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Phytochemistry and Pharmacological Activities of *Murraya koenigii* (Curry Leaves) Leaves Extracts

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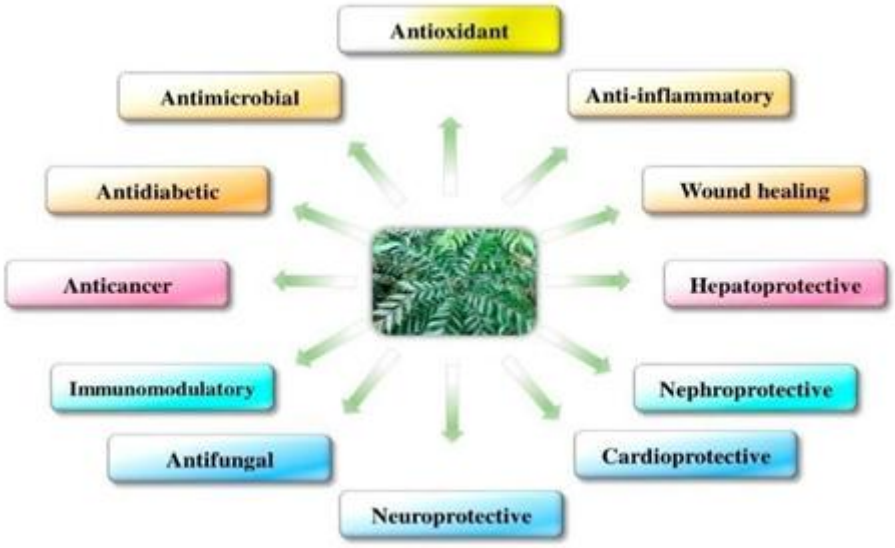
Abstract: ***In traditional medicinal therapies, several plant extracts and phytochemicals have been reported to impart remedial effects as better alternatives. Murraya koenigii (M. koenigii) belongs to the Rutaceae family, which is commonly used as a medicinally important herb of Indian origin in the Ayurvedic system of medicine. In vitro antimicrobial efficency of leaves extracts of M. koenigii was performed by disc diffusion method against six Gram positive bacterial (Bacillus cereus, Bacillus megaterium, Bacilus 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 albicans) and two fungal strains (Aspergillus brasiliensis, Candida albicans). The most suspectable bacteria strains were Bacillus subtilis and Staphylococcus aureus whereas not showing antifungal activity. The leaf extracts in organic solvants (CH3OH) showed better antimicrobial activity as compared to aqueous extracts. Result of present study shows that the leaves of M. koenigii having antimicrobial activity and can be used as natural antimicrobial agent.***

Keywords: *Murraya koenigii*, curry leaves, antimicrobial activity and antifungal activity

1. Introduction

In the present scenario of emergence of multiple drug resistance to human pathogenic organism, it is necessary to search for new antimicrobial substance from other souces including plants. Tradionationally used medicinal plants produce a variety of compounds of known therapeutic properties. Murraya koenigii (M. koenigii*) (L) Spreng (Family: Rutaceae) is usually known as "curry leaves"*. The tropical and subtropical regions in the world have large distributions of *M. koenigii [1]*. Among the 14 global species belonging to the genus of *Murraya*, only two, *M. koenigii* and *M. paniculate*, are available in India. *M. koenigii* is more important due to its huge spectrum of traditional medicinal properties. For centuries, this plant has been used in diverse forms and holds a place of pride in Indian Ayurvedic medicine, known as "krishnanimba" [2]. Different parts of *M. koenigii*, such as its leaves, root, bark, and fruit, are known to promote various biological activities. Aromatic bioactive constituents in the leaves of *M. koenigii* retain their flavor and other qualities, even after drying [3-8]. *M. koenigii* leaves are slightly bitter in taste, pungent in smell, and weakly acidic. They are used as antihelminthics, analgesics, digestives, and appetizers in Indian cookery [9-10]. The leaves are rich in mono terpenoids and susquiteterpenoids which exhibited antifungal activities [11]. *M. koenigii* has numerous disease remedial activities, for instance, different parts of the plant, such as the leaves, roots, and bark, can be prepared as tonics for inducing digestion and flatulence or as antiemetics [12-13].



Different investigations support the traditional use of the plant as an antifungal agent. In vitro antifungal activity may explain the use of curry leaves for the treatment of diarrhea, dysentery, and skin eruptions in folklore medicines [14]. Bioactive compounds of *M. koenigii* appreciably hold the ability of mycelial growth inhibition and thereby promote antifungal activity. The antifungal activity of *M. koenigii* against a wide range of pathogenic fungi has been studied. In the present paper, an attempt has been made to investigate antimicrobial screening of aqueous and solvent leaf extracts of *M. koenigii*.

Figure 1: Morphology of *Murraya koenigii (*A-Whole plant, B-leaves, C-seeds).

Phytochemistry of *M. Koenigii*

Murraya koenigii is a rich source of organic compounds with diverse chemical composition. The presence of alkaloids, flavonoids, and sterol in plant extracts prepared in solvents such as petroleum ether, ethyl acetate, chloroform, ethanol and water has been reported by various workers [**15-18]**.

Biological activities of different chemical constituents identified from different parts of *Murraya koenigii* are:

Plant parts	Chemical constituents	Biological activity	References
Stem bark	Girinimbine	Anti fungal and antibacterial	19
	Murrayanine	Anti-cancer	20
	Marmesin-1'-O-beta-Dgalactopyranoside	Anti fungal , Antimicrobial	19, 21
	Mahanine	Anti viral, Anti bacterial, Anti fungal	19
	Murrayacine	Topoisomerase I and II inhibitory activity	22
	Girinimbine	Antimicrobial	22
	Mukoeic acid	Antimicrobial	23
	Murrayazolinine	Anti-tumor	24
	Girinimbilol	Anti-oxidant	25
	Mahanine,	Anti –leukemial	26
	Pyrafoline-D and	Anti-trichomonal	27
	Murrafoline-I	Cytotoxic and induced the loss of	28
Leaves	Koenimbine	Antioxidant activity, Anti-diarrhea	29
	Koenine	Anti-oxidant	30
	Koenigine	Anti-oxidant, radical-scavenging properties	30
	Mahanimbine	Anti-oxidant	30, 31
	Murrayazolidine	Hepatoprotective	32
	Murrayazoline	Hepatoprotective	32
Root	Mukoline	Cytotoxic activity	33
Seed	Koenoline	Cytotoxic activity	33
	Kurryam,	Anti diarrheal activity	34
	Koenine	Anti diarrheal activity	34
	Koenimbine	Anti diarrheal activity	34
	Koenoline	Cytotoxic activity	33

2. Material and Methods

Plant Collection:

Fresh leaves of M. koenigii were collected randomly fromsubtropical forest in foothills of shivalik of Dehradun and Garhwal and also from subtropical forest of Ramnagar and Nanital in Uttrakhand. Few samples were also collected randomly from west Uttar Pradesh parts like Bijnor, Moradabad and Pilibhitc districts. The taxonomic identities of these plants were confirmed by Dr. Mukesh Kumar, Department of Botany, Gurukul Kangri University, Haridwar. Fresh plant material were washed under running tap water, air dried and homogenized to fine powder and stored in air tight bottle.

1. Preparation of Plant Extracts:

a. Aqueous Extraction:

10 gm of dried plant material was extracted in distilled water for 6 hours at slow heat. After every 2 hours it was filtered through eight layers od muslin cloth and centrifuged at 2000rpm for 25 min. The supernatant was collected. This procedure was repeated twice and after 8 hours, the supernatant was concentrated to make the final volume one fifth of its original volume. The extract was then autoclaved at 1110C and 15 lbs pressure and stored at 50C.

b. Solvent Extraction:

20 gm of dried leaves material was extracted with 100 ml of C2H5OH /CH3OH kept on rotator shaker for 20 hours at room temperature. Thereafter, it was filtered and centrifuged at 2000 rpm for 20 min. The supernatant was collected and solvent was evaporated to make the final volume 1/5 of the original volume. It was stored at 50C in air tight bottles for further studies.

2. Microorganism Used:

The test organismused included gram positive bacterial cultures *Bacillus cereus ATCC11778, Bacillus megaterium ATCC9885, Bacillus subtillis 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 pneumonia NCIM2719, Proteus mirabilis NCIM224, Pseudomonas pseudoalcaligenes ATCC17440 and salmonella abony NCTC6017 and fungal cultures aspergillus brasiliensis 16404, Candida albicans 10231*.

3. 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 shown at 370C for 24 hours and fungal cultures at 280C for 48 hours were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 105 CFU/ml.

All the microbial cultures were maintained at 4oC on nutrient agar slants 9 for bacteria) and MGYP slants (for yeast).

4. Preparation of test compound:

The extracts of M. koenigii were diluted in 100% DMSO and stock solution prepared of 25mg/ml.

5. Antimicrobial Assay:

Antimicrobial assay of crude extracts was carried out against nine test pathogenic strains by disc diffusion method [35]. The muller hinton agar and Sabouraud Dextrose agar plates were inoculated (106 cfu/ml) of bacterial and fungal strains respectively. The sterilized whatmann no 1 filterpaper disc of 6mm were impregnated with 1000µg/ml of extract and placed aseptically on the surface of inoculated plates with the help of sterile forceps. The standard disc impregnated with antibiotics nystatin (2µg/ml) and chloramphenicol (2 µg/ml) were used as control. The plates were incubated at 370c for 24 hours and at 280C 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 [36]. For broth dilution, 1 ml of the standardized suspension of strain (106 cfu/ml) was added to each tube containing extracts at various concentrations in soya bean casein digest medium. The tubes were incubated at 370C for 24 hours and at 280C for 48 hours for bacteria and fungi respectively and observed for visible growth. The experiment was repeated 3 times. the minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there is turbidity after incubation.

3. Result and Discussion

The antimicrobial efficacy of the leaves extracts of M. Koenigii was determined on the basis of zone of inhibition (Table 2) and minimum inhibitory concentration (table 3). In present study methanol extract was found to be effective against tested microbial strains as compared to aqueous extract. Most sensitive bacteria were S. Aureus and B. Subtilis. It shows that the leaves extracts of M. Koenigii having antimicrobial properties which are effective against diseases.

Table 2: Antimicrobial activity of leaves extracts of *M. Koenigii*

Test of Organism	Methanol Extract	Aqueous Extract	Control	
			Nystatin (2 µg/ml)	Chloramphenicol (2 µg/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

Zone of inhibition in mm:; no activity, values are average of three replicates

Table 3: Minimum Inhibitory Concentration (MIC) of leaves extracts of *M. Koenigii*

Test of 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	0.625	1.25
epidermidis		
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

Values are average of three replicates

The result from present investigation shows that the leave extracts of M. Koenigii can be used as antimicrobial agent. Study supports traditional use of curry leaves.

The present investigation concludes that leave extracts of M. Koenigii shows antimicrobial properties which confirms the use in traditional medicines to treat the disease caused by pathogens. Methanol extracts shows maxium antimicrobial activity against Staphylococcus aureus and Aspergillus brasilliensis.

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