is one of the most botanized areas of South India. A vast knowledge regarding how to use the plants against different illness may be expected to be accumulated in area where the use of plant still of great importance. The medicinal properties of those plants were studied by several workers in Tamil Nadu. Many medicinal plants are used by traditional healers for their antimicrobial properties (Hema and Mahomoodally, 2012). Climber plants are an important source of novel drugs for many diseases but to attain that numerous challenges are encountered including the procurement of climber plant material, the selection and implementation of appropriate high-throughput screening bioassays and the scale up bioactive compounds (Hogan et al., 2010). Species of the Aristolochia genus are used in traditional medicine, mainly in South America, to treat skin diseases, poisoning, wounds, worms, diarrhea, and also as emmenagogue (Wu et al., 2004). Aristolochia tagala is a rare medicinal plant. The roots of this plant are strongly aromatic and are used to treat snake bites, bone fracture, malaria, indigestion, rheumatism, toothache and various dermatological conditions. And also the roots are used to treat colic fits and bowel complaints. Due to indiscriminate harvesting of root for local medicine and trade, the species has become rare in its natural habitat (Ravikumar and Ved, 2000).

## Material and Methods

### I.Plant Material and Preparation of Plant Powder

The plant material Aristolochia tagala Cham. Was collected from the Bodi Hill North Forest, Theni District, Tamil Nadu, during the Month of December, 2017. The plant parts were carefully examined and old, insect damaged, fungus infected leaves, stems and roots were removed. The selected healthy plant leaves were spread out and shade dried in the laboratory at room temperature for 8 days. The dried plant leaf was ground to a fine powder by using an electronic blender and the powders were stored in a closed container at room temperature for further uses.

II. Extraction Of Plant Solvent Extract

Fifty grams of the powdered leaf material was separately impregnated with 300 ml of each of the solvents viz, acetone, chloroform, ethanol, methanol and aqueous. At the end of 48 h each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature. The paste like extracts were stored in pre-weighed screw cap bottles and the yield of extracts was calculated based on initial and final weight of the container. These screw cap bottles with the extracts were kept in refrigerator at 4℃. Each of the extract was individually reconstituted by using minimal amount of the extracting solvent prior to use.

Antimicrobial Activity Test (Disc Diffusion Method)

I) Disc Preparation

Disc is most convenient to use. Whatman No.1 filter paper was used for preparing the discs. Dried discs of 6 mm diameter were prepared from and sterilized in an autoclave.The filter paper discs of uniform size are impregnated with the compound (plant extract) usually consisting of absorbent paper. These dried discs were used for the test.

II) Tested Microorganisms

The antimicrobial activity of Aristolochia tagala leaf powder extract was used against two gram positive bacterial species, Staphylococcus lentus and Bacillus cereus. One gram negative bacteria, Serratia marcescens and three fungal species such as Candida albicans, Candida dubliniesis and Cryptococcus neoformans the species that were purchased from Department of Microbiology, K.AP Viswanatham Government Medical College, Tiruchirappalli, Tamil Nadu

III. Procedure

Sterile liquid Nutrient Agar (NA) medium and Potato Dextrose Agar (PDA) medium (pH 7.4 ± 2) was poured (10-15 ml) into each sterile petriplates. The growth media also seem to play an important role in the determination of the antimicrobial activity. Nutrient Agar medium appears to be the best medium to explicate the antibacterial activity, Potato Dextrose Agar medium appears to be the best medium to explicate the antifungal activity and the same was used in the present study. After solidification, 100 µl of suspension containing 108 CFU/ml of each test bacteria and fugal was spread over Nutrient Agar and Potato Dextrose Agar plates. The sterile filter paper discs (6 mm in diameter) were impregnated with 10µl of the 3 mg/ml extracts (30µg/disc) placed on the inoculated agar. Negative controls were prepared in using the same solvents. Chloramphenicol (30µg/disc) and Fluconazolecol (30µg/disc) was used as positive reference control to determine the sensitivity of the plant extract on each bacterial and fungal species. The inoculated plates were incubated at 37°C for 24hrs (bacteria) and 27°C for 48-72hrs (fungi). After indicated the presence of antimicrobial activity each assay was conducted in triplicate.

IV. Statistical Analysis

Agar disc diffusion activity was performed in three replicates under strict aseptic conditions to ensure consistency of all conclusions. Data of all experiments were statistically analyzed and expressed as Mean ± Standard Deviation [8].

## RESULT

I) Antimicrobial activity

In vitro antimicrobial activity of acetone, chloroform, ethanol, methanol and aqueous was carried out against various clinical pathogenic bacteria viz, Staphylococcus lentus, Bacillus cereus and Serratia marcescens both gram positive and gram negative. Similarly the species of fungal viz, Candida albicans, Candida dubliniesis and Cryptococcus neoformans were also used for the present investigation. (Table. 1)

The acetone extract showed greater activity against gram-positive organism than against gram-negative organism. Antibacterial activity of the acetone and ethyl acetate extracts may be due to the greater solubility of the extract in these organic solvents [De Bore, et al., 2005]. In present study the acetone solvent of Aristolochia tagala leaf extract showed the highest inhibition zone against tested bacterial and fungal such as Staphylococcus lentus (30.6 ± 0.4), Bacillus cereus (26.3 ± 0.5). The low degree zone of inhibition against Serratia marcescens (8.2 ± 0.2) Candida albicans (7.6 ± 0.3) and Candida dubliniesis (7.4 ± 0.2) No result found in acetone leaf extract against Cryptococcus neoformans. Similarly (Vaghasiya and Chanda, 2007) have reported that the maximum antibacterial activity against Staphylococcus species (29.9±0) on acetone extract of Aristolochia indica.

The chloroform solvent of Aristolochia tagala leaf extract showed the highest inhibition zone against tested bacterial and fungal such as Staphylococcus lentus (24.2 ± 0.1), Bacillus cereus (22.3 ± 0.5). The low degree zone of inhibition against Serratia marcescens (12.3 ± 0.8) and Candida albicans (7.6 ± 0.3) no result found in chloroform leaf extract against Candida dubliniesis and Cryptococcus neoformans.

The ethanol solvent of Aristolochia tagala leaf extract showed the highest inhibition zone against tested bacterial and fungal such as Bacillus cereus (25.6±0.3), Staphylococcus lentus (21.6 ± 0.7). The low degree zone of inhibition against Serratia marcescens (11.6±0.5), Cryptococcus neoformans (10.6±0.3). Candida albicans (9.6±0.9) and Candida dubliniesis (8.3±0.3)

The methanol solvent of Aristolochia tagala leaf extract showed the highest inhibition zone against tested bacterial and fungal such as Bacillus cereus (25±0.3), Staphylococcus lentus (23 ± 0.9). The low degree zone of inhibition against Serratia marcescens (16.6±0.5), Candida dubliniesis (11.2 ± 0.9) Candida albicans (10.5±0.3) and Cryptococcus neoformans (10.3±0.3) the previous reported that methanol extract of Croton macrostachyus stem bark induced antibacterial activity against K. pneumonia, E. coli, C. albicans and E. aerogenes with the zone of

Table – 1 Antibacterial screening of Leaf extracts of *Aristolochia tagala* cham on pathogenic bacteria and fungi (Disc diffusion method)

| Inhibition zone diameter in mm (mean ± SD) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tested microorganism | Acetone | | Chloroform | | Ethanol | | Methanol | | Aqueous | | Positive Control |
| | Experimental 30 µg/disc | Negative control | Experimental 30 µg/disc | Negative control | Experimental 30 µg/disc | Negative control | Experimental 30 µg/disc | Negative control | Experimental 30 µg/disc | Negative control | Chloramphenicol Fluconazole col 30 mcg/disc |
| Gram-positive bacteria | | | | | | | | | | | |
| *Staphylococcus lentus* | 30.6 ± 0.4 | - | 24 ± 0.1 | - | 23 ± 0.9 | - | 21.6 ± 0.7 | - | - | - | 28±0 |
| *Bacillus cereus* | 26.3±0.5 | - | 22.3±0.5 | - | 25±0.3 | - | 25.6±0.3 | - | - | - | 27±0 |
| Gram-negative bacteria | | | | | | | | | | | |
| *Serratia marcescens* | 8±0 | - | 12.3±0.8 | - | 16.6±0.5 | - | 11.6±0.5 | - | - | - | 25±0 |
| Fungi | | | | | | | | | | | |
| *Candida albicans* | 7.6±0.3 | - | 7.6±0.3 | - | 10.5±0.3 | - | 9.6±0.9 | - | - | - | 15.6±0.3 |
| *Candida dubliniensis* | 7±0.2 | - | - | - | 11±0.9 | - | 8.3±0.3 | - | - | - | 15.3±0.3 |
| *Cryptococcus neoformans* | - | - | - | - | 10.3±0.3 | - | 10.6±0.3 | - | - | - | 24±0.3 |

'–' represents as 'no inhibition'

inhibition between 9.0 ±1.1 mm and 14.9 ± 1.3 mm [Obey, et al., 2016]. No result found in aqueous leaf extract of Aristolachia tagala against selected tested bacterial pathogens include both gram positive and gram negative and fungal pathogens (Table-1)

## CONCLUSION

Aristolachia tagala has been used in Ayurved, unani and siddha system of medicine. The various solvent based extract of A. tagala against selected human pathogens. From the above studies it can be concluded that the antimicrobial potential of the selected plant leaf extract would be beneficial in caring several kinds of disorders.

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