ANTIMICROBIAL ACTIVITY OF AILANTHUS EXCELSA ROXB. COLLECTED FROM COIMBATORE DISTRICT, TAMIL NADU, INDIA

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ABSTRACT
Antimicrobial activity of leaf extract of Ailanthus excelsa Roxb. was studied using different solvents chloroform, ethanol, methanol and water against clinical isolates of Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and fungal strains of Aspergillus niger, Penicillium chrysogenum and Aspergillus fumigatus. The antimicrobial activity was determined by disc diffusion method. The crude plant extracts demonstrated broad spectrum activity against all the pathogens. The highest inhibitory zone was observed in ethanolic extract of Ailanthus excelsa. The antibacterial activity could be confirmed in most species used in traditional medicine in South India. Nevertheless, traditional knowledge might provide some leads to elucidate potential candidates. The present study will be successful in identifying candidate plant with different antimicrobial activity which could be further exploited for isolation and characterization of the novel phytochemicals in the treatment of infectious disease especially in light of the emergence of drug resistant microorganisms and the need to produce more effective antimicrobial agents.

Keyword: Ailanthus excelsa Roxb, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus fumigatus, Penicillium chrysogenum.

INTRODUCTION
Medicinal plants are important source for their therapeutic remedies in various ailments. Scientific experiments on the antimicrobial properties of plant components were first
documented in the late 19th century [1]. India is known for its rich diversity of medicinal plants. Nearly 70 percent of the world population is dependant on the traditional medicines for primary health care [2]. The knowledge of medicinal plants has been accumulated during the course of centuries based on different medicinal systems such as Ayurvedha, Unani and Siddha [3]. In India traditional healers used 2500 plants species and of these 100 species of plants served as regular sources of medicine [4]. Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever increasing therapeutic problem. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [5]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [6]. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents Systematic screening of them may result in the discovery of novel active compounds.

Traditional medicines are used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system [7]. The herbal medicines serve the health needs of about 80% of the world’s population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care modality [8].

In our research for new antibacterial substances from plants, in particular from Simaroubaceae family, A. excelsa Roxb is a tree of rapid growth and is called tree of heaven, leaves appear in March-April, 30-90 cm long, pinnate, the flowers, small in size, yellow in color and arranged in panicles and the fruits are formed soon after flowering [9]. The fruits ripen in May-June, just before the onset of monsoon A. excelsa is used in treatment of skin eruption and for the cure of wounds, The bark is bitter, astringent, antihelmenthic, febrifuge, appetizer, bitter tonic, taste bud stimulant, useful in treating diarrhea, amoebic dysentery, chronic giardiasis, dyspepsia, abdominal spasm anoarectal disease, haemorrhoids, fistula, fissures, ulcerative colitis as mentioned in traditional medicine [10].
MATERIAL AND METHODS

Collection and processing of Ailanthus excelsa
The plant materials were collected from Coimbatore District. The collected plants were identified and authenticated by Botanical survey of India, Coimbatore, Tamil Nadu, and India. A voucher specimen was deposited in the Department of Microbiology. PSG College of Arts and Science, Coimbatore. Fresh leaves and stem were washed thoroughly in running tap water and dried under shade. They were then finely ground in an electric blender.

Extraction of active components
About 10g of shade dried powder of plant materials was filled separately in the thimble and extracted successively with 100 ml each of chloroform, ethanol, methanol and water using Automatic Soxhlet solvent extractor for 2 hrs. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extracts was weighed and preserved at 4°C in airtight bottles until further use.

Microorganisms
The bacterial strains of Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa and the fungal strains of Aspergillus niger, Penicillium chrysogenum and Aspergillus fumigatus were obtained as clinical isolates from Coimbatore Medical College, Coimbatore, Tamil Nadu, India. The Stock cultures of bacteria were maintained at 4°C on slopes of nutrient agar and fungus was maintained on Potato dextrose agar (PDA) at 28°C.

Determination of antibacterial activity
The antibacterial activity was tested against crude ethanol, methanol, chloroform and aqueous extracts of Ailanthus excelsa by disc diffusion method. Muller Hinton agar medium was prepared and the inoculum suspensions (equivalent to 0.5 Mcfarland standard) of respective bacteria were spread uniformly over the agar plates using spreader, for uniform distribution of bacteria. Whatmann No.1 filter paper was cut into small discs of diameter 3-6 mm in size and autoclaved. Each sterile disc was loaded with 10µl (concentration) of test extract and placed on the agar plates inoculated with respective microorganisms. Later the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 h at 37°C temperature. Chloramphenicol (10 mg/ml) was used as positive control. The results were recorded by measuring the diameter of inhibition zone at the end of 24-72 h. Zone of inhibition surrounding the discs was measured using a transparent ruler and the diameter was recorded in mm.
Determination of antifungal activity

The antifungal activity was tested against crude ethanol, methanol, chloroform and aqueous extracts of *Ailanthus excelsa* by well diffusion method. Antifungal assay was carried out with fungal cultures such as *Aspergillus niger*, *Penicillium chrysogenum* and *Aspergillus fumigatus*. The test fungal cultures were grown on Potato dextrose agar plate at 28°C and maintained with periodic sub culturing and storing at 4°C. The fungal spore suspension were prepared by flooding the surface of a two-week-old culture of the individual test fungi with 10 mL of sterile phosphate buffered saline, (PBS; 10 mM phosphate buffer, 2.7 mM potassium chloride, 137 mM sodium chloride, pH 7.4). The spore suspensions were stored at 4°C for further use.

Potato dextrose agar medium was poured into a petridish, allowed for solidification and after that it was seeded uniformly with fungal spore suspension of different fungal cultures. The appropriate wells were made on agar plate by using well cutter and loaded with plant extracts and antifungal agent Fluconazole was also loaded in the center well of the agar plate that served as a control. Incubation period of 24-28 hours at 28°C was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm.

RESULTS

Antibacterial activity

Results of inhibition zones in the disc diffusion assay using crude ethanol, methanol, chloroform and aqueous extracts of *Ailanthus excelsa* showed significant zone of inhibition (ZOI) against tested bacterial organisms as compared to the standard antibiotic, chloramphenicol (10 mg/ml). Among the different solvent extracts studied methanol and ethanol showed a high degree of inhibition, followed by chloroform and aqueous extract. The methanol extracts showed 18 mm diameter zone of inhibition against *S.aureus*. This was followed by 17 and 15 mm zone of inhibition against *Pseudomonas aeruginosa* and *Bacillus Subtilis* respectively. The ethanol extract showed 16 mm diameter zone of inhibition against *Bacillus Subtilis* and *Staphylococcus aureus*. This was followed by 15 mm zone of inhibition against *Pseudomonas aeruginosa*. Chloroform extract showed moderate antibacterial activity.
against all the tested bacteria (12 – 15 mm). The aqueous extract showed minimum activity against all tested bacteria (8 – 10 mm).

Table 2 Antibacterial Activity of Ailanthus excelsa

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Plant part</th>
<th>Solvent used</th>
<th>Antibacterial activity (Zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ailanthus excelsa</td>
<td>-</td>
<td>Ethanol</td>
<td>Bc 16  Sa 16  Pa 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>Bc 15  Sa 18  Pa 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td>Bc 13  Sa 12  Pa 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>Bc 10  Sa 08  Pa 10</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>Chloromphenicol</td>
<td>Bc 22  Sa 18  Pa 19</td>
</tr>
</tbody>
</table>

Bc: Bacillus cereus; Sa: Staphylococcus aureus; Pa: Pseudomonas aeruginosa;

Antifungal activity

The antifungal activity was also studied by well diffusion method using crude ethanol, methanol, chloroform and aqueous extracts of Ailanthus excelsa using fungal strains, Aspergillus niger, Penicillium chrysogenum and Aspergillus fumigatus. In the present study, maximum antifungal activity was observed for the aqueous extract against Aspergillus niger (16mm), Penicillium chrysogenum (15mm) and Aspergillus fumigatus (14 mm). Whereas, chloroform and the alcoholic extracts showed very low zone of inhibition as compared to aqueous extract.

Table 3 Antifungal Activity of Ailanthus excelsa

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Plant part</th>
<th>Solvent used</th>
<th>Antifungal activity (Zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ailanthus excelsa</td>
<td>-</td>
<td>Ethanol</td>
<td>An 08  Pc 11  Af 09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>An 06  Pc 08  Af 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td>An 07  Pc 09  Af 05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>An 16  Pc 15  Af 14</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>Fluconazole</td>
<td>An 18  Pc 22  Af 18</td>
</tr>
</tbody>
</table>

An: Aspergillus niger; Pc: Penicillium chrysogenum; Af: Aspergillus fumigatus

DISCUSSION

Medicinal herbs possess curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more parts of these plants. There is continuous and urgent need for discovery of new
antimicrobial compounds with diverse chemical structures and novel mechanisms of action because of alarming increase in the incidence of new and re-emerging infectious diseases\cite{11}.

The presence of these biologically active compounds in the extracts has made the plant to be known of its medicinal use especially for antimicrobial activity against pathogenic organisms. Tannin has been reported to interfere with bacterial cell protein synthesis and is important in the treatment of ulcerated or inflamed tissues and also in the treatment of intestinal disorders\cite{13}. Alkaloid has also been reported to be a pain killer and saponin has managing effect against inflammation; Flavonoid is also important against inflammation and microorganisms \cite{12}. A Novel Triterpenoid Isolated from the Root Bark of Ailanthus excelsa Roxb. (Tree of Heaven), AECHL-1 as a Potential Anti-Cancer Agent\cite{13}. Few drugs of plant origin have been screened for antifertility but with only limited efficacy, whereas Ailanthus excelsa would be worth while in serving as a tool, in birth control. The extract and purified fractions of A. excelsa were strong plant growth inhibitors, therefore could be considered as potent, effective and environmentally safe agricultural pesticides \cite{14}. The Ailanthus excelsa. Pollen also contains allergenic proteins which causes for various respiratory diseases. So, the collection of plant material in specific time is necessary for the maximum rational use and utilization of plant. At last it is not wrong to say that this plant is really a plant of heaven which is due to wide scope in the treatment of serious and chronic diseases \cite{15}.

**CONCLUSION**

The inhibitory effect of the extract of Ailanthus excelsa against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by human pathogens. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum antimicrobial potential of various parts of Ailanthus excelsa, which makes the complete plant a candidate for bioprospecting for antibiotic and antifungal drugs.

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REFERENCE


