EVALUATION OF ARISTOLOCHIA BRACTEOLATA LINN. FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

The antibacterial activities of the various organic solvent extracts of Aristolochia bracteolata leaf were assayed in vitro by the Disc diffusion method against ten opportunistic bacterial pathogens such as Klebsiella pneumonia, Escherichia coli, Staphylococcus haemolyticus, Klebsiella pneumoniae DSM, Staphylococcus hominis, Staphylococcus haemolyticus, Enterobacter faecalis, Staphylococcus saprophyticus, Enterobacter faecalis Pseudomonas lutea. The zones of inhibition were compared with standard antibiotics. Among various solvent extracts tested ethanol leaf extracts exhibited high degree of inhibition followed by ethyl acetate and petroleum ether solvent extracts. The chloroform and acetone extracts did not show any antibacterial activities. The ethanol extract exhibited stronger antibacterial activity against the test bacteria. The zones of inhibition were higher in Staphylococcus hominis and Pseudomonas lutea. The chloroform and acetone extracts did not show any antibacterial activities.

KEYWORDS: Antibacterial activity, high degree of Inhibition, Aristolochia bracteolate.

INTRODUCTION

Microbial resistance to antibiotics represents a serious problem for human beings since most of the rampant killer diseases especially opportunistic infections in HIV/AIDS patients are of microbial origin and account for high proportion of mortality in underdeveloped as well as developed countries (Gundidza and Gaza, 1993; Jones, 1998; Guilletmot, 1999). The failure of these time honored antibiotics has rendered and necessities to search for have antimicrobial substances from various sources. Thus, antimicrobial research is geared towards the discovery and development of novel antibacterial agents (Alonso et al., 1995).
Traditional medicine is an important source of potentially useful new compounds for the development of antimicrobial agents. The first step towards this goal is the screening of plants used in popular medicine. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. 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 action (Amani et al., 1998; Barbour et al., 2004). The world health organization (WHO) also reports that 80% of world’s population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts.

*Aristolochia bracteolata* one of the important medicinal plants in Indian traditional system of medicine which is belonging to the family Aristolochiaceae. It is mainly uses to treat 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 gonorrhea, boils, ulcers and other skin infections (Sankaranarayanan, et al., 2010). The whole plant was used as anthelmentic, anti pyretic and anti inflammatory agents. The plant contain Aristolochic acid has many medicinal properties in various disease conditions (Kirtikar and Basu) Root extract was reported to have antibacterial activity (Dirdiri et al., 1987). The selection of the medicinal plant is based on its uses in Siddha medicine for the treatment of microbial infections. Hence, The present investigation was undertaken to investigate the antibacterial activity of various solvent xtracts of leaves of the medicinal plant *Aristolochia bracteolata* against some clinical associated pathogens.

**MATERIALS AND METHODS**

**Plant Collection**

The plant materials were collected around Thalur village of Pachamalai Hills, part of Eastern ghats of Tamil Nadu located in Tiruchirappalli District and were authenticated by Dr.S.John Britto, Director, Rapinat Herbarium, St.Joseph’s College, Trichy The leaves were separated from stems, washed in clean water, and dried at room temperature (Eloff, 1998). The dried plants were milled to a fine powder in an Electronic Blender and stored in the dark at room temperature in closed containers until required.

**Solvent extraction**

50 grams of the dried powdered plant materials (leaves) were soaked separately with 300 ml of each of the solvents *viz*. ethanol, ethyl acetate, chloroform, petroleum ether and acetone in
a soxhlet apparatus for 48 hr at 310°C until complete extraction of the materials. At the end of 48 hr each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature in order to reduce the volume. The paste like extracts were stored in pre-weighed screw capped bottles and the yield of extracts has been weighed. These screw capped bottles were kept.

**Test bacteria**

Bacterial pathogens tested in this study were isolated from clinical samples of suspected symptomatic HIV/AIDS patients. More than ten bacterial pathogens were isolated and confirmed by staining, morphological and biochemical characteristics. Among them, ten bacterial pathogens **viz.** *Klebsiella pneumonia, Escherichia coli, Staphylococcus haemolyticus, Klebsiella pneumonieae DSM, Staphylococcus hominis, Staphylococcus haemolyticus, Enterobacter faecalis, Staphylococcus saprophyticus, Enterobacter faecalis Pseudomonas lutea* were selected for antimicrobial screening test.

**Maintenance of bacterial culture**

The bacterial pathogens were inoculated on Nutrient agar slants and incubated overnight at 37°C. These cultures were stored in a refrigerator at 4°C. Fresh slant cultures were prepared every 2-3 weeks until tested for further antibacterial studies.

**Antibacterial assay**

Antibacterial activity of the above mentioned four different solvent and aqueous extracts were assayed separately using disc diffusion method (Bauer *et al.*, 1966). Petri plates containing 10 ml of Muller Hinton Agar medium were inoculated with 108 CFC/ml of each test bacteria. Sterile filter paper discs (6 mm in diameter) were impregnated with 10μl of the 3 mg/ml plant extracts (30μg/disc) placed on the surface of the medium. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. A standard disc containing chloramphenicol antibiotic drug (30μg/disc) was used as a positive control and they were incubated for 24 h. The assessment of antibacterial activity was based on the measurement of diameter of inhibition zone formed around the disc. Three independent trials were conducted.

**RESULT AND DISCUSSION**

The antibacterial activities of the various organic solvent extracts of *Aristolochia bracteolata* were assayed *in vitro* by the Disc diffusion method against ten opportunistic bacterial
pathogens. The in vitro results were observed in terms of inhibition zone around each disc caused by diffusion of antibacterial properties from the plant extract impregnated disc into the surrounding medium. As can be seen from Tables 1, among various solvent extracts tested ethanol leaf extracts exhibited high degree of inhibition followed by ethyl acetate and petroleum ether solvent extracts. The chloroform and acetone extracts did not show any antibacterial activities. In addition, the inhibition zones formed by standard antibiotic disc (chloramphenicol 30 mcg/disc) and those filter paper discs injected with respective solvents are also listed in Table 1. The diameter of inhibition zones for plant extracts were also compared with standard antibiotic. It was noted that the inhibition zones of the samples to be either less than or greater than or equal to the inhibition zones of standard antibiotics. The diameter of inhibition zones were noted in the leaf extracts (Table 1), the ethanol extract showed significant antibacterial activity against the test bacteria. The zones of inhibition were higher in Staphylococcus hominis (9.8±0.94) and Pseudomonas lutea (18 ±2.16). Moderate level of inhibition was observed against Klebsiella pneumoniae (13±2.94), Staphylococcus haemolyticus (11.6±3.09) and Enterobacter faecalis (9.0 ±0.81) followed by ethylacetate extracts were exhibited moderate inhibition on Staphylococcs saprophyticus(9±0.81) Staphylococcs hominis(8.66±1.24) and Klebsiella pneumoniae(8.0±4.1).

The ethanol extracts of Aristolochia bracteolate were effectively inhibited the growth of both gram-positive and gram-negative bacteria. Similar results were also drawn by several workers (Rabe and Vanstaden, 2000; Ates and Erdogral, 2003; Bouhadjera et al., 2005) whereby majority of the significant antibacterial activity was observed in the ethanol extracts. The other solvent extracts showed satisfactory results whereas the aqueous extracts of the plant showed nil activity. This is because most of the antibacterial principles are extracted much through the organic solvents (Chakrabarty and Brantner, 1999; Aburjai et al., 2001). In conclusion Aristolochia bracteolata crude extracts posses a broad spectrum of activity against a panel of bacterial strains responsible for opportunistic bacterial infections. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

ACKNOWLEDGEMENT

Mrs. Freeda Rose expresses her gratitude to the Management of Holy Cross College for their constant encouragement to complete this research work.
Table 1: Antibacterial activity of leaf extracts of *Aristolochia bracteolata* on pathogenic bacteria (Disc diffusion method).

<table>
<thead>
<tr>
<th>Test bacteria</th>
<th>Petroleum ether Experimental (30 mg/disc)</th>
<th>Petroleum ether Negative control</th>
<th>Chloroform Experimental (30 mg/disc)</th>
<th>Chloroform Negative control</th>
<th>Acetone Experimental (30 mg/disc)</th>
<th>Acetone Negative control</th>
<th>Ethanol Experimental (30 mg/disc)</th>
<th>Ethanol Negative control</th>
<th>Positive Control Chloramphenicol (30 mcg/disc)</th>
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<tbody>
<tr>
<td>Gram-positive</td>
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<td><em>S. haemolyticus</em></td>
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<td>-</td>
<td>-</td>
<td>11.6±3.09</td>
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<td>16±0.0</td>
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<td><em>S. hominis</em></td>
<td>4.6±3.29</td>
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<td>-</td>
<td>-</td>
<td>8.66±1.24</td>
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<td>19.8±0.94</td>
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<td><em>S. haemolyticus DSM</em></td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>8.66±1.24</td>
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<tr>
<td><em>S. saprophyticus</em></td>
<td>4.6±3.29</td>
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<td>9±0.81</td>
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<td><em>E. faecalis</em></td>
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<td>9.0±0.81</td>
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<td><em>P. lutea</em></td>
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