ABSTRACT

In the present work an attempt has been made to carry out screening for the preliminary antibacterial activity of plants used in Indian folk medicine. The aim of the study was to select an active plant extract which may be useful in developing new lead compounds to combat deadly diseases. The antibacterial activity was done by agar diffusion method against four bacterial strains, viz., gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. The plant *Plumbago zeylanica* leaves was extracted with hydro alcohol by hot decoction method, were active against gram-positive bacteria than gram-negative bacteria and the experiment revealed the potency of the hydroalcohol extract.

Keywords Antimicrobial, *Plumbago zeylanica*, minimum inhibitory concentration, hydroalcoholic extract.
INTRODUCTION
Microbial infections pose health problems throughout the world with the alarming increase in the rate of infection by antibiotic resistant microorganisms. The increasing resistance of most synthetically derived antimicrobial agents is of utmost concern. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (1). Medicinal plants, which are the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies (2). In recent years, there has been renewed interest in the treatment against different diseases as herbal drugs are generally known to be non-toxic (3). The WHO has also recommended the evaluation of the effectiveness of plants were in condition we lack safe modern drugs (4). Evaluation of plant products for pharmacological and medicinal effects is of growing interest as they contain many bioactive substances which have therapeutics potential. Evaluation of antibacterial medicinal plants is essential because phytotherapy is cheaper and locally available. There are increasing research efforts to develop herbal formulations to treat various diseases and there is a continuing need to develop herbal formulations used for treatment effectively with minimal or no side effects (5).

Since ancient times these medicinal plants play an important role in developing of newer drugs even when they are biologically active compounds are unknown, because of their effectiveness, less side effects and relatively low cost when compared to synthetic drugs. Today, Ayurveda, Homoeopathy, and Unani physicians utilize numerous species of medicinal plants that found their way a long time ago into the Hindu Materia Medica (6).

*Plumbago zeylanica* Linn (Plumbaginaceae) is a perennial herb commonly distributed in forest of the Uttarakhand, India, and cultivated in the gardens throughout India. The plant is commonly known as Ceylon leadwort (English), Chita, Chitra (Hindi) and Chitramoolam (Tamil). The root is used as laxative, expectorant, astringent, abortifacient, and in dysentery. Tincture of root bark is used as antiperiodic. The leaves are used as aphrodisiac and in scabies. Earlier chemical examination of this plant revealed that the root contains plumbagin, 3-chloroplumbagin, 2,3-biplumbagin, 6,6-biplumbagin, zeylinone, isozyelinone, chitranone, droserone, plumbagic acid, plumbazeylanone, glucose, fructose, enzymes as protease and invertase. The leaves and stem contains little or no plumbagin. The aerial parts contain naphthoquinones, sitosterol, lupeol, luponyleacetate, hentriacontane, and amino acids (7-12).
Therefore, aim of this study was to select an active plant extract which have not been screened so far and which are useful in developing new lead compounds to combat deadly diseases and so, the antimicrobial activities of *Plumbago zeylanica* leaves hydroalcoholic extract was planned to evaluate used in Indian folk medicine.

**MATERIAL AND METHODS**

**Collection of Plant Material**

Around 5kg of fresh leaves *Plumbago zeylanica* were collected during the month of November 2010 from S.V.U. Botanical Garden and authenticated by Dr. K. Madhava chetty, M.Sc., M.Ed., M.Phil., Ph.D., PG., DPD. Assistant Prof., Department of Botany, S.V.U. Thirupathi-517502, Andhra Pradesh, India. A voucher specimen (No.PCP/PCOG/14) was deposited at the Department of Pharmacognosy. The leaves were washed thoroughly with running tap water, shade dried under normal environmental temperature for 15-20 days, and pulverized to get a coarse powder and stored in an air tight container and used for studies.

**Preparation of extract**

The powdered leaves of 500mg were taken in 1 liter round bottom flask, extracted with 50% ethanol by hot decoction method for about 4hours using reflex condenser. The extract were cooled at room temperature, evaporated to dryness under reduced pressure in a hot air oven (Thermo Lab Standard 325Lt) and stored in a desicador (13).

**BACTERIAL STRAINS**

Four different bacterial strains were used in antibacterial sensitivity test. The gram positive bacteria, *Bacillus subtilis* (MTCC 7086), *Staphylococcus aureus* (MTCC 7443), gram negative bacteria *Escherichia coli* (MTCC 41) and *Pseudomonas aeruginosa* (MTCC 424) procured by microbial type culture collected from KMCH college of Pharmacy, Coimbatore, Tamilnadu, India, were used during the investigation.

**Preparation of Microorganisms (14,15)**

The cultures of the selected bacteria’s were checked for purity by doing gram staining and biochemical test and they were grown in nutrient broth at 37±1°C and maintained in nutrient agar slants at 2-8°C. Nutrient agar medium was used as bacterial culture medium in the antibacterial assays.
Preparation of inoculums (14,15)

Bacterial strains preserved in nutrient agar at 4°C were revived in nutrient broth (liquid medium) and incubated at 37±1°C overnight, and the suspensions were checked to provide ~10^5 cfu/ml.

Procedure for performing Agar Diffusion test (14,15)

The antimicrobial evaluation was carried out using the agar diffusion method. An isolated colony of each organism was transferred into an appropriate nutrient agar and propagated consecutively for 72 hours. Ten-fold serial dilutions of 72 hour-Broth cultures were made. The inoculums (1 ml of each of the microorganism culture) was added into 20 ml of molten nutrient agar and properly mixed. These were poured into sterile petri dishes and were allowed to set for 10 minutes. With the aid of dimethylsulphoxide (DMSO), standard aqueous solutions of the extract were prepared. The extract was tested in triplicate (3 discs/plate) and the plates were inoculated at 37±1°C for 48 h and average results were recorded. Serial dilutions of each of the standard solution were prepared using a 8 mm-cork borer, holes were aseptically made on the agar plates and into the holes were added solutions of the extract. The solutions were allowed to diffuse for 30 minutes in the agar medium. Test (500 µg/ml), Ciprofloxacin (500 µg/ml) as positive and DMSO (500 µg/ml) as negative control for bacteria were used. The inoculated agar petri dishes were incubated at 37±1°C (bacteria) for 48 hours. The zones of inhibition (IZD) were measured as the difference of the diameter of the circle of inhibition and the diameter of the cork borer. The minimum inhibitory concentrations (MIC) were obtained at the end of incubation. The inhibition zones produced by the extract were compared with the inhibition zones produced by a commercial antibiotic Ciprofloxacin.

Statistical Analysis

The experiments were carried out using a completely randomized design. Calculations were carried out in triplicate with their mean values and standard deviations by the formula given by Gupta (1977). Differences were considered significant at p<0.05. The statistical analyses employed SPSS ver. 13.

RESULTS AND DISCUSSION

The antibacterial potential measured in terms of zone of inhibition exhibited by the Plumbago zeylanica extract against the respective bacterial strains Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa are given in the table 1.
TABLE 1 ANTIMICROBIAL SENSITIVITY ASSAY OF CRUDE EXTRACT

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Zone of inhibition in (mm)*</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plumbago zeylanica (500µg/ml)</td>
<td>Standard (20µg/ml) Positive</td>
</tr>
<tr>
<td>Bacillus subtilis (MTCC 7086)</td>
<td>1.5±0.61*</td>
<td>1.8±0.35</td>
</tr>
<tr>
<td>Staphylacoccus aureus (MTCC 744)</td>
<td>1.2±0.45**</td>
<td>0.6±0.68</td>
</tr>
<tr>
<td>Escherichia coli (MTCC 41)</td>
<td>0.9±0.36*</td>
<td>1.2±0.83</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (MTCC 424)</td>
<td>1.6±0.54*</td>
<td>2.0±0.32</td>
</tr>
</tbody>
</table>

Each value represented mean±SE *, Activity significantly lower than standard antibiotic (p<0.05) **, Activity significantly higher than standard antibiotic (p<0.05).

Fig 1 Graph showing the minimal inhibitory concentration of Plumbago zeylanica, standard and DMSO against bacterial strains like Bacillus Subtilis, Staphylococcus Aureus, Escherichia Coli and Pseudomonas Aeruginosa.

The extract showed activity against all the tested bacteria. The maximum zone of inhibition was determined against Staphylococcus aureus. Very less zone of inhibition was against Pseudomonas aeruginosa, Escherichia coli and Bacillus subtilis.

The extract exhibited zone of inhibition ranged from 1.6-0.9mm in diameter with 500µg/ml sample and 2.0-0.6mm in diameter with 20µg/ml concentration against the test bacteria’s. The highest zone of inhibition (1.6mm) was recorded when compared to the standard (0.6mm). The extract exhibited good antimicrobial activities against the test organisms among the test bacteria, Staphylococcus aureus was found to be the most sensitive to the extract showing the highest diameter zone of inhibition of 1.2mm. The extract was also very...
effective against *Bacillus subtilis* showing zone of inhibition of 1.5mm. The extract at a concentration 500µg/ml showed larger zone of inhibition as compared to that formed by the standard Ciprofloxacin 20µg/ml.

The above results can be justified by the literature which reveals that the chemical content might be responsible for the activity. The antimicrobial agents of the plant *Plumbago zeylanica* are more polar than most of the antifungal principles. The anti-microbial properties of the plants may be attributed due to the secondary metabolites present in them. Phytococonstituents like phenolics, tannins and alkaloids is found to be effective anti-microbial substance against a wide range of micro organisms.

According to Rajani Chauhan *et al.*, The antimicrobial effect of *Plumbago zeylanica* Linn. leaf extract was evaluated on microbial strains like gram positive species *Staphylococcus aureus*, and *Bacillus subtilis* and gram negative species *Escherichia coli* and *Pseudomonas aeruginosa*. The solvent used for extraction of plant were petroleum ether, chloroform and alcohol. The alcoholic extract of leaves of *Plumbago zeylanica* shows maximum antimicrobial activity. The significant antibacterial activity of active extract was compared with standard antibiotic Amphiocillin. The antibacterial activities of the leaves were due to the presence of various secondary metabolites. The ethanolic extracts of plant leaves showed significant activity against bacterial and fungal pathogen and results were unpored with standard antibiotics such as *Ampicillin, Penicillin Streptomycin Griscofulenin, anpolenicin and flunonazole* (9).

The maximum antibacterial activity was observed in methanol extract against the *Staphylococcus aureus* (16 mm), *Bacillus substilis* (14mm) and minimum in ethanol (10 mm and 12mm respectively). The result suggests that methanol and ethanol extract shows moderate antibacterial activity (16).

The antimicrobial activity of methanol and chloroform extract showed positive results against the entire organism. The methanol extract inhibit *Streptococcus aureus, Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* at 10 µl concentration indicated by the zone of inhibition around the disc in the culture plate except that the plate containing *Pseudomonas aeruginosa*. But in 20 µl concentration the plant extract showed positive result against all the five tested samples. Chloroform leaf extract showed antibacterial activity against
Streptococcus aureus, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli. Its inhibition was moderate and lower.

The results show that the methanol extract of Plumbago zeylanica showed more inhibitory effect than the other plant extracts. This tends to show that the active ingredients of the plant parts are better extracted with methanol than Chloroform. The methanol extracts contain alkaloids, coumarins and tannins. Coumarins and tannins have antibacterial properties found that methanol was more efficient than of chloroform in extracting phytochemicals from plant materials.

The results of the present study reveals the fact that the organic solvent extracts (chloroform and methanolic extracts) exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium. The present study justifies the claimed uses of Plumbago zeylanica leave in the traditional system of medicine to treat various infections diseases caused by the microorganisms (17).

The ethanol, ethyl acetate and acetone extracts of Plumbago zeylanica have the highest inhibitory effects against Helicobactor pylori using the agar diffusion and dilution methods at the pH 1-7, having synergistic and action against Mycobacterium intracellulare, M. smegmatis, M. xenopei and M. Chelonei (18).

CONCLUSION
Plumbago zeylanica has broad spectrum of antimicrobial activities. The plant was found to possess antimicrobial activity, which was statistically significantly better than that of Ciprofloxacin. The results of the present study clearly reveal that the plant crude extract showed good antimicrobial activity and it is suggested that if the active compounds which posses antimicrobial activity if been isolated from these extract, more potency herbal drugs when compared to standard allopathic drugs can be obtained. Indian systems of medicine such as Ayurveda and Siddha uses majority of the crude drugs that are of plant origin. It is necessary that standards have to be laid down to control and check the identity of the plant an ascertain its quality before use. A detailed pharmacognostic evaluation therefore is highly essential prerequisite (19).
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REFERENCES


