EVALUATION OF ANTIMICROBIAL ACTIVITY OF LEAF AND STEM EXTRACTS OF SIDDHA MEDICINAL PLANT SIDA CORDATA

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ABSTRACT

Successive solvent extract viz., petroleum ether, chloroform, ethyl acetate, ethanol and water extracts of leaf and stem of \textit{Sida cordata} was evaluated for antimicrobial activity, against bacterial pathogens E.coli, S.aureus, Pseudomonas aeruginosa and Lactobacillus and two fungal strains Candida albicans, and Aspergillus niger by agar-well diffusion/broth dilution / Resazurin microtiter method. MIC values for the aqueous extract of leaf varied from 0.187-1.5mg/ml for bacterial isolates, comparatively the ethanol leaf extract showed higher MIC values (0.375-3mg/ml) for all the tested bacteria. Chloroform, Ethyl acetate and Petroleum ether extracts did not show any significant activity. Similarly the antibacterial activity in the different extracts of the stem was comparatively less significant. Increased zone of inhibition was observed with increasing aqueous leaf extract, highest inhibition was with the highest concentration against S.aureus, E.coli and Lactobacillus compared to the inhibition effects on these organisms by Ampicillin used as control, indicating that the active principle responsible for antibacterial activity is more soluble in water. The extracts had no inhibitory effect on fungal isolates. The above findings suggest that \textit{Sida cordata} is scientifically validate the use of this plant in the Sidda medicine. Further isolation and characterization of the active principle responsible for the antibacterial activity may be an alternate source for antibiotics.

KEYWORDS: \textit{Sida cordata}, Antimicrobial Activity, Solvent Extracts, Resazurin Microtiter Dilution Assay

INTRODUCTION

Traditional medicine is one of the oldest method of curing diseases and infections, by using various plants. A huge percentage of world’s population partially or entirely still depends on botanicals to treat human diseases and infections (Caceres et al, 1991) Whole plant and different parts of plant are used to treat various forms of diseases and infections. The use of plants whether herbs, shrubs or trees in parts or in whole for the treatment and management of diseases and disorders date back to pre-historic days (Gahlaut et al, 2012). Plant extracts have been used in folk medical practices for the treatment of various ailments since antiquity [Koppula S et al, 2010]. The medicinal properties of various plant material and extracts have been recognized since the beginning of the 5\textsuperscript{th} century.

In the era of modernization and changed environmental conditions, man frequently encounters pathogenic microorganisms causing infectious diseases. The indiscriminate use of commercially available antibiotics for the treatment of infectious diseases will results in multiple drug resistance in the microorganisms, putting new challenge before the drug industries for identification of new efficient antimicrobial compounds. Herbal drugs therapy is regarded as an important alternate, leading the researchers to focus and evaluate the traditionally recommended medicinal plants for their efficacy in various diseases (Clark AM, 1996). As reported by World Health Organization (WHO), traditional medicinal plants are the best reservoirs to develop newer pharmaceuticals (Fransworth NR et al 1985). Medicinal plants are renewable sources therefore farmers get encouraged to include them in traditional agriculture (Okanala et al 1990] Medicinal plants are
known to owe their curative potentials to certain biological active substances, which exist in parts of the plants. The substances which are referred to as active principles are phytochemical substances include terpenes, flavonoid, bioflavonoid, benzophenones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthraquinones. (Iwu et al 1999).

A rich storehouse of medicinal plants exists everywhere especially in India, which offers a vast reservoir of plants that, have been categorized (Joy.P.P 2001). Since the Vedic period in India major part of the total population still depends on the folk medicines obtained from the plant source (Srivatsa et al 1993). The ethnobotanical wealth of Indian subcontinent is one of the biggest sources of herb based with potential to be included in drug discovery programmes. Herbs and herbal medicines may substitute the conventional system of medicine in future due to lesser side effects and cost effectiveness. To ensure the commercial medicinal potential of plants depicted in Ayurveda, the antimicrobial potential need to be evaluated against present day pathogens according to new parameters to ensure there efficacy and reliability.

In the Indian medicine system the medicinal plant *Sida cordata* is in usage to cure various ailments, both the whole plant and various parts of this plant are used. Pedhi marandu is an herbal formulation used by herbal vendors of south India to treat dysentery, diarrhoea, cholera given orally for only one day (100g/day), in which *Sida cordata* is one of the components (Chitra vaidiu et al 2009) The whole plant material of *Sida cordata* is used in treatment of chronic liver disorders, 10-20 ml decoction of the whole plant is given daily for one week to relive from joint pain. The root paste along with cow’s butter is applied locally to cure piles and just root paste is applied on boils ‘k hateera’ to take out pus (Panthi.M.P et al 2009).

Our earlier phytochemical studies carried out on different solvent extracts of leaf and stem of *Sida cordata* has revealed the presence of primary metabolites like carbohydrates, amino acids, proteins etc and secondary metabolites like the alkaloids, flavonoids, tannin saponins, phenolics, terpenoids, glycosides, emodins, catechins, coumarins anthraquinones etc. (Gulnaz.A.R & Savitha.G 2013) Several reports are present on adaptogenic, hepato protective, and rejuvenation and anti ageing properties of *Sida cordata* (Gunasekaran et al 2013).Studies conducted on *Sida cordata* along with some other Indian medicinal plants have shown the antimicrobial property in the methanolic leaf extracts (Sudip Nag et al 2013).These studies give diminutive information on the antimicrobial property of the plant.

In the present study an attempt has been made to investigate the antimicrobial activity of different solvent extracts of leaf and stem extracts of *Sida cordata* by sequential extraction method against gram positive, gram negative and fungal strains, which are associated with human infections.

The plant *Sida cordata* (Burm.f.) belongs to the family Malvaceae, is distributed in India from sea level to high hills. It is a prostrate herb with medicinal value, stems rooting at the nodes commonly called as heartleaf, fan petals, has cordate leaves with auxiliary, solitary flowers, sometime the flowers are in pairs and are yellow in colour.

**MATERIALS AND METHODS**

**Plant Collection**

Fresh plant material leaves & stem of *Sida cordata* was collected from its natural habitat, from the forest region of Somawarpet in Madekeri district Karnataka. The plant was identified and authenticated at National Ayurveda Dietetics Research Institute Bangalore, (voucher no: RRCBI-11748). The collected fresh plant materials (leaves & stem) were washed in water, shade dried at room temperature and then homogenized to fine powder of 40 mesh sizes and stored in airtight bottles at 4°C.
Sample Preparation

About 100gm of each leaf & stem powder were subjected to extraction by a hot percolation method with 150ml of solvents in their increasing polarity (petroleum ether, chloroform, ethyl acetate, ethanol water respectively), in soxhlet apparatus. Each solvent extraction step was carried out for 24 hrs. After extraction, the extracts were concentrated by evaporation and stored at 4°C for further study. For each plant material, 1% stock solution was prepared with 0.1% Dimethyl sulphoxide solution. The extract was filtered using membrane filter. The extracts obtained were stored in a refrigerator at 4°C until required for use.

Antimicrobial Activity

Test Organisms

Human pathogenic organisms (bacteria and fungi) were isolated from clinical samples, are used in this study (Department of Microbiology, Farooqia dental college & hospital Mysore, India). The organisms used were* Staphylococcus aureus, Escherichia coli, Lactobacillus, Pseudomonas aeruginosa, Candida albicans and Aspergillum niger* and were identified and confirmed by clinical microbiologist.

Antimicrobial Activity by Agar Well Diffusion Method

The antimicrobial activity of the plant extracts were determined by agar well diffusion method (Barry 1980). Briefly, About 20 to 25ml of Muller Hinton agar for bacterial culture and Sabouraud dextrose agar for fungal culture (as a nutrient medium) was poured in the sterilized petridish and allowed to solidify. Wells of 6mm in diameter and 2.5-mm deep about 2cm apart were made on the surface of the solid medium using a sterile borer. The inoculums of microorganisms were adjusted to 0.5 McFarland standards, and with the help of sterile swab test organisms are inoculated in to the above petridishes.

The extracts were reconstituted by dissolving in Dimethyl sulphoxide (DMSO). Each well was filled with 0.5ml of test sample. Sterile DMSO was used as negative control, while Ampicillin & Nystatin were used as positive control for bacteria and fungi respectively. Then the plates were refrigerated for 30 min and incubated for 24 hours at 37°C for bacteria and at room temperature for fungi. After 24 hours, the plates were removed and zones of inhibition measured with Himedia antibiotic scale and the results were tabulated. Antimicrobial activities were evaluated by measuring inhibition zone diameters. Extracts with zones of inhibition greater or equal to 8-mm diameter were regarded as positive. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

Broth Dilution Assay

A dose dependent study on the inhibition of ethanol and aqueous extracts of leaf and stem of *Sida cordata* was determined using broth dilution assay.

The medium containing different concentrations of ethanol and aqueous extracts of leaf and stem of *Sida cordata* viz., 10, to 0.0195 mg/ml were prepared by serial dilution. After inoculation, the tubes were incubated for 24 hours at 37°C for bacteria and at room temperature for fungi after incubation period the tubes were read at 465nm using UV-visible Spectrophotometer (Hitachi U-200, Japan) against suitable controls.

The controls were only the broth, different concentrations of extracts without test organisms. The optical density for the test organism without any extracts was taken as 100% activity.

The absorbance values of respective test tubes with nutrient broth and different concentration of extracts were subtracted from the respective test samples. The values are the average of three experiments in triplicates.
MIC by 96 Well Resazurin Based Microtiter Dilution

Preparation of Resazurin Dye Solution (RDS)

Resazurin dye (300 mg) was dissolved in 40 ml sterile water. Vortex mixer was used to homogenize the solution. This solution was then referred as Resazurin dye solution. Resazurin is an oxidation-reduction indicator used for the evaluation of cell growth, particularly in various cytotoxicity assays (Mc Nichol 2006). It is purple non-fluorescent and non-toxic dye becomes pink and fluorescent when reduced to resorufin by oxidation-reduction within viable cells. Resorufin is further reduced to hydroresorufin (uncoloured). Resazurin reduction test has been used from decades to demonstrate bacterial and yeast contamination of milk (Bigalke 1984).

Resazurin Based Microtiter Dilution Assay (RMDA) Under aseptic conditions, 96 well micro titre plates (Tarson) were used for Resazurin based Micro titre Dilution Assay. The one of the row of microtiter plate was filled with 60 μl of test materials in 10% (v/v) DMSO or sterile water. All the wells of micro titre plates were filled with 50 μl of nutrient broth. Two fold serial dilution (through out the column) was achieved by starting transferring 60 μl test material from first row to the subsequent wells in the next row of the same column and so that each well has 60 μl of test material in serially descending concentrations. 10 μl of resazurin solution as indicator was added in each well. Finally, a volume of 10 μl was taken from bacterial suspension and then added to each well to achieve a final concentration of 5×10^6 CFU/ml. To avoid the dehydration of bacterial culture, each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each micro titre plate had a set of 4 controls: (a) a column with Ampicillin as positive control, (b) a column with all solutions with the exception of the test extract and (c) a column with all solutions except bacterial solution replaced by 10 μl of nutrient broth (d) a column with DMSO negative control. The plates were incubated in temperature-controlled incubator at 37° C for 24 hours. The colour change in the well was then observed visually. Any colour change observed from purple to pink or colourless was taken as positive. The lowest concentration of plant leaf extract at which colour change occurred was recorded as the MIC value. All the experiments were performed in triplicates.

Statistical Analysis

All the experiments were seeded in triplicate and data thus obtained was reported as mean ± standard deviation (SD).

RESULTS

Antimicrobial Activity

The antimicrobial activity of *Sida cordata* plant leaf and stem extract are shown in table 1 &2. The antimicrobial activity was determined in comparison with Ampicillin (13-16mm), antifungal activity was compared with Nystatin (13.5-14mm).

Different solvent and aqueous extracts of leaves were taken at 50 μgms concentration against six important human pathogenic microorganisms (clinical isolates) are presented in table: 1. Against E.coli the high activity was seen in aqueous (18mm) followed by Ethanol(17mm),while petroleum ether, ethyl acetate, and chloroform did not show significant activity against E.coli. For S.aureus aqueous extract showed better activity (20mm) followed by ethanol extract (15mm). For Lactobacillus also aqueous extract showed better activity (19mm) followed by ethanol extract (18mm), same was observed for pseudomonas aeruginosa, aqueous (15mm) followed by ethanolic extract (12mm) and other solvent extracts were found to be neutral. Different extracts of leaf of *Sida cordata* did not show any significant activity against fungal strains.

The antimicrobial activity of different extracts of stem of *Sida cordata*, were determined against selected microorganisms were presented in table:2, the results reveals that aqueous extracts have significant activity against *E.coli*
(16mm), S. aureus (18mm), Pseudomonas aeruginosa (11mm) and Lactobacillus (17mm) respectively at 50 μgms/well. No inhibition was observed against the fungal strains. Antimicrobial activity of ethanol extract varied greatly among the different pathogenic bacteria, highest activity was observed against lactobacillus (16mm) followed by S. aureus (15mm), E. coli (14mm), Pseudomonas aeruginosa (10mm). Antifungal activity could not be seen in different stem extracts.

Even though antimicrobial activity was found in petroleum ether, ethyl acetate, and chloroform extracts of both leaf & stem it was not significant as the zone of inhibition was found to be less than 8 mm.

**Dose Dependent Antimicrobial Activity by Broth Dilution Assay**

Results of the antimicrobial activity of the different concentrations of the extracts of leaf and stem of *Sida cordata* by broth dilution assay on the test isolates are shown in the figure 1a-d and 2a-d. The results shows that increase in the concentration of the extracts inhibit the growth all but highest percentage of inhibition was observed in *E. coli*, *S. aureus*, and *Lactobacillus* and *Pseudomonas aeruginosa* and scanty for *Candida albicans*, *Aspergillus niger* even at the highest concentration.

**MIC of Leaf & Stem Extracts Using Resazurin Based Microtiter Dilution Assay (RMDA)**

Different crude extracts of leaf and stem of *Sida cordata* were screened for their antimicrobial potential. MIC values of different leaf & stem extracts for pathogenic microorganisms are presented in table: 3 & 4.

Leaf extract of *Sida cordata* shows promising activity, the aqueous extract showed maximum activity against *S. aureus* (MIC 0.187mg/ml) followed by *E. coli* & *Lactobacillus* (MIC 0.375mg/ml) *Pseudomonas aeruginosa* (MIC 1.5mg/ml). *Lactobacillus* (MIC 0.187mg/ml) was found to be highly susceptible to ethanol extract of leaf followed by *S. aureus* (MIC 0.375mg/ml) *E. coli* (MIC 0.750) and *Pseudomonas aeruginosa* (MIC 3mg/ml) respectively.

Ampicillin was used as a positive control, showed the MIC in the range of 0.097-0.39mg/ml.

The MIC values for the aqueous extracts of stem were found to be in the following order, *S. aureus* and *Lactobacillus* (1.5mg/ml) *E. coli* and *Pseudomonas aeruginosa* (3mg/ml). The MIC value for ethanol extracts of stem was found to be 0.75mg/ml for S. aureus and lactobacillus while E. coli and Pseudomonas aeruginosa it was 4.0, 4.5mg/ml respectively.

Both aqueous & ethanol extracts of leaf and stem had no significant effect on fungal test strains compared to the MIC value exhibited by anti-fungal agent Nystatin (MIC 0.390mg/ml)

**DISCUSSIONS**

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. In recent years development of multidrug resistance in the pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases (Davies 1994).

And other problems such as toxicity of certain antimicrobial drugs on the host tissue (Idose et al 1968, Maddux & Barrere 1980] triggered interest in search of new antimicrobial substances/drugs of plant origin. Considering the rich diversity of plants, it’s expected that screening and scientific evaluation of plant extracts for their antimicrobial activity may provide new antimicrobial substances; hence the present investigation clearly reveals the antibacterial nature of this plant and suggests that this plant could be exploited in the management of diseases caused by these bacteria in human systems.
In our study the antibacterial activity was highly pronounced in aqueous extracts compared to ethanol extracts the aqueous extract of *Sida cordata* leaf showed broad spectrum of antibacterial activity against all the four bacterial clinical isolates. Based on the results obtained it can be concluded that aqueous leaf extract showed significant antibacterial activity against *S.aureus* followed by *E.coli*, *Lactobacillus*. Several types of alkaloids sterols, protein and other secondary metabolites known to exert antimicrobial activity, our earlier studies on phytochemical analysis of leaf and stem extracts of *Sida cordata* have revealed the presence of different secondary metabolites (Gulnaz.A.R & Savitha.G 2013). Moderate antimicrobial activity was recorded for ethonolic extracts against *E.coli*, low activity for *pseudomonas aeruginosa*, significant activity is seen against *lactobacillus* followed by *S.aureus*. This difference is attributed to the solubility of the active component in different solvents. Karou et al. (2007) observed that the IC50 of chloroform fraction of *Pterocarpus erinaceous* was higher than the IC50 of the petroleum ether fraction. He noted that the differences in the IC50 of the difference extracts were as a result of the differences in the solubility of the active agents in the extracting solvents.

Other two pathogens E.coli frequently associated with urinary tract infection, and Lactobacillus associated dental caries is also susceptible to water extract. However least inhibition was observed for *pseudomonas aeruginosa* associated with infant bacteria.

It was observed that different isolates exhibited varying degree of resistance to the aqueous extracts of *Sida cordata*, this indicates the presence of more than one active principle in the aqueous extract of leaf as confirmed by our earlier studies (Gulnaz.A.R & Savitha.G 2013). Hence the present findings are highly encouraging in recognising leaves of *Sida cordata* with potential antimicrobial activity, comparative efficacy with Ampicillin is also highly encouraging. Finally, it was observed that the highest concentration of the ethanolic and aqueous extract of the plant has no significant effect on the fungi isolates. They had lower zone of inhibition compared to the antifungal agents used as control. Similar result was reported by Adeleke et al. (2006). This difference in their susceptibility could be due to the structural and chemical differences in their cells and cell-wall structures. The results of present study reveal that the antibacterial potential of medicinal plants varies with the parts of the plants and solvents used for the extraction of phytoconstituents.

The active extracts serve as reservoir of potential compounds. Further studies on *Sida cordata* may lead toward discovery of novel antimicrobial compounds. Further studies in the direction for identification, isolation, purification characterization its chemical structure and assess its biological activities is in progress. Additionally the extracts were not pure compounds and in spite of this good result were obtained which merely suggests the potency of the extract as a natural product with potential antimicrobial properties. It may be concluded that the above plant *Sida cordata* is very useful plant. This plant may be used to cure some common and other various diseases. It is necessary of exploration of maximum potential of this plant in medicinal field and pharmaceutical sciences for its appropriate application.

REFERENCES

19. Panthi M.P and Chaudhary R.P,. Antibacteriactivity of some selected folklore medicinal
APPENDICES

Figure 1a: AM Activity of *S.cordata* Leaf Extracts on *E.coli*

Figure 1b: AM Activity of *S.cordata* Leaf Extracts on *S.aureus*

Figure 1c: AM Activity of *S.cordata* Leaf Extracts on *Pseudomonas aeruginosa*
Evaluation of Antimicrobial Activity of Leaf and Stem Extracts of Sidda Medicinal Plant *Sida cordata*

**Figure 1d:** AM Activity of *S.cordata* Leaf Extracts on *Lactobacillus*

**Figure 2a:** AM Activity of *S.cordata* Stem Extracts on *E.coli*

**Figure 2b:** AM Activity of *S.cordata* Stem Extracts on *S.aureus*
Figure 2c: AM Activity of *S.cordata* Stem Extracts on *Pseudomonas aeruginosa*

Table 1: In Vitro Antimicrobial Activity of Leaf Extracts of *Sida cordata* against Selected Bacterial & Fungal Strains

<table>
<thead>
<tr>
<th>S. No</th>
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<th>Water</th>
<th>Ampicillin</th>
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*values are mean +/- SD triplicates experiments. (-) means no activity less than 8mm

Figure 2d: AM Activity of *S.cordata* Stem Extracts on *Lactobacillus*

Table 2: In Vitro Antimicrobial Activity of Stem Extracts of *Sida cordata* against Selected Bacterial & Fungal Strains

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Evaluation of Antimicrobial Activity of Leaf and Stem Extracts of Sidda Medicinal Plant *Sida cordata*

Table 2: Contd.,

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*values are mean +/- SD triplicates experiments. (-) means no activity less than 8mm

Table 3: Minimum Inhibitory Concentration (MIC, mg/ml) of Leaf Extracts of *Sida cordata* against Pathogens by Resazurin Micro Titre-Plate Assay

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*values are mean +/- SD triplicates experiments. (-) means no activity, MIC values are above 5mg/ml

Table 4: Minimum Inhibitory Concentration (MIC, mg/ml) of Stem Extracts of *Sida cordata* against Pathogens by Resazurin Micro Titre-Plate Assay

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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida albicans</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.390</td>
</tr>
<tr>
<td>6</td>
<td><em>Aspergillus niger</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.390</td>
<td>-</td>
</tr>
</tbody>
</table>

*values are mean +/- SD triplicates experiments. (-) means no activity, MIC values are above 5mg/ml.