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Study for anti-infective potential of antibacterial properties of Murraya Koenigii

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Abstract: *In vitro antimicrobial efficacy of leaves extracts of Murraya koenigii was performed by disc diffusion method against six Gram positive bacterial (Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus subfava), nine Gram negative bacterial (Alcaligenes fecalis, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas pseudoalcaligenes and Salmonella typhimurium) and two fungal strains (Aspergillus brasiliensis, candida albicans). The most susceptible bacterial strains were Bacillus subtilis and Staphylococcus aureus whereas not showing antifungal activity. The leaf extracts in organic solvents (methanol) showed better antimicrobial activity as compared to aqueous extracts Results of present study shows that leaves of Murraya koenigii having antimicrobial activity and can be used for natural antimicrobial agent.*

Key Words: *Antibacterial, antimicrobial, antifungal, curry leaves, Murraya koenigii,*

I. Introduction

In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial susbstances from other sources including plants. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. *Murraya koenigii* (Rutaceae) commonly known as curry neem, is an aromatic more or less deciduous shrub or a small tree up to 6 m in height found throughout India up to an altitude of 1500 m and are cultivated for its aromatic leaves[1]. In traditional system of medicine, it is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavoring agent in curried and chutneys. The oil is used externally for bruises, eruption, in soap and perfume industry[2].Carbazole alkaloids[3], the major constituents of the plant are known to possess cytotoxic, antioxidative, antimutagenic and anti-inflammatory activities[4-5]. The leaves are rich in mono-terpenoids and sesquiterpenoids which exhibited antifungal activities[6]. In the present investigation, an attempt has been made to investigate antimicrobial screening of aqueous and solvent leaf extracts of *Murraya koenigii.*

II. Materials and methods

Plant Collection:

Fresh leaves of *Murraya koenigii* were collected randomly from the semi-arid region of Himalaya's, HRDI, Dehraun-UK and other different sources of India. The taxonomic identities of these plants were confirmed by Dr. GBS Reddy Department of R&D chemistry. Fresh plant materials were washed under running tap water, air dried and homogenized to fine powder and stored in air-tight bottles.

Preparation of Plant Extracts:

Aqueous Extraction:

10 grams of dried plant material was extracted in distilled water for 6 h at slow heat. After every two hours it was filtered through eight layers of muslin cloth and centrifuged at 2000 rpm for 25 min. The supernatant was collected. This procedure was repeated twice and after 8 h, the supernatant was concentrated to make the final volume one-fifth of the original volume. The extract was then autoclaved at 111°C and 15 lbs pressure and stored at 5°C.

Solvent Extraction:

Twenty grams of dried leaves material was extracted with 100 ml of ethanol/ methanol kept on a rotary shaker for 20 h at room temperature. Thereafter, it was filtered and centrifuged at 2000 rpm for 20 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume (Saini) et al., 2005). It was stored at 5°C in airtight bottles for further studies.

Microorganisms used:

The test organisms used included Gram positive bacterial cultures *Bacillus cereus* ATCC11778, *Bacillus megaterium* ATCC9885, *Bacillus subtilis* ATCC6633, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC12228 and *Staphylococcus subfava* NCIM2178; Gram-negative bacterial cultures *Alcaligenes fecalis* ATCC8750, *Enterobacter aerogenes* ATCC13048, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* NCIM2719, *Proteus mirabilis* NCIM224, *Proteus vulgaris* NCTC8313, *Pseudomonas aeruginosa* ATCC27853, *Pseudomonas pseudoalcaligenes* ATCC17440 and *Salmonella abony* NCTC6017 and fungal cultures *Aspergillus brasiliensis* 16404*, candida albicans* 10231.

Culture media and inoculum

Sabouraud Dextrose (SD) and soyabean Casein Digest (SCD) media (Hi media) were used for fungal and bacterial cultures, respectively. Bacterial cultures, freshly grown at 37°C for 24 hours and fungal cultures at 28°C for 48 hours were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 10^5 CFU/ml.

All the microbial cultures were maintained at 4°C on nutrient agar slants (for bacteria) and MGYP slants (for yeast).

Preparation of test compound

The extracts of *Muraya koenigii* were diluted in 100% dimethylsulphoxide (DMSO) and stock prepared of 25 mg/ml.

Antimicrobial Assay:

Antimicrobial assay of crude extracts was carried out against nine test pathogenic strains by disc diffusion method[7].The Muller Hinton Agar and Sabouraud Dextrose Agar plates were inoculated with (10^6 cfu/ml) of the bacterial and fungal strains respectively. The sterilized Whatman no. 1 filter paper disc of 6 mm. were impregnated with 1000 µg/ml of extract and placed aseptically on the surface of inoculated plates with the help of sterile forceps. The standard discs impregnated with antibiotics nystatin (2 µg/ml) and chloramphenicol (2 µg/ml) were used as control. The plates were incubated at 37°C for 24 hours and at 28°C for 48 hours for bacteria and fungi respectively. The diameter of the zone of inhibition in mm was measured. The experiment was repeated three times and the mean values calculated for the conclusion.

Minimum inhibitory concentration was determined by broth dilution method[8]. For broth dilution, 1 ml of the standardized suspension of the strain (10^6 cfu/ml) was added to each tube containing extracts at various concentrations in soyabean casein digest medium. The tubes were incubated at 37°C for 24 hours and at 28°C for 48 hours for bacteria and fungi respectively and observed for visible growth. The experiment was repeated three times. The minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there turbidity after incubation.

III. Results and Discussion

The antimicrobial efficacy of the leaves extracts of *Murraya koenigii* was determined on the basis of zone of inhibition (Table 1) and minimum inhibitory concentration (Table 2). In the present study methanol extract was found to be effective against tested microbial strains as compares to aqueous extract. Most sensitive bacteria were *S. aureus* and *Bacillus subtilis*. It shows that leaves extracts of *Murraya koenigii* having antimicrobial properties which are effective against diseases.

The results from the present investigation shows that leaves extracts of *Murraya koenigii* can be used as antimicrobial agent. Study supports traditional use of curry leaves.

IV. Conclusion

The present investigation concludes that leaves extracts of *Murraya koenigii* shows antimicrobial properties which confirms the use in traditional medicines to treat the disease caused by pathogens. Methanol extracts shows maximum antimicrobial activity against *Staphylococcus aureus* and *Aspergillus brasilliensis*.

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Table 1- Antimicrobial activity of leaves extracts of *Murraya koenigii*

Test organism	Methanol Extract	Aqueous Extract	Control	
			Nystatin (2µg/ml)	Chloramphenicol (2µg/ml)
Bacillus cereus	8.22	5.25	-	18.67
Bacillus megaterium	9.37	5.22	-	18.08
Bacillus subtilis	12.39	10.67	-	17.96
Staphylococcus aureus	10.64	8.09	-	20.22
Staphylococcus epidermidis	9.29	6.61	-	19.87
Staphylococcus subfava	7.33	3.19	-	19.00
Alcaligenes fecalis	2.55	-	-	18.76
Enterobacter aerogenes	-	-	-	20.19
Escherichia coli	7.05	3.22	-	21.64
Klebsiella pneumonia	7.76	4.13	-	19.08
Proteus mirabilis	-	-	-	17.67
Proteus vulgaris	1.68	-	-	18.01
Pseudomonas aeruginosa	4.89	1.52	-	20.31
Pseudomonas pseudoalcaligenes	3.36	1.08	-	19.63
Salmonella abony	8.86	5.45	-	22.08
Aspergillus brasiliensis	14.68	10.76	21.28	-
candida albicans	7.16	3.59	18.67	-

Zone of inhibition in mm, - : No activity, Values are average of three replicates

Table 2- Minimum Inhibitory Concentration (MIC) of leaves extracts of *Murraya koenigii*
Values are average of three replicates

Test organism	Methanol Extract (mg/ml)	Aqueous Extract (mg/ml)
Bacillus cereus	1.25	2.50
Bacillus megaterium	1.25	2.50
Bacillus subtilis	0.312	0.625
Staphylococcus aureus	0.312	0.625
Staphylococcus epidermidis	0.625	1.25
Staphylococcus subfava	0.625	-
Alcaligenes fecalis	2.50	-
Enterobacter aerogenes	-	-
Escherichia coli	2.50	-
Klebsiella pneumonia	2.50	-
Proteus mirabilis	-	-
Proteus vulgaris	-	-
Pseudomonas aeruginosa	1.25	2.50
Pseudomonas pseudoalcaligenes	-	-
Salmonella abony	1.25	-
Aspergillus brasiliensis	0.312	0.625
candida albicans	0.625	2.50