Mycopath (2015) 13(1): 13-19 **Research Article**

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

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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 cerus* 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 extracts of *E. officinalis* exhibited increased antibacterial activity around 1.8 folds (*B. cerus*), 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: A*egle 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 cerus*, *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. subtlis* 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. cerus*, *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 ^{o}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 ºC. 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 µL 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 ºC 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 ± 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. cerus*, 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

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. cerus*, 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.

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Table 1: Qualitative phytochemical analysis of certain plant extracts

Phytochemicals	*Lantana camara*		*Emblica officinalis*		*Aegle marmelos*	
	Ethanol extract	Methanol extract	Ethanol extract	Methanol extract	Ethanol extract	Methanol extract
Flavonoid	+	+	+	+	+	+
Terpinoid	+	–	+	+	+	–
Carbohydrate	+	–	+	–	–	–

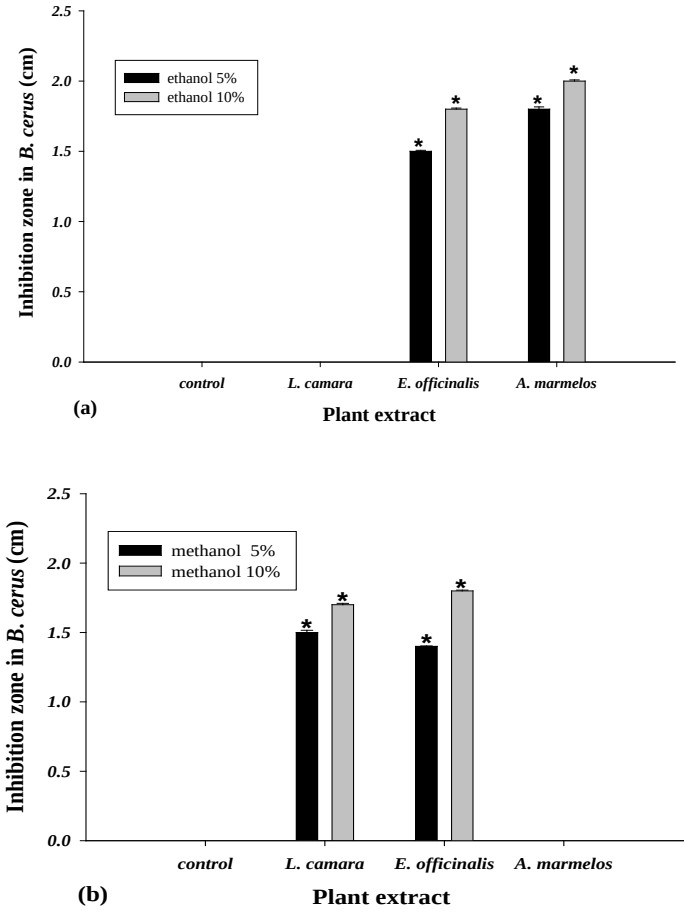

Fig. 1: Effect of ethanolic (a) and methanolic (b) plant extracts on inhibition zone of *B. cerus*.

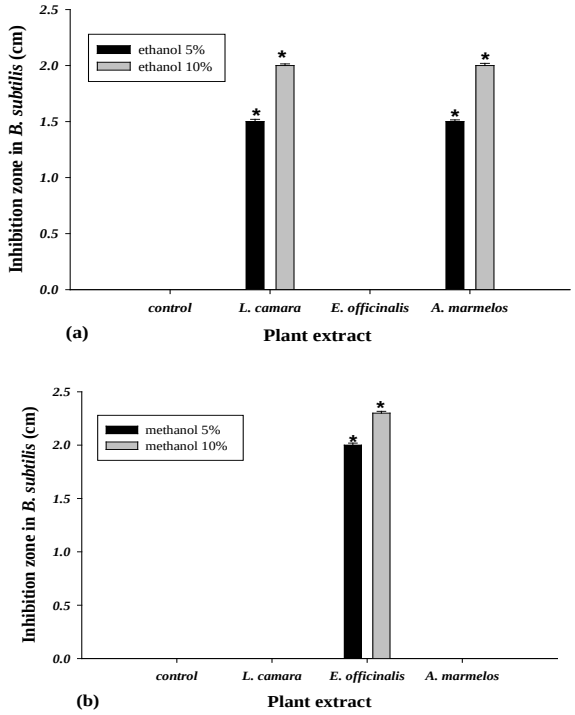

Fig. 2: Effect of ethanolic (a) and methanolic (b) plant extracts on inhibition zone of *B. subtilis*.

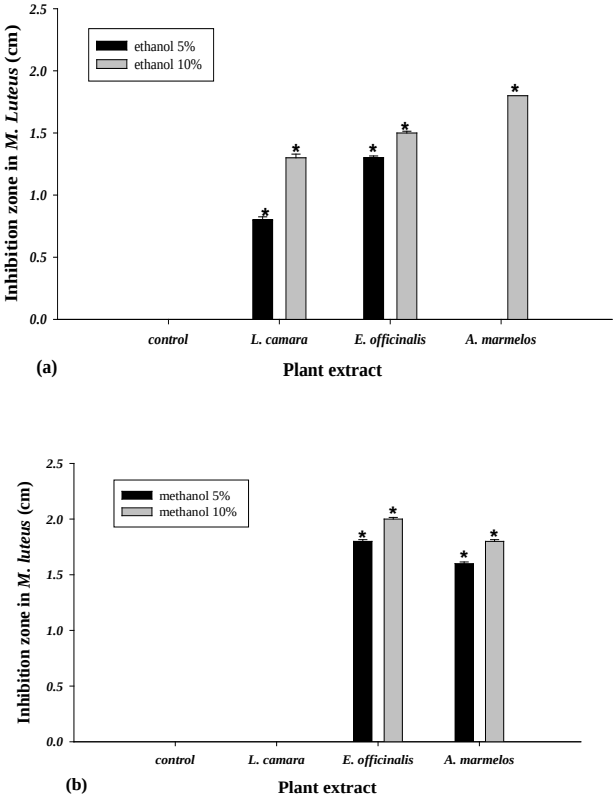

Fig. 3: Effect of ethanolic (a) and methanolic (b) plant extracts on inhibition zone of *M. luteus*.

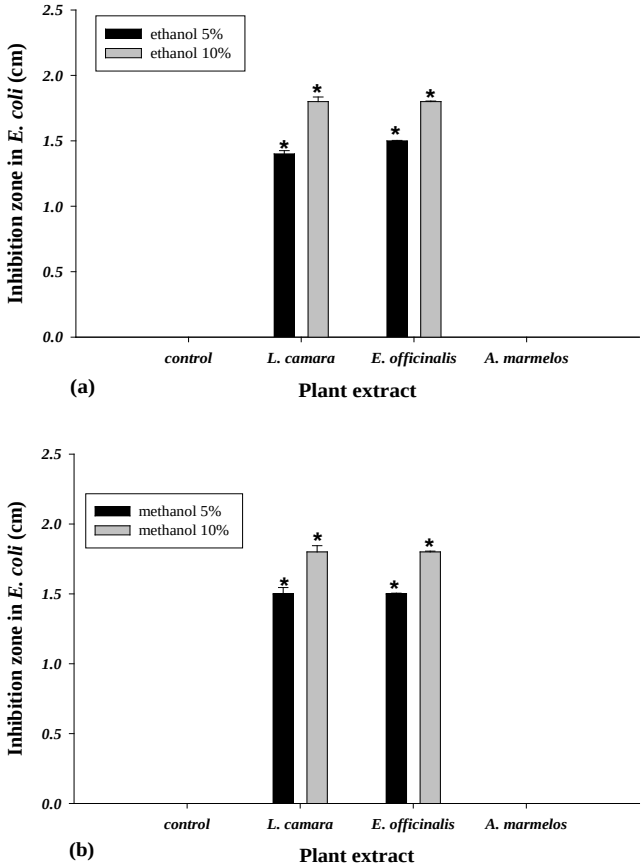

Fig. 4: Effect of ethanolic (a) and methanolic (b) plant extracts on inhibition zone of *E. coli*.

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