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Full Length Research Paper

In vitro antibacterial activity of extracts of *Mimusops elengi* against gram positive and gram negative bacteria

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The extracts of *Mimusops elengi* L. (family: Sapotaceae) bark, fruit and seed were evaluated for antibacterial activity by using spectrophotomertic method against gram positive and gram negative strains viz. *Nocardia asteroids* NRRL 174, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* PCSIRB 248, *Bacillus licheniformis* NCL 2024, *Proteus mirabilis* ATCC 29425 and *Salmonella typhimurium* ATCC 14028. The minimum inhibitory concentration (MIC) of all active extracts of *M. elengi* was calculated using macro dilution method. The fruit and seed extracts were found inactive, while stem bark extracts showed antibacterial activity against all 6 bacterium. The ethyl acetate extract exhibited the highest % age inhibition (84.5 % age, MIC = 0.6 mg/ml) against *B. subtilis*. The aqueous methanol (2:8) extract also showed significant results with 74.9% age inhibition (MIC = 0.9 mg/ml) against *N. asteroids.* The results were compared with standard antibacterial drugs (streptomycin and ampicillin).

Key words: Antimicrobial activities, medicinal plants, *Mimusops elengi.*

INTRODUCTION

The search of biologically active compounds from plants has always been of great interest to scientists looking for new sources of useful drugs against infectious diseases. Approximately 25% of all prescriptions sold in the United States are for natural products (Cespedes et al., 2006). In the recent years, infections have increased to a great extent and antibiotic resistance becomes an ever-increasing therapeutic problem (Austin et al., 1999). Natural products of higher plants may provide a new source of antimicrobial agents with possibly novel mechanism of action (Hamil et al., 2003; Machado et al., 2003; Motsei et al., 2003; Barbour et al., 2004).

The genus *Mimusops* (family: Sapotaceae) consists of 30 species of which, *Mimusops schimperi* A. Rich., *Mimusops laurifolia* Forssk. and *Mimusops elengi* Linn. are widely distributed throughout tropical and subtropical regions of Asia (Watt, 1908; Friis, 1981). *M. elengi* is an ornamental tree with sweet-scented flowers and grows wild in the southern India, Burma and Pakistan. The plant finds an important place in the indigenous system of and its various parts are used as a febrifuge, astringent, purgative and stimulant (Nadkarni, 1976). The saponins of fruit are reported to have anti-inflammatory activity. The studies conducted using bark of the plant has shown dose-dependent inhibition of gastric lesions against ethanol-induced gastric ulcer (Payal et al., 2003). The pounded seeds pasted with oil are used for the treatment of obstinate constipation (Nusrat et al., 1995a). The seeds are known to contain triterpenes and triterpenoid saponins (Nigam et al., 1992; Nusrat et al., 1995b; Sahu et al., 1996, 1997; Sen et al., 1995; Lavaud et al., 1996; Catherine et al., 1996). Previous studies on *M. elengi* were mainly focused on the extraction and structure elucidation of individual constituents. Moreover, no significant research carried out on its efficiency in preventing infectious diseases. Therefore, study was undertaken to explore new herbal antimicrobial agents on the extracts of different parts of *M. elengi*.

MATERIALS AND METHODS

Materials

Methanol, chloroform, acetone, dichloromethane, ethyl acetate, ethanol and dimethyl sulphoxide (DMSO) were purchased from

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Table 1. Antibacterial activity of different extracts of *M. elengi.*

Microbes	Percentage inhibition											
	MBM[a]	MBMW[b]	MBMC[c]	MBA[d]	MBAW[e]	MBD[f]	MBC[g]	MBEA[h]	MBET[i]	MBEW[j]	MFM[k]	Mim[l]
P. Mirabilis	72.3±0.9	70.4±2.3	66.8±2.4	56.3±2.3	59.7±1.6	40.1±1.8	70.9±2.0	70.3±2.0	64.6±2.3	61.3±1.6	-	-
M. luteus	67.2±1.2	63.3±2.0	58.3±2.1	57.4±2.1	55.2±2.5	57.2±2.3	30.2±2.1	71.5±2.4	68.6±2.0	62.1±2.3	-	-
B. icheniformis	57.3±1.2	48.4±1.9	53.4±1.3	39.3±1.5	35.6±2.0	46.9±2.3	60.5±1.6	58.3±0.8	57.8±1.1	55.7±0.9	-	-
N. asteroids	56.4±1.8	74.9±1.7	53.6±1.3	43.4±1.8	41.2±2.3	36.3±1.1	60.6±0.9	51.1±1.2	55.6±1.4	46.7±1.0	-	-
S. typhimorium	69.2±1.2	65.2±1.2	71.2±1.2	67.6±1.2	62.3±2.1	37.2±0.8	71.2±0.7	56.3±1.4	64.3±1.2	67.3±0.7	-	-
B. subtilis	70.1±0.8	74.5±0.9	79.3±1.2	68.3±0.7	64.4±1.2	56.3±1.4	67.4±1.6	84.5±1.5	69.4±0.8	67.9±0.6	-	-

[a]Bark in methanol; [b]Bark in methanol-water 8:2; [c]Bark in methanol-chloroform 1:1; [d]Bark in acetone; [e]Bark in acetone-water 7:3; [f]Bark in dichloromethane; [g]Bark in chloroform; [h]Bark in ethyl acetate; [i]Bark in ethanol; [j]Bark in ethanol-water 8:2; [k]Fruit in methanol; [l]Seed in methanol; -= No activity.

Panerac (ACS Grade). Agar-agar and nutrient broth from Merck while potato dextrose agar (PDA) from Oxoid (England). Streptomycin and ampicillin were purchased from Smith-Kline (USA).

Plant material

M. elengi fruit, seed and stem bark were collected in June 2006 from Government College University campus Lahore, Pakistan. The plant was identified by Dr. Zaheer-ud-Din Khan (Taxonomist) of Botany, GCU Lahore where a voucher specimen was deposited (036-GCU-BOT-06).

Preparation of crude plant extract

Powdered air dried stem bark (100 g for each solvent) was subjected to sequential extraction using solvents of increasing polarities to afford dry extracts of dichloromethane 0.43 g ,chloroform (9.27 g), ethyl acetate (1.23 g), acetone (11.02 g), methanol-chloroform (1:1, 8.64 g), methanol (9.06 g), ethanol (9.21 g), acetone-water (7:3, 9.32 g), methanol-water (8:2, 8.25 g) and ethanol-water (8:2, 9.04 g). Powdered air dried fruit and seed (100 g each) were extracted in methanol leading to crude extracts of fruit (23.61 g) and seed (10.28 g).

Microorganisms

Microorganisms were obtained from Biochemistry laboratory, GC University, Lahore. Bacterial strains were maintained at 4°C on nutrient broth agar.

Antimicrobial assay

The antimicrobial assay was performed as described by Janssen et al. (1987) and Vagi et al (2005). Extracts and standards were dissolved in DMSO to make a concentration of 100 mg/ml for extracts and 2 mg/ml for standards. 50 ml sterilized nutrient broth medium was taken in culture flask and 1 ml of the extract was added. The mixture was inoculated with 1 ml of bacterial suspension (1 x 10^8 cfu/ml). Streptomycin and ampicillin were usedas positive references. The flasks were incubated at 37°C for 24 h and antibacterial activity was determined by measuring the optical density by using spectrophotometer at 550 nm. The minimum inhibitory concentration (MIC) was calculated against each bacterial strain using broth macrodilution method. The experiments were conducted in triplicate.

Statistical analysis

The data was analyzed using one-way analysis of variance (ANOVA) for repeated measurements. The Duncan's multiple range tests was used to determine differences at each point. Differences at each point were considered significant at $P \leq 0.05$.

Table 2. Percentage inhibition of standard drugs against different microorganisms.

	Percentage inhibition	
	Streptomycin	**Ampicillin**
P. mirabilis	98.3 ± 0.1	94.2 ± 1.1
B. subtilis	98.1 ± 1.3	95.4 ± 1.8
B. licheniformis	97.3 ± 1.5	96.2 ± 1.3
N. asteroids	98.2 ± 1.6	92.5 ± 1.4
S. typhimorium	97.1 ± 1.1	93.4 ± 1.0
M. luteus	97.6 ± 1.0	94.2 ± 0.8

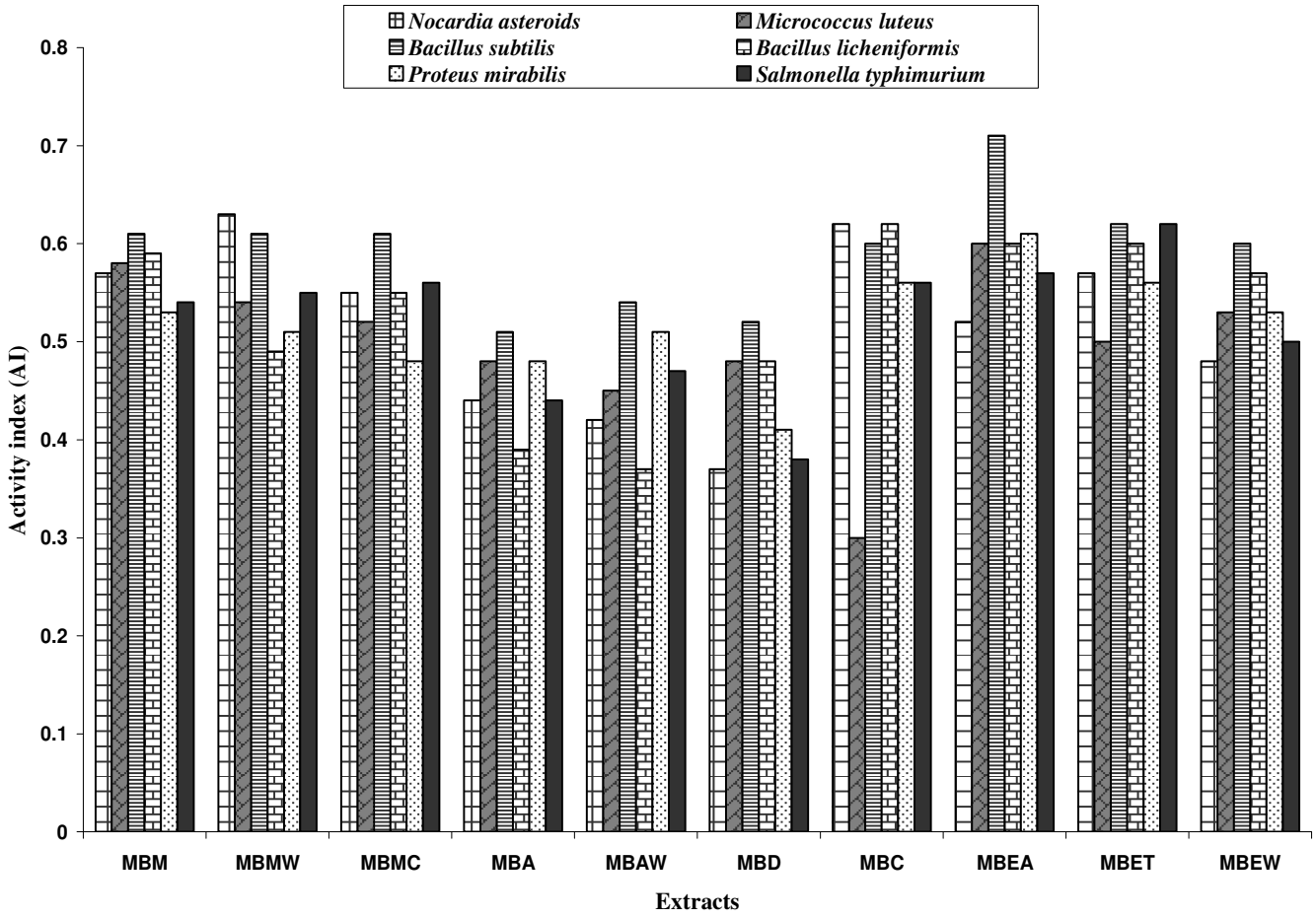

Figure 1. Activity index of antibacterial activity of different extracts of *M. elengi* against streptomycin.

RESULTS

Antimicrobial activity

The antibacterial activity of 11 different extracts of *M. elengi* was checked against 6 different microorganisms and the results are summarized in Table1 and 2.

Activity index

Activity index was calculated (Singh et al., 2002) for both antibacterial standard drugs and results are shown in

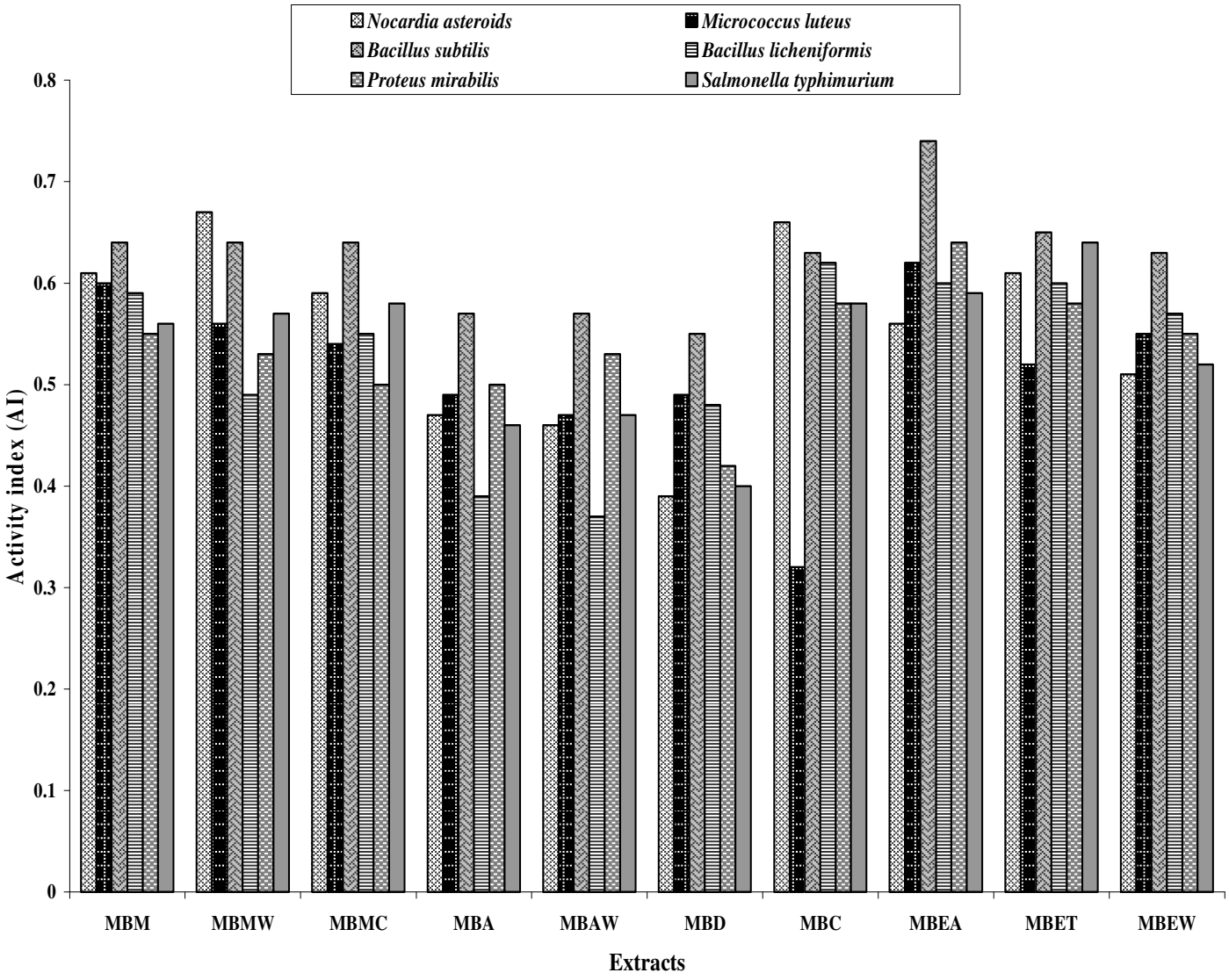

Figure 2. Activity index of antibacterial activity of different extracts of *M. elengi* against ampicillin.

Table 3. Minimum inhibitory concentration (MIC) of different extracts of *M. elengi*.

	MIC (mg/ml)									
Microbes	**MBM[a]**	**MBMW[b]**	**MBMC[c]**	**MBA[d]**	**MBAW[e]**	**MBD[f]**	**MBC[g]**	**MBEA[h]**	**MBET[i]**	**MBEW[j]**
P. Mirabilis	3.0±0.1	9.2±0.5	3.1±0.4	1.5±0.3	5.2±0.9	4.8±0.7	3.1±0.7	5.2±0.6	4.1±0.4	6.1±0.6
M.luteus	3.1±0.3	2.8±0.3	8.1±0.7	4.2±0.7	5.2±0.9	6.7±0.7	7.8±0.5	4.1±0.6	9.4±0.8	8.6±1.0
B.Licheniformis	4.0±0.4	4.9±0.6	5.1±0.8	6.9±0.8	8.1±0.8	5.9±0.5	4.6±0.7	3.7±0.7	5.4±0.6	6.2±0.3
N.asteroids	4.2±0.4	0.9±0.1	5.7±0.6	6.1±0.4	8.2±0.5	5.0±0.6	5.6±0.4	6.1±0.3	3.4±0.7	3.4±0.7
S. typhimorium	4.6±0.3	3.2±0.2	4.7±0.5	2.8±0.3	3.4±0.3	4.7±0.7	3.1±0.7	4.1±0.6	3.8±0.4	6.1±0.3
B. subtilis	2.7±0.8	3.4±0.2	3.8±0.3	4.1±0.5	2.5±0.4	3.2±0.4	4.7±0.3	0.6±0.1	1.8±0.2	3.8±0.6

[a]Bark in methanol; [b]Bark in methanol-water 8:2; [c]Bark in methanol-chloroform 1:1; [d]Bark in acetone; [e]Bark in acetone-water 7:3; [f]Bark in dichloromethane; [g]Bark in chloroform; [h]Bark in ethyl acetate; [i]Bark in ethanol; [j]Bark in ethanol-water 8:2.

Figures 1 and 2.

Determination of MIC values

The minimum inhibitory concentration MIC was calculated against each bacterialstrain using broth macrodilution method and results are shown in Table 3.

DISCUSSION

The results indicate that the extracts of *M. elengi* have antibacterial potential and can be used in the treatment of infectious diseases caused by resistant microorganisms. The extracts of different parts of *M. elengi* inhibited the growth of microorganisms with various degrees. The bark extracts showed significant antibacterial activities in similar fashion while the seed and fruit extracts were almost inactive against all tested organisms. The minimum inhibitory concentration (MIC) of all active extracts of *M. elengi* was calculated using macro dilution method. The ethyl acetate extract showed the highest percentage age inhibition (74.5 % age, MIC = 0.6 mg/ml) against *B. subtilis*. The aqueous methanol (2:8) extract also showed significant results with 84.9 age inhibition (MIC = 0.9 mg/ml) against *N. asteroids,* comparable to standard antibacterial drugs (streptomycin and ampicillin). The results also confirmed that the gram-positive bacterial strains were more susceptible to the plants extracts as compared to gram negative bacteria. This is in agreement with the fact that gram-positive bacteria have only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971). Antibacterial activity of the seed extract of *M. elengi* has been recently reported by Hazra et al. (2007). But the seed extract was completely inactive against the bacterial strains used in our experiment.

The solvents used in the extraction procedure were found to have pronounced effect on the solubility of the antibacterial compounds (Hugo et al., 2005). Therefore, it may suggested that methanol-water (8:2) and ethyl acetate are the effective solvents for the extraction of antibacterial compound from stem bark of *M. elengi*. Further research has to be carried out on these extracts in order to purify and identify active components with the view of their use for *in vivo* studies

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