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In-vitro antibacterial activity on human pathogens and total phenolic, flavonoid contents of *Murraya paniculata* Linn. leaves

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ABSTRACT

Objective: To deals with *in-vitro* antibacterial analysis of *Murraya paniculata* Linn. (Rutaceae) leaves extract on human pathogens in different solvent and determination of total phenolic and flavonoid contents in petroleum ether, methanol, ethanol and hydro-alcoholic extract of the plant. **Methods:** These extracts were tested against various human pathogens for antimicrobial activity which was evaluated by disc diffusion method and minimum inhibitory concentration (MIC) and minimum bactericidal concentration was calculated by micro dilution method. Phenolic content was estimated by using folin ciocalteau reagent and flavonoids by using aluminium chloride reagent against quercetin equivalent. **Results:** The methanolic extract of leaf showed marked antibacterial activities against gram-positive and gram-negative bacteria. Methanolic extract of *Murraya paniculata* leaf contain higher phenolic content (24.80±0.64) followed by ethanolic fraction (15.40±0.38), pet. Ether (13.50±0.96) and hydro-alcoholic (9.06±1.13). Flavonoid content was found to be maximum in pet. ether extracts (3.38±1.89). **Conclusions:** *Murraya paniculata* leaves posses antibacterial properties against human pathogens with high content of phenolic and flavonoids, which have supportive action of antibacterial activity. Studying plant based antimicrobial properties provides additional information in developing nature antibiotics and discovering the alternative of antimicrobial drugs for the treatment of infectious disease.

1. Introduction

Bacteria are extremely pathogenic causing serious human infection and widespread use of commercially available antibiotics led to developed resistance or ability to produce substances which block the action of antibiotics or change their target and produce undesirable side effects. Tuberculosis, malaria and gonorrhea infection are just a few disease that have become harder to treat with antibiotics[1]. Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects. The screening of plant extracts for antimicrobial activity has shown that plants signify a potential source of new antibacterial agent[2]. Phenolic compounds are one of the main secondary metabolites, present in the plants and these are an integral part of the human diet. Phenolic compounds are commonly known for their antioxidant, anti-inflammatory and antimicrobial activities[3]. Flavonoids, the most common group of polyphenolic compounds that are found ubiquitously in plants kingdom. More than 5000 flavonoids have been identified in nature. Flavonoids are most commonly known for their antioxidant activity and reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity[2].

Murraya paniculata (*M. paniculata*) Linn., commonly known as orange jasmine or honey bush (Kamini in Hindi) belongs to the family Rutaceae. It is distributed throughout India, Bangladesh, tropical Srilanka to Myanmar, southern China and Taiwan, Thailand and eastwords throughout the Malesian region to northeastern Australia and Caledonia. The leaves are stimulant and astringent; they are reportedly used in the form of an infusion to treat diarrhoea and dysentery in the Philipines. The powder leaves are applies to cuts to promote healing; there decoction is taken

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internally to treat dropsy. Among the baigas of northestern Madhya Pradesh, the crushed leaves are made in to a paste and mixed with molasses to make tablets that are taken orally to treat joint pain; the leaves, cooked with mustard or sesame oil along with dried ginger, are applied externally to relieve inflamed joints. The warm leaf paste is applied externally to promote the healing of broken bones among the Paudi Bhuinya in northern Orissa. In the Gandhamardn hills of Orissa, the leaves and twigs are boiled to make a bath that is used to relieve stomach-ache in children and rheumatic pains in adults. It is reported to have anti-diabetic[4], anti-nociceptive and anti-inflammatory[5], anti-diarrhoeal, oxytocic, anti-fertility and *in-vitro* antioxidant properties[6] and also hydro-alcoholic extract of *M. paniculata* having no toxicity on rodents[7].

In view of the importance of *M. paniculata* as health remedy we set out to investigate the antibacterial activity of its leaf extracts against some human pathogenic bacteria and also find out total phenolic and flavonoid content present in plant leaves extract.

2. Material and methods

2.1. Plant material and extraction

The plant *M. paniculata* Linn were collected from botanical garden of National Botanical Research Institute, Lucknow, India. The collected plant materials washed with distilled water and dried under control condition of (30±2) ℃. Powdered plant materials exhaustively extracted with methanol, ethanol, petroleum ether and 50% ethanol (Plant material and solvent ratio-1X: 3X). The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and dried under reduced pressure in lyophilizer (Lobconco, USA).

2.2. Test microorganism

A total of eight bacterial strains *viz. Escherichia coli* (*E. coli*) ATCC 25922, *Klebsiella pneumoniae* (*K. pneumonia*), *Salmonella typhi* (*S. typhi*) MTCC 3216, *Enterococcus faecalis*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Shigella flexinerrii*, *S. aureus* (*S. aureus*) ATCC 25323, *Shigella sonneii* were used in the investigation for antimicrobial assay. All cultures preserved at Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India which were obtained from American Type Culture Collection, MTCC and clinical strain. The fresh bacterial broth cultures were prepared in normal saline before the screening procedure.

2.3. Antimicrobial susceptibility test

The paper disc diffusion method was used for antibacterial assay[3]. Muller Hinton agar (MHA, Hi-media, Mumbai, India) plates were prepared by pouring 15 mL of molten media into sterile petriplates. Briefly, 1.0 mL of an 18 h culture of bacteria adjusted to 0.5 MacFarland standards in sterile saline to achieve concentration of 10^7 CFU/mL. This suspension was spread on the surface of MHA agar plates. The concentrations of extract 200 mg/mL were put on 6 mm sterile disc of Whatman filter paper no.1. The disc was then placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37 ℃ for 24 h. Standard disc of antibiotics 6 mm in diameter were used as positive control. At the end of incubation, inhibitions zones were examined around the disc and measured with transparent ruler in millimeters. All the experiments were performed in triplicate.

2.4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

It is defined as the lowest concentration of the compound which will inhibit the growth of microorganism. MIC was determined by micro-dilution method[8,9] using serially diluted (2 folds) plant extracts according to the National Committee for Clinical Laboratory Standards. We found that methanolic extract posses high inhibition zone, so we set out MIC and MBC only for methanolic extract. MIC of the extract was determined by dilution of methanolic extract with 200 mg/mL concentrations. Specifically, 0.1 mL of standardized inoculums ($1-2\times10^7$ cfu/mL) was added in each tube. The plates were incubated aerobically at 37 ℃ for 18-24 h. The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control were regarded as MIC. The highest dilution that yielded no bacterial was taken as MBC.

2.5. Determination of total phenolic content

Total phenolic concentration in different extract was determined by spectrophotometric method using Folin ciocalteau reagent. Briefly, 5 mL of distilled water, 0.5-1.0 mL of sample, and 1.0 mL of Folin ciocalteau reagent was added to a 25 mL flask. Next 10 mL of 7% sodium carbonate solution was added followed by distilled water. Solution mixture was allowed to stand at room temperature for 15 min; following absorbance was recorded at 750 nm. Total phenolic content was standardized against gallic acid and expressed as milligram per liter of gallic acid equivalents (GAE). The linearity range for this assay was determined as 0.5-5.0 mg/L GAE (R^2=0.999), giving an absorbance range of 0.050-0.555 absorbance units[10].

2.6. Determination of total flavonoid content

Total flavonoid content determined spectrophotometrically using aluminium chloride (2%). Absorbance was recorded at 415 nm after 10 min against a blank sample consisting of 5 mL of sample and 5 mL of methanol without aluminium chloride. The total flavonoid content was determined comparing with a standard curve of quercetin at 0-50 μg/mL. Average of three different readings were used and then expressed in μg quercetin equivalent flavones per mg extract[11].

3. Results

The results of antibacterial activities of petroleum ether extract, methanolic extract, ethanolic extract and hydro-alcoholic extract were presented in Figure 1. The zones of inhibition produced by petroleum ether extract, methanolic extract, ethanolic extract and hydro-alcoholic extract were ranged from 8-12, 9-14, 8-11, and 8-11 mm

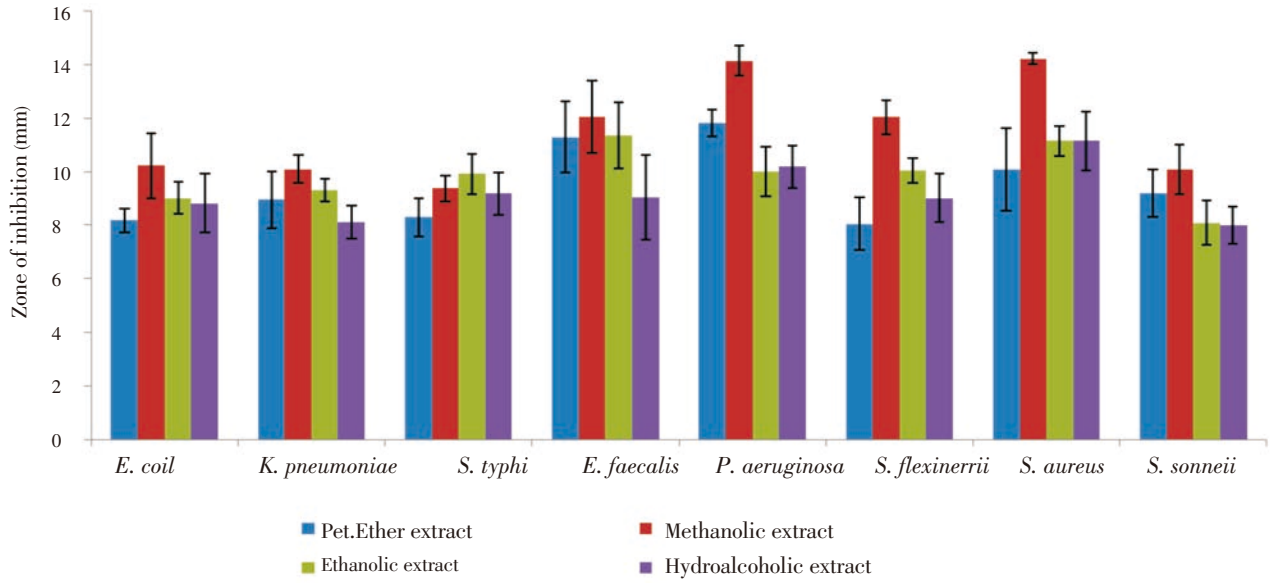

Figure 1. Zone of inhibition of *M. paniculata* extract (200 mg/mL) in different solvent.
Values are mean±SEM of 3 experiments in each group.

at a concentration of 200 mg/mL. The ethanol and hydro-alcoholic extract showed mild to moderate activity against human pathogenic bacteria. But the methanolic extract of *M. paniculata* showed highest antibacterial activity (14 mm) against *P. aeruginosa*. MIC and MBC of the methanolic extract were within the range of 3.125 mg/mL and 50 mg/mL. Methanolic extract showing minimum MIC *i.e.* 3.125 mg/mL against *P. aeruginosa* ATCC 27893 followed by *S. aures* ATCC 25323 and *E. coli* ATCC 25922 (Figure 2).

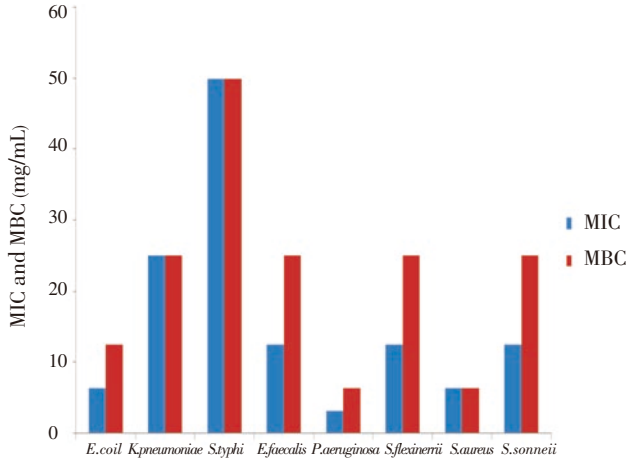

Figure 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic extract of *M. paniculata*.
Values are mean±SEM of 3 experiments in each group.

Estimation of total phenolic and total flavonoid content showed that methanolic extract showed maximum phenolic content in μg gallic acid equivalents followed by Pet. Ether, ethanolic and hydroalcholic extract. Pet. Ether extract showed maximum flavonoids content followed by methanolic extract in μg of quercetin equivalents. The amount of total phenolic and flavonoids for the test samples are summarized in Table 1.

Table 1
Total phenolic and flavonoid content of different extract of *M. paniculata* Linn.

Extract	Solubility	Phenolic content μg gallic acid equivalents (GAE)	Total flavonoid content μg of quercetin equivalents (QE)
Petroleum Ether	DMSO	13.5±0.96	3.38±1.89
Methanol	DMSO	24.8±0.64	2.11±0.23
Ethanol	DMSO	15.4±0.38	1.62±0.18
Hydro-alcoholic	DMSO	9.06±1.13	1.80±0.21

Values are mean±SEM of 3 experiments in each group.

4. Discussion

The *in-vitro* antibacterial activity of the *M. paniculata* extract was tested against the series of standard and clinical isolates of different gram positive and gram negative pathogens were investigated. Similarly activity of plant extract against test organism was expressed graphically in form of diameter of zone of inhibition. *M. paniculata* extract reported to have alkaloids, flovonoids, phenolic compounds, carbohydrate, proteins and amino acids[12]. Reasons accounting for antibacterial activity of plant extracts may be either due to the nature of its biological active components like alkaloids, flavonoids and phenolic compouns, which are all reported for antimicrobial activities[13]. Some significant infections should not be treated with single antibiotic, due to that bacteria can rapidly develop resistance when such a single antibiotic is used. According to different reports, multiple drug resistances to *Pseudomonas aeruginosa* are spreading in the world and making the therapeutic management of these patients more problematic[14]. Individual bacterial sensitivity pattern was done to assess its MIC value, against the standard antibiotics. MIC of methanolic extract for different organism ranged between

3.125 mg/mL to 50 mg/mL. Methanolic extract showing minimum MIC against *P. aeruginosa* ATCC 27893 followed by *S. aures* ATCC 25323 and *E. coli* ATCC 25922. The different extracts exhibited considerable level of inhibition against various human pathogens as compare to standard drug. This is suggestive of the presence of some metabolite in the extract with almost similar mechanism of action to that of standard drug used in antibacterial activity.

Flavonoids are classified under phenolic groups in plants which have been known to possess antimicrobial activity. The mechanisms of flavonoids that are antimicrobial can be classified as the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism[15]. Estimation of total phenolic and total flavonoid content showed that methanolic leaf extract was having maximum phenolic content in μg Gallic acid equivalents followed by followed by Pet. Ether, ethanolic and hydroalcholic extract. Pet. Ether extract was having maximum flavonoid content followed by methanolic extract μg of quercetin equivalents. Flavonoid present in the extract seems to possess antioxidant activity through their scavenging or chelating process which contains hydroxyl functional groups. Antimicrobial properties of phenolic compounds have been reported. The mechanism of action involves the alteration of the permeability of the cell membrane that could result in the uncoupling of oxidative phosphorylation, inhibition of active transport, and loss of pool metabolites due to cytoplasmic membrane damage. Also, the presence of hydroxyl group in phenolic compound might influence their antimicrobial effectiveness by binding to the active site of enzymes, form hydrogen bonds with enzymes and alter their metabolism, and also the lipid solubility and the degree of steric hindrance of the phenolic compounds might determine their antimicrobial activity[16]. Many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites[17].

The antibacterial effect of the different extract of *M. paniculata* Linn. leaves against different human pathogenic bacterial strains shown to contain wide spectrum of activity, introduce the plant as a potential candidate for drug development for the treatment of ailments caused by human pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] González MJ, Marioli JM. Antibacterial activity of water extracts and essential oils of various aromatic plants against Paenibacillus larvae, the causative agent of American Foulbrood. *J Invertebrate Pathol* 2010; **104**: 209-213.

[2] Sakunpak A, Panichayupakaranant P. Antibacterial activity of Thai edible plants against gastrointestinal pathogenic bacteria and isolation of a new broad spectrum antibacterial polyisoprenylated benzophenone, chamuangone. *Food Chem* 2012; **130**: 826-831.

[3] Klančnik A, Piskernik S, Jeršek B, Možina SS. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *J Microbiol Methods* 2010; **81**: 121-126.

[4] Gautam MK, Gupta A, Rao CV, Goel RK. Antihyperglycemic and antioxidant potential of *Murraya paniculata* Linn. leaves: a preclinical study. *J Pharm Res* 2012; **5**: 1334-1337.

[5] Wu L, Li P, Wang X, Zhuang Z, Farzaneh F, Xu R. Evaluation of anti-inflammatory and antinociceptive activities of Murraya exotica. *Pharma Biol* 2010; **48**: 1344-1353.

[6] Gautam MK, Gangwar M, Singh A, Rao CV, Goel RK. *In-vitro* antioxidant properties of *Murraya paniculata* (L.) leaves extract. *Invent J Ethnopharmacol*. [Online] Available from http://www.inventi.in/Article/pep/692/12.aspx. [Accessed in 2012].

[7] Gautam MK, Singh A, Rao CV, Goel RK. Toxicological evaluation of *Murraya paniculata* (L.) leaves extract on rodents. *Am J Pharm Toxicol* 2012; **7**(2): 62-67.

[8] Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protocol* 2008; **3**: 163-175.

[9] Gangwar M, Kumar D, Tilak R, Singh TD, Singh SK, Goel RK, et al. Qualitative phytochemical characterization and antibacterial evaluation of glandular hairs of *Mallotus philippinensis* fruit extract. *J Pharm Res* 2011; **4**: 4214-4216.

[10] Sharma AK, Mayank Gangwar M, Tilak R, Nath G, Sinha ASK, Tripathi YB, et al. Comparative *in vitro* antimicrobial and phytochemical evaluation of methanolic extract of root, stem and leaf of *Jatropha curcas* Linn. *Pharmacog J* 2012; **4**(30): 34-40.

[11] Hossain MA, Shah MD, Gnanaraj C, Iqbal M. *In vitro* total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant *Tetrastigma* from Sabah. *Asian Pac J Trop Med* 2011; **4**(9): 717-721.

[12] Gautam MK, Goel RK. Exploration of preliminary phytochemical studies of leaves of *Murraya paniculata* (L.). *Int J Pharm Life Sci* 2012; **3**(8): 1871-1874.

[13] Kazłowska K, Hsu T, Hou CC, Yang WC, Tsai GJ. Anti-inflammatory properties of phenolic compounds and crude extract from *Porphyra dentata*. *J Ethnopharmacol* 2010; **128**(1):123-130.

[14] Al-Bayati FA. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. *J Ethnopharmacol* 2008; **116**: 403-406.

[15] Hendra R, Ahmad S, Sukari A, Shukor MY, Oskoueian E. Flavonoid analyses and antimicrobial activity of various parts of *Phaleria macrocarpa* (Scheff.) Boerl fruit. *Int J Mol Sci* 2011; **12**: 3422-3431.

[16] Salawu SO, Ogundare AO, Ola-Salawu BB, Akindahunsi AA. Antimicrobial activities of phenolic containing extracts of some tropical vegetables. *Afric J Pharm Pharmacol* 2011; **5**(4): 486-492.

[17] Haidari M, Ali M, Casscells SW, Madjid M. Pomegranate (Punicagranatum) purified polyphenol extract inhibits influenza virus and has a synergistic effect with oseltamivir. *Phytomedicine* 2009; **16**: 1127-1136.