IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF LAUNAEA PINNATIFIDA CASS LEAVES
SANTOSH.G.N AND PARAMJYOTHI S.*
Department of Biochemistry, Gulbarga University, Gulbarga-585106, Karnataka, India.

ABSTRACT
The aim of the study was to investigate the antimicrobial activity of various extracts of Launaea pinnatifida Cass leaves against some clinically isolated bacterial and fungal strains. Preliminary antimicrobial activity was evaluated by agar disc diffusion method. Minimum inhibitory concentration (MIC) was determined by tube dilution method, while minimum bactecidal concentration (MBC) and a minimum fungicidal concentration (MFC) were determined by agar diffusion method. Ethanolic extract (EE) exhibited highest sensitivity against Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi and moderate sensitivity against Bacillus subtilis and Vibrio cholera. While the distilled water extract (DWE) showed the maximum zone of inhibition against Pseudomonas aeruginosa compared to EE. Petroleum ether extract (PEE) exhibited the moderate inhibition while chloroform extract (CE) showed lowest inhibition. The DWE showed sensitivity against Aspergillus niger and Aspergillus flavus. The MIC and MFC values of 1000, 1200, 1400 and 1500 µg/ml exhibited by different extracts showed the moderate sensitivity while the extracts that showed the MIC and MFC values of 1600, 1800 and 2000 µg/ml was found to be the least sensitive against the fungi. The study confirmed that the EE possessed potential antimicrobial activity than the DWE when compared to other extracts. The differentiating activities of these extracts encourage to develop novel broad spectrum antimicrobial substances in future.

KEY WORDS: Launaea pinnatifida, Antimicrobial activity, MIC, MBC, MFC.

1. INTRODUCTION
The exploration of the plants as a source of natural remedy for the treatment of various diseases dated back to prehistory around the globe in their own traditions. Natural products have been playing a pivotal role in replacing the synthetic drugs due to their severe adverse effects. Over 25% of prescribed medicines are derived from plant sources (Newman, 2000). Infectious diseases has been a serious concern for the health, pharmaceutical and government sectors all around the world (accounting for 50,00 deaths every day), due to huge increase in the number of multi drug resistance among the emerging and re-emerging pathogens to the modern drugs or antibiotics (Franklin and Snow 1989; Prescott et al 2002). It is reported that most antibiotics derived from microorganisms, one to three antibiotics are launched every year (Clark, 1996). In many developing countries medicines are too expensive and hence the phytochemicals will find the arsenal of antimicrobial drugs prescribed by the physicians (Cowan, 1999). Plants are the cost effective and safer alternative source of antimicrobial agents (Pretorious and watt 2001; Sharif and Banik 2006; Doguhari, 2007). The use of plant extracts and phytochemicals, both with known antimicrobial properties can be a great significance in the therapeutic treatments. In the past few years, a number of studies have been carried out to prove the efficiency of the plants possessing the antimicrobial properties (Almagboul, 1985; Artizzu, 1995; Izzo 1995; Ikram and Inamul, 1984). The antimicrobial compounds derived from the secondary metabolism in plants may inhibit microbial growth by the different mechanisms than those presently used antimicrobial agents and may have significant clinical value in the treatment of resistant microbe (Elloff, 1998).

Launaea pinnatifida Cass (Asteraceae) is found in India along the coastal regions from Bengal to Ceylon and Madras to Malabar, where it serves as sand binders with other plants. It is commonly called as paathri, kneekhowa, almirao, (Nadkarni, 1982) and locally called as hatarki or hakkarki. It is a perennial procumbent herb with characteristic odour and slightly bitter in taste. It is reported to be tonic, soporific and diuretic and used as substitute for taraxacum. Leaves

*Corresponding author
Department of Biochemistry,
Gulbarga University,
Gulbarga-585106, Karnataka (India).
Telephone: 00918472248819 Fax: 0091847225632.
Email: paramjyothig@gmail.com

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are eaten during famine. Herb is fed to buffaloes as a galactagogue (Kiritikar and Basu, 2001). In the present investigation we have made an effort to show that this plant possesses antimicrobial property.

2. MATERIAL AND METHODS
Collection of plant material:
The fresh leaves of Lamnae pinmatifida Cass (Asteraceae) were collected from the farmland of Saradagi village, 24 km south of Gulbarga district, Karnataka (India) during the flowering month of Oct-Nov 2008. The plant was identified and authenticated by Prof. Y. N. Seetaram, Department of Botany, Gulbarga University, Gulbarga. A voucher specimen HGUG/SN-76 is deposited in this department.

Extraction of plant material:
The shade dried leaves were powdered to 22-mesh size and then subjected to successive soxhlet extraction with petroleum ether (40°-60°C), chloroform, ethanol and distilled water until the solvents became colourless. Then the extracts were further evaporated to dryness under vacuum and refrigerated for future use. In the study of antimicrobial activity, extracts were dissolved in dimethyl sulphoxide (DMSO). The corresponding concentrations were expressed in terms of μg of extract per ml of solvent (μg/ml).

Test organisms:
Five bacterial strains namely, Salmonella typhi, Bacillus subtilis, Vibrio cholera, Staphylococcus aureus, Pseudomonas aeruginosa and five different fungal strains namely, Aspergillus niger, Aspergillus flavus, Aspergillus terreus, Penicillium notatum and Cladosporium were collected from Department of Microbiology, M. R. Medical College, Gulbarga, India. The bacterial strains were grown in Mac Conkey agar plates at 37°C and maintained on nutrient agar slants, while fungi were grown at 30°C and maintained in saboraud glucose agar slants.

Determination of phytochemical constituents
Various preliminary phytochemical analysis were carried out to detect the phytoconstituents present in Lamnae pinmatifida Cass leaves (Evans, 2002; Harborne, 1992).

Preliminary screening for antimicrobial activity
The test was performed by disc diffusion assay as per NCCLS, 1993. The nutrient agar plates containing an inoculum size of 106 cfu/ml for bacteria and 2 × 105 spores for fungi on Saboraud glucose agar plates were used (Mandal, 2000). Previously prepared extract impregnated disc (6mm in diameter) at the concentrations of 200 μg/ml for bacterial and 2000 μg/ml for fungal strains were placed aseptically on sensitivity plates with appropriate controls. Ciprofloxacin (200 μg/ml) and griseofulvin (200 μg/ml) were used as standard antibacterial and antifungal antibiotics respectively. Plates were incubated at 37°C for 24 hours for bacteria and 30°C for 72 hours for fungal spores (Mandal, 2000). Sensitivity was recorded by measuring the clean zone of growth inhibition on agar surface around the disc.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal concentration (MFC)
MIC was determined by tube dilution method for each of the test organism in triplicates (Doughari, 2006). To 0.5 ml of varying concentrations of the extracts (0 – 200 μg/ml for bacterial strains and 0 - 2000 μg/ml for fungal strains), 2ml of nutrient broth was added and then a loopful of test organism previously diluted to 0.5 McFarland turbidity standard for (Bacterial isolates) and 106 cfu/ml (for fungal strains) was introduced to the tubes. The procedures were repeated on the test organisms using standard antibiotics ciprofloxacin (for bacteria) and griseofulvin (for fungi). A tube containing nutrient broth only seeded with the test organisms served as control. Tubes containing bacterial cultures were then incubated at 37°C for 24 hours for bacteria and 30°C for 72 hours for fungal spores. After incubation the tubes were examined for microbial growth by observing the turbidity. To determine the MBC and MFC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar (for bacteria) and Saboraud glucose agar (for fungi) by streaking. Plates inoculated with bacteria and fungi were then incubated at 37°C for 24 hours and 30°C for 72 hours respectively. After incubation the concentration at which no visible growth was seen was noted as MBC (for bacteria) and MFC (For fungi).

3. RESULTS
The preliminary phytochemical analysis of all the four extracts revealed the presence of alkaloids, steroids, oils, fats, carbohydrates, proteins, flavonoids,
terpinoids, saponins and phenolics. The in vitro antimicrobial activity of PEE, CE, EE and DWE is presented in table 1. The EE has showed maximum zone of inhibition of 14.02 mm on against *Staphylococcus aureus*. This was followed by *Salmonella typhi* (12.39 mm), *Pseudomonas aeruginosa* (11.68 mm), *Bacillus subtilis* (11.21 mm) and *Vibrio cholera* (10.43 mm). However, DWE showed the maximum zone of inhibition of 12.01 mm against *Pseudomonas aeruginosa*. This was followed by *Staphylococcus aureus* (11.24 mm), *Bacillus subtilis* (10.89 mm), *Salmonella typhi* (10.71 mm) and *Vibrio cholera* (7.99 mm). The PEE exhibited moderate antibacterial activity while EE the lowest. Activities of various extracts were found to be comparable to that of standard antimicrobial agent ciprofloxacin. The data presented in table 1 also indicate that the various extracts of *Launaea pinnatifida* leaves not only possess antibacterial but also antifungal activity. In this case also it was the EE and DWE which exhibited the highest antifungal activity with the maximum zone of inhibition of 12.08 mm against *Aspergillus niger* and 11.64 mm against *Cladosporium*, respectively. The PEE exhibited the moderate activity while CE showed the lowest activities of various extracts were found to be comparable to that of standard antifungal agent griseofulvin.

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and minimum fungal concentration (MFC) is presented in table 2. EE showed highest sensitivity against *Pseudomonas aeruginosa* recording 10 μg/ml of both MIC and MBC. This was followed by *Staphylococcus aureus* with 10 μg/ml MIC and 25 μg/ml MBC and *Salmonella typhi* with 50 μg/ml for both MIC and MBC. However, *Bacillus subtilis* and *Vibrio cholera* were found to be moderately sensitive. DWE showed sensitivity against both *Staphylococcus aureus* and *Salmonella typhi* with 50 μg/ml MIC and 75 μg/ml MBC and moderate sensitive against *Pseudomonas aeruginosa* with 100μg/ml MIC and 175 μg/ml MBC. *Vibrio cholera* and *Bacillus subtilis* showed least sensitivity of 150μg/ml MIC and resistant to DWE with 200 μg/ml and above in case of MBC. The PEE exhibited moderate sensitivity of 100 μg/ml MIC and MBC against *Staphylococcus aureus* and 100 μg/ml MIC and 125μg/ml MBC was seen in case of *Salmonella typhi*. Least sensitivity was found against *Vibrio cholera* of 175μg/ml MIC and MBC while *Pseudomonas aeruginosa* was resistant showing 200 μg/ml MIC and MBC. The CE showed almost the moderate sensitivity of 100 μg/ml MIC and MBC against *Staphylococcus aureus* and 150 μg/ml of MIC and MBC against *Salmonella typhi*. Resistance was observed in both *Vibrio cholera* and *Pseudomonas aeruginosa* (MIC- 200 μg/ml and MBC-200 μg/ml). *Bacillus subtilis* was resistant for both PEE and CE showing 200 μg/ml MIC and MBC. The MIC and MFC of EE were found to be highest sensitive against *Aspergillus niger* and *Aspergillus flavus* showing 200 μg/ml MIC and MFC. Whereas *Aspergillus terreus* (400 μg/ml MIC and MFC), *Cladosporium* (800 μg/ml MIC and MFC) and *Penicillium notatum* (600 μg/ml MIC and 800 μg/ml MBC) exhibited moderate sensitivity. The DWE showed moderate sensitivity against *Aspergillus niger* showing 400 μg/ml MIC and 600 μg/ml MFC while *Aspergillus flavus* showed 600 μg/ml MIC and 800 μg/ml MFC respectively. The MIC and MFC values of 1000, 1200, 1400 and 1500 μg/ml exhibited by different extracts signifies the moderate sensitivity while the extracts that showed the MIC and MFC values of 1600, 1800 and 2000 μg/ml is the less or least sensitive against the fungi.

**DISCUSSION**

Herbal medicines play a pivotal role in treatment of pathogenic diseases which has been gaining the multiple drug resistance even after launching higher antibiotics. Plants have been used since ages for its effective source as antimicrobial agents. WHO has asked that countries should interact with the traditional medicine to identify and exploiting the aspects of providing the safe and effective remedies for alignments of both microbial and non-microbial origins (technical report series 1978). The antimicrobial activities of various plants have been reported by many researchers (Shariff, 2001). Phytoconstituents present in the plants namely alkaloids, flavonoids, triterpenoids, tannins etc are rendering greater opportunity for expansion of modern chemotherapies against wide range of microorganisms (Lutterodt, 1999).
In present study a variety of Gram positive, Gram negative bacteria and fungal strains were subjected for screening of antimicrobial effect of various extracts of the plant to perceive the antimicrobial spectrum as well as to authenticate the ethnomedicinal claims. The result of this study clearly signifies that the different extract of *Launaea pinnatifida* leaves have a varied antimicrobial activity against the tested organisms. Amongst them the EE exhibited the significant inhibitory effect followed by DWE which showed both the high and moderate susceptibility of the pathogens. PEE and the CE showed the moderate susceptibility as well as resistance towards some organisms. The MIC values of this active plant extract in this study were lower than MBC values suggesting that the plant extracts were bacteriostatic at lower concentration and bactericidal at higher concentration. The EE exerted greater activity than the corresponding extracts at same concentration. These observations may attribute to two reasons; firstly the nature of the biologically active components (alkaloids, flavonoids, saponins, coumarins, anthraquinone and tannins) which could be enhanced in ethanol. It has been documented that these bioactives are well known for their antimicrobial activity (Tsheshe, 1970). Secondly the stronger extraction capacity of ethanol could have produced greater bioactive constituents as detected in the phytochemical analysis, which may be responsible for antimicrobial activity. This study not only shows the scientific basis for the therapeutic uses of traditional plants but also confirms the ethnomedicinal claims of the plant.

### 4. CONCLUSION

The results of this investigation reveals that the ethanolic extract of *Launaea pinnatifida* leaves has exerted highest potential antimicrobial activity followed by the distilled water extract. The differentiating activities against these varieties of pathogens will encourage a novel broad spectrum antimicrobial formulation in future to combat the multi drug resistant pathogens. Further research is under progress on establishing the potential antimicrobial agents from the isolated and purified compounds of this plant.

### Table 1: Preliminary antimicrobial activity of various leaves extracts of *Launaea pinnatifida* Cass leaves

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Zone of inhibition diameters in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PEE (200 μg/ml)</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>10.2 ± 0.03</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>7.11 ± 0.17</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>6.12 ± 0.01</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>6.76 ± 0.01</td>
</tr>
<tr>
<td>Penicillium brevicompactum</td>
<td>7.34 ± 0.07</td>
</tr>
</tbody>
</table>

**PEE-** Petroleum ether extract, **CE-** Chloroform extract, **EE-** Ethanolic extract, **DWE-** Distilled water extract. All values are expressed as mean ± S.E.M of three replications.

### Table 2: Minimum inhibitory concentration (MIC) of various leaves extracts of *Launaea pinnatifida* Cass leaves

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>MIC</th>
<th>MBC</th>
<th>MIC</th>
<th>MBC</th>
<th>MIC</th>
<th>MBC</th>
<th>DWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>100</td>
<td>100</td>
<td>150</td>
<td>150</td>
<td>10</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>100</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>100</td>
<td>125</td>
<td>104</td>
<td>104</td>
<td>50</td>
<td>50</td>
<td>50</td>
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<tr>
<td>Penicillium brevicompactum</td>
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<td>175</td>
<td>200</td>
<td>200</td>
<td>100</td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

Mean values from three replicates are recorded. **MIC-** Minimum Inhibitory Concentration, **MBC-** Minimum Bactericidal Concentration, **MFC-** Minimum fungicidal Concentration.
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