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Antimicrobial and Free Radical Scavenging Activity of Different Solvent Extracts of *Mangifera indica* L. Seeds

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Abstract: The aim of the present study was to evaluate antibacterial and antioxidant activity of different solvent extracts of *Mangifera indica* L. seeds. The seeds of *Mangifera indica* L. were successively extracted in petroleum ether, chloroform, ethyl acetate, acetone and methanol. All obtained extracts were evaluated for antimicrobial and free radical scavenging (DPPH) activity. The *in vitro* antimicrobial activity was done by agar disc diffusion method at a concentration of 600 µg disc⁻¹ against 5 Gram-positive bacteria, 7 Gram-negative bacteria and 3 fungal strains. The antioxidant potential was evaluated using scavenging of DPPH radical. Maximum antibacterial activity was shown by methanol extract followed by acetone extract. Acetone (IC₅₀ = 11 µg mL⁻¹) and methanol (IC₅₀ = 12 µg mL⁻¹) extract also showed DPPH scavenging activity which was comparable with that of standard ascorbic acid (IC₅₀ = 11.4 µg mL⁻¹). This study has highlighted the potentiality of antibacterial and antioxidant properties of *M. indica* seeds.

Key words: *Mangifera indica*, antimicrobial, antioxidant, DPPH, methanol extract

INTRODUCTION

There is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. In addition, bacterial adaptation to antibiotics and increase in antibiotic resistance over the past decade has generated a considerable worldwide public health problem (Andersson, 2003). Plants contain numerous biologically active compounds, many of which have antimicrobial properties (Cowan, 1999). Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds (Chah *et al.*, 2006; Nair and Chanda, 2006; Parekh and Chanda, 2007a).

Free radicals are often generated as by products of biological reactions or from exogenous factors. The involvement of free radicals in the pathogenesis of a large number of diseases is well documented. A potent scavenger of free radicals may serve as a possible preventive intervention for the diseases (Gyamfi *et al.*, 1999). A large number of naturally occurring compounds such as flavonoids, catechins, lignans and phenolic acids contained in edible plants and medicinal herbs have antioxidant properties. Dietary antioxidant intake has been associated for example with reduced risk of cardiovascular diseases, cancer and neurodegenerative diseases (Nair *et al.*, 2007).

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Mangifera indica L. is commonly grown in many parts of the world belongs to the family Anacardiaceae. *Mangifera indica* L., a plant widely used in the traditional medicinal systems of India, has been reported to possess antiviral, antibacterial and anti-inflammatory activities (Makare *et al.*, 2001). The seeds have been used for anti-diarrhoeal activity in Indian traditional medicine (Sairma *et al.*, 2003). The young and the unripe fruits of mango are acidic in taste and utilized for various culinary purposes. The ripe fruits are used in preparing various processed products such as squash, nectar, jam, cereal flakes, custard powder, baby food and toffee etc. (CSIR, 1962). The leaves showed antibacterial activity (Bbosa *et al.*, 2007) however there are hardly any reports on the antimicrobial and antioxidant activity of mango seed. The aim of this study was to determine the antimicrobial and antioxidant activity of the seed extracts of *M. indica*.

MATERIALS AND METHODS

Plant Material

Fresh seeds of *Mangifera indica* L. (kesar) were collected in the month of June 2008, from Talala (Gir), Junagadh, Gujarat, India. The taxonomic identity of the plant was confirmed by Dr. N.K. Thakrar, Department of Biosciences, Saurashtra University, Rajkot, India. Fresh seeds were washed, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction

The dried powder of *M. indica* was extracted in a series of solvents of increasing polarity. Petroleum ether, chloroform, ethyl acetate, acetone and methanol were used for the extraction. Ten grams of dried powder of *M. indica* was taken in 100 mL of solvent in a conical flask, plugged with cotton wool and then kept on a rotary shaker for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth. Remaining residue was dried and used for extraction in another solvent. Filtrate was centrifuged at 5000 g for 10 min. The supernatant was collected and the solvent was evaporated. The dried extract was stored at 4°C in airtight bottles.

Microorganisms

The tested microorganisms were obtained from National Chemical Laboratory, Pune, India. Amongst fifteen microorganisms investigated five were Gram positive bacteria (*Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC12228, *Bacillus cereus* ATCC11778, *Bacillus subtilis* TCC6633, *Micrococcus flavus* ATCC10240) while seven were Gram negative bacteria (*Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* NCIM2719, *Proteus mirabilis* NCIM2241, *Proteus vulgaris* NCTC8313, *Salmonella typhimurium* ATCC23564, *Citrobacter freundii* ATCC10787) and three fungus strains were *Candida albicans* ATCC2091, *Candida tropicalis* ATCC4563 and *Cryptococcus luteolus* ATCC32044. The bacteria were grown in the nutrient broth and maintained on nutrient agar slants at 4°C, while fungal strains were grown in sabourad broth and maintained on MGYB slants at 4°C.

Antimicrobial Assay

The antimicrobial assay was performed by agar disc diffusion method (Bauer *et al.*, 1996; Parekh and Chanda, 2007b). The molten Mueller Hinton Agar (HiMedia) was inoculated with 200 μ L of the inoculum (1×10^8 cfu) and poured into the sterile Petri plates (Hi-media). The disc (0.7 cm) (Hi-Media) was saturated with 20 μ L of the extract ($600 \mu\text{g disc}^{-1}$) in 100% dimethyl

sulphoxide (DMSO) and air dried. Thereafter the discs were introduced on the upper layer of the seeded agar plate. Piperacillin (100 µg disc⁻¹), Amikacin (30 µg disc⁻¹), Fluconazole (10 µg disc⁻¹) and Amphotericin-B (100 units disc⁻¹) were used as positive controls. Paper discs loaded with 20 µL of DMSO served as negative control. The plates were incubated at 37°C for all the bacterial strains while that of fungal strains were incubated at 28°C for 48 h. The experiment was done three times and the mean values are presented. The antimicrobial activity was evaluated by measuring the inhibition zones.

DPPH Free Radical Scavenging Assay

The free radical scavenging of the extracts of *M. indica* was measured using the modified method of McCune and Johns (2002) was used. The extracts were dissolved in methanol. One milliliter of the extract was added to 1 mL of a solution of DPPH radicals (0.3 mM) dissolved in methanol. The mixture was shaken vigorously and allowed to stand for 10 min at room temperature. The absorbance of the resulting solution was measured at 517 nm. The radical scavenger activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC₅₀). The IC₅₀ value for each sample was determined graphically by plotting the percentage disappearance of DPPH as a function of the sample concentration.

RESULTS AND DISCUSSION

Disc diffusion methods are extensively used to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of substances to be examined (Sambath-Kumar *et al.*, 2006). In this study, 5 Gram-positive, 7 Gram-negative and three fungal strains were used to screen the possible antimicrobial activities of different solvent extracts of *M. indica* seeds.

The extractive yield of petroleum ether, chloroform, ethyl acetate, acetone and methanol was 13, 2.9, 0.8, 1.8 and 5%, respectively. The maximum yield was in petroleum ether while minimum was in ethyl acetate. None of the extracts, showed activity against the fungal strains studied. Antifungal activity is not common in medicinal plants. For example 23 extracts of 12 Cuban plants, used in traditional medicine failed to inhibit the growth of yeast (Marting *et al.*, 1996). Petroleum ether extract also did not show any activity against Gram positive and Gram negative bacterial strains investigated.

The antibacterial activity was different with different solvent extracts. Minimum antibacterial activity was with chloroform extract followed by ethyl acetate, acetone and methanol extracts (Table 1). Maximum activity was in methanol extract. This trend was

Table 1: Antimicrobial activity of *mangifera indica* L. seed extracts in different solvents

Extracts/antibiotics	Diameter of inhibition zones (mm)											
	Gram positive bacteria					Gram negative bacteria						
	SA	SE	BC	BS	MF	PA	EC	KP	PM	PV	ST	CF
Chloroform (600 µg disc ⁻¹)	11	10	10	-	17	-	10	9	10	10	10	10
Ethyl acetate (600 µg disc ⁻¹)	12	13	15	-	19	10	15	12	11	15	11	10
Acetone (600 µg disc ⁻¹)	12	14	16	8	20	11	14	15	12	17	12	11
Methanol (600 µg disc ⁻¹)	13	14	16	8	22	12	15	17	12	17	12	11
Amikacin (30 µg disc ⁻¹)	14	22	19	32	24	21	15	24	23	14	15	12
Piperacillin (100 µg disc ⁻¹)	20	16	17	15	30	7	17	29	20	-	23	19

SA: *Staphylococcus aureus*, SE: *Staphylococcus epidermidis*, BC: *Bacillus cereus*, BS: *Bacillus subtilis*, MF: *Micrococcus flavus*, PA: *Pseudomonas aeruginosa*, EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, PM: *Proteus mirabilis*, PV: *Proteus vulgaris*, ST: *Salmonella typhimurium*, CF: *Citrobacter freundii*, Zone of inhibition includes disc diameter (7 mm), n = 3, -: No activity, negative control did not show any activity

observed in all the Gram positive and Gram negative bacterial strains investigated. Ahmad *et al.* (1998), Ellof (1998) and Parekh *et al.* (2005) reported that methanol is a better solvent than ethanol or hexane for consistent extraction of antimicrobial substances from medicinal plants. The most susceptible Gram positive bacteria was *M. flavus* with inhibition zone of 22 mm (methanol extract) and 20 mm (acetone extract) while most susceptible Gram negative bacteria *P. vulgaris* with 17 mm inhibition zone with methanol and acetone extracts. The most resistant bacteria was Gram positive *B. subtilis* with almost no antibacterial activity.

The antibacterial activity of different extracts was comparable with standard antibiotics. Generally Gram-negative bacteria are more resistant than Gram-positive bacteria because of the complexity of the cell wall of Gram negative bacteria but in the present study, the *M. indica* seed extracts in different solvents showed activity against both Gram positive and Gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. Some antibiotics have become almost obsolete because of the problem of drug resistance (Ekpendu *et al.*, 1994) and the consequences of drug resistance implies that new drugs must be sought for and to treat diseases for which known drugs are no longer useful.

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). The extract of plant is allowed to react with the stable radical DPPH, in methanol solution. The reduction capability of DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by an antioxidant (AH) 10 min, as follows:

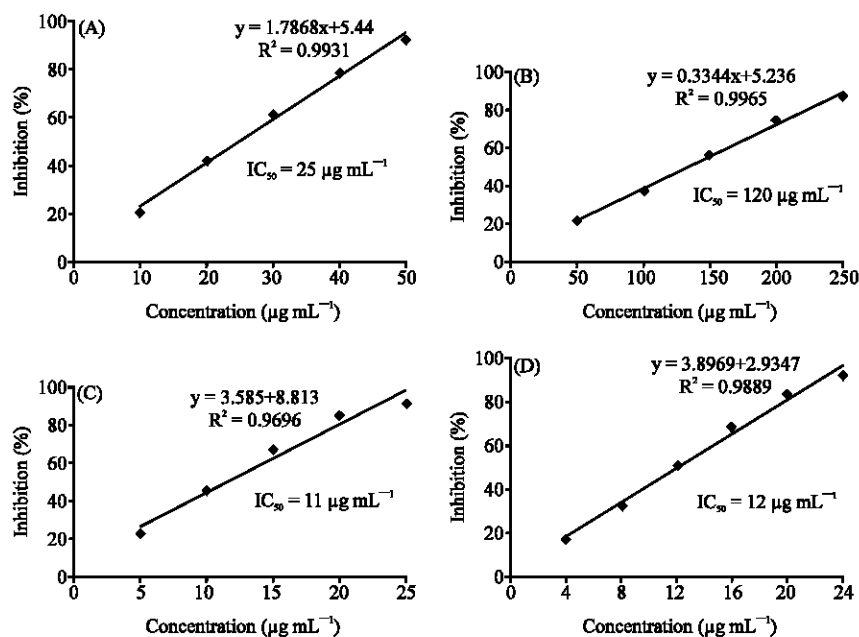
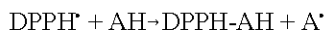


Fig. 1: DPPH radical scavenging activity of seed extracts of *M. indica* (A) ethyl acetate extract (B) chloroform extract (C) acetone extract and (D) methanol extract

The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% (IC₅₀) is a parameter widely used to measure the antioxidant activity (Sanchez-Moreno *et al.*, 1998). A lower IC₅₀ value corresponds with a higher antioxidant activity.

All the four extracts of *M. indica* seeds exhibited different extent on antioxidant activity. The IC₅₀ values of acetone (11 µg mL⁻¹) and methanol (12 µg mL⁻¹) extracts were almost equal to or better than standard ascorbic acid (11.4 µg mL⁻¹) while that of ethyl acetate and chloroform were 25 and 120 µg mL⁻¹, respectively. The IC₅₀ values for all the four extracts are shown in Fig. 1A-D. DPPH radical scavenging activity of acetone and methanol extracts of *M. indica* seeds revealed very high potency considering the fact that the free radical quenching properties were only from the crude extracts. Such high free radical scavenging properties of the crude extracts are shared by few other plants (Gulcin *et al.*, 2003; Harish and Shivanandappa, 2006). In the longer term, plant species or their active constituents identified as having high levels of antioxidant activity *in vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radical induced tissue damage.

CONCLUSION

It is interesting to note that the extracts are not pure compounds and in spite of it good results were obtained which only suggests the potency of these extracts. Hence *M. indica* seed extract could be used as a guide in our continuing search for new natural products with potential medicinal properties. The results of the present study indicate that *M. indica* extracts can be used as easily accessible source of natural antioxidants and antimicrobics. The potential for developing antimicrobials from higher plants is rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobials and antioxidants have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic compounds.

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