

Antibacterial Potency and Synergistic Effect of Certain Plant Extracts against Food-Borne Diarrheagenic Bacteria

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ABSTRACT

In the present study, aqueous and ethanolic extracts of leaves of six commonly available medicinal plants, *Balanites aegyptiaca* (L.) Del. (Balanitaceae), *Hyptis suaveolens* Poit. (Lamiaceae), *Lawsonia inermis* L. (Labiaceae), *Leucas aspera* L. (Lamiaceae), *Lobelia nicotianaefolia* Roth. ex. Roem. & Schult. (Lobeliaceae) and *Phyllanthus madraspatana* L. (Euphorbiaceae), individually and in combinations were tested in crude form for their antibacterial activity against five different diarrheagenic bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* O157:H7 (enterohemorrhagic *E. coli*, EHEC), *Salmonella enteritidis* and *Listeria monocytogenes*. Ciproflaxacin (20 µg) was used as antimicrobial standard. The highest antimicrobial activity was recorded in both crude aqueous leaf extract (CALE) and crude ethanolic leaf extract (CELE) of *L. nicotianaefolia* when all the extracts were tested individually. The zone of inhibition (IZ) of 2.2 cm against *S. aureus* and *S. enteritidis*, 2.3 cm against *B. cereus* and *L. monocytogenes* were observed in CELE of *L. nicotianaefolia*. CALE and CELE of *H. suaveolens* and *P. madraspatana* when tested individually showed least IZ against test organisms (IZ, 0-1.2 cm). Synergistic activity of CALE and CELE of selected plant leaves, in combination of two, three, four, five and six against test organisms ranged from 0-2.8 cm zone of inhibition. The highest IZ of 2.8 cm was observed against *S. aureus* in CELE combination of *B. aegyptiaca* + *L. nicotianaefolia*. The IZ range of 2.6-2.8 was recorded for standard antibiotic, ciprofloxacin against test organisms. The combined or synergistic activity of CALE and of CELE also showed closer IZ to that of standard antibiotic, against food-borne diarrheagenic bacteria, there is a scope to develop effective combination of antimicrobial agents in purified form.

Keywords: antimicrobial activity, aqueous extracts, diarrheagenic bacteria, medicinal plants, solvent extracts

Abbreviations: CALE, crude aqueous leaf extract; CELE, crude ethanolic leaf extract; EHEC, enterohemorrhagic *E. coli*; IZ, inhibition zone; MH, Muller-Hinton

INTRODUCTION

The concern towards the use of traditional medicine and medicinal plants now-a-days is given much importance in developing countries for the maintenance of good health. The traditionally used rural herbal remedies and folklore remedies have been found to be effective against microorganisms (Karuppusamy *et al.* 2002a; Akinpelu and Onakoya 2006; Koné *et al.* 2007). Moreover, in these societies, herbal remedies have become more popular in the treatment of minor ailments, and also on account of the increasing costs of personal health maintenance. A multitude of plant compounds (often of unreliable purity) is readily available over-the-counter from herbal suppliers and natural-food stores, and self-medication with these substances in common place (Cowan 1999).

Considerable mortality and morbidity has been caused by widely distributed food-borne pathogens such as diarrheagenic serotypes of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella enteritidis* and *Listeria monocytogenes*. There are more than 1.3 billion annual cases of human salmonellosis worldwide with three million deaths (Pang *et al.* 1995; Baumler *et al.* 2000). Among the various diarrheagenic serotypes of *E. coli*, enterohemorrhagic *E. coli* O157:H7 is implicated in a large number of food-borne outbreaks in many parts of the world, including developed nations, and this serotype has low infective dose (Doyle 1991; Mead *et al.* 1999; Gopinathan *et al.* 2005). *L. monocytogenes* has been isolated from various environ-

ments and is reported to cause 25% of all the deaths resulting from food-borne outbreaks in the world annually (CDC 1995). These bacteria have broad host range and have often been isolated from humans with diarrhea.

The epidemiology of food-borne diseases is changing and reports from different parts of the world indicate that strains of resistant food-borne pathogens have emerged as public health problem. The illnesses as a result of eating foods contaminated with bacteria and/or their toxins range from stomach upset to more serious symptoms such as diarrhea, fever, vomiting, abdominal cramps and dehydrations (Slutsker *et al.* 1998; Adak *et al.* 2002). Food-borne diseases associated with *S. enteritidis*, *S. aureus*, *B. cereus* and *E. coli* have been reported in Australia, Canada, Japan, United States, European countries, Nigeria and South Africa and these pathogens and newer food-borne pathogens have shown raised resistance to antimicrobial agents in practical use (Slutsker *et al.* 1998; Akinyemi *et al.* 2000; Farzana and Hameed 2006). In view of resistance patterns of pathogenic bacteria against antimicrobial agents, there is an urgent need for new antimicrobial products that too from plant materials is essential. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Karuppusamy *et al.* 2001a, 2001b; Reddy *et al.* 2001; Karuppusamy *et al.* 2002b; Ateb and Erdourul 2003; Parekh and Chanda 2007; Doughari *et al.* 2008). Studies on traditional medicinal plants have provided the path to the discovery of many effective drugs (Karuppusamy *et al.* 2002a, 2002c). Ample evidence of the

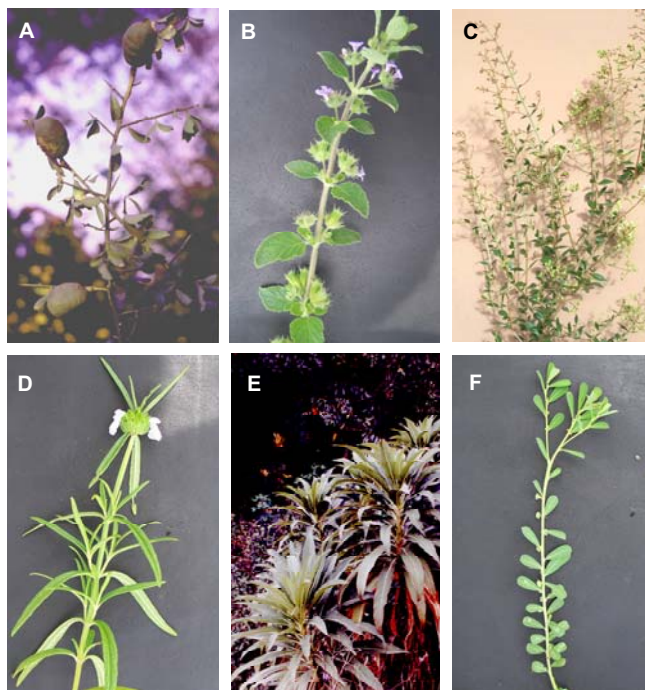


Fig. 1 Plants used in the study. (A) *Balanites aegyptiaca* (L.) Del. (B) *Hyptis suaveolens* Poit. (C) *Lawsonia inermis* L.; (D) *Leucas aspera* L.; (E) *Lobelia nicotianaefolia* Roth. ex. Roem. & Schult.; (F) *Phyllanthus madraspatana* L.

use of individual plant extracts as antibacterial agents are available, but the works on the combined use of extracts of two or more plants is very limited (Prakash *et al.* 2006a, 2006b). Based on above facts, the present study has been carried out to find out an alternative medicine for controlling food-borne diarrheagenic bacteria by using combinations of six different medicinal plant extracts.

MATERIALS AND METHODS

Collection and identification of plants

In the present study, the leaves of six medicinal plants (Family) namely, *Balanites aegyptiaca* (L.) Del. (Balanitaceae), *Hyptis suaveolens* Poit. (Lamiaceae), *Lawsonia inermis* L. (Lathyraceae), *Leucas aspera* L. (Lamiaceae), *Lobelia nicotianaefolia* Roth. ex. Roem. & Schult. (Lobeliaceae) and *Phyllanthus madraspatana* L. (Euphorbiaceae) (Fig. 1) were collected in and around Dindigul, Salem and Kanchipuram Districts, South India and the identification was confirmed using standard local floras (Gamble and Fischer 1957; Matthew 1983).

Preparation of crude leaf extracts

The crude extracts of the leaves of all the six plant species were prepared separately using ethanol (95%) and distilled water as described below.

Solvent extraction

The collected leaves of the plants were immediately transported to the laboratory and individually washed with tap water, blotted with filter paper and spread over news paper for air drying under shade. After complete dryness, the leaves of each plant were powdered using a mixer grinder. A known quantity of leaf powder (50 g) of each plant was taken in a 250 ml conical flask and added with 100 ml of ethanol (95%). The ethanol-leaf powder mixtures were kept at room temperature for 48 hours and rapidly stirred using glass rod every 8 h.

After 48 h, the extract of each plant was filtered through Whatman No. 1 filter paper to exclude the leaf powder. Then each filtrate was concentrated using vacuum evaporator. A greasy final

material (crude ethanolic-leaf extract, CELE) obtained for each plant leaves was transferred to screw cap bottles, labeled and stored under refrigerated (4°C) condition till use.

Aqueous extraction

For aqueous extraction, 10 g of air-dried powder of each plant leaves was placed in 100 ml distilled water and boiled for 6 h. At 2-h intervals, it was filtered through eight layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and concentrated to make the final volume one-fourth of the original volume. A greasy final material (crude aqueous-leaf extract, CALE) obtained for each plant was transferred to screw cap bottles, labeled and stored under refrigerated (4°C) condition until use.

Preparation of stock and test solutions

By using digital electronic balance, 200 mg of each CALE and CELE was carefully taken in a standard measuring flask and 5 ml of ethanol was added to dissolve the CELE and 5 ml of distilled water for CALE respectively. One to two drops of emulsifier (Triton-X100) were added to completely dissolve both CALE and CELE. Then each extract was made up to 200 ml by adding distilled water and stored under refrigerated (4°C) condition till use. This formed the stock solution of 1000 ppm.

Bacterial susceptibility testing

Bacterial culture

Five food-borne pathogenic species, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* O157:H7 (enterohemorrhagic *E. coli*, EHEC), *Salmonella enteritidis* and *Listeria monocytogenes* were used for the bacterial susceptibility test. The organisms were maintained on agar slope at 4°C and sub-cultured for 24 h before use. These organisms were originally obtained from the Microbial Type Culture Collection (MTCC) of Institute of Microbial Technology (IMTECH), Chandigarh, India.

Antibacterial assay

Standardized inoculums of each bacterium, i.e., 1 to 2 × 10⁷ CFU (Colony Forming Units)/ml with 0.5 McFarland standard was introduced onto the surface of sterile Muller-Hinton (MH) agar plates and a sterile glass spreader was used for even distribution of inoculums. A sterile paper disc previously soaked in known concentration of extracts (20 µg/ml/disc) was carefully placed at the centre of the seeded and labeled MH agar. Sterile paper discs containing physiological saline alone was served as control. For each test solution, three replicates were maintained. Ciprofloxacin at 20 µg/disc was used as an antibiotic reference standard. The CALE and CELE test extracts were individually tested at a concentration of 1000 ppm against test organisms. The crude aqueous leaf extracts were mixed in equal proportions in combination of two, three, four, five or six extracts. For comparison, individual plant extracts (CALE & CELE) were also tested for antibacterial activity. The same procedure was followed for the preparation of different combinations of CELE. Whatman No.1 filter paper discs (5 mm diameter) were dipped in each test solution, evaporated to dryness in hot air oven and used for antibacterial assay. The plates were incubated aerobically at 37°C and examined for zone of inhibition after 24 h. Each zone of inhibition was measured with a ruler and compared with the control (Bauer *et al.* 1966).

RESULTS AND DISCUSSION

In this study six commonly available medicinal plants used by traditional medical practitioners in South India were tested against food-borne pathogenic bacteria. The result of antibacterial susceptibility testing showed that all the bacterial pathogens, *S. aureus*, *B. cereus*, *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* were highly susceptible to Ciprofloxacin with average diameter zone of inhibitions of 2.7, 2.8, 2.6, 2.7, and 2.6 cm, respectively (Table 1).

Table 1 Effect of antibacterial reference standard on food-borne diarrheagenic bacteria.

Antibiotic	Concentration ($\mu\text{g}/\text{disc}$)	Test bacteria	Inhibition zone (cm)
Ciprofloxacin	20	<i>Staphylococcus aureus</i>	2.7
		<i>Bacillus cereus</i>	2.8
		<i>Escherichia coli</i> O157:H7	2.6
		<i>Salmonella enteritidis</i>	2.7
		<i>Listeria monocytogenes</i>	2.6

Table 2 Effect of crude aqueous (CALE) and ethanolic extracts (CELE) of selected plant leaves on food-borne diarrheagenic bacteria (24 h). SA= *S. aureus*, BC = *B. cereus*, EC = *E. coli* O157:H7, SE = *S. enteritidis* and LM = *L. monocytogenes*.

Plant species	Inhibition zone ^a (IZ) in cm									
	CALE					CELE				
	SA	BC	EC	SE	LM	SA	BC	EC	SE	LM
<i>Balanites aegyptiaca</i> (L.) Del.	0.9	1.4	0.8	1.0	1.1	1.6	2.0	1.4	1.6	1.7
<i>Hyptis suaveolens</i> Poit.	-	0.7	-	-	0.8	-	1.0	-	-	1.1
<i>Lawsonia inermis</i> L.	0.7	1.1	0.8	0.7	-	1.0	1.4	0.8	0.7	0.8
<i>Leucas aspera</i> L.	0.8	0.8	1.3	0.9	1.2	1.2	1.4	1.7	1.3	1.6
<i>Lobelia nicotianaefolia</i> Roth. ex. Roem. & Schult.	1.5	1.9	1.1	2.0	1.6	2.2	2.3	1.8	2.2	2.3
<i>Phyllanthus madraspatana</i> L.	-	0.8	-	0.9	1.2	-	1.0	0.8	1.0	0.8

^a Values are mean of three replicates.**Table 3** Synergistic activity of aqueous (CALE) and ethanolic extracts (CELE) of selected plant leaves, in combination of two, against food-borne diarrheagenic bacteria (24 h). SA = *S. aureus*, BC = *B. cereus*, EC = *E. coli* O157:H7, SE = *S. enteritidis* and LM = *L. monocytogenes*.

Combination of plant extracts tested ^a	Inhibition zone ^b (IZ) in cm									
	CALE					CELE				
	SA	BC	EC	SE	LM	SA	BC	EC	SE	LM
A+B	0.8	1.2	-	-	1.5	0.7	1.9	-	-	2.0
A+C	1.8	2.1	1.2	1.5	1.2	2.3	2.4	2.0	1.8	2.1
A+D	1.5	1.4	2.0	1.7	2.1	2.4	2.3	2.5	2.3	2.0
A+E	1.7	2.0	2.2	2.3	2.4	2.5	2.2	2.0	2.4	2.6
A+F	-	-	-	0.8	-	-	0.8	-	1.0	1.1
B+C	-	0.8	-	-	-	-	1.1	-	-	0.9
B+D	-	0.8	0.8	-	0.7	-	1.4	1.0	0	1.3
B+E	1.6	2.2	1.4	1.8	2.0	1.3	2.5	1.0	1.8	2.4
B+F	-	0.8	-	-	1.0	-	0.8	-	-	1.2
C+D	1.8	1.6	1.1	0.9	0.8	2.1	2.5	1.8	1.8	2.2
C+E	1.8	2.1	2.0	2.4	1.8	2.5	2.2	2.0	2.5	2.3
C+F	-	1.2	-	1.2	-	-	1.0	0.8	1.5	0.9
D+E	2.1	2.4	2.0	2.6	2.2	2.6	2.4	1.9	1.9	2.5
D+F	-	-	-	0.7	1.5	-	1.2	1.0	0.8	-
E+F	0.9	1.4	-	1.6	0.9	0.9	2.4	1.2	1.9	2.4

^a A = *B. aegyptiaca*, B = *H. suaveolens*, C = *L. inermis*, D = *L. aspera*, E = *L. nicotianaefolia* and F = *P. madraspatana*.^b Values are mean of three replicates.

Leaf extracts, CALE and CELE when tested individually for their antibacterial activity, showed various degrees of activity (**Table 2**). The leaf extracts of *L. nicotianaefolia* showed comparatively a high degree of activity followed by *B. aegyptiaca* and *L. aspera*. The diameter of IZ was more than 2.0 cm for *L. nicotianaefolia* CELE against *S. aureus*, *B. cereus*, *S. enteritidis* and *L. monocytogenes* and 1.8 cm diameter of IZ against EHEC. The lowest antimicrobial activity was shown by CALE and CELE of *H. suaveolens* followed by *P. madraspatana*. Both CALE and CELE of *H. suaveolens* showed no activity against *S. aureus*, *E. coli* O157:H7 and *S. enteritidis*.

The acceptable standard diameter zone of inhibition for sensitive organism for the standard antibiotic ciprofloxacin is >21 mm (NCCLS 1993). However, for the plant extracts, the average zone of inhibitions observed against these food-borne diarrheagenic bacteria ranged from 0.7 to 2.0 cm in aqueous extracts and 0.7 to 2.3 cm in ethanolic extracts. These values fall within the range of resistant and/or intermediate sensitive when compared with control antibiotic. Although, the low values recorded for the plant extracts may be attributed to the fact that the extracts being in crude form, contain very small amounts of bioactive compounds. At the same time, several workers have reported bioactivity of crude extracts of medicinal plants within such range of diameter zone of inhibitions (Olukoya *et al.* 1993; Ogbeche *et al.* 1997; Akinyemi *et al.* 2006). Even drug resistant pathogens are susceptible to plant extracts. For example, as re-

ported by Voravuthikunchai and Mitchell (2008), drug resistant *Helicobacter pylori* was found to be susceptible to seven medicinal plants (out of 24 extracts from 13 kinds of Thai herbs), in which *Punica granatum* and *Quercus infectoria* showed hopeful results. The present study was in conformity with these previous findings.

The antibacterial activities of extracts in combination of two plants showed different degrees of IZ as shown in **Table 3**. The average diameter of >2.0 cm IZ was observed in the following CALE combinations and CELE combinations: *B. aegyptiaca* + *L. nicotianaefolia*, *L. inermis* + *L. nicotianaefolia* and *L. aspera* + *L. nicotianaefolia*. The lowest antibacterial activity was observed in combinations of *B. aegyptiaca* + *H. suaveolens*, *B. aegyptiaca* + *P. madraspatana*, *H. suaveolens* + *L. inermis*, *H. suaveolens* + *L. aspera*, *H. suaveolens* + *P. madraspatana*, *L. inermis* + *P. madraspatana* and *L. aspera* + *P. madraspatana* and rest of the combinations showed moderate antimicrobial activity. An IZ of 2.8 cm was observed in CELE combination of *B. aegyptiaca* + *H. suaveolens* + *L. nicotianaefolia* against *S. aureus* (**Table 4**). CALE in combination of *B. aegyptiaca* + *H. suaveolens* + *L. inermis* had no inhibitory effect over all the bacterial species tested in the study, whereas the CELE of same combination showed 2.4, 2.1, 1.9, 1.5 and 1.1 cm IZ against *B. cereus*, *L. monocytogenes*, *S. enteritidis*, *E. coli* O157:H7 and *S. aureus* respectively. The CALE and CELE combination of *H. suaveolens* + *L. inermis* + *P. madraspatana* individually showed low antimicrobial activity.

Table 4 Synergistic activity of aqueous (CALE) and ethanolic extracts (CELE) of selected plant leaves, in combination of three, against food-borne diarrheagenic bacteria (24 h). SA = *S. aureus*, BC = *B. cereus*, EC = *E. coli* O157:H7, SE = *S. enteritidis* and LM = *L. monocytogenes*.

Combination of plant extracts tested ^a	Inhibition zone ^b (IZ) in cm									
	CALE					CELE				
	SA	BC	EC	SE	LM	SA	BC	EC	SE	LM
A+B+C	-	1.5	-	-	-	0.9	1.5	-	-	1.2
A+B+D	-	-	-	-	-	1.1	2.4	1.5	1.9	2.1
A+B+E	0.8	1.8	1.4	2.2	2.6	2.8	2.4	2.1	2.5	2.0
A+B+F	-	1.2	-	-	0.9	-	1.9	0.8	1.3	1.9
B+C+D	-	1.9	1.1	1.0	-	-	2.4	1.5	0.9	2.6
B+C+E	1.6	2.3	1.5	1.8	1.9	1.8	2.7	1.9	2.1	2.5
B+C+F	-	1.0	-	-	-	-	0.9	-	-	0.9
C+D+E	1.6	2.1	1.8	1.5	1.6	2.3	2.5	2.6	2.1	2.4
C+D+F	-	0.9	-	1.1	0.8	0.9	1.8	1.5	2.1	0.9
D+E+A	1.5	1.8	1.4	2.1	2.4	2.5	2.4	1.9	2.4	2.1
D+E+B	-	1.1	-	-	1.6	0.8	1.7	0.8	0.9	1.4
D+E+F	-	0.9	-	1.0	1.4	-	2.2	2.4	1.8	1.7
E+F+A	1.3	1.9	1.1	1.6	2.0	1.8	2.4	2.1	1.9	2.3
E+F+B	1.4	1.8	-	1.2	1.6	1.1	2.2	1.2	1.7	1.5
E+F+C	1.6	2.0	1.4	1.2	1.8	1.6	2.1	1.5	1.6	1.8
F+A+C	-	1.3	-	1.2	1.5	-	1.7	0.9	1.5	1.6
F+A+D	-	-	-	1.1	1.4	0.9	1.8	1.5	1.8	1.4

^aA = *B. aegyptiaca*, B = *H. saiveolens*, C = *L. inermis*, D = *L. aspera*, E = *L. nicotianaefolia* and F = *P. madraspatana*.

^bValues are mean of three replicates.

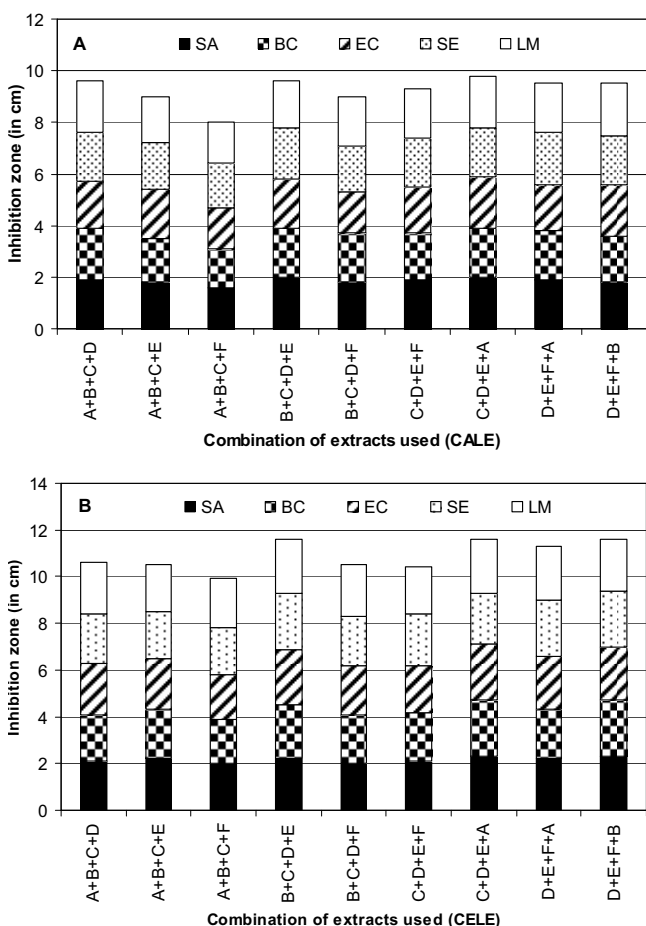


Fig. 2 Synergistic activity of aqueous (CALE) and ethanolic extracts (CELE) of selected plant leaves, in combination of four, against food-borne diarrheagenic bacteria (24 h). (A) CALE; (B) CELE; for abbreviations see legend of Table 4.

The average IZ of 1.9 and 2.2 cm were observed in CALE and CELE combinations of four extracts (Figs. 2A, 2B). A minimum of 1.7 cm and a maximum of 2.4 cm diameter zones of inhibition were resulted in the extracts of four plants. The range of IZ in CALE combinations of five plants recorded was between 1.5 and 2.0 cm and for CELE, it was 1.9 and 2.4 cm (Figs. 3A, 3B). Synergistic activity of selected plant leaves, in combination of six, against food-

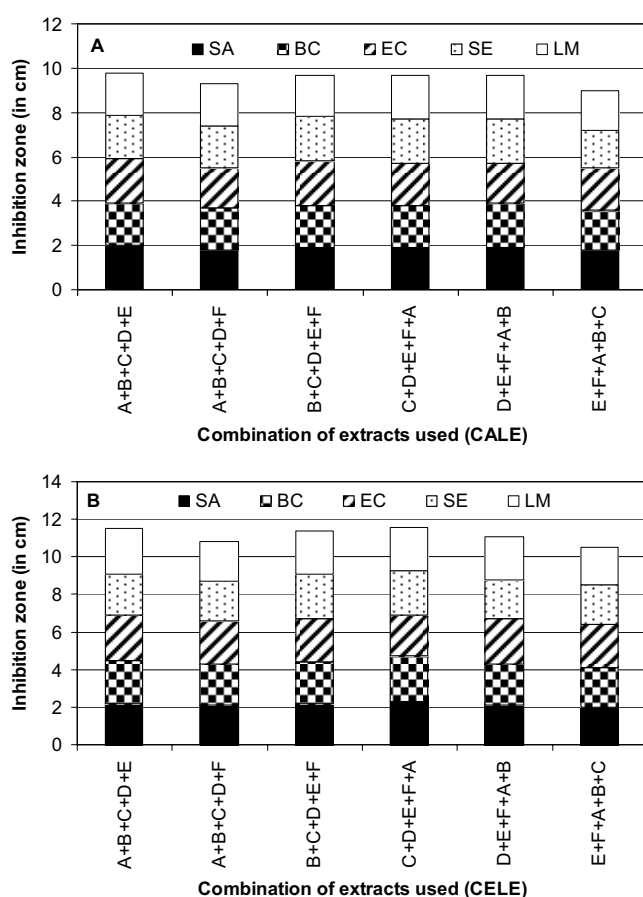


Fig. 3 Synergistic activity of aqueous (CALE) and ethanolic extracts (CELE) of selected plant leaves, in combination of five, against food-borne diarrheagenic bacteria (24 h). (A) CALE; (B) CELE; for abbreviations see legend of Table 4.

borne diarrheagenic bacteria also showed elevated antimicrobial activity and the range of IZ falls between 1.8 and 2.1 cm for CALE and 2.3 and 2.5 cm for CELE (Fig. 4).

The results showed that both aqueous and ethanol extracts in combination of four, five and six plants exerted good antibacterial activity against all the bacterial pathogens, *S. aureus*, *B. cereus*, *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* with CELE exerting more activity. This suggests that these plant extracts, when used tradition-

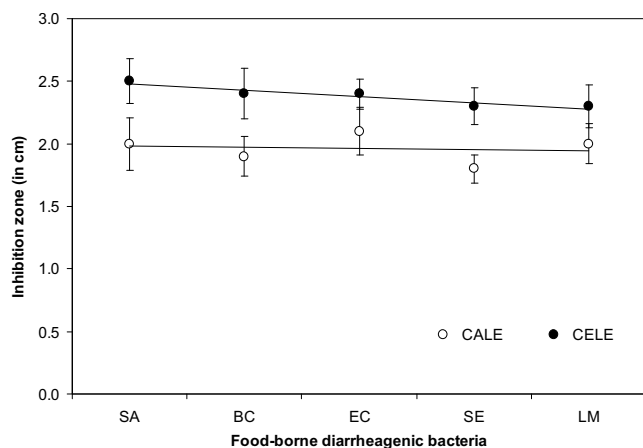


Fig. 4 Synergistic activity of aqueous (CALE) and ethanolic extracts (CELE) of selected plant leaves, in combination of six, against food-borne diarrhegenic bacteria (24 h). Error bars indicate \pm S.D. For abbreviations see legend of Table 4.

ally as antimicrobials inhibit bacterial growth without necessarily killing the bacteria and since most of the traditional preparations lack specific concentrations, this may thus account for the use of large quantity of the extracts by traditional medical practitioners for the treatment. Firstly, the bioactive constituents in these plant extracts may be enhanced in the presence of ethanol. Secondly, the stronger extraction capacity of ethanol may be responsible, such that more active ingredients may be present in the ethanol extracts.

Karuppusamy *et al.* (2002a) reported that 133 medicinal plant species belonging to 66 families were sold as raw drugs by street herbal vendors and they were prescribed for more than 25 ailments. The results on the antimicrobial formulations of these traditional medicinal plants showed that all the formulations were effective against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans* with different zones of inhibition. The hexane extracts and water extracts of *kalimbugal* (ointments) and *podimarundhugal* (medicinal preparations in powder form) showed least antimicrobial activity when compared with ethanol and petroleum-ether extracts. A well-defined antimicrobial activity of ethanol and petroleum-ether extracts of '*kalimbugal*' and '*podimarundhugal*' which were formulated using combinations of 5-10 raw medicinal plants and their parts sold by street herbal vendors in Tamil Nadu, South India. For example, ethanolic extract of '*kayakalimbu*' (wound healing ointments applied externally on wounds) procured from traditional healers *en suite* the combination of fruits (*Aegle marmelos* and *Punica granatum*), bulbs (*Cyperus rotundus*), roots (*Sida cordifolia*), seeds (*Celastrus paniculatus*) and leaves (*Cissampelos pareira*, *Diospyrus microphylla*, *Eclipta prostrata*, *Phylla nodiflora* and *Piper betle*) showed 20.5, 21.2, 18.9, 19.1 and 25.1 mm zone of inhibition against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans*. Similarly, in the present study also ethanolic extracts showed higher activity than aqueous extracts. The enhanced antimicrobial activity could probably be due to one or more components present in different plants and/or plant parts, which synergistically act upon the microbes and excel a good activity index. The present study also falls in line with the results of Karuppusamy *et al.* (2002a).

Prakash *et al.* (2006b) reported that the ethanolic leaf extracts of *Catharanthus roseus*, *Lawsonia inermis* and *Chrysanthemum odoratum* showed least activity against methicillin resistant *Staphylococcus aureus* (MRSA) when used individually. Whereas, the combination of these three plant-extracts exerted a higher activity of 26 mm zone of inhibition followed by *C. roseus* + *L. inermis* (23 mm) and *L. inermis* + *C. odoratum* (20 mm) extract combinations against MRSA. As observed from the results of the present study, extract combinations with *L. nicotianaefolia* showed

good antimicrobial activities. *L. nicotianaefolia* is a tall erect herb with a hollow stem, the leaves are large and look like tobacco and the inflorescence is a long cylindrical raceme with large flowers. All the parts of this plant consists bitter alkaloids, which are used in several medicinal systems (Jain 1983). Presence of bitter alkaloids in the leaves of *L. nicotianaefolia* could be the reason for the effectiveness of the plant. The ecology, habitat and regeneration pattern of this important medicinal plant in Palani Hills, Tamil Nadu, India has been reported by Karuppusamy *et al.* (2001c).

Balanites aegyptiaca (L.) Del. (syn. *B. roxburgii* Planch) is a small tree armed with strong sharp spines, often ending arrested branchlets; ashy-grey foliate, grey bark and yellowish white wood of peculiar structure; fruits – a large fleshy oily one seeded drupe. Flowers small, green, fragrant, in axillary cymes. The zone of inhibition of 30, 24, 15 and 18 mm were recorded in the ethanol extracts of leaves, stem bark, root bark and fruits of *B. aegyptiaca* respectively (Karuppusamy *et al.* 2002b). In the present study also ethanolic extracts of *B. aegyptiaca* leaves in combination exerted synergistic activity against food-borne diarrhegenic bacteria.

Tiwari *et al.* (2005) reported that chloramphenicol and tea extract in combination inhibited the growth of *S. dysenteriae* at 2.5 μ g/ml chloramphenicol (MIC 5 μ g/ml) and 5.094 mg/ml black tea extract (MIC 9.089 mg/ml). Tea extract showed synergistic activity with chloramphenicol and other antibiotics like gentamycin, methicillin and nalidixic acid against test strains. Synergistic microbial growth inhibition by black tea extract and antibiotics could be attributed to the presence of dual binding sites on the bacterial surface for antibiotic and tea extract. Liu (2003) opined that the additive and synergistic effects of phytochemicals in fruit and vegetables are attributed to the complex mixture of phytochemicals present in whole foods. The data obtained by Pereira *et al.* (2007) demonstrated that the use of olive leaves as nutraceuticals may lower the risk of microbial infections, particularly in the intestinal and respiratory tract, mainly due to the protective action provided by its phenolic compounds. The use of leaf extracts is recommended to achieve health benefits due to the additive and synergistic effect of phytochemicals present in whole extract. These studies strongly support the present findings on the synergistic effect of crude extracts of the plants used. In conclusion, there is a possibility of using plant extracts in combinations against food-borne diarrhegenic bacteria as has been observed from the results. There is a scope to use combined ethanolic extracts of *B. aegyptiaca*, *H. sauveolens*, *L. inermis*, *L. aspera*, *L. nicotianaefolia* and *P. madraspatana* against food-borne diarrhegenic bacteria.

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REFERENCES

- Adak GK, Long SM, O'Brien SJ (2002) Trends in indigenous food borne disease and deaths, England and Wales, 1992-2000. *Gut* 51, 832-841
- Akinpelu DA, Onakoya TM (2006) Antimicrobial activities of medicinal plants used in folklore remedies in south-western. *African Journal of Biotechnology* 5, 1078-1081
- Akinyemi KO, Bayagbon C, Oyefolu AOB, Akinside KA, Monigbeyin EA, Coker AO (2000) Antibacterial screening of five indigenous Nigerian medicinal plants against *S. typhi* and *S. paratyphi*. *Journal of Nigerian Infection Control Association* 3, 30-33
- Akinyemi KO, Oluwa OK, Omomigbehin EO (2006) Antimicrobial activity of crude extracts of three medicinal plants used in South-West Nigerian folk medicine on some food borne bacterial pathogens. *African Journal of Traditional, Complementary and Alternative Medicines* 3, 13-22
- Ateb DA, Erdourul OT (2003) Antimicrobial activities of various medicinal and commercial plant extracts. *Turkish Journal of Biology* 27, 157-162
- Bauer AW, Kirby WMN, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clin-*

- nical Pathology 45, 493-496
- Baumler AJ, Hargis BM, Tsolis RM** (2000) Epidemiology: Tracing the origins of *Salmonella* outbreak. *Science* 287, 50-52
- Centre for Disease Control and Preservation** (1995) *Escherichia coli* O157:H7 and *Listeria monocytogenes* outbreak linked to commercially distributed dry cured salami. *Morbidity and Mortality Weekly* 44, 157-160
- Cowan MM** (1999) Plant products as antimicrobial agents. *Clinical Microbiology Review* 12, 564-582
- Doughari JH, El-Mahmood AM, Tyoyina I** (2008) Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L). *African Journal of Pharmacy and Pharmacology* 2, 7-13
- Doyle MP** (1991) *Escherichia coli* O157:H7 and its significance in foods. *International Journal of Food Microbiology* 12, 289-302
- Farzana K, Hameed A** (2006) Resistance pattern of clinical isolates of *Staphylococcus aureus* against five groups of antibiotics. *Journal of Research (Science)* 17, 19-26
- Gamble JS, Fischer CEC** (1957) *Flora of the Presidency of Madras* (Vols I-III), Authority of Secretary of State for India in Council, London, 1389 pp
- Gopinathan R, Kang G, Asokan V, Arunakirinathan N, Vasanthan G** (2005) Evidence of verocytotoxigenic *Escherichia coli* (VTEC) in livestock of Tamil Nadu – an explorative study. *Indian Journal of Animal Science* 75, 1241-1243
- Jain SK** (1983) *Handbook of Medicinal Plants*, National Book Trust, New Delhi, 123 pp
- Karuppusamy S, Karmegam N, Rajasekaran KM** (2001a) Antimicrobial screening of Asclepiadacean medicinal plants of Dindigul District, Tamil Nadu, South India. *Journal of Ecotoxicology and Environmental Monitoring* 11, 47-51
- Karuppusamy S, Rajasekaran KM, Karmegam N** (2001b) Potential alkaloid source from *Alstonia venenata* (Apocynaceae) – A promising antimicrobial and mosquito larvicidal agent. *Journal of Ecotoxicology and Environmental Monitoring* 11, 173-176
- Karuppusamy S, Karmegam N, Rajasekaran KM** (2001c) Eco-biology of a medicinal plant, *Lobelia nicotianaefolia* in Palani Hills. *Geobios* 28, 165-166
- Karuppusamy S, Rajasekaran KM, Karmegam N** (2002a) Evaluation of phytomedicines from street herbal vendors in Tamil Nadu, South India. *Indian Journal of Traditional Knowledge* 1, 26-39
- Karuppusamy S, Rajasekaran KM, Karmegam N** (2002b) Antibacterial activity of *Balanites aegyptiaca* (L) Del. *Journal of Ecotoxicology and Environmental Monitoring* 12, 67-68
- Karuppusamy S, Rajasekaran KM, Karmegam N** (2002c) Medicinal plant resources of Dindigul District of Tamil Nadu [Diversity, domestication trends, cultivation and conservation]. *Journal of Nature Conservation* 14, 151-158
- Koné WM, Atindehou KK, Kacou-N'Douba A, Dosso M** (2007) Evaluation of 17 medicinal plants from Northern Côte d'Ivoire for their *in vitro* activity against *Streptococcus pneumoniae*. *African Journal of Traditional, Complementary and Alternative Medicines* 4, 17-22
- Liu RH** (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition* 78 (Suppl.), 517S-520S
- Matthew KM** (1983) *The Flora of Tamil Nadu Carnatic*, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India, 1034 pp
- Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV** (1999) Food related illness and death in the United States. *Emerging Infectious Diseases* 5, 607-625
- National Committee for Clinical Laboratory Standards** (1993) Dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard (2nd Ed), NCCLS Document M7-A3, 209 pp
- Ogbeche AK, Ajayi GO, Onyeneta P** (1997) Antibacterial activities of the leaf extract of *Ageratum conyzoides*. *Nigerian Quarterly Journal of Hospital Medicine* 7, 397-399
- Olukoya DK, Idika N, Odugbemi T** (1993) Antimicrobial activity of some medicinal plants from Nigeria. *Journal of Ethnopharmacology* 39, 69-72
- Pang T, Bhutta ZA, Finlay BB, Altwegg M** (1995) Typhoid fever and other salmonellosis: a continuing challenge. *Trends in Microbiology* 3, 253-255
- Parekh J, Chanda SV** (2007) *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish Journal of Biology* 31, 53-58
- Pereira AP, Ferreira ICFR, Marcelino F, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA** (2007) Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. cv. Cobrançosa) leaves. *Molecules* 12, 1153-1162
- Prakash M, Ananda Kumar S, Karmegam N** (2006a) Synergistic effect of selected medicinal plant extracts against certain bacteria. *Indian Journal of Applied Microbiology* 6, 52-53
- Prakash M, Karthikeyan V, Karuppusamy S, Karmegam N** (2006b) Synergistic activity of certain plants extracts against methicillin resistant *Staphylococcus aureus* (MRSA). *Journal of Ecotoxicology and Environmental Monitoring* 16, 387-390
- Reddy PS, Jamil K, Madhusudhan P** (2001) Antibacterial activity of isolates from *Piper longum* and *Taxus baccata*. *Pharmaceutical Biology* 39, 236-238
- Slutsker L, Alterkruse SF, Swerdloe DL** (1998) Food borne diseases: Emerging pathogens and trends. *Infectious Diseases and Clinical Journal of North America* 12, 199-216
- Tiwari RP, Bharti SK, Kaur HD, Dikshit RP, Hoondal GS** (2005) Synergistic antimicrobial activity of tea and antibiotics. *Indian Journal of Medical Research* 122, 80-84
- Voravuthikunchai SP, Mitchell H** (2008) Inhibitory and killing activities of medicinal plants against multiple antibiotic-resistant *Helicobacter pylori*. *Journal of Health Science* 54, 81-88