Biological activity and phytochemical screening of different extracts of Sida cordata (Burm.F.) borssum root

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Abstract
In the present study was designed to evaluate antimicrobial, anthelmintic activity and phytochemical analysis of different extracts of Sida cordata (Burm. f.) borssum root extract was prepared using different solvents like chloroform, petroleum ether, ethylacetate, ethanol, and water (aqueous extract). By using agar well diffusion method, the antibiotic activity of different extract was evaluated and minimum inhibitory concentration (MIC) by 96 well resazurin based microtiter dilution the test organisms were Escherichia coli, Staphylococcus aureus, Lacto bacillus Pseudomonas aeroginosa, Candida albicans, Aspergillus niger. Among the different the extracts (ethanolic and aqueous) showed significant antimicrobial activity against Staphylococcus aureus, Escherichia coli, Lactobacillus, Pseudomonas aeroginosa, Candida albicans and Aspergillus niger. Against S.aureus, aqueous extract showed better activity (20mm, MIC 0.187 mg/ml) followed by Ethanol extract (15mm with MIC 0.390 mg/ml), further the aqueous extract showed activity against Pseudomonas aeruginosa, (15mm, MIC 1.5 mg/ml) followed by Ethanol extract (12mm with MIC 0.375 mg/ml) and same was observed for Lactobacillus (19mm). In terms of anthelmintic activity against Phereitima posthuma (Indian earthworm) aqueous extract of the root exhibited significant activity in the concentration of 100mg/ml with 22.0 and 39 minutes for paralysis and death, respectively and that of standard drug Albendazole was 20 and 38.00 minutes. The aqueous extract showed significantly better effect comparable to standard and hence higher anthelmintic activity.

Keywords: Sida cordata, Antimicrobial Resazurin, Anthelmintic, Phereitima posthuma.

Introduction
Human beings are using plants for the treatment of various diseases since thousands of years.1,2 As pre the World Health Organization reports majority of the world populations still depends on traditional medicines for their psychological and physical health requirements,3 mainly because of the two reasons first they cannot afford the products of Western pharmaceutical industries,4 second with their side effects and lack of healthcare facilities.5

Secondary metabolites are derived from plant source and constitute an important source of microbicides, pesticides and many pharmaceutical drugs from a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases.6 Compounds of secondary metabolites have no apparent function in a plant’s primary metabolism, but often have an ecological role, as pollinator attractants, represent chemical adaptations to environmental stresses or serve as chemical defense against micro-organisms, insects and higher predators and even other plants (allelochemicals). Frequently secondary metabolites are accumulated by plants in smaller quantities than the primary metabolites7,8 compared to primary metabolites, secondary metabolites are synthesized by specialized cell types and at various and distinct developmental stages, which makes difficult for extraction and purification. Hence all commercially available secondary metabolites are having high value-low volume.

Sida cordata (Burm.f.) Borssum (Family: Malvaceae) is a small weed found throughout India, usually on the road sides and other waste places. It is a non-woody herb with hairs and slender with trailing branches, hispid sparingly, procumbent frequently and sometimes roots are seen at the nodes showing characteristic of a perennial plant. Flowers and fruits mainly during September to November or almost throughout the year depending upon the habitat conditions.

Ethnomedicinal Uses
The root of the plant tastes sweet, bitter, sour and acid. It has property of astringent, febrifuge, stomachic, tonic and thermogenic. It has good medicinal property as a diuretic for the treatment of uropathy. Its bark of the root is also used for the treatment of gonorrhea and leucorrhoea. It has property of analgesic, haemostatic and wound healing. The decoction of its root is known to be used for dysentery. The root paste is known to be used to cure boils and wounds.9 Its paste of the leaves is used to apply as a poultice in cuts and bruises, and also used in ophthalmic conditions.

One of our earlier studies on antimicrobial, anthelmintic activity and phytochemical analysis of different extracts of Sida cordata (Burm.f.) Borssum leaf and stem had proved its efficacy10 as per the lay claims Sida cordata (Burm.f.) Borssum root is used a traditional medicine to cure various diseases hence
present studies are undertaken to give a scientific validation to these lay publications.

Materials and Methods

Plant Collection: The Fresh plant *Sida cordata* was collected from its natural habitat, from the forest region of Somawarpet in Madekeri Kodagu district Karnataka. The plant was identified and authenticated at National Ayurveda Dietetics Research Institute Bangalore, (voucher no: RRCBI-11748). The roots were separated from the plants, washed thoroughly under tap water, shade dried at room temperature and then homogenized to fine powder of 40 mesh sizes and stored in airtight bottles at 4°C.

Extraction of Plant Material: The dried powder of the root of about 150gm was subjected to hot percolation extraction using 150ml different solvents with increasing in polarity like Petroleum ether(A), Chloroform(B), Ethyl acetate(C), Ethanol(D), and Water (E) by using Soxhlet apparatus. Solvents until the solvents turn colorless followed by concentrating the same by evaporation and it stored in dessicator at 4°C until further use.

Antimicrobial Activity

Test Organisms: Human pathogenic organisms (bacteria and fungi) were isolated from clinical samples, are used in this study (the samples were collected from Department of Microbiology, Farooqia Dental College & Hospital Mysore, India and were identified and confirmed by Microbiologist.). The organisms used were *Esherichia coli*, *Staphylococcus aureus*, *Lactobacillus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*.

Antimicrobial activity and MIC of leaf & stem extracts using Resazurin based Microtiter Dilution assay (RMDA): The antimicrobial activity of different crude *Sida cordata* root extracts are shown in Fig. 2 the antimicrobial activity was determined in comparison with Ampicillin (13-18mm), antifungal activity was compared with Nystatin (14-14.5mm). Different crude extracts Root of *Sida cordata* were screened for their antimicrobial potential.

Anthelmintic Activity

Worm Collection: *Pheretima posthuma* (Indian earthworm) were collected from Vermiculture tank Maharani’s Science College for Women, Mysore, Karnataka, India. The worms are washed with normal saline & water to remove all fecal matter and were identified by the HOD Department of Zoology, Maharani’s Science College for Women, Mysore, Karnataka, India.

*In vitro* anthelmintic activity of different extracts of *Sida cordata* (burm.f.) Borss root: Among the different extracts was tested in comparison with the standard drug Albendazole and observation was done based on paralysis and death of the parasite.

Phytochemical Screening: The primary metabolites like proteins, carbohydrates and fixed oils, fats, etc and the secondary metabolites like, alkaloids, flavonoids, saponins, phenolics, tannins volatile oils, terpenoids, glycosides etc were assessed in the leaf and stem extracts of *sida cordata* as per the standard procedures.11

Results

Percentage Yield

![Fig1. Percent yield in different solvents](image)

| Solvents | % of Yield |
|----------|-----------|
| A        | 1         |
| B        | 2         |
| C        | 4         |
| D        | 6         |
| E        | 8         |

Fig. 1:

Antibacterial & Antifungal Activity

MIC value of different solvent and aqueous extracts of roots were taken at 50µgms concentration against six important human pathogenic microorganisms (clinical isolates).

- Aqueous extract has shown 17mm with MIC of 0.187mg.ml followed by ethanol with 16mm and MIC of 0.195mg/ml against *E. coli*. The MIC value was not significantly evident against *E. coli* with petroleum ether, ethyl acetate, and chloroform extracts. Aqueous extract has shown better value at 20mm with MIC of 0.187mg/ml followed by ethanol extract with 15mm with an MIC value of 0.390mg/ml against *S.aureus*, and similarly aqueous extract has shown better activity with 15mm with an MIC of 1.5mg.ml followed by ethanolic extract with 12mm with an MIC value of 0.375 against *Pseudomonas aeruginosa* (Table 1). Similar results were seen against *Lactobacillus* with 18mm and 19mm for aqueous and ethanolic extract respectively (Fig 2). No activity was seen against fungal strains.
Table 1: Minimum inhibitory concentration (MIC, mg/ml) of different extracts of *Sida cordata* (burm.f.) Bors. by Resazurin micro titre-plate assay.

| Source       | *E. coli*       | *S. aureus*     | *P. aeruginosa* | *L. bacillus* | *C. albicans* | *A. niger* |
|--------------|-----------------|-----------------|-----------------|--------------|--------------|-----------|
| Std (Ampicillin/Nystatin) | 0.195±0.02c | 0.097±0.04a | 0.390±0.1c | 0.195±0.1c | 0.390±0.0c | 0.390±0.0c |
| A            | 6.0±02          | 3.0±0.23        | 6.0±0.5        | 6.0±0.38     | -            | -         |
| B            | 3.0±0.29        | 1.5±01          | 6.0±0.29       | 6.0±0.23     | -            | 3.0±02    |
| C            | 1.5±0.14a       | 3.0±0.49        | 3.0±0.28       | 3.0±0.27     | 1.5±0.19     | 3.0±0.6   |
| D            | 0.195±0.0f      | 0.375±0.1a      | 3.0±0.15       | 1.5±0.15     | 0.750±012    | 1.5±010   |
| E            | 0.187±0.23c     | 0.187±0.31b     | 1.5±0.2a       | 0.75±0.13a   | 0.375±0.0c   | 0.195±0.39c |

A-Petroleum ether, B-Chloroform, C-Ethyl acetate, D-Ethanol, E-Water/ Aqueous. : (-) No Activity

The values are means of triplicates ± standard deviation, the values followed by different superscript differ significantly a $p<0.001$, b $p<0.01$, c $p<0.05$.

**Anthelmintic Activity**

Aqueous extract of the root showed significant anthelmintic activity at the concentration of 100mg/ml for paralysis and death at 22.0 and 39 minutes respectively comparable to standard drug Albendazole with 20.0 and 38.00 for death and paralysis respectively (Fig 3 & 4).

**Phytochemical Analysis**

The qualitative phytochemical analysis of crude extracts revealed the presence of most of these compounds which are tabulated in Table-2. Phytochemicals were extracted by using various solvent. All extracts showed presence of coumarins. Alkaloids, phenols and emodins were present in all extracts but not in ethyl acetate, petroleum ether, and chloroform extracts respectively. Ethyl acetate, ethanol and water extract showed presents of tannins, saponins, flavonoids and glycosides (Table 2).
Table 6: Phyto chemical analysis of different extract of Sida cordata (burm. f.) Borss.root

| Phyto Compounds        | Test              | A  | B  | C  | D  | E  |
|------------------------|-------------------|----|----|----|----|----|
| Alkaloids               | a. Mayer’s test   | +  | +  | -  | +  | +  |
|                        | b. Wager’s test   | +  | +  | -  | +  | +  |
|                        | c. Dragondoff’s test | +  | -  | -  | +  | +  |
|                        | d. Hager’s test   | +  | +  | -  | +  | +  |
| Phytosterols/triterpenoids | a. Liebermann test | -  | -  | -  | -  | -  |
|                        | b. Salkowski test | -  | -  | -  | -  | -  |
| Saponins               | a. Froth test     | -  | -  | +  | +  | +  |
|                        | b. Foam test      | -  | -  | +  | +  | +  |
| Tannins                | Gelatin test      | -  | -  | +  | +  | +  |
| Flavonoids             | a. Alk.reagent test | -  | -  | +  | +  | +  |
|                        | b. Lead acetate test | -  | -  | +  | +  | +  |
| Glycosides             | Borntrager’s test | -  | -  | +  | +  | +  |
| Fixed oil/fat          | Spot test         | -  | -  | -  | -  | -  |
| Phenol                 | Ferric chloride test | -  | +  | +  | +  | +  |
| Gum                    |                   | -  | -  | -  | -  | -  |
| Volatile oil           |                   | -  | -  | -  | -  | -  |
| Coumarin               |                   | +  | +  | +  | +  | +  |
| Emodin                 |                   | +  | -  | +  | +  | +  |
| Anthocyanin            |                   | -  | -  | -  | -  | -  |
| Anthraquinones         | Sulphuric acid Test | -  | -  | -  | +  | +  |
| Catechins              | Erhlish test      | -  | -  | -  | +  | +  |

(A-Petroleum ether, B-Chloroform, C-Ethyl acetate, D-Ethanol, E-Water,+: Present, -: Absent)

Discussion

The antimicrobial activity of the extracts might be due to the presence of flavonoids alkaloids and antimicrobial substance. Better antimicrobial activity was observed in root extracts compared to the leaf and stem extracts, which may be due to the interference of pigments and phenolic with the antimicrobial activity of these extracts. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The aqueous extracts of the whole plant showed significant anthelmintic activity in comparison with standard drug which could be due to the presence of tannins and other secondary metabolites. Phytochemicals are the reliable sources in healing of different health problems. Preliminary phytochemical screening is a fundamental step in the detection of bioactive compounds present in plants and it may lead to discovery of novel environmentally friendly natural chemicals and drug.

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