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Synergistic Antibacterial Activity of Four Medicinal Plants Collected from Dharapuram Taluk of Tiruppur District, South India

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ABSTRACT

Aqueous and ethanol extracts of leaves (1000 ppm) of four important medicinal plant species, *Aegle marmelos* (Rutaceae), *Albizia amara* (Mimosoideae), *Cassia auriculata* (Caesalpinoideae) and *Cissus quadrangularis* (Vitaceae) has been tested individually and in combination for their antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis*. Results showed higher antibacterial activity in combination of extracts of the medicinal plants studied. The aqueous leaf extracts of *C. auriculata* and ethanolic leaf extracts of *C. quadrangularis* showed 1.8 and 1.7 cm zone of inhibition (ZI), respectively against *E. coli* and *B. subtilis* while tested individually. Whereas, the combination of aqueous leaf extracts of *C. auriculata* +*C. quadrangularis* (1:1) showed 2.5 cm ZI against *E. coli*. Similarly, the highest antibacterial activity of 3.0 cm ZI was observed against *B. cereus* in combination of aqueous leaf extracts of all the four plants. This study clearly demonstrates the synergistic activity of plant extracts against different bacteria.

Key words: Antimicrobial, medicinal plants, plant extracts, synergistic activity

INTRODUCTION

It is well known fact that the medicinal plants are the resources of promising drugs for many diseases. The biological and pharmacological properties of many plants are still unknown. World-over, the scientists are exploring the possibilities of utilizing or finding out pharmacologically active compounds from medicinal plants. For example, screening of medicinal plants for their phytochemicals, antioxidant, anticancer and antimicrobial activities is the prime concern for finding out an effective phytochemically active principle (Ayyanar and Ignacimuthu, 2008; Agbafor *et al*., 2011; Roy *et al*., 2011; Vinoth *et al*., 2011; Mishra and Tripathi, 2011). Majority of these kind of works are concerned with the study of aqueous or solvent extracts of plant parts and testing them individually for selective pharmacological activities, such as antibacterial (Mishra and Mishra, 2011), hepatoprotective (Dhanasekaran and Ganapathy, 2011), hypoglycemic and hypolipidemic activities (Sharma *et al*., 2007). Recent studies show that the plant extracts in combination of two or more are exhibiting effective antimicrobial activity against a wide range of microorganisms including drug resistant bacteria (Prakash *et al*., 2006a, b; Karmegam *et al*., 2008). The medicinal plants selected for the present investigation viz. *Aegle marmelos*, *Albizia amara*, *Cassia auriculata*

Table 1: Medicinal plants used in the present study and their biological activities

Plant name (Family)	Reported activity	References
Aegle marmelos (L.) Corr. (Rutaceae)	Hypoglycemic and hypolipidemic effect	Sharma *et al.* (2007)
	Fruit gum as binding material for tablets	Patil *et al.* (2010)
	Antihyperglycemic and antidyslipidemic agent	Narendra and Sweta (2007)
	Insect repellent activity	Mishra and Tripathi (2011)
Albizia amara (Roxb.) Boiv.	Hepatoprotective	Dhanasekaran and Ganapathy
(Fabaceae, Sub-family: Mimosoideae)		(2011)
	Antimicrobial activity	Neogi *et al.* (2008)
	Antioxidant activity	Kumar *et al.* (2008)
Cassia auriculata L.	Antioxidant activity	Kumaran and Karunakaran (2007)
(Fabaceae, Sub-family: Caesalpinoideae)	Microbicidal activity	Prakash (2006)
	Medicinal and pharmacological activities (review)	Ayyanar and Ignacimuthu (2008)
	Antioxidant activity	Kumar *et al.* (2008)
	Antidiabetic and hypolipidemic effect	Uma Devi *et al.* (2006)
Cissus quadrangularis L. (Vitaceae)	Analgesic, anti-inflammatory and venotonic effects	Panthong *et al.* (2007)
	Antioxidant and antimicrobial activity	Murthy *et al.* (2003)
	Medicinal and pharmacological activities (review)	Ayyanar and Ignacimuthu (2008)

and *Cissus quadrangularis* exhibit various biological activities (Table 1). However, their activity in combined form is unavailable. Hence the present study has been carried out to study the synergistic antibacterial activities of the medicinal plants, *A. marmelos*, *A. amara*, *C. auriculata* and *C. quadrangularis* collected from Dharapuram Taluk, Tiruppur District, Tamil Nadu.

MATERIALS AND METHODS

Collection and identification of plants: In the present study, the leaves of the medicinal plants (Family) namely, *Aegle marmelos* (L.) Corr. (Rutaceae), *Albizia amara* (Roxb.) Boiv. (Fabaceae, Sub-family: Mimosoideae), *Cassia auriculata* L. (Fabaceae, Sub-family: Caesalpinoideae) and *Cissus quadrangularis* L. (Vitaceae) (Fig. 1) were collected in and around Dharapuram Taluk, Tiruppur District, South India and the identification was confirmed using standard local floras (Matthew, 1983).

Preparation of crude leaf extracts: The crude extracts of the leaves of all the four 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 h 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) obtained for each plant was transferred to screw cap bottles, labeled and stored under refrigerated (4°C) condition till use.

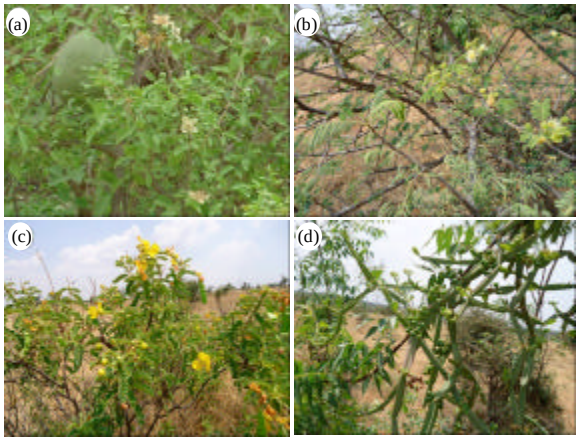

Fig. 1(a-d): Plants used in the study: (a) *Aegle marmelos*, (b) *Albizia amara*, (c) *Cassia auriculata* and (d) *Cissus quadrangularis*

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) 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 aqueous and ethanolic leaf extracts was carefully taken in a standard measuring flask and 5 mL of ethanol was added to dissolve the ethanolic-leaf extract and 5 mL of distilled water for aqueous leaf extract respectively. One to two drops of emulsifier (Triton-X100) were added to completely dissolve both aqueous and ethanol extracts. 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: Six bacterial species, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis* were used for the antibacterial activity 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 (MTTC) of Institute of Microbial Technology (IMTECH), Chandigarh, India.

Antibacterial assay: Standardized inoculums of each bacterium, i.e., 1 to 2×10^7 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. Amoxycillin at 10 μg disc^{-1} was used as an antibiotic reference standard. The aqueous and ethanolic 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 (aqueous and ethanol) were also tested for antibacterial activity. The same procedure was followed for the preparation of different combinations of aqueous leaf extracts. 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, four commonly available medicinal plants used by traditional users in South India were tested against six different bacteria. The result of antibacterial susceptibility testing showed that all the bacteria, *S. aureus*, *B. cereus*, *P. aeruginosa*, *E. coli*, *S. typhi* and *B. subtilis* were highly susceptible to amoxicillin with average diameter zone of inhibitions (ZI) of 2.9, 3.2, 3.1, 2.9, 3.3 and 3.2 cm, respectively (Table 2). Aqueous and ethanolic leaf extracts when tested individually for their antibacterial activity, showed various degrees of activity (Table 3). The ethanolic leaf extracts of *C. auriculata* showed comparatively a high degree of activity followed by *C. quadrangularis* and *A. marmelos*. The diameter of ZI was 1.8 cm for *C. auriculata* ethanolic extract against *B. cereus*. The lowest antimicrobial activity was shown by aqueous leaf extract of *A. amara* (Table 3). The studies on screening the extracts of specific plant parts alone obtained by many workers fall in line with the present investigation: Different solvent extracts of *Zapoteca portoricensis* (Agbafor *et al*., 2011), antibacterial ester from root bark extracts of *Vitellaria paradoxa* (Garba and Salihu, 2011), methanolic leaf extracts of *Anogeissus leiocarpus*

Table 2: Effect of antibacterial reference standards on selected bacteria

Standard antibiotic	Concentration (μg disc^{-1})	Test bacteria	Zone of inhibition (cm)
Amoxycillin	10	*Staphylococcus aureus*	2.9
		Bacillus cereus	3.2
		Pseudomonas aeruginosa	3.1
		Escherichia coli	2.9
		Salmonella typhi	3.3
		Bacillus subtilis	3.2

Table 3: Effect of crude aqueous and ethanolic extracts of selected plant leaves on different bacteria (24 h)

	Zone of Inhibition (ZI) in cm											
	Aqueous leaf extract						Ethanolic leaf extract					
Plants species	SA	BC	PA	EC	ST	BS	SA	BC	PA	EC	ST	BS
A. marmelos (A)	0.7	1.2	1.0	0.9	-	1.3	1.0	0.9	1.5	1.1	0.8	0.8
A. amara (B)	-	0.9	-	-	-	1.2	1.0	1.2	-	0.9	-	1.4
C. auriculata (C)	1.0	1.5	0.8	1.4	1.2	1.6	1.7	1.8	1.2	1.6	1.1	0.9
C. quadrangularis (D)	1.2	0.9	-	1.7	0.8	-	1.0	1.3	0.9	-	-	-

SA: *S. aureus*, BC: *B. cereus*, PA: *P. aeruginosa*, EC: *E. coli*, ST: *S. typhi* and BS: *B. subtilis*. Values are mean of three replicates

Table 4: Synergistic activity of aqueous and ethanolic extracts of selected plant leaves in combination of two against bacteria

	Zone of Inhibition (ZI) in cm											
	Aqueous leaf extract						Ethanolic leaf extract					
Combination of plant extracts tested	SA	BC	PA	EC	ST	BS	SA	BC	PA	EC	ST	BS
A+B	-	1.5	0.8	-	-	2.0	1.4	1.5	0.9	1.0	-	1.8
A+C	1.3	2.1	1.1	0.9	-	2.3	1.5	2.0	1.5	1.9	1.0	-
A+D	1.8	1.0	-	2.1	-	1.0	1.2	1.7	1.0	1.5	-	-
B+C	-	1.6	-	-	-	1.9	2.1	2.5	-	2.1	0.9	2.1
B+D	-	1.5	-	1.2	-	1.0	1.2	1.8	1.6	-	-	1.2
C+D	2.3	0.9	-	2.5	1.5	0.8	2.5	2.2	1.0	-	-	1.9

SA: *S. aureus*, BC: *B. cereus*, PA: *P. aeruginosa*, EC: *E. coli*, ST: *S. typhi* and BS: *B. subtilis*; (A) *A. marmelos*, (B) *A. amara*, (C) *C. auriculata* and (d) *C. quadrangularis*; Values are mean of three replicates

Table 5: Synergistic activity of aqueous and ethanolic extracts of selected plant leaves in combination of three against bacteria

	Zone of Inhibition (ZI) in cm											
	Aqueous leaf extract						Ethanolic leaf extract					
Combination of plant extracts tested	SA	BC	PA	EC	ST	BS	SA	BC	PA	EC	ST	BS
A+B+C	1.0	2.7	1.5	-	-	1.8	1.6	1.9	0.8	2.3	1.9	-
A+B+D	-	2.3	-	1.7	-	0.6	1.3	1.5	1.0	-	0.9	2.3
B+C+D	-	1.5	-	0.8	1.5	2.5	2.8	2.1	1.6	-	-	2.6
A+C+D	2.5	1.2	-	2.0	2.0	2.1	1.5	2.4	1.1	-	2.1	-

SA: *S. aureus*, BC: *B. cereus*, PA: *P. aeruginosa*, EC: *E. coli*, ST: *S. typhi* and BS: *B. subtilis*. Values are mean of three replicates

(Ichor and Ekoja, 2011) and antibacterial activity of *Artemisia dracunculus* essential oil against multi-drug resistant *Acinetobacter baumannii* (Jazani *et al*., 2011).

The antibacterial activities of extracts in combination of two plants showed different degrees of ZI as shown in Table 4. The average diameter above 2.0 cm ZI was observed in the following aqueous and ethanolic leaf extract combinations: *A. marmelos* +*C. auriculata*, *A. amara* +*C. auriculata* and *C. auriculata* +*C. quadrangularis* followed by other combinations. A ZI of 2.7 cm was observed in ethanolic leaf extract combination of *C. auriculata* +*C. quadrangularis* against *S. aureus* (Table 4). The highest ZI of 2.8 cm against *S. aureus* was observed in ethanolic leaf extract combination of *A. amara* +*C. auriculata* +*C. quadrangularis* (1:1:1) (Table 5). The combination of aqueous extracts of all the four plants in equal proportion showed a maximum of 3.0 cm ZI against *B. cereus* followed by 2.4 cm ZI against *B. subtilis* (Fig. 2). These findings are in coherence with the study reported earlier on synergistic activity of six different plants against pathogenic bacteria by Karmegam *et al*. (2008). Synergistic activity of aqueous and ethanolic extracts 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 ZI of 2.8 cm was observed against *S. aureus* in ethanolic leaf extract combinations of *Balanites aegyptiaca* +*Lobelia nicotianaefolia* (Karmegam *et al*., 2008). Similarly, 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* (2.3 cm) and *L. inermis* +*C. odoratum* (2.0 cm) extract combinations against MRSA.

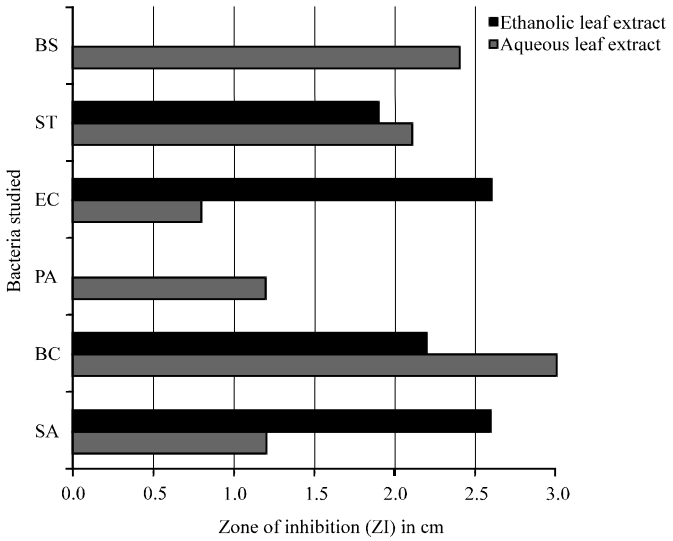

Fig. 2: Synergistic activity of aqueous and ethanolic extracts of selected medicinal plant leaves, in combination of four, against different bacteria (24 h). Values are mean of three replicates (Refer Tables for abbreviations)

CONCLUSION

The antimicrobial activity of aqueous and ethanolic leaf extracts of *A. marmelos*, *A. amara*, *C. auriculata* and *C. quadrangularis* showed lower inhibition zones when used alone than that of the extract combinations. The ZI reached 3.0 cm against certain bacteria by the combination of aqueous extracts of all the four plants used in the study, indicating the high potential of combined use of plant extracts against pathogenic microorganisms. There is a possibility of using plant extracts in combinations against pathogenic bacteria as has been observed from the results.

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