

Antibacterial Activity of *Mimusops elengi* Leaf, Seed and Bark Extracts Alone and in Combination with Antibiotics against Human Pathogenic Bacteria

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Abstract

Aims: The current study determines the antibacterial activity of 'Bakul' (*Mimusops elengi*) leaf, seed and bark extracts against gram-negative clinical bacterial isolates as well as the standard bacterial strains.

Methods: The disc diffusion method was followed to determine the antibacterial activity of *M. elengi* leaf, seed and bark extracts against the clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The antibiotic susceptibility of the bacterial isolates was determined by disc diffusion.

Results: The concentration dependent activity of the extracts against the bacteria was recorded with zone diameter of inhibition 7-21 mm. The extracts in combination with antibiotics (cefepodoxime, gentamycin and ciprofloxacin) had growth inhibitory indices (GIs), 0.5-0.6, 0.5-0.89 and 0.73-0.82 against *E. coli*, *K. pneumoniae* and *Pr. vulgaris* clinical isolates, respectively, while for the standard strains (*E. coli*, *K. pneumoniae* and *Ps. aeruginosa*) the GIs ranged 0.56-0.86. Most of the extracts were tested positive for the presence of flavonoids, glycosides, steroids, terpenoids, quinone and phenol.

Conclusion: The *M. elengi* leaf, seed and bark extracts, in combination with antibiotics, had synergistic interactions against all the standard bacterial strains and *Pr. vulgaris* clinical isolate, while both synergistic and additive interactions were recorded against *E. coli* and *K. pneumoniae* clinical isolates.

Keywords: *Mimusops elengi*; Ethanolic extracts; Antibacterial activity; Phytochemical; Pathogenic bacteria

Introduction

The huge numbers of infectious diseases caused by the gram-negative bacteria that are resistant to many commonly used antibiotics are the causes of great concern to the clinicians as well as the microbiologists. Phytomedicines, prepared from various plant materials, such as Ayurvedic traditional medicine, are comparatively safe, inexpensive and have less antagonistic effects. The leaf, bark, fruit and seeds of *Mimusops elengi* possess several medicinal properties, viz., astringent and tonic in dental diseases and uterine disorders [1-4]. This plant has also been reported for analgesic, diuretic, antiulcer, antipyretic, anti-inflammatory and antimicrobial activities [5-8].

In rural areas of developing countries, like India, herbal materials are in use as the primary source of medicines [9]. Nearly 80% of the people in developing countries use traditional drugs for the purpose of primary health maintenance [10]. Among the plant species occurring worldwide [11], only a very less percentage has been investigated phytochemically. The medicines of plant origin used by the medical practitioners are in the form of extract of the whole plants or part of the plants. Some of the effects elaborated by the plant extracts used in the traditional medicine include antiviral, antitumor, antimicrobial, and having central nervous system effect [12]. The plants possess bioactive components of therapeutic value to cure several health

disorders of humans [13]. The research interest on the antimicrobial activity of plant extracts is a raising one because of the current problems with bacterial antibiotic resistance, and the use of phytochemicals as natural antimicrobials is gaining popularity [14].

One such important traditional medicinal plant is *M. elengi* belonging to the *Sapotaceae* family, called as 'Bakula' in Bengali and it is well known in Ayurvedic medicine. All the parts of *M. elengi* have medicinal properties, and the leaves are reported to be used in the treatment of bacterial diseases by tradition [15]. The pharmacognostic and phytochemical screening reports on *M. elengi* stem bark has been documented [16]. Recently, estimation of triterpene acids using from *M. elengi* stem bark has been published [17,18]. Antimicrobial, antiviral and hepatoprotective and cytotoxic activities of *M. elengi* are well accepted because of the wealth of scientific literature supporting these effects [19]. The aqueous and ethanol extracts *M. elengi* leaves have been tested against *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica* serovar Typhimurium and *Bacillus cereus*. The ethanol extracts had greater activity than the aqueous extracts of all the remedial plants [20]. The *M. elengi* leaf extracts showed great antioxidant activity in different solvent like n-hexane, dichloromethane and methanol compared to different standard antioxidants [21]. Both enzymatic and non-enzymatic antioxidant activities were conducted by Kalaiselvi *et al.* [22] in assessing the antioxidant properties of *M. elengi*. The antibacterial activity of silver nanoparticles is known [23], and the biogenic silver

nanoparticles produced by *M. elengi* fruit (pericarp) and flower extracts have been reported as excellent antimicrobials against gram-positive as well as gram-negative bacterial strains [24,25]. Therefore, in the present study, the ethanolic extracts of leaf, seed and bark of *M. elengi* were evaluated phytochemically and tested against six different bacterial strains to identify the potentiality of antibacterial activity.

Materials and Methods

Bacterial strains

The clinical isolates of *Escherichia coli*, *Klebsiella pneumonia* and *Proteus vulgaris* were used in the current study, and the standard strains included were *E. coli* ATCC 25922, *K. pneumonia* MTCC 7407, *Pseudomonas aeruginosa* ATCC 27853.

Plant parts and preparation of extracts

Various parts of Bakul plant, *M. elengi*: leaf, seed (from mature fruits) and bark of were collected from naturally grown wild plants at Raiganj of Uttar Dinajpur district, West Bengal (India). The plant materials were washed repetitively with distilled water and shed-dried, and grinded thereafter using a grinding machine.

The granules prepared from different parts of the plant, 25 g (leaf and seed) and 20 g (bark), were soaked separately in 100 ml of ethanol for a period of 48 h with manual shaking [26]. The extracts thus obtained were sieved through sterilized Whatman No.1 paper filter after straining through an autoclaved cheese cloth, and stored at 4°C for further use. The concentrations of the ethanolic Bakul (*M. elengi*) leaf extract (BLE) and seed extract (BSE) in the stock solutions were 250 µg/µl, and bark extract (BBE) was 200 µg/µl.

Phytochemical analysis

The plant extracts were screened qualitatively for different types of bioactive components (phenol, quinone, flavonoids, steroids, terpenoids and glycosides) using the protocol of Radhakrishnan *et al.* [27].

Antibiotic susceptibility

The test bacterial strains were screened for susceptibility to antibiotics (Hi-Media, India): cefpodoxime (CPD; 10-µg/disc), gentamycin (GEN; 10-µg/disc) and ciprofloxacin (CIP; 5-µg/disc) following disk diffusion method [28], as per the recommendation of CLSI guidelines [29]. The inoculated nutrient agar plates with impregnated antibiotic discs were incubated at 35°C for 24 h, and the zone diameter of inhibition (ZDI) values obtained around each of the antibiotic discs were measured.

Antibacterial activity of plant extract

The Antibacterial activities of BLE, BSE and BBE were determined by disc diffusion method, as mentioned earlier [30]. In this process, on sterile nutrient agar plate young broth culture of the test bacteria was swabbed and dried, and 4 sterilized discs, each of 6 mm diameter (prepared in the laboratory from Whatman's No. 1 filter paper), were placed on 4 different sectors and marked. Thereafter, the discs were soaked with four different concentrations: 20, 35, 50 and 65 µl/disc, equivalent to 5, 8.75, 12.5 and 16.25 mg/disc for BLE and BSE, while 4, 7, 10 and 13 mg/disc for BBE. The ZDIs were recorded and interpreted

according to the CLSI criteria [31], for resistance and sensitivity of the isolates.

Combined antibacterial activity

The combined antibacterial activity of antibiotic and plant extract was determined following the protocol mentioned earlier [32]. Briefly, on nutrient agar plates, swabbed inoculated with test bacteria, three sectors were prepared and marked with BLE, BSE and BBE, and the antibiotic discs: CPD (10-µg/disc), GEN (10-µg/disc) and CIP (5-µg/disc) were placed. On each of the antibiotic disc, 20 µl (i.e., 5 mg/disc for BLE and BSE, and 4 mg/disc for BBE) was dropped, soaked and dried properly for about 30 min at room temperature. The ZDIs were measured for each combined action against the bacteria tested.

Growth inhibitory indices

The GII (growth inhibitory indices) values were calculated following the formula stated earlier [33], in order to interpret the various effects of antibiotic-plant extract interaction. The synergistic, additive or antagonistic activities, if any, in between the two of the antimicrobial agents (plant extract and antibiotic) were defined with GIIs > 0.5, 0.5 and < 0.5, respectively [32].

Statistical analysis

The t-test was used in order to compare the antibacterial activity (in terms of ZDIs) of the antibiotics (CPD, GEN and CIP) alone and in combination with plant extracts (BLE, BSE and BBE), against the test bacterial isolates. The p-value of < 0.10 was considered significant.

Results

The antibacterial activity of the plant extracts is shown in Table 1. The BLE, BSE and BBE, tested at various concentrations, against *E. coli* (ATCC 25922), *E. coli* (clinical), *K. pneumoniae* (MTCC 7407), *K. pneumonia* (clinical), *Ps. aeruginosa* (ATCC 27853) and *Pr. vulgaris* (clinical) had ZDIs 7-21mm. The BBE displayed ZDIs 12, 14, 20 and 21mm at 4, 7, 10 and 13 mg/disc, respectively, against *K. pneumonia* (MTCC 7407), and BSE had antibacterial activity against *K. pneumonia* (clinical) with ZDIs 7, 8, 9 and 10 mm at 5, 8.75, 12.5 and 16.25 mg/disc, respectively. The BLE, BSE and BBE had ZDIs 10-14 mm for *E. coli* (clinical) and 8-14 mm for *E. coli* ATCC 25922 strain. In case of *Ps. aeruginosa*, BSE exhibited ZDIs 10-17 mm at 5-16.25 mg/disc, respectively; against *Ps. aeruginosa* BBE showed ZDIs 8-18 mm at 4-13 mg/disc.

The antibiotic susceptibility test results are presented in Table 2. The *E. coli* (clinical), *K. pneumonia* (clinical) and *Ps. aeruginosa* were resistant to CPD, while *E. coli* (ATCC 25922), *K. pneumonia* (MTCC 7407) and *Pr. vulgaris* (clinical) were sensitive to CPD having ZDIs 10, 18 and 17 mm, respectively. The CIP showed activity, with ZDIs 20-30 mm, for the test bacteria except the *E. coli* clinical isolate (6 mm); the ZDIs ranged 10-28 mm and 6-30 mm, due to GEN and CIP, respectively.

The combined antibacterial activity of the plant extracts and antibiotics against the test bacterial isolates is presented in Figure 1. The growth inhibitory indices (GIIs) of combined action are represented in Table 3.

Bacterial Isolates	Extracts Name	Zone Diameter of Inhibition(mm)			
		20µl	35µl	50µl	65µl
<i>E. coli (clinical)</i>	Leaf	10	12	13	14
	Seed	10	12	13	14
	Bark	10	11	12	13
<i>K. pneumoniae (clinical)</i>	Leaf	10	11	12	13
	Seed	7	8	9	10
	Bark	9	11	13	13
<i>Pr. vulgaris (Clinical)</i>	Leaf	8	10	11	12
	Seed	10	11	12	14
	Bark	10	11	12	13
<i>E. coli (ATCC 25922)</i>	Leaf	10	11	12	13
	Seed	10	12	13	14
	Bark	8	10	12	14
<i>K. pneumoniae (MTCC 7407)</i>	Leaf	10	11	12	14
	Seed	10	11	12	12
	Bark	12	14	20	21
<i>Ps. aeruginosa (ATCC 27853)</i>	Leaf	8	10	11	14
	Seed	10	12	13	17
	Bark	8	10	16	18

Table 1: Antibacterial activity of ethanolic leaf, seed and bark extract of *M. elengi*.

Bacterial Isolates	Antibiotic	Zone Diameter of Inhibition (mm)
<i>E. coli (clinical)</i>	CPD	6
	GEN	10
	CIP	6
<i>K. pneumoniae (Clinical)</i>	CPD	6
	GEN	21
	CIP	26
<i>Pr. vulgaris (Clinical)</i>	CPD	17
	GEN	18
	CIP	30
<i>E. coli (ATCC 25922)</i>	CPD	10
	GEN	18
	CIP	26
<i>K. pneumoniae (MTCC 7407)</i>	CPD	18
	GEN	16
	CIP	20
<i>Ps. aeruginosa (ATCC 27853)</i>	CPD	6
	GEN	28
	CIP	30

Table 2: Antibiotic susceptibility test results for clinical and standard bacterial strain; CPD: cefpodoxime, GEN: gentamycin; CIP: ciprofloxacin.

GIs	Bacterial Isolates						
	Antibiotic + Plant extract	<i>E. coli (clinical)</i>	<i>K. pneumonia (clinical)</i>	<i>Pr. vulgaris (clinical)</i>	<i>E. coli ATCC 25922</i>	<i>K. pneumonia MTCC 7407</i>	<i>Ps. aeruginosa ATCC 27853</i>
CPD+BLE		0.563	0.5	0.8	0.6	0.75	0.71
GEN+BLE		0.5	0.71	0.73	0.714	0.65	0.78
CIP+BLE		0.563	0.72	0.79	0.81	0.73	0.79
CPD+BSE		0.563	0.77	0.78	0.6	0.714	0.56
GEN+BSE		0.6	0.79	0.82	0.79	0.69	0.79
CIP+BSE		0.5	0.89	0.75	0.86	0.8	0.8
CPD+BBE		0.5	0.6	0.74	0.61	0.67	0.5
GEN+BBE		0.6	0.73	0.78	0.77	0.61	0.78
CIP+BBE		0.563	0.74	0.775	0.823	0.66	0.79

Table 3: Growth inhibitory indices (GII) from the combined action of antibiotics and plant extracts against the test bacterial isolates. CPD: cefpodoxime; GEN: gentamicin; CIP: ciprofloxacin; BLE: Bakul leaf extract; BSE: bakul seed extract; BBE: bakul bark extract.

Glycosides, steroids, terpenoids and quinone were presented in the BLE, BSE and BBE whereas along with the above phytoconstituents, presence of flavonoids was seen in the BSE.

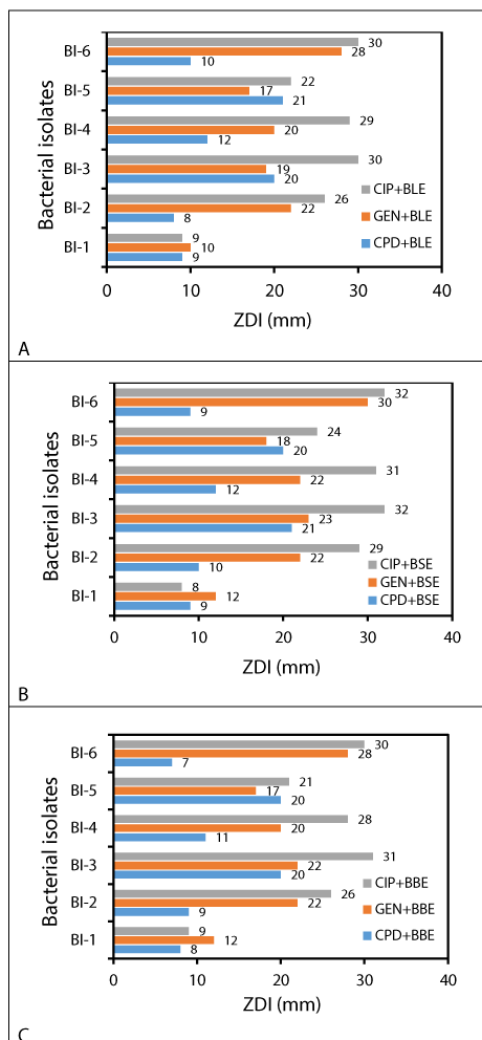


Figure 1: Combined susceptibility test results A: leaf extract; B: seed extract; C: bark extract; ZDI (zone diameter of inhibition), BI-1: *E. coli* (clinical); BI-2: *K. pneumoniae* (clinical); BI-3: *Pr. vulgaris* (clinical); BI-4: *E. coli* (ATCC 25922); BI-5: *K. pneumoniae* (MTCC 7407); BI-6: *Ps. aeruginosa* (ATCC 27853); CPD: cefpodoxime, GEN: gentamicin, CIP: ciprofloxacin, BLE: bakul leaf extract; BSE: bakul seed extract; BBE: bakul bark extracts. The p-values ranged 0.22-0.42 when the difference between antibacterial activity of antibiotic alone and in combination with plant extracts, were considered.

Discussion

The therapeutic role of plants in the treatment of human health disorders of several kinds has been recorded worldwide [9]. Among 122 compounds, 80%, used in the ethnomedical purposes, have been derived from 94 plant species [11]. The ethyl acetate, hexane,

methanol, and ethanol *M. elengi* extracts had antibacterial activity against the dental caries causing bacteria *Streptococcus mutans* isolated from the patients [34]. The acetone, petroleum ether, methanol and water extracts of *M. elengi* bark have been tested for their antibacterial activity against dental infection causing bacteria: *Staph. aureus*, *Str. mutans*, *Str. salivarius*, *Str. sanguinis*, and fungus (*Candida albicans*) by well diffusion method [35,36]. *M. elengi* leaf extracts had antibacterial activity against *Bacillus subtilis* and *Trichoderma viride* [37]. In the current study, three different extracts (BLE, BSE and BBE) showed antibacterial activity against *E. coli*, *K. pneumoniae*, *Ps. aeruginosa*, *Pr. vulgaris*. The concentration dependant antibacterial activities, as indicated by the ZDI due to the action of BLE (250 µg/µl), BSE (250 µg/µl) and BBE (200 µg/µl) alone, and in combination with antibiotics (CPD, GEN and CIP), have been recorded. The ZDIs were increased from 8 mm to 14 mm, when the leaf extract concentration was increased from 5 mg/disc to 16.25 mg/disc; for seed extracts ZDI was 7 mm at 5 mg/disc, which was increased to 17 mm at the highest concentration (16.25 mg/disc), while the bark extracts had highest ZDI (21 mm) at 16.25 mg/disc and lowest (8mm) at 5mg/disc against the test bacterial isolates.

Lalitha *et al.* [38] reported that the aqueous extract of *M. elengi* (leaf) had a strong antibacterial activity having ZDIs 27 mm and 24 mm against *E. coli* and *Str. pneumoniae*; the extract showed moderate growth inhibitory activity against *Ps. aeruginosa*, *Vibrio cholerae* and *Salmonella typhi*. In the present investigation, BLE showed antibacterial activity with ZDIs 14, 13, 13, 14, 12 and 14 mm, against *E. coli* (clinical), *E. coli* ATCC 25922, *K. Pneumoniae* (clinical), *K. pneumoniae* MTCC 7407, *Ps. aeruginosa* ATCC 27853 and *Pr. vulgaris* (clinical), respectively in presence of 65 µl of the extracts (16.25 mg) per disc. The aqueous extract of *M. elengi* (leaf) was, thus, found more effective than the ethanolic extract. Kannadhasan *et al.* demonstrated that, the antibacterial activity of two different extracts of *M. elengi* leaf having ZDIs 8 – 10 mm and 11 mm against *K. pneumoniae* and *E. coli*, respectively [39].

Lalitha *et al.* reported the top ZDI of GEN (36 mm) against *E. coli* [38]. The susceptibility to chloramphenicol and trimethoprim for *S. enterica* serovar Typhi isolates was determined with resistivity to both chloramphenicol and trimethoprim [40]. The study conducted by Reddy and Joss, utilized the gram-positive bacteria: *Bacillus cereus* (MTCC-1305), and *Staph. aureus* (MTCC 96), and gram-negative bacteria: *Enterobacter faecalis* (MTCC 5112), *Salmonella paratyphi* (MTCC 735), *E. coli* (MTCC 729), *K. pneumoniae* (MTCC 109), *Ps. aeruginosa* (MTCC 647), *Pr. vulgaris* (MTCC 426) and *Serratia marcescens* (MTCC 86), and showed top ZDI (36 mm) against *E. coli* and *K. pneumoniae* [41]. Padhi and Mahapatra utilized *E. coli* (MTCC 40), *Staph. aureus* (MTCC 87), *Staph. epidermidis* (MTCC 2639), *Ps. aeruginosa* (MTCC 424), *Str. pneumoniae* (MTCC 237) and *Pr. vulgaris* (MTCC 426), and found highest susceptibility of *E. coli* to tetracycline (ZDI: 32 mm) [15]. The standard strains of *E. coli* DSM 1103 and *Staph. aureus* ATCC 25923 were sensitive to ampicillin and CIP [42]. In the present investigation, CPD, GEN and CIP were used to determine antibiotic susceptibility tests, where CIP showed greater ZDI (30 mm) against *Pr. vulgaris*, compared to the other antibiotics. The action of antibiotics can be stimulated by the plant extracts (BLE, BSE and BBE), as has been reflected in this study. In the present study calculated GIIs ranged 0.5-0.89, and thus, all test result showed synergistic and additive activities, but not antagonistic. However, no significant difference between the action of antibiotic alone and in combination was seen (p= 0.22-0.42).

The presence of alkaloids, carbohydrates, glycosides, tannins and phenolic compounds, steroids, saponins and flavonoids in the aqueous *M. elengi* leaf extract has been reported by Padhi and Mahapatra [15]. Lalitha et al. observed cardiac glycosides, tannins, alkaloids, flavonoids, saponins, and reducing sugar present in BLE [38]. Dichloromethane, The *M. elengi* seed extracts prepared with petroleum ether, ethyl acetate and ethanol had antibacterial activity against *E. coli*, *B. subtilis* and *S. enterica* serovar Typhi, as has been reported by Hazra et al. [43]. The phytochemicals detected in BLE included β -carotene and glucose, quercitol and hentriacontane, β -sitosterol, β -sitosterol- β -D-glucoside, D-mannitol and quercetin [44-46]. *M. elengi* bark has been shown to contain alkaloids, saponins, tannin and ash forming inorganic salts [47,48]. In this study, the presence of glycosides, steroids, terpenoids and quinone was noted in BLE and BBE, and flavonoids, glycosides, steroids, terpenoids and quinone in BSE. The findings of the current study, along with the reports made by the others, the *M. elengi* might be useful in the preparation of cost effective new antimicrobials, alone and in combination with antibiotics, in combating bacterial antibiotic resistance.

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