Antimicrobial Activity of Root of *Mangifera indica*

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Summary

Mangifera indica Linn (Anacardiaceae) is well known for curing a variety of ailments such as abscesses, broken horn, tumour, snakebite, stings, datura poisoning, heat stroke, anthrax, blisters and wounds in the mouths. Chloroform and ethanolic extracts were screened for antimicrobial activity against bacteria and fungi.
The antimicrobial activity was carried out using different dilutions of different extracts (5mg/ml, 10mg/ml, 25mg/ml and 50mg/ml) against gram positive strains (*Staphylococcus aureus, Micrococcus luteus, Bacillus subtilus)* and gram negative ones (*Pseudomonas aeruginosa, Escherichia coli),* and Fungi like *Candida albicans* and *Aspergillus niger* by the cup-plate assay method and minimum inhibitory concentrations (MICs).
The minimum inhibitory concentration of chloroform extract against bacterial strains *staphylococcus aureus, Bacillus subtilis, Micrococcus luteus* was 6.25µg/ml and against*, Pseudomonas aeruginosa and Escherichia coli* was 3.12µg/ml and the minimum inhibitory concentration of ethanolic extract against bacterial strains S*taphylococcus aureus, Bacillus subtilus* was observed 3.12µg/ml and against *Pseudomonas aeruginosa, Micrococcus luteus, Escherichia coli* was 6.25µg/ml. The minimum inhibitory concentration (MIC) of the chloroform extract was observed 12.5µg/ml and ethanolic extract was found 6.25µg/ml against *Candida albicans*.
Both the extracts chloroform and ethanolic extracts shows the significant zones of inhibition for all microorganisms studied and no MIC was found against Aspergillus *niger*.

Keywords: *Mangifera indica,* Antimicrobial activity, MIC, Microorganisms

Introduction

Medicinal plants are of important therapeutic aid for various ailments. It is estimated that an amount of 20,000 species from several families are useful for these purposes [1]. Furthermore, about 80% of the world population is dependent (wholly or partially) on plant-based drugs [2]. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century [3]. Naturally occurring antimicrobials can be derived from plants, animal tissues, or microorganisms [4]. The shortcomings of the drugs available today propel the discovery of new pharmacotherapeutic agents in medicinal plants [5].

In the present study we investigated the medicinal potential of the plant namely *Mangifera indica* Linn belonging to the *Anacardiaceae* family. Medicinally the plant is used in ophthalmia and eruption, hemorrhage of uterus, lungs or intestine [6]. The ripe fruit is laxative, diuretic. The dried mango peel can be used as a fuel for biogas plant. The all parts are used to treat abscesses, broken horn, tumour, snakebite, stings, datura poisoning, heat stroke, anthrax, blisters and wounds in the mouths. The seed kernel extracts have antibacterial activity against *Bacillus subtilis, Staphylococcus albus and Vibrio cholerae* [7, 8, 9] and antifungal activity [8]. An alcoholic extract of the seed kernel of *Mangifera indica* has anti-inflammatory activity [107]. Mangiferin was found to be effective in controlling herpes simplex virus type 2, in vitro [11, 12]. The induction of interferon release from the macrophages. The mangiferin have Immunomodulatory activity [12, 13, 14]. A 50% ethanolic extract of the leaves has hypoglycemic activity. *Mangifera indica* is rich sources of phenolic components, terpenoids, Saponins, hydrocarbons, Vitamins and carotenoids, some fatty acids and Essential oil. We herein report the antimicrobial activity of the crude extracts from the root of *Mangifera indica* against a wide range of microorganisms which cause infectious diseases.

Material and Methods

Collection of plant material: The root of *Mangifera indica* Linn was collected freshly from Sonipat (Haryana) in the month of December 2008 depending upon its easy availability. It was authenticated by Dr. H.B. Singh, at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (letter no. NISCAIR/RHMD/Conslt/2008-09/1121/152). The root of *Mangifera indica* was subjected to shed drying and further crushed to powder, and then the powder was passed through the mesh 40.

Preparation of extracts: The collected plant material was dried in the shade and ground to a powder. The dried and ground plant material (1.0 kg) was successively extracted with the solvents like chloroform and ethanol for 72 hours each. Both the solvent extracts were concentrated to dryness under reduced pressure. The obtained extracts were stored in a refrigerator at 4^0C until use.

Microbial strains: Four strains of bacteria and two strains of fungi from the Microbial Type Culture Collection (MTCC, IMTECH), Institute of Microbial Technology Sector – 39A, Chandigarh – 160036, INDIA, were tested: *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 737), *Bacillus subtilis* (MTCC 441), *Micrococcus luteus* (MTCC *106), *Escherichia coli* (MTCC 443), *Candida albicans* (MTCC 3017), and *Aspergillus niger* (MTCC 1344). All the strains were stored at freeze temperature until use.

Antimicrobial assays: The antimicrobial activity was evaluated by Cup-Plate method.
I. Culture media: Nutrient agar (NA) (Himedia) containing bromocresol purple was used for the activation of *Bacillus* species, while NA was used for other bacteria. Sabouraud glucose agar (Himedia) was used for the activation of the fungi. The Nutrient agar was used in sensitivity assay. Nutrient broth was used for MIC determination.
II. Chemicals for antimicrobial assay: Ciprofloxacin and Nystatin (Central Drug House (P). LTD., New Delhi-110002., India) were used as positive reference standards (RA) for all bacterial and fungi strains respectively. The dimethylsulfoxide (DMSO) (Qualigenis) was used as solvent for the tested samples.
III. Preparation of inoculums: Bacterial inoculums were prepared by growing freeze-dried cells in Nutrient Broth for 24 h at 37^0C. Slants were prepared by streaking of these cell suspensions and sub culturing was done by using the same broth to provide initial cell counts of about 10^4 CFU/ml did sub culturing and incubated at 37^0C for required time. The

filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 25^0C for seven days and the spores were collected using sterile doubled distilled water and homogenized.

IV. *Preparation of test sample:* The chloroform and ethanolic extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to obtain the different concentrations (5 mg, 10 mg, 25 mg and 50 mg per ml). Negative controls were used 10% aqueous dimethylsulfoxide (solvent control). Ciprofloxacin and Nystatin were used as positive reference standards having a concentration 5 µg per ml for all bacterial and fungi strains.

V) Cup-plate method assay: Petri plates were prepared by pouring 30 ml of Nutrient Agar Medium for all the bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spreaded and allowed to dry for 10 min. Three wells or cavities were made in agar containing each Petri dish by a sterilized steel borer. To these cavities standard and test compound solutions were filled. All the work was carried out under aseptic conditions for microbial assay. The plates for the bacteria were incubated at 37^0C ± 1^0C for 24 hours. The fungal strains *Candida albicans* and *Aspergillus niger* were incubated at 25^0C for 72 hours and seven days respectively. The antimicrobial potential of test compound was determined on the basis of mean diameter of zone of inhibition around the wells in millimeters. Each assay was carried out in the form triplicate three times. The results are shown in the Tables 1 & 2.

VI) Minimum Inhibitory Concentration (MIC): The experiment was according to two fold serial dilution method. The stock solution of test solutions (extracts) was prepared at concentration of 100µg/ml in nutrient broth and serially diluted up to five times. Six assay tubes were taken for screening of minimum inhibitory concentration of each strain. In the first tube 1ml of the sterilized nutrient broth was inoculated and then 1ml of the test compound solution was added and thoroughly mixed to concentration of 50µg/ml. Further dilutions of this solution were made by inoculating 1ml from first tube into second assay tube serially and 0.1 ml of each test inoculums were added in each tube and were done in duplicate. The procedures were conducted under aseptic conditions.

The inoculated tubes were kept at 37^0C ± 1^0C at 24 hours for bacterial assay and seven days for fungi (*Aspergillus niger*) & three days for fungi (*Candida albicans*) at 25^0C ± 0.1^0C during the incubation period. After the incubation period, tubes were removed and observed for any deposits or turbidity in the solution and shaken to suspend bacteria and fungi that might have been settled down. These concentrations were observed & assumed as minimum inhibitory concentration (MIC). The results are shown in the Tables 3 & 4.

Results And Discussion

Chloroform and ethanolic extracts were screened against bacteria and fungi. Both the shows activity against all bacterial strains (*Staphylococcus aureus, Micrococcus luteus, Bacillus subtilus, Pseudomonas aeruginosa,* and *Escherichia coli)* [Table 1].

The chloroform and ethanolic extracts shows activity against *Candida albicans* but no activity against *Aspergillus niger* [Table 2]. The results of the both extracts were correlated with standard drug and show that both of the extracts shows good activity against all bacteria and one fungus.

The minimum inhibitory concentration of chloroform extract against bacterial strains *staphylococcus aureus, Bacillus subtilis, Micrococcus luteus* was 6.25µg/ml and against, *Pseudomonas aeruginosa and Escherichia coli* was 3.12µg/ml and the minimum inhibitory concentration of ethanolic extract against bacterial strains S*taphylococcus aureus, Bacillus subtilus* was observed 3.12µg/ml and against *Pseudomonas aeruginosa, Micrococcus luteus, Escherichia coli* was 6.25µg/ml [Table 3]. The minimum inhibitory concentration (MIC) of the chloroform extract was observed 12.5µg/ml and ethanolic extract was found 6.25µg/ml

against *Candida albicans* and no minimum inhibitory concentration was found against *Aspergillus niger* [Table 4].

In conclusion, although the activities displayed by the chloroform & ethanolic extracts of *Mangifera indica* root are significant, the results reported here, render this species interesting for future research.

Table 1: Antibacterial activity of chloroform and ethanolic extracts of root of *Mangifera indica*

Extracts	Conc. (mg/ml)	Cup-plate method (inhibition zone, mm)				
		S. A.	M. L.	B. S.	P. A.	E. Coli
Chloroform	5 10 25 50	17±0.1 19±0.0 20±0.05 23± 0.05	18±0.11 19±0.05 20±0.1 21±0.11	17± 0.11 18±0.05 18±0.0 19±0.0	15±0.05 18±0.0 18± 0.0 19±0.0	12±0.1 14±0.05 21±0.05 22±0.1
Ethanol	5 10 25 50	- - 20±0.05 21±0.05	15±0.05 17±0.05 22±0.06 28±0.1	15±0.11 18±0.1 25±0.1 28±0.2	18±0.1 22±0.1 25±0.1 27±0.1	14±0.1 17±0.2 20±0.1 23±0.1
Ciprofloxacin	5 µg/ml	26 ± 0.051	14 ± 0.068	32 ± 0.024	25 ± 0.035	22 ± 0.056

S. A. –Staphylococcus aureus, M. L. – Micrococcus luteus, B. S. – Bacillus subtilus,

P. A. – Pseudomonas aeruginosa, E. coli **-** *Escherichia coli*;

- Sign shows no zone of inhibition

Table 2: Antifungal activity of chloroform and ethanolic extracts of root of *Mangifera indica*

Extracts	**Conc.(mg/ml)**	**Cup-plate method (inhibition zone, mm)**	
		CA	AN
Chloroform	5	15±0.0	-
	10	16±0.0	-
	25	17±0.0	-
	50	18±0.0	-
Ethanol	5	-	-
	10	-	-
	25	-	-
	50	-	-
Nystatin	5µ/ml	12.2±0.08	11.47±0.065

CA- Candida albicans, AN – Aspergillus niger; - Sign shows no zone of inhibition

Table 3: The results showed the MIC for antibacterial activity

Microorganism	**Extract**	**Serial dilution (µg/ml)**					
		50	25	12.5	6.25	3.12	1.56
Staphylococcus aureus	CHF	-	-	-	-	+	+
	Eth.	-	-	-	-	-	+
Micrococcus luteus	CHF	-	-	-	-	+	+
	Eth.	-	-	-	-	+	+
Pseudomonas aeruginosa	CHF	-	-	-	-	-	+
	Eth.	-	-	-	-	+	+
Bacillus subtilus	CHF	-	-	-	-	+	+
	Eth.	-	-	-	-	-	+
Escherichia coli	CHF	-	-	-	-	-	+
	Eth.	-	-	-	-	+	+

- No growth; + Growth; CHF – Chloroform extract; Eth. – Ethanol extract
Stock solution = 100 µg/ml

Table 4: The results showed the Minimum Inhibitory Concentration for antifungal activity

Microorganism	Extract	Serial dilution (µg/ml)					
		50	25	12.5	6.25	3.12	1.56
Candida albicans	CHF	-	-	-	+	+	+
	Eth.	-	-	-	-	+	+
Aspergillus niger	CHF	+	+	+	+	+	+
	Eth.	+	+	+	+	+	+

- No growth; + Growth

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