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Research article

In vitro Antibacterial Activity and Phytochemical Analysis of *Mangifera indica L* flower Extracts against Pathogenic Microorganisms

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Keywords

- *MangiferaIndica*
- Antibacterial activity
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Abstract

To identify the *in vitro* antibacterial activity, minimum inhibitory concentration and phytochemical content of *Mangifera indica* flower extract against six bacterial strains. Antibacterial activity of *Mangifera indica* was performed with different solvents (aqueous, ethanol, methanol and acetone) against various human pathogens viz., *Staphylococcus saprophyticus*, *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Salmonella typhi*. Preliminary phytochemical analysis was carried with potent methanolic extract. The ethanolic and methanolic extract of *Mangifera indica* flower can be used as potential antibacterial sources. The methanolic extract showed a maximum zone of inhibition (23±1.00 mm) against *S. typhi* and minimum zone of inhibition (13.00±1.21mm) with ethanolic extract against *Staphylococcus aureus*. Further, the ethanolic extract observed in maximum (21±1.00 mm) against *S. typhi* and minimum (15±0.57mm) zone of inhibition against *Staphylococcus aureus*. In addition to the phytochemical analyses were showed the presence of phenol, flavonoides, steroids, soapanins, tannins, carbohydrateand proteins. The experimental result revealed that the usage of these plants traditionally for medicinal purposes.

INTRODUCTION

For many years, various parts of plants such as leaves, flowers, barks etc. are used as medicine; only recently the synthetic drugs used to treat different infections have same chemical constituents as identified in plants [1]. According to WHO, a medicinal plant could be any plant that contains substances which can be obtained from its different parts and can be applied for beneficial purposes or can be predecessor for the production of useful drugs [2]. In recent times, due to the antioxidative, antimicrobial and other health promoting properties, there has been increasing interest in the study of bioactive compounds from mango pulp, peels, seeds, leaves, flowers, and stem bark making consumption of mangoes and its derived products a healthy habit [3,4].

Magnifera indica L, plant belonging to the family Anacardiaeceae commonly called as mango, consisting about sixty genera and six hundred species [5]. It is one of the most popular tropical fruit bearing trees in the world [6]. The aqueous leaf extract of *M. indica* has been reported to be rich in polyphenols amongst which mangiferin stem, bark and leaf extracts of *M. indica L.* possess several pharmacological activities including antioxidant, analgesic, antidiabetic, antiinflammatory, antitumor, immunomodulatory and anti-HIV effects, which have been extensively studied by several authors and proposed as the bioactive principle [3] Besides mangiferin, there is a wide array of other polyphenols and microelements both known and unknown in the leaves and stem bark extracts of the mango, all of which play a role in their pharmaceutical potential.

Acute as well as chronic infections are caused by one of the most common and important urinary pathogen *E. coli* [7]. 70-90% urinary tract infections (UTIs) can cause by uropathogenic *Escherichia coli* (UPEC) strains. The clinical management of UTI is complicated by the increasing incidence of infections caused by multidrug resistant E. coli strains. Common drugs used for UTI are Ampicillin, Chloramphenicol, Kanamycin, Nalidixic acid, Norfloxacin, Nitrofurantoin, Trimethoprim- Sulfamethoxazole (SMP-SMX), Streptomycin etc.

Increased resistance towards these antibiotics has been reported in the pathogenic isolates from the outpatient clinics,

therefore most of the commonly used antibiotics are not effective for the treatment [8]. Now a day's people rely on herbal medicines to overcome the problems of antibiotic resistance and Multidrug resistant pathogens [9]. Natural plants as herbal remedies are being employed to prevent and cure several illnesses vary in different communities [1]. These herbal plants are largely raw source for the production of modern antibiotics. The aim of this study to prepare extract in different solvent for antimicrobial and phytochemical analysis.

MATERIALS AND METHODS

Collection of plant material

Flowers from mango tree which are disease free are collected. Until processed, samples were stored at 4 °C in polythene bags. Collected materials were washed intap water, rinsed in distilled water twice and shade dried forfive days in open air then crushed using mortar andpestle.They were then reduced to powder at high speed and then stored inairtight closed bottles for two days before used for analysis. Fifty grams of all the fresh samples were stored for juice preparation.

Microorganisms

Bacteria cultures of *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, Methicillin-resistant *Staphylococcus aureus, Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* were used in this study. The strainswere maintained on agar slant at 4 °C and activated at 37 °C for 24 h on nutrient agar (Sigma-Aldrich, Germany) before any susceptibility test.

PREPARATION OF FLOWER EXTRACT

Aqueous extraction

Solution of 30 mL of 0.01 mol/L HCl containing 0.15 mol/L NaCl was used to dissolve in ten grams of dry powder of samples. Cheese cloth was used to remove the residue by filtration. The filtrate was then centrifuged at 8100 Xg, for 5 min. These extracts were subjected to antibacterial activity experiments and protein determination [10].

Methanol extraction

Ten grams of powdered sample was dissolved in 100 mL of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 r/min for 24 h. Till the final volume was reduced to one fourth of the original volume of the solvent used, thesupernatant was collected slowly and evaporated in wide mouthed evaporating bowls at room temperature for 2-3 days [11].

Ethanol extraction

Ten grams of powdered sample was dissolved in 100 mL of Ethanol in a 250ml conical flask, kept on a rotary shaker at 190-220 r/min for 24 h. After the reduction of final volume to one fourth of the original volume of the solvent used, the supernatant was collected slowly and evaporated the ethanol in wide mouthed evaporating bowls at room temperature for 2-3 days.

Acetone extraction

Ten grams of sample was dissolved in 100 mL of Acetone. This solution kept on a rotary shaker at 190-220 r/min for 24 h, till the final volume was reduced to one fourth of the original volume of the solvent used, the supernatant was collected slowly and evaporated in wide mouthod evaporating bowls at room temperature for 3 days.

Media preparation

Distilled water of 1000 mL was taken and thirty eight grams of Müller-Hinton agar (Hi-Media, India) were dissolved in it and bring to boil. Agar was then autoclaved for 15 min at 121 °C and left to cool at room temperature. Once the LB medium was cooled (about 45 °C), it was poured into Petri dishes. Each Petri dish was left on the flat surface for 30-40 minuntil completely set.

Antibacterial activity

Well diffusion method was used for antibacterial activity assay. For all bacteria strains, overnight culture grown in broth was adjusted to an inoculum's density of 1 OD at 660nm, culture containing $3.7_{x}10^{8}$ colony forming unit. Further, 20 µL was spread onto 20 mL of sterile agar plates by using a sterile cotton swab. The surface of the medium was dried for about 3 min.Three sterile cylinders (diameter 6 mm) were gently placed at equidistance from each other in a triangular form on the inoculated media. A 200 µL of the each extract solutions was then introduced into the cylinders. Normal saline was used as the negative control while Gentamycin was used as the positive control. The plates were then incubated at 37 °C for 24 h after which microbial growth was determined by measuring thediameter of the inhibition zone (mm) using a transparent scale. Each extract was analyzed in triplicate, themean values are presented.

Determination of minimal inhibitory concentration (MIC)

The MIC's of the extracts were estimated using a two-fold serial agar media dilutions method [12,13]. A stock solution of the flower extract (200 mg/mL), was prepared as the highest concentration which was then dilutedtwo-fold with distilled water and the dilution process wasrepeated down to 1.6 mg/mL. From each of the dilutions, an aliquot was introduced into 25 mL of agar media solution,vortexed and poured onto the plates, inoculated with 100µL of the test organisms, left to solidifyand incubated at 37 °C for 24 h.After which the plateswere evaluated for the presence or absence of growth.Gentamycin and saline water were used as positive andnegative controls respectively. MIC values weredetermined as the lowest concentration of the mango flower extractwhere absence of visible growth was recorded. All testswere carried out in triplicates.

PHYTOCHEMICAL ANALYSIS

Test for alkaloids

Methanolic and ethanolic extract of 0.1 ml was dissolved separately in 5 mL of distilled water and then filtered. A volume of 2 mL of each filtrate from each sample were stirred with 5 mL of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1 mL was taken individually into 2 test tubes. To the first portion (1 mL), few drops of Dragendorff's reagent were added. Occurrence of orange-red precipitate was taken as positive. To the second 1 mL, Mayer's reagent was added and appearance

of buff-colored precipitate was taken as positive test for the presence of alkaloids [13].

Molisch's test for Carbohydrates

Half ml of methanolic and ethanolic extract was dissolved separately in 5 mL of distilled water and filtered. Few drops of Molisch's reagent were added to each solution, after this addition of 1 mL of concentrated H_2SO_4 by the side of the test tube. The mixture was allowed to stand for 2 min before diluting with 5 mL distilled water. Formation of a red or dull violet colour at the interphase of the two layers is considered as positive test [14].

Liebermann-Burchard test for steroids

At the beginning 2 mL of acetic acidis used to dissolve, 0.2 ml of methanolic and ethanolic extract of each sample. Concentrated H_2SO_4 is added carefully after the solutions were cooled well in ice. Color development from violet to blue or bluish-green indicated the presence of a steroidal ring [14].

Test for Protein

About 0.5 ml methanolic and ethanolic extract was treated with few drops of concentrated HNO3 formation of yellow indicates the presence of proteins.

Shinoda's test for flavonoids

About 0.5 ml methanolic and ethanolic extractwas dissolved in 5 mL of distilled water separately, warmed and then filtered. Magnesium sulphate solution was then added to the filtrate followed by few drops of concentrated HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids [15].

Test for saponins

3 mL of distilled water was further added in 1 ml methanolic and ethanolic extract and shaken vigorously for about 5 min. Frothing which persisted on warming was taken as an evidence for the presence of saponins [14].

Test for tannins

10 mL of distilled water was stirred with about 0.5 ml methanolic and ethanolic extract separately and then filtered. Few drops of 1% ferric chloride solution were added to 2 mL of each filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins [14].

Test for Diterpenes

About 0.5 ml methanolic and ethanolic extract were dissolved in water and treated with 10 drops of copper acetate solution, formation of emerald green colour indicates presence of diterpenes.

Thin layer chromatography (TLC)

The TLC method is used to separate the compound presentin the crude extract. The separation of thecompound also depends on the type of the solvent [16].The extract with a concentration of 1mg/ml wasplotted on the TLC plate and dried. It was run with different ratios of the solvent (Chloroform: ethylacetate) the spots were identified both in the UV light and by using the iodine fumes. The R_f value was calculated using the formula:

R_f value = Distance travelled by the solute /Distance travelled by the solvent.

Statistical analysis

The results were analyzed by using standard deviation (SD) statistical methods of M S Excel [17].

RESULTS

The results of the antibacterial activity suggested that, the maximum zone of inhibition of Mango flowers was observed inmethanolic extract against *Salmonella typhi* followed by, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis, Escherichia coli,* Methicillin-resistant *Staphylococcus aureus,* and minimum zone of inhibition was observed against *Staphylococcus aureus.* Further, the maximum zone of inhibition was observed in ethanolic extract against *Salmonella typhi* (Figure 1) additionally, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus, Escherichia coli, salmonella typhi* and minimum zone of inhibition was observed in ethanolic extract against Methicillin-resistant *Staphylococcus aureus.* Moreover, the maximum zone of inhibition was observed in aquous extract against *Salmonella typhi* and in acetone extract against *Staphylococcus saprophyticus* (Table 1). The zone diameter standard and equivalent minimal inhibitory concentration listed in (Table 2) [18].

For *M. indica*'s methanolic and ethanoloc extract, the MIC was equal to .16±00 and .32±00mg/mL respectively on *Salmonella typhi*. 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, fortherapy of infectious diseases in human.

In addition, the quantitative phytochemical estimation were showed the presence of phenol, flavonoides, steroids, soapanins, tannins, carbohydrate, proteins and absence of Alkaloids and Diterpines was observed in methanolic and ethanolic extract (Table 3).

Due to the lack of knowledge about the specific chemical composition of the crude plant extracts, TLC served as qualitative methods. Methanolic extracts of *M. Indica flower* with good antimicrobial spectrum and MIC were subjected to TLC assay,

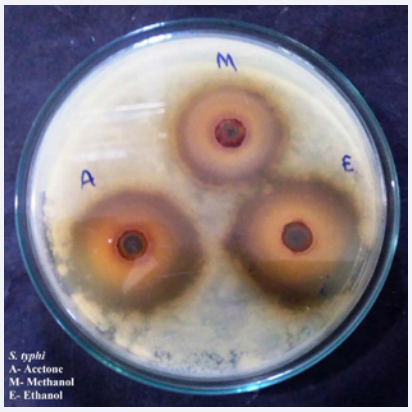

Figure 1 The antagonistic effect M. Indica's flower extracts on the growth of *Salmonella typhi.*

Table 1: Antibacterial activity of various extracts against pathogenic bacterial species tested by disc diffusion assay (Values are mean inhibition zones (mm) ± SD of three replicates).

Pathogenic Bacterial Strain	Aqueous	Ethanol	Methanol	Acetone
Staphylococcus saprophyticus	14±0.577	18±1.00	21±0.57	13±0.57
Staphylococcus aureus	13±1.00	15±0.57	13±0.57	09±1.00
Staphylococcus epidermidis	10±1.15	19±1.52	20±1.00	14±1.00
Methicillin-resistant *Staphylococcus aureus*	10±1.00	16±1.15	15±0.57	11±0.57
Salmonella typhi	17±1.00	21±1.00	23±1.00	10±0.57
Escherichia coli	12±0.57	18±1.00	17±1.00	11±1.00

Table 2: Zone diameter interpretative Standards and equivalent minimal inhibitory concentration (MIC) according to CLSI document [18].

Antibiotics	Zone diameter interpretative standards (mm)			MIC (µg/mL)	
	R	I	S	R	S
Ciprofloxacin (CIP) (5 µg)	≤15	16–20	≤21	≤4	≤1
Cefoxitin (FOX) (30 µg)	≤14	15–17	≤18	≤32	≤8
R– resistant; I – intermediate; S – susceptible					

Table 3: Phytochemical screening of methanol and ethanol extracts (-: Absent; +: Present; ++: Moderately present; +++: Abundantly present).

Phytochemical	Methanol extract	Ethanol extract
Carbohydrate	+++	+++
Steroid	+++	+
Tannins	+++	++
Saponins	++	++
Flavonoids	+++	+
Phenol	++	+++
Alkaloids	_	_
Protein	+++	++
Diterpines	_	_

and the results were presented in (Figure 2). The extract of *M. Indica* showed one or several inhibition spots under the TLC development system (Chloroform: ethyl acetate, 5:1 v/v), and the Rf values of the spot was 0.81.

DISCUSSION

India is place of rich and diverse flora of flowering medicinal plants. In every culture from ancient times plants have been used for medicinal purposes. About 80% of the world population role on the use of traditional medicine concomitantly based on plant materials therefore medicinal plants play a vital role in human health care. Dash *et al*. and Verma and Kumar reported the antibacterial with methanol and acetone extract against *Trigonella fo*enum and *Coriandrum sativum* against *Pseudomonas sp*. *Shigella dysentiriae*, *Salmonella typhi* and *E. coli* [19, 20]. Khan et al., 2011 reported the antibacterial activity with the *Melia azedarach* against *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus sp*, *Enterococcus faecalis*, *B. subtilis*, *E. coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *P. aeruginosa*, *S. typhi*, *Shigellaboydii*, *Shigelladysenteriae*, *Shigellaflexneri* and *Plesiomonasshigelloides* [21]. The present learn also made an effort to identify the phytochemical constituents analysis and the results showed the presence soluble sugars, reducing sugar, amino acid, proteins, lipids, chlorophyll, phenol, ortho-dihydroxy phenols and this phytochemical constituents previously reported with several biological properties [22]. Similarly Koche *et al*. reported the phytochemical analysis with the *Ocimum sanctum*, *Hyptis suaveolens*, *Croton viscose*, *Malachra capitata*, *Physalis minima*, *Cleme viscose*, *Galphimia glauca* and *Tephrosiavillosa* [23] and Hussain *et al*., and Koche *et al*., reported the phytochemical analysis through the *Ranunculus arvensis, Equisetum ravens*, *Carathamus lanatus* and *Fagonia critica*. Moreover, several species of Apocynaceae family plants has been widely used as main ingredient in traditional medicine [24,23]. Hence, the presently studied *C. roseus* plant extract could be of considerable infers to the development of new life saving drugs. However, further research is required to isolate the bioactive principle of this species as well as further studies on its bio efficiency against human pathogens.

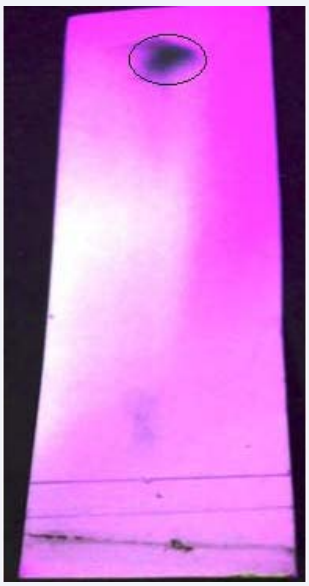

Figure 2 Thin layer chromatography of *M. Indica*s methanolic extract tested against pathogenic bacteria.

It can be concluded from the present findings that, the methanolic extract of flower extract of *Mangifera indica* collected from the Madhya Pradesh region was showed potential antimicrobial activity source for various infects. Further, studies is need to be conform identify the particular compounds to use as a drug as main ingredient in the traditional medicine.

CONCLUSION

The experimental study concluded that flower extract of *Mangifera indica* has shown the presence ofvarious bioactive phytochemicals in the plant extracts. These phytochemicals have been reported to produce some physiological and therapeutic effects in the human body. Demonstration of good antibacterial activity and MIC of methanol extract against *S. typhi* is an indication that there is possibility of alternative antibiotic substances in these flower extract for the development of newer antibacterial agents. Thus the authors suggest that further work aimed at establishing the mechanism of action of these plants should be carried out.

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REFERENCES

1. Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr J Biotechnol. 2007; 6: 1690-1696.
2. Nunez-Selles AJ. Antioxidant therapy: Myth or reality? Brazil Chem Soc.2005; 16: 699-710.
3. Junaid SA, Olabode AO, Onwuliri FC, Okwori AEJ, Agina SE. The antimicrobial properties of Ocimum gratissimum extracts on some selected bacterial gastrointestinal isolates. Afr J Biotechnol. 2006; 5: 2315-2321.
4. Masibo M, He Q. Major mango polyphenols and their potential significance to human health. Comprehensive Rev Food Sci Food Safety. 2008; 7: 309-319.
5. Akinpelu DA, Onakoya TM. Antimicrobial activities of medicinal plants used in folklore remedies in South-Western. Afr J Biotechnol. 2006; 5: 1078-1081.
6. Kabuki T, Nakajima H, Araia M, Ueda S, Kuwabara Y, Dosako S. Characterization of novel antimicrobial compounds from mango (Mangiferaindica L.) kernel seeds. J Food Chem. 2000; 71: 61-66.
7. Jellheden B, Norrby RS, Sandberg T. Symptomatic urinary tract infection in women in primary health care. Bacteriological, clinical and diagnostic aspects in relation to host response to infection. Scand J Prim Health Care. 1996; 14: 122-128.
8. Zhanel GG, Karlowsky JA, Low DE, Hoban DJ. Antibiotic resistance in respiratory tract isolates of Haemophilus influenzae and Moraxella catarrhalis collected from across Canada in 1997-1998. J Antimicrob Chemother. 2000; 45: 655-662.
9. Ndip RN, Ajonglefac AN, Wirna T, Luma HN, Wirmum C, Efange SMN. In-vitro antimicrobial activity of Ageratum conyzoides (Linn) on clinical isolates of Helicobacter pylori. Afr J Pharm Pharmacol. 2009; 3: 585-592.
10. Franco OL, Murad AM, Leite JR, Mendes PA, Prates MV, Bloch C Jr. Identification of a cowpea gamma-thionin with bactericidal activity. FEBS J. 2006; 273: 3489-3497.
11. Harbone JB. Phytochemical Methods. Chapman and Hall Ltd. London. 1973; 49-188.
12. Russell AD, Furr FR. The antibacterial activity of a new chloroxylenol preparation containing ethylenediamine tetraacetic acid. J Applied Bact. 1977; 43: 253-260.
13. Gulluce M, Sokmen M, Daferera D, Agar G, Ozkan H, et al. In vitro antibacterial, antifungal and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of Saturejahortensis L. J Agric Food Chem. 2003; 51: 3958-3965.
14. Sofowora A. Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicine in Africa, Sofowora A. 2nd Edn. Spectrum Books Ltd. Ibadan Nigeria. 1990; 3: 134-156.
15. Trease GE, Evans WC. Pharmacognosy. 15th Edn, Saunders Publishers. London. 2003; 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.
16. Gauthier MJ, Lafay B, Christen R, Fernandez L, Acquaviva M, Bonin P. Marinobacter hydrocarbonoclasticus gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. See comment in PubMed Commons below Int J Syst Bacteriol. 1992; 42: 568-576.
17. Bhat RS, Al-Daihan S. Antimicrobial activity of Litchi chinensis and Nepheliumlappaceum aqueous seed extracts against some pathogenic bacterial strains. J King Saud Univ Sci. 2014; 26: 79-82.
18. Sader HS, Flamm RK, Jones RN. Antimicrobial activity of daptomycin tested against Gram-positive pathogens collected in Europe, Latin America, and selected countries in the Asia-Pacific Region. 2011; Diagn Microbiol Infect Dis. 2013; 75: 417-422.
19. Dash BK, Sultana S, Sultana N. Antibacterial activities of methanol and acetone extracts of fenugreek (Trigonellafoenum) and coriander (Coriandrumsativum). Life Sci Med Res. 2011.
20. Verma SK, Kumar A. Therapeutic uses of Withania somnifera (Ashwagandha) with a note on withanolides and its pharmacological actions. Asian J Pharm Clin Res. 2011; 4: 1-4.
21. Khan AV, Ahmad QU, Mir MR, Shukla I, Khan AA. Antibacterial efficacy of the seed extracts of Melia azedarach against some hospital isolated pathogenic bacterial strains. Asian Pacific J Trop Biomed. 2011; 6: 452-455.
22. Mbaebie BO, Edeoga HO, Afolayan AJ. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of Schotia latifolia Jacq. Asian Pac J Trop Biomed. 2012; 2: 118-124.
23. Koche D, Shirsat R, Imran S, Bhadange DG. Phytochemical screening of eight traditionally used ethnomedicinal plants from Akola district (MS) India. Int J Pharm Bio Sci. 2010; 1: B-253-B-256.
24. Hussain I, Ullah R, Khurram M, Ullah N, Baseer A, et al. Phytochemical analysis of selected medicinal plants. Afr J Biotechnol. 2013; 10: 7487-7492.

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