PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 10 (10): 925-929, 2011
ISSN 1680-5194

Studies on *in vitro* Antibacterial, Antifungal Property and Antioxidant Potency of *Murraya paniculata*

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Abstract: In this investigation, *Murraya paniculata* was analyzed for its antibacterial, antifungal and antioxidant properties. The antibacterial property of *Murraya paniculata* was studied against different bacteria include *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Enterobacter aerogenes* and *Shigella flexineri* and showed growth inhibition activity at concentrations ranging of 300-500 mg. The antifungal property of aqueous, ethanol and hexane extracts of *Murraya paniculata* was studied by agar well diffusion method and we observed that only at 500 mg, it showed positive inhibitory effect. The antioxidant effect of those extracts was also studied against α-tocopherol as a control. From the results, Ethanol extract at the concentration of 500 µg/ml showed 67.77% antioxidant activity against 500 µg/ml of α-tocopherol which showed 72.24% as a standard reference.

Key words: Antifungal, antioxidant, antibacterial, *Murraya paniculata*

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann *et al*., 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy *et al*., 2001; Ateb and ErdoUrul, 2003). Much work has been done on ethno medicinal plants in India (Maheshwari *et al*., 1986; Negi *et al*., 1993). Interest in a large number of traditional natural products has increased (Taylor *et al*., 1996). Plants are the sources of natural pesticides that make excellent leads for new pesticide development (Arokiyaraj *et al*., 2008; Gangadevi *et al*., 2008; Satish *et al*., 2008; Brindha *et al*., 2009; Jagadish *et al*., 2009; Milind Pande *et al*., 2009; Shanmugavalli *et al*., 2009; Swarna Latha and Neelakanta Reddy, 2009; Vetrivel Rajan *et al*., 2009).

Murraya paniculata belongs to the family of Rutaceae, which is a large family of trees, shrubs and climbers recognized easily from aromatic or lime-like smell of the broken twigs or fruits or of the crushed leaves. Some constituents of essential oils, such as citronella and bergamot, are obtained by distillation from plants of this family and many species are used in native medicine.

Murraya paniculata which belong to Rutaceae family is one of the two genus species that can be found in many parts of India. The plant which also well known as "kemuning" or orange jasmine also known as Chinese box in America and Canada (Bailey, 1978). The leaves are rather lather and dark shiny green. Their root bark is used as an anodyne or local anesthetic for the treatment of gout, contusion and bone ache (Kinoshita *et al*., 1989).

The ground bark of *Murraya paniculata* is used in mixture of a drink and as antidote in snake bites and rubbed on the bitten limb. The ground bark of the root is eaten and rubbed on body to cure body ache. The powdered leaves is used as an application to fresh cuts and decoction of the leaves is drunk in dropsy. The leaves and roots of the plant are used in folk medicine for the treatment of stomachache, toothache and gout (Rahman *et al*., 1997) and treatment of diarrhea, dysentery and useful against rheumatism, cough and hysteria (Sastri, 1962; Chopra *et al*., 1956; Ghani, 2003). It is also reported that it is used to treat cuts, joint pain, body aches (Parotta, 2001) and venereal disease (Kinoshita and Firman, 1996). Previous studies have reported several flavonoids and coumarins from the leaves and roots of *M. paniculata* (Sukari *et al*., 2003; De Silva *et al*., 1980).

The aim of the present investigation is to study the antibacterial and antifungal effect of aqueous, ethanol and hexane extract of *Murraya paniculata* and also the assessment of its antioxidant potency.

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Table 1: Antibacterial activity of aqueous extract of *Murraya paniculata*

		Diameter of the zone of inhibition (mm)			
Aqueous extract	Concentration in µg	*E. coli*	*P. mirabilis*	*S. typhi*	*E. aerogenes*
Murraya paniculata	100	10±0.4	NS	NI	NI
	200	12±0.5	10±0.4	NS	NS
	300	15±0.3	13±0.7	11±0.5	NS
	400	19±0.6	16±0.8	13±0.3	13±0.8
	500	22±0.7	20±0.6	16±0.4	17±0.5
Streptomycin	100	24±0.2	21±0.8	19±0.3	22±0.4
Chloramphenicol	100	25±0.2	21±0.4	20±0.7	21±0.8

NS = Non Significant value (<10 mm), NI = No Inhibition

Table 2: Antibacterial activity of ethanolic extract of *Murraya paniculata*

		Diameter of the zone of inhibition (mm)			
Ethanolic extract	Concentration in µg	*E. coli*	*P. mirabilis*	*S. typhi*	*E. aerogenes*
Murraya paniculata	100	NS	NI	NI	NS
	200	NS	NS	NS	NS
	300	10±0.6	10±0.6	13±0.4	11±0.7
	400	14±0.6	15±0.8	15±0.6	13±0.8
	500	17±0.3	18±0.2	17±0.4	17±0.5
Streptomycin	100	21±0.2	21±0.9	20±0.8	22±0.8
Chloramphenicol	100	23±0.2	20±0.4	21±0.4	20±0.3

NS = Non Significant value (<10 mm), NI = No Inhibition

Table 3: Antibacterial activity of hexane extract of *Murraya paniculata*

		Diameter of the zone of inhibition (mm)			
Hexane extract	Concentration in µg	*E. coli*	*P. mirabilis*	*S. typhi*	*E. aerogenes*
Murraya paniculata	100	NI	NI	NI	NS
	200	NS	NI	NS	NS
	300	NS	11±0.4	NS	NS
	400	12±0.2	14±0.7	14 ± 0.8	10±0.8
	500	15±0.3	17±0.3	16±0.5	12±0.5
Streptomycin	100	22±0.4	19±0.9	19±0.8	19±0.8
Chloramphenicol	100	21±0.2	20±0.6	20±0.4	21±0.8

NS = Non Significant value (<10 mm), NI = No Inhibition

MATERIALS AND METHODS

Preparation of plant extract: *Murraya paniculata* used in this study were collected from Thirukkalukundram, Kanchipuram District, TamilNadu, South India. The plant was identified by the experts of Centre for Advanced Studies in Botany, University of Madras, Guindy campus, Chennai and a voucher specimen was deposited in our departmental laboratory. The collected plant sample was refluxed in running tape water for 1-2 h and shade dried at room temperature for 15-20 days. Aqueous, ethanolic and hexane extract of *Murraya paniculata* was prepared using soxhlet apparatus (Hoffman *et al*., 2004) for about 24 h. The extract was distilled and concentrated *in vacuo* with addition of $CaCl_2$. Lyophilized aqueous fractions were further used to test for the antifungal, antibacterial and antioxidant properties.

Microbial cultures: *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Enterobacter aerogenes* and *Aspergillus niger* were purchased from IMTECH, Chandigar, India. Solvent and other chemicals which were used during this study were purchased from Himedia, Merck and s.d. Fine-Chemicals, Mumbai.

Antibacterial activity assessment: The antibacterial activity of *Murraya paniculata* was evaluated by agar well diffusion method (Chung *et al*., 1990). Muller Hinton agar medium was prepared and poured into the petri dishes. Then it was inoculated with a swab of bacterial culture and spread throughout the medium uniformly with a sterile cotton swab. Using a sterile cork borer (10 mm diameter) wells were made in the agar medium. The test compound was introduced into the wells and all the plates were incubated at 37°C for 24 h. The experiment was performed five times under strict aseptic conditions. Sensitivity of the organism was determined by measuring the diameter of the zone of inhibition. Each assay was repeated for five times and the mean value was taken for analyses. The control experiment was carried out with the antibiotics such as streptomycin and chloramphenicol. The final results were tabulated (Table 1, 2 and 3).

Table 4: Antifungal activity of different extracts of *Murraya paniculata*

		Diameter of the zone of inhibition (mm) of *A. niger*		
Material	Concentration in µg	*Hexane extract*	*Aqueous extract*	*Ethanolic extract*
Murraya paniculata	100	NI	NI	NI
	200	NS	NI	NS
	300	11±0.8	10±0.8	NS
	400	13±0.4	12±0.5	12±0.6
	500	16±0.6	15±0.3	15±0.7
Fluconazole	100	20±0.5	18±0.6	19±0.5

NS = Non Significant value (<10 mm), NI = No Inhibition

Table 5: Antioxidant activity of aqueous, ethanolic and hexane extracts of *Murraya paniculata*

	% of inhibition of lipid peroxidation				
Extract	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
Water	11.18	21.35	38.77	45.35	59.13
Ethanol	23.13	36.50	45.32	52.67	67.77
Hexane	20.58	28.35	35.67	45.35	50.13
α-Tocopherol	27.54	38.62	50.75	61.08	72.24

Antifungal activity assessment: The aqueous, ethanolic and hexane extracts of *Murraya paniculata* were screened for antifungal activity by agar well diffusion method (Perez *et al*., 1990) with sterile cork borer of size 6.0 mm. The cultures of 48 h old grown on Potato Dextrose Agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02 ml) of inoculum was introduced to molten PDA and poured into a petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method aqueous, ethanolic and hexane extracts of *Murraya paniculata* were introduced serially in the concentrations of 100, 200, 300, 400 and 500 mg. Incubation period of 24-48 h at 28°C was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured in mm. The control experiment was carried out with Fluconazole. The final results were tabulated (Table 4).

Antioxidant activity assessment: The antioxidant activity of aqueous, ethanolic and hexane extracts of *Murraya paniculata* were determined by ferric thiocyanate method (Mistuda *et al*., 1996). 10 mg of each extract was dissolved separately in 99.5% of ethanol and various concentrations (100, 200, 300, 400 and 500 µg/mL) were prepared. A mixture of a 2 mL of sample in 99.5% ethanol, 2.052 mL of 2.51% linoleic acid in 99.5% ethanol, 4 mL of 0.05 M phosphate buffer (pH 7.0) and 1.948 mL of water was placed in a vial with a screw cap and placed in an oven at 60°C in the dark. To 0.1 mL of this sample solution 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate was added. After the addition of 0.1 mL of 2 x 10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red color developed was measured in 3 min at 500 nm (Matook and Hashinaga, 2005). The control and standard were subjected to the same procedures as the sample, except that for the control, only solvent was added and for the standard, sample was replaced with the same amount of α-tocopherol (reference compound) (Ali Yildirim *et al*., 2001). The inhibition of lipid peroxidation in percentage (Table 5) was calculated by following equation:

$$\text{Inhibition (\%)} = 1 - (A1/A2) \times 100$$

Where:
A1 = Absorbance of the test sample
A2 = Absorbance control reaction

RESULTS AND DISCUSSION

Antibacterial activity: From Table 1, 2 and 3 it is very clear that the aqueous, ethanolic and hexane extracts of *Murraya paniculata* showed growth inhibition activity at the concentrations of 300-500 mg. *E. coli* and *P. mirabilis* were sensitive to aqueous extracts of *Murraya paniculata* at higher concentrations (400 and 500 mg) rather than ethanolic and hexane extracts. *S. typi* and *E. aerogenes* showed moderate resistance to all extracts when compared to others (Table 1, 2 and 3). It was observed that in both ethanolic and hexane extracts of *Murraya paniculata*, bacterial strains are not highly susceptible even at high concentration than aqueous extract.

Antioxidant activity: The antioxidant activity of the aqueous, ethanolic and hexane extracts of *Murraya paniculata* were determined by Ferric Thiocyanate (FTC) and the values are presented in Table 5. FTC method was used to determine the amount of peroxide formed

and that react with ferrous chloride ($FeCl_2$) to form a reddish ferric chloride ($FeCl_3$) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity of extract increases. Aqueous, ethanolic and hexane extracts at various concentration (100, 200, 300, 400 and 500 in µg/mL), showed antioxidant activities in a concentration dependent manner. Ethanol extract at the concentration of 500 µg/mL showed 67.77%, an antioxidant activity at the concentration of 500 µg/mL of α-tocopherol (72.24%), the reference compound. The aqueous and hexane extracts of *Murraya paniculata* also have showed some significant level of inhibition of lipid peroxidation. It has been observed that the extract exhibited moderate antioxidant activity with the increase in concentration.

Antifungal activity: The antifungal potency of different extracts of *Murraya paniculata* was observed against *Aspergillus niger* using Fluconazole as a control compound. From the results, we observed that all the extracts (aqueous, ethanolic and hexane) of *Murraya paniculata* showed moderate antifungal potency. *Aspergillus niger* was not susceptible to the extracts in mild concentrations (<300 mg) and susceptible only at higher concentrations (400-500 mg).

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