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Original Research Article

Antimicrobial Activity of Ethanolic Leaf Extracts of Selective Medicinal Plants against Food-borne Pathogenic Bacteria

T. Sivakami[1] and M. Jayakumar[2]*

[1]*Department of Biochemistry, Directorate of Distance Education, Vinayaka Missions University, Ariyanoor, Salem- 636 308, Tamil Nadu, India*
[2]*Department of Chemical Engineering, Institute of Technology, Haramaya University, Dire Dawa, Ethiopia*

Corresponding author.

Abstract

In the present study, ethanolic leaf extracts of *Abrus precatorius* L., *Barleria buxifolia* L., *Blepharis maderaspatensis* (L.) Heyne ex Roth. and *Sphaeranthus indicus* L. were tested for their antibacterial activity against food-borne pathogenic bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* O157: H7, *Salmonella enteritidis* and *Listeria monocytogenes* using disc diffusion technique. Ciprofloxacin (20 µg/disc) was used as standard control. The test concentration of each extract was set as 0, 125, 250, 500 and 1000 ppm. The results showed a higher zone of inhibition (ZI) of2.2 cm in 1000 ppm concentration of leaf extract of *Sphaeranthus indicus* against *Listeria monocytogenes* followed by *Abrus precatorius* against *Bacillus cereus* (ZI=2.1 cm)>*Blepharis maderaspatensis* against *Salmonella enteritidis* (ZI=2.0)> *Barleria buxifolia* against *Salmonella enteritidis* (ZI=1.7cm).

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Introduction

Medicinal plants are the reservoirs of medicines for different ailments, especially for the treatments associated with microbial pathogens. Now-a-days, growing concern is given to the food borne pathogenic bacteria because of their easy spread and antibiotic resistance. The results of the study conducted by Hao Van et al. (2007) confirmed that the role of raw food as a reservoir of antibiotic resistance bacteria that contained a pool of mobile genetic elements, which are ready to disseminate antibiotic resistance genes to other human pathogens and so constitute a problem for human health. Diarrheagenic *Escherichia coli* is considered world-wide as a common pathogenic bacteria of symptomatic and persistent diarrhea (Kaur et al., 2010). The resistance of food-borne pathogenic bacteria has shown considerable increase due to the use of antibiotics in several routes including the animals of human food source.

Medicinal plants are major source of drugs for several microbial diseases including food-borne pathogenic bacteria. The resistance pattern of food-borne pathogenic bacteria to synthetic antimicrobials may be overcome by utilizing medicinal plants which serve us an alternate source of natural way of remedy. Several studies conducted world-wide clearly indicated that the medicinal plants in raw or compounded form or extracts of different parts may serve as an excellent source of

antimicrobial agents in controlling varieties of microbial pathogens. Karmegam et al. (2008 and 2012) reported that the medicinal plant extracts in crude form alone and in combined form possesses the potential of inhibiting bacteria, especially food-borne diarrheagenic bacteria. Indu et al. (2006) reported that the antibacterial activity of extracts of south Indian spices, *Allium sativum* (garlic), *Myristica fragrans* (nutmeg), *Zingiber officinale* (ginger), *Allium cepa* (onion) and *Piper nigrum* (pepper) had 20 different serogroups of *Escherichia coli*, 8 serotypes of *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila.* Similarly, spices and herbs are reported to possess antibacterial effects against bacteria isolated from frozen meat (Akrayi, 2014). Considering the above facts, the present study has been aimed to investigate the antibacterial activity of leaf extracts of selected medicinal plants against food-borne pathogenic bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* O157: H7, *Salmonella enteritidis* and *Listeria monocytogenes*.

Materials and methods

The leaves *Abrus precatorius* L. (Fabaceae), *Barleria buxifolia* L. (Acanthaceae), *Blepharis maderaspatensis* (L.) Heyne *ex* Roth. (Acanthaceae) and *Sphaeranthus indicus* L. (Asteraceae) (Fig. 1) were collected in Salem district, Tamil Nadu, India and the identification was confirmed using standard local floras (Gamble and Fischer, 1957; Matthews, 1983). The leaves and fruits collected were transported to the laboratory for further processing.

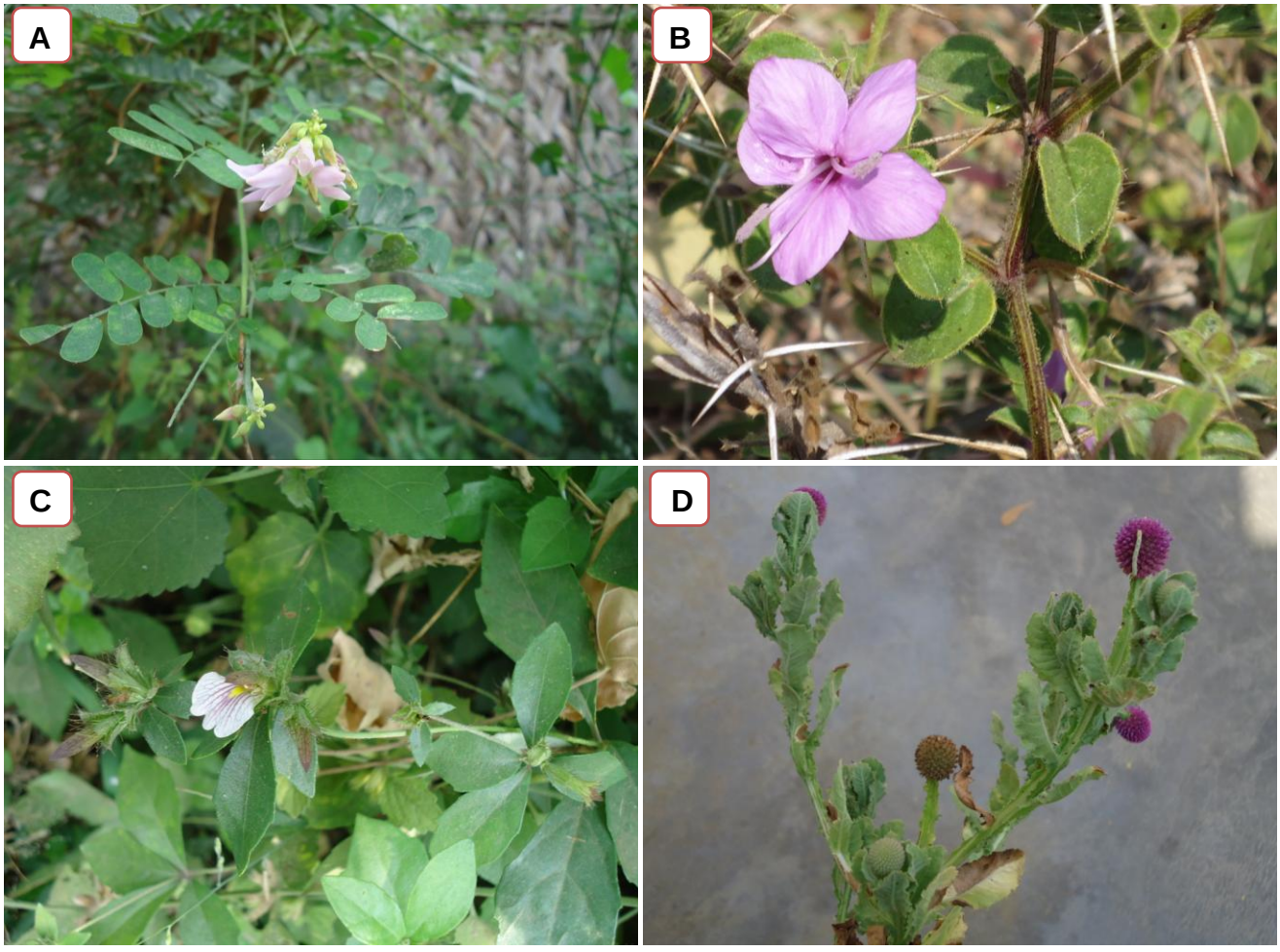

Fig. 1: Medicinal plants used in the study. (A) *Abrus precatorius*, (B) *Barleria buxifolia*, (C) *Blepharis maderaspatensis* and (D) *Sphaeranthus indicus*.

The cold extraction procedure was used for extracting leaves with solvents as per the procedure given below (Prakash and Karmegam, 2012; Vigneshwari et al., 2014). The leaves collected were individually washed with tap water, blotted with filter paper and spread over news paper for air drying under shade. After complete

dryness, the leaves and fruits were powdered using a mixer grinder. A known quantity of the powder (100 g) of each plant was taken in a 250 ml conical flask and added with 100-200 ml of ethanol individually for leaves and fruits. The solvent-powder mixtures were kept at room temperature for 48 hrs and rapidly stirred using glass rod every 8 hrs. After 48 hrs, the extract of each plant leaves was filtered through Whatmann No.1 filter paper to exclude the powder/particles. Then each filtrate was kept in beaker on a water bath at 45ºC until the solvent gets evaporated. A greasy final material (crude extract) obtained was transferred to screw cap tubes and stored under refrigerated condition till use.

By using digital electronic balance, 200 mg of each crude extract was carefully taken in a standard measuring flask and 5 ml of ethanol was added to dissolve the extract and 1-2 drops of emulsifier (Triton-X100) was added to completely dissolve the extract. Then it was made up to 200 ml by adding distilled water. This forms the stock solution of 1000 ppm (i.e., 1mg/ml), from which different concentrations of test solutions, 125, 250, 500 and 1000 ppm were prepared and used for antibacterial assay. Disc diffusion method of antibacterial assay was used to test the sensitivity of selected test organisms to the ethanolic extracts adopting the method of Bauer et al. (1966). Each extract (100 µl) was applied to filter paper discs (Whatman No. 1) measuring 6 mm diameter and allowed to dry before being placed on the agar plate.

The test bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* O157: H7, *Salmonella enteritidis* and *Listeria monocytogenes* maintained in the Laboratory of Department of Biotechnology, VMKV Engineering College, Salem, originally obtained from the Microbial Type Culture Collection (MTTC) of Institute of Microbial Technology (IMTECH), Chandigarh were used for the present study. The Petri plates of 100mm diameter with nutrient agar media were swabbed with broth culture of the test bacteria in separate plates by using sterile swab. Over this, prepared antimicrobial discs were placed under aseptic conditions. Three discs of each extract were placed in triangle. Ciprofloxacin (20 µg/disc) was used as standard antibiotic. Also the discs without plant extract were also maintained as control. The plates were then incubated at 37ºC for 24 hrs and the zone of inhibition (ZI) was measured in diameter (cm) around the discs and recorded. The assays were performed with three replicates.

Results and discussion

The antibacterial activity of ethanolic leaf extracts of selected medicinal plants showed concentration dependent activity, i.e., lower antibacterial activity in low concentration and higher activity in higher concentration. The standard antibiotic, ciprofloxacin (20 µg/disc) showed a range of 2.6-3.0 cm zone of inhibition (ZI) against the test bacteria (Table 1).

The leaf extract of *Abrus precatorius* showed a maximum ZI of 2.1 cm in 1000 ppm against *Bacillus cereus* followed by 1.8 cm ZI against *Escherichia coli* O157: H7 and 1.7 cm ZI against *Salmonella enteritidis*. The least antibacterial activity was found in 125 ppm where it did not show any ZI. *Staphylococcus aureus* showed sensitivity only in 1000 ppm concentration (ZI=1.1 cm) (Table 1).

Antibacterial activity of ethanolic leaf extracts of *Barleria buxifolia* was found to the least active among the four different leaf extracts of medicinal plants tested in the present study (Table 2). No ZI or least ZI (0.8-1.2 cm) was recorded in 125, 250 and 500 ppm of extracts of *Barleria buxifolia* against test organisms, and it showed a highest ZI of 1.7 cm against *Salmonella enteritidis* followed by *Bacillus cereus* (ZI=1.3 cm) and *Listeria monocytogenes* (ZI=1.2 cm).

Table 1. Antibacterial activity of ethanolic leaf extracts of *Abrus precatorius* L.

Bacteria tested	Zone of inhibition (cm)[#]					
	Std.*	0 ppm[$]	125 ppm	250 ppm	500 ppm	1000 ppm
Bacillus cereus	2.6	-	-	0.9	1.4	2.1
Staphylococcus aureus	2.8	-	-	-	AD	1.1
Escherichia coli O157: H7	2.7	-	AD	1.3	1.5	1.8
Salmonella enteritidis	3.0	-	-	0.8	1.2	1.7
Listeria monocytogenes	2.9	-	-	AD	0.9	1.2

[#] - Values are mean of three replicates; [$] - Control (without extract); *Std. - Standard antibiotic, Ciprofloxacin (20 µg/disc); AD – Around the disc.

Table 2. Antibacterial activity of ethanolic leaf extracts of *Barleria buxifolia* L.

Bacteria tested	Zone of inhibition (cm)[#]					
	Std.*	0 ppm[$]	125 ppm	250 ppm	500 ppm	1000 ppm
Bacillus cereus	2.6	-	-	AD	0.9	1.3
Staphylococcus aureus	2.8	-	-	-	-	AD
Escherichia coli O157: H7	2.7	-	-	-	-	AD
Salmonella enteritidis	3.0	-	-	0.8	1.2	1.7
Listeria monocytogenes	2.9	-	-	AD	0.8	1.2

[#] - Values are mean of three replicates; [$] - Control (without extract); *Std. - Standard antibiotic, Ciprofloxacin (20 µg/disc); AD – Around the disc.

The highest ZI of 2.0 cm was observed in ethanolic leaf extracts of *Blepharis maderaspatensis* against *Salmonella enteritidis* followed by 1.9 cm against *Listeria moncytogenes*, 1.6 cm against both *Staphylococcus aureus* and *Escherichia coli* O157: H7 and the least activity was found against *Bacillus cereus* (ZI=1.1 cm) (Table 3). In 125 ppm, no activity was found and in 250 ppm very low activity was seen. *Bacillus cereus* showed resistance to 125, 250 and 500 ppm concentration of *Blepharis maderaspatensis*.

The ethanolic leaf extract of *Sphaeranthus indicus* was the only extract which showed some activity against test bacteria, except *Escherichia coli* O157: H7 at 125 ppm concentration (Table 4). The ZI of 0.8, 1.1, 1.4 and 1.7 cm was found in 125, 250, 500 and 1000 ppm concentration of *Sphaeranthus indicus* leaf extracts and it also showed the highest ZI (2.2 cm) against *Listeria moncytogenes.* Similar range of results has been reported in a variety of medicinal plants in single and combined form against food-borne pathogenic bacteria (Karmegam et al., 2008). Kalpana and Prakash (2015) reported that the leaf and fruit extracts of *Capparis sepiaria* with similar trend of results against bacteria. Karmegam et al. (2012) highlighted that the combined extract forms of medicinal plants were effective against bacteria. The present investigation shows that the extracts of *Sphaeranthus indicus* is possessing potential antibacterial activity followed by *Blepharis maderaspatensis* which may be useful for finding out antimicrobial drugs against food-borne pathogenic bacteria after proper screening and testing.

Table 3. Antibacterial activity of ethanolic leaf extracts of *Blepharis maderaspatensis* (L.) Heyne ex Roth.

Bacteria tested	Zone of inhibition (cm)[#]					
	Std.*	0 ppm[$]	125 ppm	250 ppm	500 ppm	1000 ppm
Bacillus cereus	2.6	-	-	-	AD	1.1
Staphylococcus aureus	2.8	-	-	AD	1.0	1.6
Escherichia coli O157: H7	2.7	-	-	AD	1.2	1.6
Salmonella enteritidis	3.0	-	-	0.8	1.1	2.0
Listeria monocytogenes	2.9	-	-	1.0	1.5	1.9

[#] - Values are mean of three replicates; [$] - Control (without extract); *Std. - Standard antibiotic, Ciprofloxacin (20 µg/disc); AD – Around the disc.

Table 4. Antibacterial activity of ethanolic leaf extracts of *Sphaeranthus indicus* L.

Bacteria tested	Zone of inhibition (cm)[#]					
	Std.*	0 ppm[$]	125 ppm	250 ppm	500 ppm	1000 ppm
Bacillus cereus	2.6	-	0.8	1.1	1.4	1.7
Staphylococcus aureus	2.8	-	AD	0.8	1.0	1.2
Escherichia coli O157: H7	2.7	-	-	AD	0.9	1.1
Salmonella enteritidis	3.0	-	0.9	1.2	1.5	1.9
Listeria monocytogenes	2.9	-	AD	1.0	1.6	2.2

[#] - Values are mean of three replicates; [$] - Control (without extract); *Std. - Standard antibiotic, Ciprofloxacin (20 µg/disc); AD – Around the disc.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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