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Full Length Research Paper

Antibacterial activity of *Mangifera indica* L. seeds against some human pathogenic bacterial strains

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Antibacterial activity of methanol extract of *Mangifera indica* L. seeds was done against 41 clinically isolated and 20 standard bacterial strains. Clinical bacterial strains were isolated from different specimens like blood, urine, catheter, stool and pus. Antibacterial activity was done by agar disc diffusion method at two different concentrations (600 and 1200 µg/disc). Preliminary qualitative phytochemical study was carried out for the presence of alkaloids, flavonoids, tannins, cardiac glycosides, steroids and saponins. Total phenol content and flavonoid content were also measured. The extract showed potent antibacterial activity against all the clinically isolated bacterial strains and most of the standard bacterial strains which was comparable with that of standard antibiotics studied. Phenols were present in higher amount than flavonoids. The qualitative analysis showed only presence of tannins. It can be stated that the antibacterial activity may be due to the presence of tannin and higher amount of total phenol content. The present study supports the folkloric use of *M. indica* and it can be an effective potential candidate for the development of new strategies to treat bacterial infections.

Key words: *Mangifera indica,* antibacterial, clinical isolates, phytochemical amalysis.

INTRODUCTION

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used anti-microbial agents is increasing with an alarming frequency (Ge et al., 2002; Nair and Chanda, 2005; Neogi et al., 2008). Surveys have revealed that almost no group of antibiotics has been introduced to which resistance had not been observed (Eloff, 2000). In addition to this problem, antibiotics are sometimes associated with adverse side effects on the host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immuno-suppression and allergic reactions (Al-Jabri, 2005). The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indis-criminate use of broad-spectrum antibiotics, immune-suppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Ng, 1994; Dean and Burchard, 1996; Gonzalez et al., 1996). Examples include methicillin-resistant staphy-lococci, pneumococci resistant to penicillin and macrolides, vancomycin-resistant Enterococci as well as multi-drug resistant gram-negative organisms (Norrby et al., 2005). There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross-infection (Voravuthikunchai and Kitpipit, 2005; Sung and Lee, 2007).

Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care (Farnsworth et al., 1985; Akinyemi et al., 2005). *Mangifera indica* (Anacardiaceae) grows in the tropical and subtropical region and its parts are commonly used

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in folk medicine for a wide variety of remedies (Coe and Anderson, 1996; Yogisha and Raveesha, 2009). Different pharmacological activities like antioxidant (Stoilova et al., 2005), radioprotective (Jagetia and Baliga, 2005), anti-allergic (Rivera et al., 2006), antiviral (Guha et al., 1996), antidiabetic (Bhowmik et al., 2009), etc from different parts of *M. indica* are reported.

In the present study antibacterial activity and preliminary phytochemical analysis were carried out in methanol extract of *M. indica* seeds.

MATERIALS AND METHODS

Plant material

Fresh seeds of *M. indica* were collected Gujarat region, India. Seeds were washed in tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction

Ten grammes (10 g) of air-dried powder was placed in 100 ml of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190 - 220 rpm for 24 h. After 24 h, the supernatant was discarded and petroleum ether was evaporated from the powder. This dry powder was then placed in 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190 - 220 rpm for 24 h. After 24 h, the extract was centrifuged at 5000 g for 10 min, the supernatant was collected, methanol was evaporated and the dry extract was stored at 4°C in airtight bottles. The extraction was done at least three times (every time fresh material was taken) and the mean values of extractive yields are presented (Vaghasiya and Chanda, 2007).

Microorganisms

The standard microorganisms (American Type Culture Collection) were obtained from National Chemical Laboratory, Pune, India and clinically isolated microorganisms were obtained from Department of Microbiology, Smt. N.H.L. Municipal Medical College, Sheth V.S. General Hospital, Ahmedabad and Spandan Diagnostics, Rajkot. The bacterial strains were grown in the nutrient broth and maintained on nutrient agar slants at 4°C.

Preparation of the extract for antimicrobial assay

Dry methanol extract was dissolved in 100% dimethylsulphoxide (DMSO) for antimicrobial study at two different concentrations (600 and 1200 µg/disc).

Antibiotics

Six antibiotics were used for antibiotic susceptibility study on identified (ATCC or NCTC or NCIM) and clinically isolated microorganisms. All antibiotic discs were purchased from Hi-Media, Bombay, India. The names and concentration of the antibiotics are as follows: Amikacin (10 mcg), Cefaclor (30 mcg), Ciprofloxacin (10 mcg), Imipenem (10 mcg), Methicillin (5 mcg), Tetracycline (10 mcg).

Antibacterial assay

The antibacterial assay was performed by agar disc diffusion method (Bauer et al., 1966; NCCLS, 2003; Parekh and Chanda, 2007). The molten Mueller Hinton Agar (HiMedia) was inoculated with 200 µl of the inoculum (1×10^8 cfu) and poured into the sterile Petri plates (Hi-media). The disc (7 mm in diameter, Hi-Media) was saturated with 20 µl of the extract and discs were air dried. Thereafter the discs were introduced on the upper layer of the seeded agar plate. Paper discs loaded with 20 µl of DMSO served as negative control. Six standard antibiotics were used as positive controls. The plates were incubated at 37°C for all the bacterial strains. The experiment was carried out three times and the mean values are presented. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition in mm.

Preliminary qualitative phytochemical study

Preliminary qualitative phytochemical study was carried out for the presence of alkaloids (Dragondroff's reagent, Wagner's reagent, Mayer's reagent), flavonoids (Shinoda test), tannins, cardiac glycosides (Keller-kiliani test), steroids (Liebermann-Burchard reaction) and saponins (Frothing test) (Harbone, 1998).

Total phenol determination

Total phenolic content of the extract was determined by Folin Ciocalteu reagent method (Mc Donald et al., 2001) with some modifications. Plant extract (1 ml) was mixed with Folin Ciocalteu reagent (0.1 ml, 1 N), and allowed to stand for 15 min. Then 5 ml of saturated Na_2CO_3 was added. The mixtures were allowed to stand for 30 min at room temperature and the total phenols were determined spectrophotometrically at 760 nm. Gallic acid was used as a standard. Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of extracted compound).

Flavonoid determination

Aluminium chloride colorimetric method (Chang et al., 2002) with some modifications was used to determine flavonoid content. Plant extract (1 ml) in methanol was mixed with 1 ml of methanol, 0.5 ml aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature; then the absorbance was measured at 415 nm. Quercetin was used as standard. Flavonoid content is expressed in terms of quercetin equivalent (mg g^{-1} of extracted compound).

RESULTS AND DISCUSSION

The use of antimicrobial agents is critical to the successful treatment of infectious diseases. Although there are numerous classes of drugs that are routinely used to treat infections in humans, pathogenic microorganisms are constantly developing resistance to these drugs (Al-Bari et al., 2006) because of indiscriminate use of antibiotics (Gibbons, 1992; Rahman et al., 2001). The use of higher plants and preparations made from them to treat infections is a longstanding practice in a large part of the population, especially in the developing countries, where there is dependence on traditional medicine for a variety of ailments (Ahmad and Mohammad, 1998). Interest in

Table 1. Biochemical identification of clinically isolated Gram positive bacteria.

Organism	NA	McA	BA	Sp	C	CA	AP	BEA	MR	VP	U	AU	M	L	S	Ar
Ent-1 (B)	TT	LFT	NH	Cocci	-	ND	ND	+	-	-	ND	+	-	+	+	+
Ent-2 (W)	TT	LFT	NH	Cocci	-	ND	ND	+	-	-	ND	+	+	+	+	-
S. alb-1 (B)	LO	LFT	BH	Cocci	+	ND	+	ND	+	-	-	+	-	-	+	-
S. alb-2 (B)	LO	LFT	BH	Cocci	+	ND	+	ND	+	-	+	+	-	+	-	-
S. alb-3 (B)	LO	LFT	BH	Cocci	+	ND	+	ND	+	-	-	+	-	+	+	-
S. alb-4 (P)	LO	LFT	BH	Cocci	+	ND	+	ND	+	-	-	-	-	+	+	-
S. aur-1 (P)	LO	LFT	BH	Cocci	+	+	+	ND	+	-	+	+	+	+	+	-
S. aur-2 (P)	LO	LFT	BH	Cocci	+	+	+	ND	+	-	+	+	+	+	+	-
S. aur-3 (St)	LO	LFT	BH	Cocci	+	+	+	ND	+	-	+	+	+	+	+	-
S. cit-1 (P)	LO	LFT	BH	Cocci	+	ND	+	ND	+	-	-	-	-	+	+	-
S. cit-2 (P)	LO	LFT	BH	Cocci	+	ND	+	ND	+	-	+	+	-	-	+	-
S. cit-3 (U)	LO	LFT	BH	Cocci	+	ND	+	ND	+	-	-	+	-	+	-	-
S. cit-4 (U)	LO	LFT	BH	Cocci	+	ND	+	ND	+	-	+	+	-	+	+	-
Staph-1 (B)	LO	LFT	BH	Cocci	+	+w	+	ND	+	-	+	+	-	+	+	-
Staph-2 (P)	LO	LFT	BH	Cocci	+	+w	+	ND	+	-	+	+	-	-	+	-
Staph-3 (St)	LO	LFT	BH	Cocci	+	+w	+	ND	+	-	-	+	-	+	-	-
Staph-4 (St)	LO	LFT	BH	Cocci	+	+w	+	ND	+	-	+	+	-	+	+	-

NA, Nutrient agar; McA, MacConkey's agar; BA, Blood agar; Sp, Shape; C, Catalase test; CA, Coagulase test; AP, Alkaline phosphates; BE, Bile esculine agar; MR, Methyl Red test; VP, Voges Proskauer's test; U, Urease test; AU, Arginine utilization test; M, Mannitol test; L, Lactose test; S, Sucrose test; Ar, Arabinose test; TT, Tiny transparent; LFT, Lactose fermenting tiny colony; NH, Non haemolysis; LO, Large opaque colony; BH, β haemolysis; ND, Note done; +W, Weak positive; B, Blood; C, Catheter; P, Pus; St, Stool; W, Wound; U, Urine; Ent, *Enterococci* species; S. alb, *Staphylococcus albus*; S. aur, *Staphylococcus aureus*; S. cit, *Staphylococcus citrous*; Staph, *Staphylococcus* species; Kleb, *Klebsiella* species.

plants with antimicrobial properties increased because of current problems associated with the antibiotics (Emori and Gaynes, 1993; Pannuti and Grinbaum, 1995). Recently, the antimicrobial effects of various plant extracts against certain pathogens have been reported by a number of researchers (Ahmed and Beg, 2001; Erasto et al., 2004; Nair et al., 2007; Carneiro et al., 2008; Liasu and Ayandele, 2008; Parekh and Chanda, 2008).

Disc diffusion method is the most widely used procedure for testing antimicrobial susceptibility (Kumar et al., 2006). The disc diffusion procedure (Kirby-Bauer method) has been accepted by the Food and Drug Administration (FDA) and as a standard by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). In recent years, secondary plant metabolites (phytochemicals) with antibacterial potency have been actively investigated as alternatives to and/or in combination with antibiotics in the therapy of bacterial infections (Sato et al., 1995; Liu et al., 2001).

The results of antibacterial activity of methanol extract of *M. indica* against 41 clinically isolated bacterial strains and 20 standard bacterial strains are shown in Tables 1, 2, 3 and 4. The methanol extract showed 100% antibacterial activity against all the clinically isolated bacterial strains studied. The antibacterial activity was comparable with that of standard antibiotics. Amongst the Gram positive bacteria studied (Table 1), *Enterococci* species showed maximum antibacterial activity which was comparable with some of the standard antibiotics studied. Amongst the Gram negative bacteria studied (Table 2), *E. coli* from urine sample (E. coli-16) showed maximum antibacterial activity which was higher than all antibiotics studied. Antibacterial activity against *Salmonella typhimurium* was almost similar to that of all antibiotics studied.

The antibacterial activity was more against Gram positive bacteria than Gram negative bacteria. Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria (Oboh et al., 2007; Nair and Chanda, 2007; Costa et al., 2008; Khan et al., 2008). These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure (Essawi and Srour, 2000). The complex nature of cell wall structure of Gram negative bacteria may inhibit the passage of the active compound through the Gram negative cell wall.

Result of phytochemical analysis of methanolic extract of *M. indica* is shown in Table 5. Qualitative phytochemical analysis showed presence of high amount of tannis, very low amount of steroids and other phytoconstituents were absent. On the quantitative basis also, total phenols were present in large amounts while flavonoids were present in very low quantity.

Table 2. Biochemical identification of clinically isolated Gram negative bacteria.

Organism	GS	Sp	Mot	I	MR	VP	C	U	H_2S	N	G	S	L	X
E. coli-1(F)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	+	+
E. coli-2 (P)	-ve	Rod	M	+	+	-	-	-	-	+	+	-	+	+
E. coli-3 (P)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	-	+
E. coli-4 (St)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	-	+
E. coli-5 (St)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	+	+
E. coli-6 (St)	-ve	Rod	M	+	+	-	-	-	-	+	+	-	+	+
E. coli-7 (St)	-ve	Rod	M	+	+	-	-	-	-	+	+	-	+	+
E. coli-8 (St)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	+	+
E. coli-9 (St)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	+	+
E. coli-10 (St)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	-	+
E. coli-11 (w)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	+	+
E. coli-12 (w)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	+	+
E. coli-13 (U)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	+	+
E. coli-14 (U)	-ve	Rod	M	+	+	-	-	-	-	+	+	-	+	+
E. coli-15 (U)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	-	+
E. coli-16 (U)	-ve	Rod	M	+	+	-	-	-	-	+	+	+	+	+
Kleb-1 (B)	-ve	Rod	NM	-	-	+	+	+	-	+	+	+	+	+
Kleb-2 (B)	-ve	Rod	NM	-	-	+	+	+	-	+	+	+	+	+
Kleb-3 (B)	-ve	Rod	NM	-	-	+	+	+	-	+	+	+	-	+
Kleb-4 (C)	-ve	Rod	NM	-	-	+	+	+	-	+	+	-	+	+
Kleb-5 (P)	-ve	Rod	NM	-	-	+	+	+	-	+	+	+	+	+
Kleb-6 (w)	-ve	Rod	NM	-	-	+	+	+	-	+	+	+	-	+
Kleb-7 (U)	-ve	Rod	NM	-	-	+	+	+	-	+	+	+	+	+
Prot-1 (w)	-ve	Rod	AM	+	+	-	+	+	+	+	+	+	-	+

GS, Gram's stain; Sp.,Shape; Mot., Motile; NM, Non motile; AM, Activiely mobile; I, Indol test; MR, Methyl red test, VP, Voges Proskauer's test, C, citrate test; U, Urease test; H_2S, Hydrogen sulphid; M, Maltose test; L, Lactose test; S, Sucrose test; B, Blood; C, Catheter; P, Pus; St, Stool; w, Wound; U, Urine; E. coli, *Escherichia coli*; Kleb, *Klebsiella* species; Prot, *Proteus* species.

Table 3. Antibacterial activity of methanol extract of *M. indica* seed against clinically isolated and standard strains of Gram positive bacteria.

Organism	MIM		Antibiotics					
	600 µg	1200 µg	M	Ak	T	I	Cj	Cf
Ent-1 (B)	19	21	-	25	31	30	16	35
Ent-2 (W)	16	16	-	16	32	21	16	20
S. alb-1 (B)	9	14	-	16	11	18	12	-
S. alb-2 (B)	11	13	-	19	10	40	-	-
S. alb-3 (B)	13	13	-	10	8	15	-	-
S. alb-4 (P)	10	14	-	10	-	16	-	-
S. aur-1 (P)	11	13	15	10	20	30	-	-
S. aur-2 (P)	11	13	15	10	24	32	-	-
S. aur-3 (St)	12	14	12	-	12	24	19	8
S. cit-1 (P)	12	18	-	21	21	40	26	20
S. cit-2 (P)	11	14	-	16	20	30	30	23
S. cit-3 (U)	13	15	-	14	20	30	22	20
S. cit-4 (U)	11	15	-	20	20	35	25	-
Staph-1 (B)	14	14	-	13	28	15	10	15
Staph-2 (P)	12	13	17	10	20	42	20	-
Staph-3 (St)	13	14	12	11	20	30	13	9
Staph-4 (St)	12	14	12	17	16	21	16	-

Table 3. Contd

Bacillus cereus ATCC11778	15	15	-	11	17	36	20	19
Bacillus megaterium ATCC9885	8	-	14	23	19	41	12	27
Bacillus subtilis ATCC6633	9	10	16	15	21	37	35	30
Cornybacterium rubrum ATCC14898	14	15	15	16	20	24	27	16
Micrococcus flavus ATCC10240	13	12	10	20	26	37	30	21
Staphylococcus aureus ATCC25923	13	14	20	12	15	25	25	15
Staphylococcus aureus ATCC29737	13	15	16	13	19	25	20	14
Staphylococcus epidermidis ATCC12228	14	15	18	16	25	38	22	24

B, Blood; C, Catheter; P, Pus; St, Stool; W, Wound; U, Urine; Ent, *Enterococci* species; S. alb, *Staphylococcus albus*; S. aur, *Staphylococcus aureus*; S. cit, *Staphylococcus citrous*; Staph, *Staphylococcus* species; E. coli, *Escherichia coli*; Kleb, *Klebsiella* species; Prot, *Proteus* species; M, Methicillin; Ak, Amikacin; T, Tetracycline; I, Imipenem; Cj, Cefaclor; Cf, Ciprofloxacin; no activity.

Table 4. Antibacterial activity of methanol extract of *M. indica* seed against clinically isolated and standard strains of Gram negative bacteria.

Organism	MIM		Antibiotics					
	600 µg	1200 µg	M	Ak	T	I	Cj	Cf
E. coli-1(F)	10	11	-	-	18	11	-	-
E. coli-2 (P)	9	10	-	13	-	20	-	-
E. coli-3 (P)	10	11	-	15	-	19	-	-
E. coli-4 (St)	9	10	-	15	17	30	9	26
E. coli-5 (St)	10	11	-	13	9	14	-	-
E. coli-6 (St)	10	11	-	14	15	15	-	21
E. coli-7 (St)	11	10	-	16	-	19	-	-
E. coli-8 (St)	9	9	-	18	10	20	-	-
E. coli-9 (St)	9	10	-	15	-	20	-	-
E. coli-10 (St)	12	15	-	15	-	20	-	-
E. coli-11 (W)	9	10	-	13	9	17	-	-
E. coli-12 (W)	9	10	-	17	8	20	-	-
E. coli-13 (U)	10	10	-	25	11	20	13	12
E. coli-14 (U)	10	11	-	20	24	16	11	-
E. coli-15 (U)	8	10	-	18	9	25	-	-
E. coli-16 (U)	15	17	-	15	-	16	-	-
Kleb-1 (B)	9	10	-		10	19	-	-
Kleb-2 (B)	9	10	-	15	13	15	-	-
Kleb-3 (B)	9	9	-	-	15	20	-	-
Kleb-4 (C)	10	11	-	20	9	21	-	-
Kleb-5 (P)	9	11	-	8	10	11	-	-
Kleb-6 (W)	10	11	-	13	12	16	-	-
Kleb-7 (U)	10	11	-	15	-	20	10	-
Prot-1 (W)	9	10	-	9	10	9	-	-
Citrobacter freundii ATCC10787	15	16	-	8	15	25	8	13
Enterobacter aerogenes ATCC13048	-	-	-	16	11	19	-	11
Klebsiella aerogenes NCTC418	13	14	-	-	10	30	16	10
Proteus mirabilis NCIM2241	13	13	10	18	24	31	12	-
Proteus morganii NCIM2040	11	14	-	10	15	30	21	11
Proteus vulgaris NCTC8313	14	15	-	11	15	15	-	18
Pseudomonas pictoruim NCIB9152	12	11	14	20	25	27	23	23
Pseudomonas stutgeri ATCC17588	14	15	-	28	25	38	-	30
Pseudomonas syringae NCIM5102	11	11	-	-	20	30	21	-
Pseudomonas testosteroni NCIM5098	-	-	-	12	15	11	-	25

Table 4. Contd

Salmonella typhimurium ATCC23564	16	17	-	15	18	16	17	15
Escherichia coli ATCC25922	-	-	-	21	22	30	24	31

B, Blood; C, Catheter; P, Pus; St, Stool; W, Wound; U, Urine; E. coli, *Escherichia coli*; Kleb, *Klebsiella* species; Prot, *Proteus* species; MIM, Methanol extract of *M. indica*; M, Methicillin, Ak, Amikacin; T, Tetracycline; I, Imipenem; Cj, Cefaclor; Cf, Ciprofloxacin; - means no activity.

Table 5. Phytochemical study of *M. indica* seed.

Phyto-constituents	Result
Qualitative analysis	
Alkaloids	
Dragondroff's reagent	-
Wagner's reagent	-
Mayer's reagent	-
Tannins	+++
Cardiac glycosides	-
Steroids	+
Flavonoids	-
Saponins	-
Quantitative analysis	
Total phenol (mg/g)	427.28 ± 10.5
Flavonoids (mg/g)	6.36 ± 0.3

Conclusion

The methanol extract of *M. indica* seeds showed antibacterial activity against all the clinically isolated bacterial strains and most of the standard bacterial strains which was comparable with that of investigated antibiotics. The results of the present study suggest that the *M. indica* seed extract possess compounds with antibacterial properties that can be explored as a viable, alternative source to commercially available antibiotic drugs. Further studies are needed to isolate and characterize the major active constituent of methanolic extract of *M. indica* seed and test it on other microorganisms and against various infections, where in the information procured would further serve as a strong evidence for the plant as potent antimicrobial agent.

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