International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.8, pp 112-117, 2015

Antibacterial activity of *Murraya koeniigi* leaves against Urinary Tract Infection causative pathogens

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Abstract: The main aim of the study is to evaluate the antibacterial activity of aqueous and ethanolic extract of *Murraya koeniigi*(Curry leaves) against Urinary Tract Infection causative pathogens. The extracts were tested for their antibacterial activity against UTI causative pathogens viz.,P.aeruginosa ,K.pneumoniae and E.coli by agar diffusion method. The results of antibacterial activity revealed that both the extracts exhibited maximum to moderate inhibitory activity against all test pathogens. Maximum antibacterial activity by ethanolic extract were exhibited against E.Coli followed by K.pneumoniae and P.aeruginosa whereas aqueous extract showed moderate sensitivity on *E. coli* and *K. peumoniae*, while, least sensitivity was recorded on *P. aureginosa*. Phytochemical analyses were performed by standard methods. The results showed positive response for significant secondary metabolites. Thus extracts of *Murraya koeniigi* can be used as an potent therapeutic agent agent UTI and their preventions.
Keywords: Antibacterial activity, UTI, *Murraya koeniigi,* agar diffusion method.

Introduction

Microbial infections are the major cause of morbidity and mortality in the developed and developing countries. Although a number of antimicrobial agents are available for the treatment and management of infectious diseases, some microbes develop resistance to many antibiotics(1).Hence, there is a need to exploit new bioactive compounds with high safety index. Natural plant products have been used for therapeutic purposes since time immemorial and their use is of a greater demand nowadays. Majority of the users rely on herbal medicines for health care because the other treatment options available are more expensive and they are often thought to be more associated with serious side effects. Contrary to synthetic drugs, antimicrobials of plant origin are not associated with many side effects (2) and have an enormous therapeutic potential to treat many infectious diseases. There is therefore, continuous and urgent need to discover new antimicrobial compounds from plant sources with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases is required(3).

Urinary tract infection (UTI) is the second most common infectious disease prevailing in community medical practice. Worldwide, about 150 million people are diagnosed with UTI each year. More than 95% of urinary tract infections are caused by a single bacterial species. *E. coli* is the most frequent infecting organism in acute infection(4). Klebsiella, Staphylococci, Enterobacter, Proteus, Pseudomonas, and Enterococci species are more often isolated from inpatients, whereas there is a greater preponderance of *E. coli* in an outpatient population (5).Thus UTI requires an immediate attention and cure worldwide which if provided by an plant product without any side effects would be more beneficial to human kind.

Table 1: Taxonomy of Plant

Kingdom	*Plantae*
Division	Magnoliophyta
Class	Magnoliospida
Order	Sapindales
Family	Rutaceae
Genus	Murraya
Species	*MurrayaKoenigii*

Murraya koenigii is an aromatic shrub or small tree found throughout India and mainly cultivated for its aromatic leaves(Table 1).*Murraya koenigii* is a highly valued plant for its characteristic aroma and medicinal value. It is an important export commodity from India as it fetches good foreign revenue. The plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, anti-ulcer, positive inotropic and cholesterol reducing activities (6).Thus in the present research the antimicrobial properties of the leaf extracts of *Murraya koenigii* against three different bacterial isolates that commonly causes Urinary Tract Infections was determined by using Agar well diffusion method(7).

Materials and Methods

Collection of plant material

The leaves of *Murraya koenigii* were collected from local gardens at Chennai, Tamil Nadu, and further air dried in shade for preparation of extracts.

Ethanolic extract preparation

10 grams of air dried *Murraya koenigii* powder was extracted with 100ml of Organic solvent (Ethanol) and kept on rotary shaker at 190-220 rpm for 24 hours. The supernatant was collected and solvent was evaporated to make the final volume one – fourth of the original volume and stored at 40 C in air tight bottles(8)

Aqueous extract preparation

The aqueous extract is prepared by soaking 100grams of air dried *Murraya koenigii* powder in 200 ml of distilled water for 12hours.The extracts were filtered using Whatman filter paper.(9)

Phytochemical screening

The qualitative tests were carried out in both the extract of *Murraya koenigii* using standard

Procedures(10-12).Both the extacts were analysed for the presence of significant secondary metabolites viz Alkaloids, Tannins, Flavonoids ,Cardiac glycosides,Steroids and Saponin.

Test Microorganism

Urinary Tract Infection (UTI) causing bacterial cultures *viz*., Escherichia coli (E. coli), Pseudomonas aeruginosa (P.aeruginosa), Klebsiella pneumonia (K.pneumoniae)were maintained on Nutrient Agar(NA) slants at 4°C.For further study, cultures have been grown in Nutrient Broth (NB) for 24hrs as overnight cultures.

Preparation of inoculums

The pure cultures of bacteria were grown on nutrient agar slants and incubated at 37°C for 24hrs. Nutrient broth and the slants were stored at 4°C and maintained in active state by regular sub-culturing for further use.

Agar well diffusion method:

The modified agar well diffusion method was employed [13].The Nutrient Agar was prepared and poured into different petri plates. The plates were made to solidify in a laminar air flow. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures and spread evenly on the plate by using cotton swab. After 20 min, the wells were filled with different concentrations of samples (20mg/ml, 40mg/ml and 60mg/ml). The control wells were filled with Ampicillin (30mg/ml). All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zones were noted.

Determination of minimum inhibitory concentration (MIC)

The Minimum inhibitory concentration method was applied on extracts that proved their high efficacy against microorganisms by the agar well diffusion method. The highest dilution of a plant extract that still retains an inhibitory effect against the growth of a microorganism is known as MIC(14). Selected plant extracts were subjected to a serial dilution (25 mg/ml to 0.37 mg/ml) using sterile nutrient broth medium as a diluents. In a 96-well titre plate 20 µl of an individual microorganism and 20 µl of selected plant extract were loaded and inoculated at at room temperature. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as the MIC value of the extract. A control experiment was run in parallel to study the impact of the solvent alone (without plant extracts) on growth of the five test organisms. Ethanol was diluted in a similar pattern with sterile nutrient broth followed by inoculation and incubation.

Results

Antibacterial activity results obtained in the present study revealed that both ethanolic and aqueous leaf extract of *Murraya koenigii* possess potential antibacterial activity against Urinary Tract Infectious bacterial species *viz.,* E. coli,K.pneumoniae ,P.auregenosa (Table 2). Secondary metabolites in both the extacts were qualitatively assessed and phytochemical screening revealed the presence of significant components responsible for its effective antibacterial activity(Table 3) .The antibacterial efficacy of the extracts of *M.koenigii* leaves was quantitatively assessed on the basis of inhibition zone in mm by agar well diffusion method and minimum inhibitory concentration by broth dilution method.The test organisms were inoculated with pure antibiotics-Ampicillin, to compare the efficacy of leaf extract for their antimicrobial properties. In the present investigation, ethanolic extract of *Murraya koenigii* showed higher sensitivity to all pathogens,whereas aqueous extract showed moderate sensitivity on *E. coli* and *K. peumoniae*, and least sensitivity for *P. aureginosa*.For ethanol extract, maximum zone of inhibition was found to be 26 mm, 20 mm, and 9 mm at the concentration of 60mg/ml of plant extract against *E.coli*, *K. pneumoniae and P. aureginosa* respectively. The results showed that increase in concentration of extract increased the zone of inhibition(Fig 1).The aqueous extract (60mg/ml) also showed satisfactory inhibition against *E. coli* (20 mm), and *K.pneumoniae* (11 mm)(Fig 2). Comparison study of ethanol and aqueous extracts for their antibacterial activity against UTI pathogens revealed that ethanolic extract has high inhibition capability than aqueous extract(Fig 3).The present findings indicated that both the extracts of *Murraya koenigii* were effective against tested pathogens at a dose of 60mg/mL of the extract showing its higher efficacy.

Table:2 Zone of inhibition of *Murraya koenigii* extracts in varying concentration(mm)

Bacterial Isolates	Ethanol extract				Aqueous extract			
	20mg	40mg	60mg	Amp	20mg	40mg	60mg	Amp
E. coli	18	24	26	20	12	16	20	20
K. pneumoniae	13	17	20	20	7	8	11	20
P. aureginosa	6	8	9	12	0	0	0	10

Table 3. Phytochemical analyses of leaf extracts of *Murrayakoenigii*

Phytochemical constituents	Results
Alkaloids	+
Flavonoids	+
Glycosides	-
Terpenes	-
Saponins	+
Steroids	+
Tannins	+

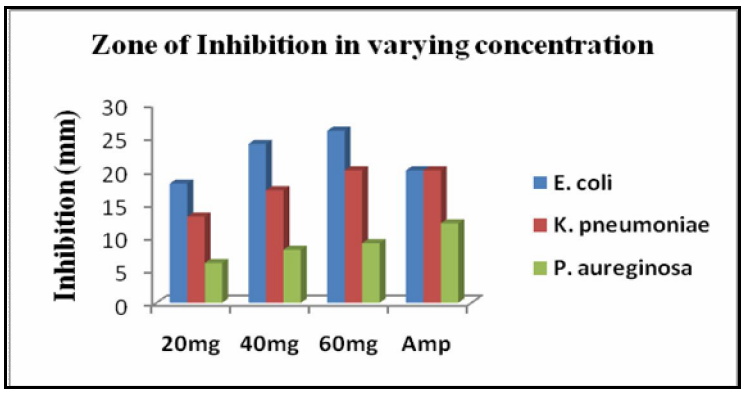

Fig 1:Antibacterial activity by Ethanolic extract of *Murraya koenigii*

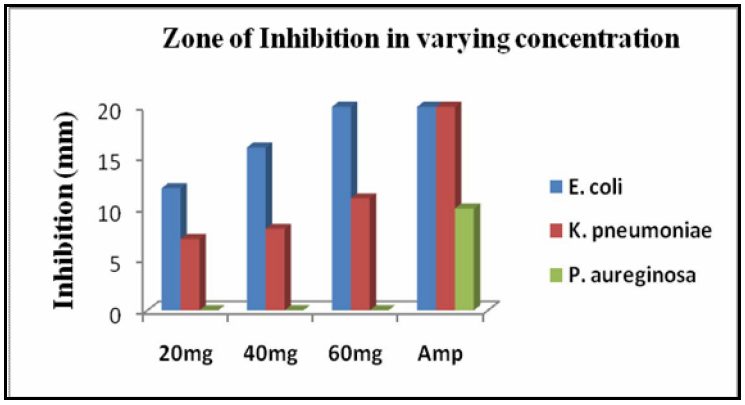

Fig 2:Antibacterial activity by Aqueous extract of *Murraya koenigii*

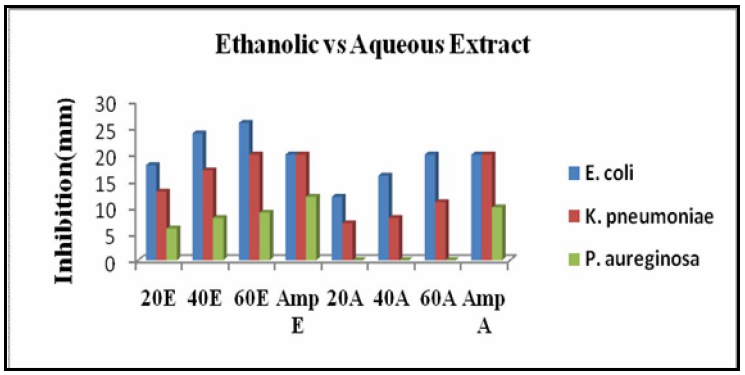

Fig 3: Comparison of Ethanolic and Aqueous extract of *Murraya koenigii*

Discussion

According to World Health Report on infectious diseases, overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management(15).Further acquaintance with different ethnic groups has contributed to the development of research on natural products, to the increase in knowledge about the close relationship between the chemical structure of a certain compound and its biological properties (16). For these reasons, medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. Since, the main concern of the general public and science is in finding new natural and therapeutically active agents; scientists all over the globe have started screening plants for searching new antimicrobial agents.[17].The numerous polyphenolic compounds, triterpenoids present in *Murraya koenigii* may account for the observed antibacterial effect. Tannins, Flavonoids, Saponins, Alkaloids and other chemical compounds present in the plant are speculated to account for the observed inhibition effect against UTI causing pathogens [18]. In classifying the antibacterial activity as Gram-Positive or Gram negative,it would generally be expected that a much greater number would be active against Gram-positive than Gram-negative bacteria(19), however, in our study all the three Gram negative bacteria were effectively inhibited by ethanolic extracts of *Murraya koenigii* .

Conclusion

Results obtained from this study, indicated that, ethanolic extracts *of Murraya koenigii* showed the strongest antibacterial activity than the commercially available antibiotics (Ampicillin). It was revealed that presence of a wide range of bioactive compounds in polar solvent extract of this plant was responsible for the inhibition of the Urinary Tract Infection pathogens. It was seen that ethanolic extract of *Murraya koeniigi* has highest zone of inhibition values for each test organisms showing that it is most effective as compared to aqueous extracts. Thus, *Murraya koeniigi* can be used as an strong antimicrobial agents in new drugs for the therapy of infectious diseases caused by UTI pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and for further pharmacological evaluation.

References

1. Ahmad I, Aqil F. In vitro efficacy of bioactive extracts of 15 medicinal plants against ESbL-producing multidrug-resistant enteric bacteria. Microbiol Res 2007; 162: 264-275
2. Mukherjee P. K, Wahile A (2006). Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J Ethnopharmacol., 103: 25-35.
3. Barbosa L. N, Rall V. L, Fernandes A. A, Ushimaru P. I, da Silva Probst I, Fernandes A, Jr. (2009). Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathog. Dis., 6: 725-728.
4. Ronald A (2002). The etiology of urinary tract infection: Traditional and emerging pathogens. *Am J Med.*; 113: Suppl 1A: 14S-9S.
5. Jacobs L. G (1996). Fungal urinary tract infections in the elderly: Treatment guidelines. *Drugs Aging*; 8: 89-96.
6. Shrinivasan K (2005). Plant foods in the management of diabetes mellitus: spices as beneficial antidiabetic food adjuncts. Int. J. Food Sci. Nutr.; 56(6): 399-414.
7. Arulselvan P, Senthilkumar G. P, Sathish Kumar D, Subramanian S (Oct 2006). "Anti-diabetic effect of Murrayakoenigii leaves on streptozotocin induced diabetic rats". *Pharmazie***61** (10): 874–7. PMID 17069429.
8. Harborne JB;Phytochemical Methods: A Guide To Modern Technique of Plant Analysis.3rd ed .London, England: Chapman and Hall;1998:114-118.
9. H.O. Edeoga, D. E. Okwu, Phytochemical constituents of some Nigerian medicinal Plants; African Journal of Biotechnology Vol. 4 (7), pp. 685-688 July 2005
10. Sofowora AE (1993). Medicinal Plants and Traditional Medicines inAfrica.. 2nd edition. Spectrum Books, Ibadan, Nigeria. p. 289.
11. Trease E.G., Evans W.C 1978. Pharmacognosy.11th Edition, Balliere Tindall, London. 115-222.
12. Harborne JB;Phytochemical Methods: A GuideTo Modern Technique of Plant Analysis.3rded .London, England: Chapman and Hall;1998:114-118.

13. Perez C, Pauli M, Bazevque P: An antibiotic assay by the agar well diffusion method. Acta Biologiae et Medicine Experimentalis 1990, 15:113-115.
14. Misra SB, Dixit SN. Antifungal properties of leaf extract of Ranunculus sceleratus. L. Experientia 1978; 34: 1442-1443.
15. Metwally A M, Omar A A, Harraz F M, El Sohafy S M ;Phytochemical investigation and antimicrobial activity of Psidium guajava L. leaves Pharmacogn Mag 2010;6: 212-218.
16. Jasmine. R, Daisy.P and Selvekumar. B.N., 2007. In vitro Efficacy of Flavonoids from Eugenia jambolana Seeds Against ESL-Producing Multidrug-Resistant Enteric Bacteria. Research Journal of Microbiology. 2 (4):369–374.
17. Viegas, C.; Bolzani, V.S. (2006). Os produtos naturais e a química medicinal moderna. Quím. Nova, 29: 326-337.
18. Priyadarshini S S, Vadivu, Jayshreeet N ;Hypolipidaemic and Renoprotective study on the Ethanolic & Aqueous extracts of leaves of Ravenala madagascariensis Sonn. on alloxan induced diabetic rats.International J Pharm Sci 2010;2: 44-50.
19. McCutcheon AR, Roberts TE, Gibbons E, Ellis SM, Babiuk LA, Hancock REW, Towers GHN (1995). Antiviral screening of British Columbian medicinal plants. J. Ethnopharmacol. 49: 101-110.

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