PRELIMINARY SCREENING OF ETHNOMEDICINAL PLANTS FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT: Based on the ethno botanical survey and the related literature, 285 solvent extracts from 57 ethnomedical plants belonging to 55 genera and 34 families were subjected to preliminary screening for antibacterial activity against *Escherichia coli* and *Bacillus subtilis* using agar well diffusion method at concentrations of 5 & 2.5 mg/ml. Among 285 plant extracts, some exhibited very effective activity, some exhibited effective activity, some exhibited moderate and some exhibited weak activity and no activity was observed in some extracts against *E. coli* and *B. subtilis*. Among the plants tested, maximum activity was observed in all the solvents extracts of *Argemone mexicana*, *Allium sativum*, *Lantana camara*, *Tephrosia purpurea* and *Withania somnifera*. The results of the present study indicate that the extracts of different parts of 57 ethnomedical plants have more potential of antibacterial activity and are concentration dependent. The demonstration of broad spectrum of the above said 5 plants may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control.

INTRODUCTION: Microorganisms are frequently developing resistance to common drugs and antibiotics and this pose an enormous threat to the treatment of a wide range of serious infections. In the present scenario of emergence of drug resistance to human pathogenic organisms; this has necessitated a search for new antimicrobial substances from other sources including plants. In all regions of the World, history shows that medicinal plants have always held an important Place. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity. There are several reports on the antimicrobial activity of different herbal extracts. The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world.

Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information, and a few attempts were made on inhibitory activity against certain pathogenic bacteria and fungi. As a result, antibacterial therapy is playing a greater role in health care and the screening of traditional plants in search of novel antibacterials are now more frequently performed. The selection of crude plant extracts for screening programs has the potential of being more...
successful in initial steps than the screening of pure compounds isolated from natural products\textsuperscript{18}.

In the present study, 57 ethno medicinal plants of Hyderabad Karnataka Region have been selected for antibacterial screening against human pathogens namely \textit{E. coli} and \textit{B. subtilis}. Among the tested plants, \textit{A. mexicana}, \textit{W. somnifera}, \textit{T. purpurea}, \textit{L. camara} and \textit{A. sativum} showed profound antibacterial activity. The demonstration of broad spectrum of the above said five plants may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control.

\textbf{MATERIAL AND METHODS:}

\textbf{Collection of plant material:}

Based on the ethno botanical survey and the related literature, 57 ethno medicinal plants belonging to 55 genera and 34 families were collected in fresh bags from different localities of Hyderabad Karnataka region during the month of June, July, August and late September and brought to laboratory. The different plant parts like, leaves, roots, flowers, fruits and stem bark collected were initially rinsed with distilled water to remove soil and other contaminants, shade dried using tray under controlled temperature at 37\textdegree C for week.

\textbf{Extraction of plant material by Soxhlet apparatus:}

All these parts of plants were powdered using mechanical pulverize and powdered materials were preserved in the sterilized polythene bags until further use. For extraction of crude drugs, 250g of shade dried powdered plant material was weighed and subjected to successive Soxhlet extraction with different solvents such as Petroleum ether, Chloroform, Ethyl acetate, Methanol and Distilled water (Aqueous) in the order of increasing polarity of solvents for a period of 18-22 h. The extracts obtained were concentrated to dryness in evaporating dish at 40\textdegree C and stored the dried extract at 4\textdegree C in the refrigerator until further use.

For preparing drug solution, Petroleum ether, Chloroform, Ethyl acetate, Methanol and Aqueous crude extracts were dissolved in DMSO and used as a reference compound (Positive control-500\textmu g/ml) for bacterial activity. DMSO was used as negative control. These solutions were preserved at 4\textdegree C until further use. The \textit{in vitro} antibacterial (\textit{E. coli} and \textit{B. subtilis}) activity of 57 ethno medicinal plants was carried out by adopting the agar well diffusion technique\textsuperscript{19}.

\textbf{Test microorganisms and preparation of inoculums:}

The pure axenic cultures of bacteria viz., \textit{E. coli}, \textit{B. subtilis} were procured from the stock culture of Department of Botany, Gulbarga University, Kalaburagi, and were further maintained on nutrient agar slants at 4\textdegree C until further use. For preparation of inoculums, 48 h old bacterial culture grown in nutrient broth (Himedia, M002) at 37\textdegree C and maintained on nutrient agar slants at 4\textdegree Cwas used for experimental studies.

\textbf{Antibacterial activity:}

The assay was conducted by agar well diffusion method. About 15 to 20 ml of nutrient agar medium was poured in the sterilized Petri dishes and allowed to solidify. Bacterial lawn was prepared using 5 days old culture strain. The bacterial strains were suspended in a saline solution (0.85\% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). 1 ml of bacterial strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4mm diameter were punctured in the culture medium. Required fractions of extracts were added to the wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37\textdegree C. After incubation for 18 h, the plates were observed for zones of inhibition.

The diameter of zone of inhibition was measured and expressed in millimeters. Dimethyl Sulphoxide (DMSO) was used as a negative control. Streptomycin for bacteria was used as positive control (500\textmu g/ml). The experiments were conducted in triplicates.

\textbf{RESULTS:}

\textbf{Preliminary screening of ethno medicinal plants for antibacterial activity:} 285 solvent extracts from
57 ethnomedicinal plants were subjected to antibacterial screening against two bacteria namely, *E. coli* and *B. subtilis* using agar well diffusion method at concentrations of 5 & 2.5 mg/ml. The results are given in Table 1.

**Screening against *Escherichia coli* and *Bacillus subtilis***: Among the 285 plant extracts, 77 and 48 extracts exhibited very effective activity against *E. coli* and *B. subtilis*. 67 and 68 extracts exhibited effective activity. 63 and 58 extracts exhibited moderate activity and 67 and 95 extracts exhibited weak activity against *E. coli* and *B. subtilis*. No activity was observed in 11 and 16 extracts against *E. coli* and *B. subtilis* (Table 2). Among the plants tested, maximum activity was observed in all the solvents extracts of five medicinal plants namely, *A. mexicana*, *A. sativum*, *L. camara*, *T. purpurea* and *W. somnifera*. The effectivity was also observed in different solvent extracts of other plants also but the above mentioned five plants showed strong antibacterial activity against both the test strains in all the solvent extracts. There was no inhibition recorded from the negative control (DMSO), while the standard drug, Streptomycin, significantly inhibited (21.00±0.00 to 31.00±0.00mm) the growth of the test organism. (Table 1).

**TABLE 1: PRELIMINARY ANTIMICROBIAL (ANTIBACTERIAL) SCREENING OF ETHNO MEDICINAL PLANTS OF HYDERABAD KARNATAKA REGION**

![Table 1](https://example.com/table1.png)

**Source**: Sharanappa and Vidyasagar, IJPSR, 2015; Vol. 6(9): 3928-3935.
<table>
<thead>
<tr>
<th>Species</th>
<th>Growth Rate</th>
<th>Percentage Increase</th>
<th>TDV</th>
<th>MVD</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crassulaceae (Leaf)</td>
<td>b</td>
<td>10.00 ± 0.57</td>
<td>11.53 ± 0.57</td>
<td>11.55 ± 0.57</td>
<td>16.46 ± 0.57</td>
</tr>
<tr>
<td>Butea monosperma</td>
<td>a</td>
<td>11.53 ± 0.57</td>
<td>13.33 ± 0.57</td>
<td>13.33 ± 0.57</td>
<td>15.66 ± 0.57</td>
</tr>
<tr>
<td>(Lam.) Taub.</td>
<td>b</td>
<td>10.00 ± 0.57</td>
<td>11.53 ± 0.57</td>
<td>11.55 ± 0.57</td>
<td>16.46 ± 0.57</td>
</tr>
<tr>
<td>Fabaceae</td>
<td>a</td>
<td>11.53 ± 0.57</td>
<td>13.33 ± 0.57</td>
<td>13.33 ± 0.57</td>
<td>15.66 ± 0.57</td>
</tr>
<tr>
<td>(Stem bark, Seed,Flower).</td>
<td>b</td>
<td>10.00 ± 0.57</td>
<td>11.53 ± 0.57</td>
<td>11.55 ± 0.57</td>
<td>16.46 ± 0.57</td>
</tr>
<tr>
<td>Calotropis procera (L.)</td>
<td>a</td>
<td>10.00 ± 0.57</td>
<td>11.53 ± 0.57</td>
<td>11.55 ± 0.57</td>
<td>16.46 ± 0.57</td>
</tr>
<tr>
<td>(Asclepiadaceae)</td>
<td>b</td>
<td>10.00 ± 0.57</td>
<td>11.53 ± 0.57</td>
<td>11.55 ± 0.57</td>
<td>16.46 ± 0.57</td>
</tr>
<tr>
<td>(Root, Latex).</td>
<td>a</td>
<td>10.00 ± 0.57</td>
<td>11.53 ± 0.57</td>
<td>11.55 ± 0.57</td>
<td>16.46 ± 0.57</td>
</tr>
<tr>
<td>Capparis ceylanica L.</td>
<td>b</td>
<td>0.57 ± 0.57</td>
<td>1.15 ± 0.57</td>
<td>1.15 ± 0.57</td>
<td>1.22 ± 0.57</td>
</tr>
<tr>
<td>Capparaceae (Root).</td>
<td>a</td>
<td>0.57 ± 0.57</td>
<td>1.15 ± 0.57</td>
<td>1.15 ± 0.57</td>
<td>1.22 ± 0.57</td>
</tr>
</tbody>
</table>

**Note:** The table above shows the growth rate, percentage increase, TDV, and MVD for various plant species. The density values are also provided, indicating the concentration or mass per unit volume. The table entries are in terms of ± values, indicating the range of expected outcomes.
   a. 11.33 ± 0.88 
   b. 11.20 ± 0.88 

   a. 08.50 ± 0.88 
   b. 07.20 ± 0.88 

   a. 07.66 ± 0.88 
   b. 06.66 ± 0.88 

   a. 16.66 ± 0.57 
   b. 14.66 ± 0.57 

   a. 08.50 ± 0.57 
   b. 07.50 ± 0.57 

   a. 08.66 ± 0.57 
   b. 07.66 ± 0.57 

   a. 10.66 ± 0.57 
   b. 09.66 ± 0.57 

43. *Mangifera indica* L. *Anacardiaceae* (Leaf).
   a. 12.66 ± 0.57 
   b. 12.33 ± 0.57 

44. *Musas paradisiaca* L. *Musaceae* (Leaf).
   a. 09.33 ± 0.57 
   b. 08.66 ± 0.57 

   a. 11.66 ± 0.57 
   b. 11.00 ± 0.57 

46. *Phyllanthus emblica* L. *Euphorbiaceae* (Fruit).
   a. 12.33 ± 0.57 
   b. 11.66 ± 0.57 

47. *Phyllanthus niruri* L. *Euphorbiaceae* (Leaf).
   a. 09.33 ± 0.57 
   b. 08.66 ± 0.57 

   a. 09.00 ± 0.57 
   b. 08.66 ± 0.57 

   a. 09.00 ± 0.57 
   b. 08.66 ± 0.57 

   a. 11.00 ± 0.57 
   b. 09.33 ± 0.57 

51. *Solanum nigrum* L. *Solanaceae* (Fruit).
   a. 13.66 ± 0.57 
   b. 13.66 ± 0.57 

   a. 19.66 ± 0.57 
   b. 18.66 ± 0.57 

   a. 12.33 ± 0.57 
   b. 11.66 ± 0.57 

54. *Tribulus terrestris* L. *Zygophyllaceae* (Fruit).
   a. 11.5 ± 0.57 
   b. 11.0 ± 0.57 

55. *Tridax procumbens* L. *Asteraceae* (Whole plant).
   a. 11.33 ± 0.57 
   b. 11.00 ± 0.57 

   a. 20.33 ± 0.57 
   b. 19.66 ± 0.57 

   a. 11.33 ± 0.57 

- Table values in the image are not legible but seem to include various measurements or values related to the plants listed. The values are not clearly visible due to the image quality.
DISCUSSION: In the present study, Petroleum ether, ethyl acetate and methanol extracts of A. sativum showed very effective activity against all the tested strains. However, Seema Bhadauria and Padma Kumar reported effective activity in aqueous extract. A study by Esimone et al., reported that it is worthy to note that in most cases of infection, a combination of antimicrobial activity and one or more other biological effects, such as immunomodulation, could be responsible for overall effect of a natural product. It is therefore likely that a combination of these biological effects of garlic and ginger and the demonstrated antimicrobial effect may explain its usefulness in the management of oropharyngeal infections.

Some in-vitro studies confirmed anti-bacterial activity of garlic extract. A study by Ankari et al., reports the garlic extract for anti-bacterial, antifungal, anti-parasitic and anti-viral activities. It has been reported that the use of fresh garlic is more effective for antimicrobial activity than that from old garlic. The antibacterial properties of crushed garlic have been known for a long time. Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against the species of Escherichia, Streptococcus, Klebsiella, Proteus, Bacillus and Clostridium. Analysis of steam distillations of crushed garlic cloves performed over a century ago showed a variety of allyl sulfides compounds responsible for antibacterial activity of crushed garlic cloves.

In the present study, all the five solvent extracts of A. mexicana root at 5mg/ml concentration showed effective activity against both the tested strains with strong activity especially in ethyl acetate extracts. The inhibition zones against bacterial and fungal strains were comparable to those elicited by streptomycin. However, a study by Yashwant Bais et al., reported that the methanolic leaf extracts of A. mexicana did not show inhibitory action against bacteria, but was found to be very good against C. albicans as compared to other yeast strain.

In the present study, the leaf extracts of L. camara in all the solvent extracts especially in ethyl acetate showed potent antibacterial activity against both the tested strains. However, in another study by Parivuguna et al., reports that the extract of flower, leaf, stem and root of L. camara showed antibacterial activity against E. coli, P. aeruginosa, S. aureus and S. saprophyticus. The antibacterial activity was determined by disc diffusion method, tube dilution technique, agar well diffusion method, micro distillation method. It was observed that strains of E. coli & S. aureus were more susceptible to essential oil. L. camara flower extract possess strong antibacterial activity of all few types, yellow lavender, red and white L. camara. L. camara leaves evaluated for antimicrobial & antifungal activity.

The essential oil of L. camara tested against 7 bacteria & 8 fungi, showed wide spectrum of antifungal activities. An investigation of acetone extracts of leaves of L. camara and L. rugosa showed growth inhibitory effects against (E. coli, P. aeruginosa, E. faecalis and S. aureus) bacteria, with MIC values varying from 0.39 mg/mL to 6.3 mg/mL. The essential oil of the leaves of L. camara has been examined for antibacterial activity by the microdilution test. The inhibitory activity was seen against the multi-resistant strains E. coli (MIC 512 µg/mL) and S. aureus (MIC 256 µg/Ml). In the present study, the leaf extracts of T. purpurea in all the solvent extracts at 5mg/mL concentration showed significant antibacterial activity against all the test organisms.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Test strains</th>
<th>Very effective</th>
<th>Effective</th>
<th>Moderate</th>
<th>Weak</th>
<th>No activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Escherichia coli</td>
<td>77</td>
<td>67</td>
<td>63</td>
<td>67</td>
<td>11</td>
</tr>
<tr>
<td>02</td>
<td>Bacillus subtilis</td>
<td>48</td>
<td>68</td>
<td>58</td>
<td>95</td>
<td>16</td>
</tr>
</tbody>
</table>

P= Petroleum ether extract, C= Chloroform extract, E= Ethyl acetate extract, M= Methanol extract, A= Aqueous extract, C= Control (DMSO), S= Streptomycin, NA= No Activity, 1= 5 mg , 2 = 2.5 mg , a= Escherichia coli, b= Bacillus subtilis.
A study by Thetwa et al., 38 tested the seed extracts of *T. purpurea* for their antimicrobial properties against some human, animal and plant pathogenic organisms and reported that the seed extract showed good results.

In another study, Kumar et al., 39 evaluated antimicrobial activity of ethanolic extract of *T. purpurea* roots by disc diffusion and broth dilution methods and reported the significant activity. Ethanolic extract showed better antibacterial activity against *S. aureus, E. coli, P. aeruginosa* and *B. subtilis* 40. However, Sachin Parashram Venkatraman 41 reported potent activity in pet ether seed extracts of *T. purpurea*.

The present study reports the potent antibacterial activity of root extracts of *W. somnifera* in ethyl acetate solvent extracts against both the test strains used in the study and the inhibitory effect was comparable with that of streptomycin. However, in another study, Ethanol, acetone, Iso propyl alcohol, toluene and hexane extract of different aerial parts (leaf and flower) of *W. somnifera* was evaluated by Premlata Singariya et al., 42 and reported that the extract of *W. somnifera* significantly inhibited 6 important bacteria and two fungi (*C. albicans* and *A. flavus*) to varying degrees. Leaf extracts of *W. somnifera* in different polar solvents showed highest activity in terms of inhibition zone, activity index, MIC, MBC/MFC and total activity.

The inhibitory effect was very identical in magnitude and comparable with that of standard antibiotics Gentamycin. Pranay Jain and Rishabh Varshney 43 reported the antimicrobial activity of aqueous and methanolic extracts of *W. somnifera* (Ashwagandha) with highest in aqueous extract against *E. coli, P. aeruginosa, S. aureus, S. mutans* and *C. albicans*. The present study also revealed very effective antibacterial activity in *M. indica* against *E. coli* and *B. subtilis* in petroleum ether and ethyl acetate extract. Similarly, *C. longa* was found effective in petroleum ether and ethyl acetate extracts.

**CONCLUSION:** Since plants produce a variety of compounds with antibacterial properties, it is expected that screening programs for some under-represented target such as antibacterial activity may yield candidate compounds for developing new antibacterial drugs. In the present study, the extracts of total 57 plant species belonging to 55 genera and 34 families were screened for their antibacterial activity, among which ethyl acetate extracts of *A. mexicana, W. somnifera, T. purpurea* and *L. camara* and Petroleum ether and methanolic extract of *A. sativum* showed significant antibacterial potential against test microbes.

The demonstration of broad spectrum of all the five plants may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. The extracts that showed strong antibacterial activity are worth of further investigation in order to isolate and identify the active compounds.

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