Evaluation of antibacterial activity of certain plant extracts against some bacterial strains

Khushbu Verma¹, Archana Kumari², Vinay Sharma¹ and *Afroz Alam¹

¹Department of Bioscience and Biotechnology, Banasthali University, Banasthali-304022, Rajasthan, India, ²Environmental and Industrial Biotechnology Division, The Energy and Resources Institute (TERI), New Delhi, India- 110 003
*Corresponding author’s email: afrozalamsafvi@gmail.com

Abstract

In the present study, methanolic and ethanolic extracts of three plant species namely Lantana camara L., Aegle marmelos (L.) Corr. and Emblica officinalis L. were evaluated for their antibacterial activity against four bacterial strains namely Bacillus subtilis Cohn, Bacillus cereus Farkland and Farkland, Micrococcus luteus (Schr.) Cohn and Escherichia coli (Mig.) Castellani and Chalmers. Two concentrations of extracts viz. 5 and 10% of all plants were used to check their antibacterial activity. Both ethanol and methanol extracts showed considerable antimicrobial potential against given microbes however, ethanol extract was found more effective than the methanol extract. E. officinalis had more antimicrobial activity than the other two plants extracts. Both Ethanolic and methanolic extract s of E. officinalis exhibited increased antibacterial activity around 1.8 folds (B. cereus), 1.5-2.0 folds (M. luteus) and 1.8 -2.0 folds (E. coli) as compared to control. In case of B. subtilis, only methanolic extract of E. officinalis showed around 2.0- 2.5 folds increased antibacterial activity.

Keywords: Aegle marmelos, antibacterial activity, Emblica officinalis, Escherichia coli, Lantana camara, Micrococcus luteus.

Introduction

Pathogenic bacterial strains have always been considered as a major cause of diseases in humans. Although pharmaceutical companies have formed many new antibiotics, resistance to these antibiotics (Adwan and Mhanna, 2008), but Multi-drug resistant (MDR) bacterial problem is gradually more limiting the efficiency of current antibiotics and considerably making treatment failure (Hancock, 2005). This issue has now become a global concern. Due to the increased resistance to antibiotics, there is a serious need to extend new antimicrobial mediators. Plants have long been investigated as the potential source of new mediator.

Plants contain many compounds which exhibit antimicrobial activity against harmful microbes (Srivastava et al., 1996). The plant kingdom has been the best source of remedies for curing a variety of disease and pain caused by microbes. This is why medicinal plants have played a key role in the worldwide maintenance of health. Natural products of higher plants are an important source of therapeutic agents; therefore, many research groups are currently screening the different biological activities of plants and antimicrobial activity is an important one of biological activities (Satyavati et al., 1976). The search for plants with antimicrobial activity has grown in importance in recent years, due to a growing about an increase in the rate of infection caused by antibiotic-resistant microorganisms (Sharma et al., 2009). Bacterial strains Bacillus subtilis, Bacillus cereus, Micrococcus luteus (Gram-positive) and E. coli (Gram negative) are very harmful microbes. These bacterial strains play a role as pathogen to human health, excluding B. subtilis. These bacterial strains cause many infections. But B. subtilis only contaminate the food. To control these microbes many pesticides like antibiotics etc. were used but caused several harmful effects on human health and the environment. To avoid these affects plants used as antimicrobial agent to control these harmful microbes (Nair et al., 2005; Khan and Khar, 2015). In the present study, antimicrobial activity of three plant species namely L. camara, A. marmelos and E. officinalis were evaluated against bacterial strains viz. B. subtilis, B. cereus, M. luteus and E. coli.

Material and Methods

Preparation of microbial strain for experiment

Bacterial cultures were collected from the Department of Bioscience and Biotechnology,
Banasthali Vidyapith, India and were revived by inoculating the flask containing the Nutrient Broth medium \((B. subtilis, B. cereus)\) and Luria Bertani broth medium \((E. coli, M. luteus)\). This was done under aseptic conditions under Laminar Air Flow. These flasks were incubated in the incubator shaker \((37 ^{\circ}C)\) for 24 h. Turbidity observed and this indicates the presence of bacterial strains. Revived culture strains were stored in a cold room to inhibit their over growth.

**Extraction of plants**

Leaves of \(L. \) camara, \(A. \) marmelos, and \(E. \) officinalis were collected from Krishi Vigyan Kendra, Banasthali Vidyapith, Rajasthan. For antimicrobial assays, extraction of plants was done with the help of organic solvents \((60\% \) ethanol, methanol). Leaves of all plants were dried for a week. Dried leaves were ground with the help of electric grinder. For extraction of plants, 10gm of each plant leaves powder was dissolved in 100 mL of each solvent. The extract was filtered and stored at 4 \(^{\circ}C\). 5\% and 10\% concentration of all the three plants extracts were made from the filtrates. Plant extracts with these concentrations were applied against all bacteria strains.

**Phytochemical screening**

The extracts of all the three plants were subjected to qualitative analysis for phytochemicals. Carbohydrates were tested using Molisch’s test, and proteins were tested by Xanthoproteic test \((Sadasivam \) and Manickam, 1996).

Flavonoids were tested by Shinoda reagent test \((Trease \) and Evans, 1989). In this test, 1-2 fragments of metallic magnesium were mixed with 3-4 mL of extract, and 0.5 mL of concentrated HCl was added to it. The color change was recorded after 5 min incubation.

Terpenoids were screened by Salkowski test \((Ayoola et al., 2008)\). 2ml of extract was mixed in 2 mL chloroform, followed by the careful addition of 1 mL conc. \(H_2SO_4\) in order to form a distinct layer with reddish brown coloration at the interface.

**Antibacterial bioassays**

Antibacterial assays were done by the disc diffusion method. In this method, sterile discs were dipped in 5\% and 10\% of ethanol \((60\%)\) and methanol extract of each plant for half an hour. NA \((Nutrient \) Agar medium) and LB \((Luria \) Bertani agar medium) was prepared and poured in Petri plates. 20 \(\mu\)L of bacteria culture poured and spread on media. Immediately the sterile discs of both plant extract concentrations were placed on the surface of NA and LB dispersion plates inoculated with bacteria culture. Blank sterile discs were used negative control and streptomycin antibiotic discs were used as positive control. For bacteria, plates were incubated at 36 \(^{\circ}C\) for 24 h. Inhibition zones were recorded as diameter of growth free zones, including the diameter of disc, at the incubation period.

**Statistical analysis**

All experiments were performed in triplicates. Values in the text and figures indicate mean values \(\pm \) SD. t-test was used for statistical study of differences among control and treatments and the level of significance was \(P \leq 0.05\).

**Results and Discussion**

Phytochemical screening for \(L. \) camara, \(A. \) marmelos and \(E. \) officinalis leaves extract exposed the presence of flavonoid, terpenoids and carbohydrate compounds. In the present study qualitative phytochemical analysis of the different solvent extracts such as methanol and ethanol of the leaves in \(L. \) camara, \(A. \) marmelos and \(E. \) officinalis indicated the presence of these secondary metabolites \(Table \) 1).

In case of \(B. \) cereus, methanol extract of \(E. \) officinalis showed significant \((P \leq 0.05)\) inhibition with around 1.8 folds against this strain at both 5 and 10\% concentration in comparison to control; while ethanol extract of all the three plants at both concentration confirmed significant antibacterial activity \(Fig. \) 1). On the other hand, only \(E. \) officinalis methanol extract caused 2.0–2.5 folds significant \((P \leq 0.05)\) inhibition against \(B. \) subtilis in comparison to control; although ethanol extract of \(L. \) camara, \(A. \) marmelos confirmed significant \((P \leq 0.05)\) 1.5–1.8 and 1.4–1.8 folds antibacterial activity, respectively, against \(B. \) subtilis \(Fig. \) 2).

In case of \(M. \) luteus, methanol extract of both \(A. \) marmelos and \(E. \) officinalis exhibited around 1.6–2.0 folds increased antibacterial activity \((P \leq 0.05)\) against this strain while 10\% ethanol extract of \(A. \) marmelos and 5, 10\% ethanol extract of \(L. \) camara showed significant inhibition \(Fig. \) 3). Methanol and ethanol extract of both \(E. \) officinalis and \(L. \) camara exhibited 1.8-20 folds antibacterial activity against \(E. \) coli, although \(A. \) marmelos showed no inhibition \(Fig. \) 4).

Previously, there are many reports about antibacterial activity of plants against many pathogenic bacteria \((Jinga et al., 2005; Saeed and Tariq, 2007)\). Jinga et al. \((2005)\) observed antibacterial activity of \(E. \) officinalis against
Evaluation of antibacterial activity of certain plant extracts

Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Bacillus cereus, Alcaligenes faecalis and Salmonella typhimurium. This plant showed strong activity against all the tested bacterial strains. Recently, some more reports have suggested antibacterial activity of E. officinalis (Javale and Sabnis, 2010; Hossain et al., 2012; Patil et al., 2012; Philip et al., 2012; Usha et al., 2012). From the present study and earlier reports, it has been shown that E. officinalis plant has compounds that show antimicrobial activity.

About L. camara, some reports suggested its antimicrobial role against microbes (Juliani et al. 2002; Kasali et al., 2002; Rajakaruna et al., 2002). Ganjawala et al. (2009) also observed antimicrobial activity of L. camara where leaf and flower ethyle acetate extracts exhibited considerable inhibition zones from 10–21 and 9–15 mm, respectively. Some recent reports also support the antibacterial capacity of this plant (Saraf et al., 2011; Agrawal et al., 2012). In the present study, ethanol and methanol extracts of L. camara also showed antimicrobial activity against microbes. Many reports have revealed the presence of the presence of terpenoids, steroids, and alkaloids chemical compounds (Sharma and Sharma, 1989; Siddiqui et al., 1995) which play important role in antimicrobial activity.

In this study, A. marmelos ethanol extract was more effective than methanol extract against microbes at 5% and 10% concentrations. A. marmelos have compounds that show antimicrobial activity. Dabur et al. (2007) also observed antimicrobial activity of this plant by micro broth dilution assay in which this plant methanol extract showed antimicrobial activity in range of 75–1200 µg mL⁻¹. Some recent reports have suggested its antibacterial role against many bacterial culture (Kothari et al., 2011; Pandey and Mishra, 2011).

From this study, it is concluded that ethanol and methanol extracts of all the plants showed antimicrobial potential against given microbes. However, ethanol extracts were found more effective than the methanol extracts against the selected bacterial strains. Among all selected plants, ethanol extract of E. officinalis showed maximum antimicrobial potential against all the bacterial strains. It was reported approximately 1.8 folds in case of B. cereus, 1.5–2.0 folds against M. luteus and for E. coli it was 1.8–2.0 folds as compared to control. However, in case of B. subtilis, methanol extract of E. officinalis showed remarkable and antibacterial activity of about 2.0–2.5 folds. On the basis of these observations, it can be concluded that both ethanol and methanol extracts of locally grown E. officinalis have significant antimicrobial properties and can be used as a controlling agent against these bacterial strains.

**Acknowledgement**

The authors are grateful to Professor Aditya Shastri, Vice Chancellor, Banasthali University, Rajasthan, India for his kind support to this research work.

**References**


**Table 1:** Qualitative phytochemical analysis of certain plant extracts

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th><em>Lantana camara</em></th>
<th><em>Emblica officinalis</em></th>
<th><em>Aegle marmelos</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol extract</td>
<td>Methanol extract</td>
<td>Ethanol extract</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpinoid</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

**Fig. 1:** Effect of ethanolic (a) and methanolic (b) plant extracts on inhibition zone of *B. cerus*.
Evaluation of antibacterial activity of certain plant extracts

Fig. 2: Effect of ethanolic (a) and methanolic (b) plant extracts on inhibition zone of *B. subtilis*.

Fig. 3: Effect of ethanolic (a) and methanolic (b) plant extracts on inhibition zone of *M. luteus*.
Fig. 4: Effect of ethanolic (a) and methanolic (b) plant extracts on inhibition zone of *E. coli*.


