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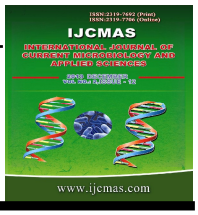

Original Research Article

Antibacterial Properties of *Mangifera indica* flower extracts on Uropathogenic *Escherichia coli*

P.Poongothai[1] and S. Rajan[2*]

[1]Research and Development Center, Bharathiyar University, Coimbatore
[2]Department of Microbiology, M.R.Government Arts College, Mannargudi, Tamil Nadu, India
Corresponding author

K e y w o r d s

Uropathogenic *Escherichia coli;* antimicrobial activity; MIC; Phytochemical screening.

A B S T R A C T

Uropathogenic *Escherichia coli* are the major cause of urinary tract infection. Modern combined antibiotics are used for the treatment of UTI, which cause various side effects and creates resistance among bacterial pathogens. To overcome these problems peoples turned towards indigenous medicinal system like siddha. Medicinal plants are the backbone of these indigenous system of medicine. *Mangifera indica* is commonly called as Mango. Traditionally dried mango flowers are astringent and given for diarrhoea, chronic dysentery, catarrah of the bladder. Antibacterial activity and qualitative phytochemical screening were done for aqueous and methanolic extracts by making use of standard methods. Antibacterial activity of *Mangifera indica* flower extracts were determined using disk diffusion, agar dilution methods. The results showed that the flower extracts *of M. indica* have antimicrobial activity against *Uropathogenic E.coli.* Methanol extracts showed the highest inhibition zone diameter of 22.6±1.2 mm. The plant extracts were shown to have a MIC 180±050µg/ml for aqueous extract and 055±025 µg/ml for methanolic extract. Phytochemical screening of the extracts revealed the presence of phenolic compounds, flavonoids and tannins in both the extracts tested, which are known to inhibit bacterial growth by different mechanisms from those of synthetic drugs. These phyto-constituents may be responsible for the *M. indica* antibacterial activity.

Introduction

E. coli is one of the most important urinay pathogen which causes acute as well as chronic infections (Jellheden *et al.*, 1996).The uropathogenic *Escherichia coli* (UPEC) strains are responsible for the majority of urinary tract infections (UTIs) that occur in 70-90%. The clinical management of UTI is complicated by the increasing incidence of infections caused by multidrug resistant *E. coli strains.* Ampicillin, Chloramphenicol, Kanamycin, Nalidixic acid, Nitrofurantoin, Streptomycin, Norfloxacin, Trimethoprim-Sulfamethoxazole (SMP-SMX) etc are commonly used for the treatment of UTI. Now a day, most of the commonly used

antibiotics are not effective for the treatment. The antibiotic resistance studies using urinary tract isolates of *E. coli* from the outpatient clinics have been reported to have shown increased resistance to these antibiotics (Zhanel *et al.,* 2000). To overcome the problems of antibiotic resistance and Multidrug resistant pathogens, people now rely on herbal medicine (Ndip *et al.,* 2006)

Plant derived products have been used for therapeutic purpose before the introduction of modern rugs (Lima *et al.,* 2006). WHO gave emphasis on the need to include traditional remedies within national drug policies as these plants serve as the best sources of a variety of drugs. It is important to study plants so that a better understanding of their properties, safety and efficacy is derived for improved benefit. In India, self-medication is common using herbs as well as synthetic antibiotics (Eisenberg, 1993). The reasons can be attributed to easy accessibility and affordability of plants compared to commercial drugs. Contrary to the belief that natural medicine has no ill effects (Lima *et al.,* 2006).

To address such challenges, plants must be investigated to validate and standardize their dosages. An estimated 74% of pharmacologically active plant derived components were discovered after following up on ethnomedicinal use (Ncube *et al.,* 2007). Thus, medicinal plants can be regarded as the richest bio-resource of drugs of modern medicine, folk medicine and chemical entities for synthetic drugs. There are many drugs in clinical use today that were discovered from the plants (Vanwyk *et al.,* 2002). There is little or no doubt that ethnographic research can provide important clues leading to new drugs for the modern pharmacies.

The emergence of multi-resistant bacteria to antimicrobial drugs has increased the need for new antibiotics or modifications of older antibiotics (Tollefson and Miller, 2000). One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agent (Mathur *et al.,* 2011). The new compound may actually be more effective than the parent compound. Since resistance is based on structural recognition, the new compound may not be recognized by resistance factors. Plants are regarded as cheaper and safe alternative source of drugs. An investigation of the antibacterial activity of Flower extracts of *Mangifera indica* on *Uropathogenic Escheichia coli* was carried out with a view to screen for phyto-chemical compounds and determine susceptibility of the bacterium.

Materials and Methods

Preparation of plant material

Flowers were collected from a *Mangifera indica tree*. The flowers were washed thoroughly with water and then air dried at room temperature for five days. After drying, the flowers were ground into powder and then sieved using a sieve. 500 grams of powdered plant were transferred into airtight containers and stored at room temperature.

Extraction of the crude extracts from flower powder

Plant active components were extracted using the cold extraction method (Fransworth, 1988). Water and methanol were used for the extraction. To 500ml each of pure methanol and sterile distilled water were added 50g portions of the

flower powder in sterile conical flasks and allowed to soak at room temperature for 48 hours. A shaker set at 120 rpm was used to improve extraction of phyto-chemicals. The filtrate was obtained by means of a vacuum filter pump. Filtering was repeated three times with same plant material until the solution was clear. The filtrate was evaporated in a weighed flask, with a water bath set at 40°C. A small proportion of dry extracts was stored for phyto-chemical analysis. Remaining portion of the extracts were used for antibacterial assay. Extracts were reconstituted by re-dissolving in DMSO. The final filtrates were filter-sterilized by using syringe filter with a pore size of 0.45µm. Sterile extracts obtained were stored separately in labelled, sterile capped bottles, in a refrigerator at 4°C before use during the antibacterial sensitivity tests.

Phytochemical analysis of plant extracts

The flower extracts were evaluated for the presence of phytochemical compounds using standard methods (Aiyelaagbe and Osamudiamen, 2009). Phytochemical examination was carried out separately for both the extracts.

Determination of antibacterial activity

Antimicrobial activity was performed by standard methods like the disk diffusion method on Mueller Hinton agar and MIC was calculated using drug dilution methods. Cells used for antibacterial assays are harvested at log phase while they are most active.

Preparation of inoculums

Direct colony suspension method was used to make a suspension of *E. coli*.Three to four colonies from overnight grown (18 hours) were suspended in saline using a sterile loop. The turbidity standard was shaken vigorously before use, and used to make a visual comparison with the density of the suspension against a white background with black lines. Density of the suspension was adjusted to 0.5 Mcfarland either by adding sterile saline. The standardized culture was used within 15 minutes of preparation for sensitivity tests.

Disc diffusion test

Antibacterial activities of the extracts were tested on Mueller-Hinton agar by disc diffusion method. Six Mueller Hinton agar plates were used for two extracts. To maintain sterile conditions this procedure was done in a laminar flow cabinet. The inoculum was spread evenly over the entire surface by swabbing in three directions using sterile cotton swab. Inoculated plates were allowed to dry for ten minutes before depositing the disks (Anusha *et al.,* 2009). Sterile paper disc having a diameter of 10 mm, were impregnated with different concentrations of extracts 50µg/disc, 100µg/disc, 200µg/disc and 250µg/disc. Paper discs were placed on the agar plate using sterile forceps. Six filter paper disks were placed on each plate and were placed at the same distance from each other and the edge, to prevent overlapping of inhibition zones. Sensitivity discs were pressed with forceps to make complete contacts with the surface of the medium. Plates were kept at room temperature for 30 minutes (pre-diffusion time), inverted and incubated at 37°C for 24 hours, in an aerobic atmosphere. A pair of divider was used to mark the diameter of the zone and a transparent ruler was used to measure the distance in mm. The experiment was repeated three times for each extract and

the mean diameter was taken. Oxytetracycline discs were used as a positive control and DMSO impregnated discs were used as negative control (Annonymous, 1999).

Determination of MIC using agar dilution method

Agar dilution method was used to find out Minimal Inhibitory Concentration. Stock concentration of various plant extract was prepared by making use of DMSO : Methanol, in the ratio of 1:1 which in turn was diluted with equal volume of phosphate buffered saline, pH 7. Mueller Hinton agar was prepared, sterilized and kept ready in molten condition. 20ml of the molten media was taken and was mixed with known concentration of different extracts / fractions and were added in different tubes. This mixture was swirled carefully for complete mixing of extract and media and poured on to the plate. After getting solidified it was inoculated with the test organism and standard organism. The plates were incubated at 37°C for 24 hours. MIC was recorded based on the growth of the organisms.

Result and Discussion

The efficiency of extraction was found to be 15.2% for water and 22.4% for methanol. Final Extract colour were also observed, methanol extracts showed a blackmild yellow colour, after vaporisation of solvent. Water extraction yielded brown colour extract.

The result revealed that the extracts of *M. indica* possess good antibacterial activity against *E. coli*. Flower extracts inhibited the growth of uropathogenic *E. coli* and the inhibition zones ranged from 10.2±2.3 mm to 22.6±1.2mm at different concentration of extracts (50μg/ disc to 250 μg/ disc). The aqueous extracts were less potent than the antibiotic whereas methanolic extract showed better result at 250 μg/ disc concentration. Negative Controls showed that solvents without extracts had no inhibitory effect on bacterial growth. The mean inhibition zone diameter of the aqueous and methanolic extracts to uropathogenic *E. coli* are shown in table 1.

There was a significant difference in mean zone of inhibition between positive control and plant extracts ($P < 0.05$). There was no zone of inhibition around disks submerged in solvents only (Negative control).To determine the extent of antibacterial activity, the extracts were subjected to MIC assay by serial two-fold dilution method of extracts and then dilution methods for inhibitory concentration assays. Results revealed that methanolic extract yielded MIC at 055±025μg/ml concentrations. Similarly aqueous extract produced 180±050 μg/ml concentrations (Table 2).

Phytochemical compounds were almost common in flower extracts. However water soluble compounds were not present in methanolic extracts. Results from phytochecimal analysis are shown in table 3. Terpenoids, flavonoids, phenolic compounds, tannins, lignin and carbohydrates were found in both the extracts. Saponins were indicated in water extracts only. Steroids and alkaloids were present only in methanolic extracts.

The basic parameters influencing the quality of an extract are plant parts used as a starting material, the solvent used for extraction, the extraction technology and

Table.1 Antibacterial activity of *Mangifera indica linn flowers* extracts Against MDR Uropathogenic *Escherichia coli*

S. No	Extract	Zone of inhibition (mm / disc)				
		Positive control	50µg	100µg	200µg	250µg
1	Aqueous extract	20.4±0.4	10.2±2.3	11.8±1.7	16.4±2.3	18.9±0.5
2	Methanolic extract	21.6±4.5	11.3±1.3	13.6±1.1	18.7±0.8	22.6±1.2

Table.2 MIC values of *Mangifera indica flower* extracts against Uropathogenic *Escherichia coli*

S. No	MIC Value µg/mL	
	Aqueous extract	*Methanolic extract*
1	180±050	055±025

Table.3 Qualitative Phytochemical analysis of Mangifera indica flower extracts

S. No	Test	Aqueous Extract	Methanolic extract
1	Alkaloids	Negative	Positive
2	Steroids	Positive	Positive
3	Terpenoids	Positive	Positive
4	Flavonoids	Positive	Positive
5	Saponins	Positive	Negative
6	Phenolic compounds	Positive	Positive
7	Coumarins	Positive	Positive
7	Tannins	Positive	Positive
8	Lignin	Positive	Positive
9	Phlobatannins	Negative	Negative
12	Cardiac glycosides	Positive	Negative
14	Carbohydrates	Positive	Positive

sterilisation method (Ncube *et al*., 2007). These findings on extraction potential of the different solvents are consistant with previous investigation, in which the percentage yield of methanol extract was higher than that of water extracts (Elloff, 1998). The observed differences in the extract yields of different solvents might be ascribed to the fact that the extract has different solubility or to the polarity of the solvent. Different extractable components were present in different quantities within the extract. Phytochemical analysis conducted on *M. indica* extracts revealed the presence of tannins, flavonoids, steroids, saponins, glycosides and resins among others. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and are thought to be responsible for coagulating the wall proteins of pathogenic organisms.

Thus, *M. indica* containing this compound may serve as a potential source of bioactive compounds in the treatment of infectious diseases. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane bound enzymes such as the ATPase and phospholipase (Li *et al.,* 2003). They also serve as health promoting compounds as a result of their anion radicals (Hausteen, 1983). These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments. Alkaloids were also detected and their common biological property is cytotoxicity (Nobori *et al.,* 1994).

The flower extracts of *M. indica* had significant antibacterial potency against the test organism. This result may suggest that all extracts possess compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs, for therapy of infectious diseases in human. Methanol extracts had an inhibition zone diameter of 22.6±1.2mm, which is higher than to a standard antibiotic, hence we suggest their effectiveness as antimicrobials from the plant. The active components in the crude extract may be acting synergistically to produce antimicrobial effects (Elloff, 1998). , the disparity between the activities of the extracts and the standard antimicrobial drug , may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotic (Olajuyigbe and Afolayan, 2012). Thus a standard drug had the highest zone of inhibition than aqueous extracts.

Most of the identified components with antimicrobial activity extracted from plants are aromatic or saturated organic compounds which are more soluble in polar solvents such as water and methanol. However water extracts were less potent. This can be attributed to the presence of water-soluble compounds such as polysaccharides and polypeptides, which are commonly more effective as inhibitors of pathogen adsorption and have no real impact as antimicrobial agents (Ncube *et al.,* 2007).

The antibacterial activity demonstrated by water extract provides the scientific bases for the use of water extracts in traditional treatment of diseases. There are also reports in literature that methanol is a better solvent for consistent extraction of antimicrobial substances for medicinal plants (Elloff, 1998). This may be attributed to two reason, firstly, the nature and potentiality of biologically active components (alkaloids, steroids, flavonoids, essential oils biterpenoids), which could be enhanced in the presence of methanol. Secondly, the stronger extraction capacity of methanol could have produced greater number or amount of active constituents responsible for antibacterial activity (Jeyachandran *et al.,* 2010). This is also proved in our study in which methanol extracts exhibited the highest antibacterial activity against *E. coli* compared to other extracts. The contents of active ingredients in plant materials have been shown to fluctuate constantly with the genetic heterogeneity of a plant species, differences in soil condition, variation in seasonal cycle, climatic influences, age of plant, alteration in weather, sun and shade fluctuations.

The Flower extracts were found to have antibacterial activity against uropathogenic *E. coli*. The stem bark extracts of *M. indica* contains several phytocompounds. There is need for lead compounds from the

plant extracts to be isolated so that they can serve as templates for the production of new antibiotics.

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