International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 4 Issue 8 ǁ August 2015 ǁ PP.19-24

Studies about Murraya Koenigii (Quality Estimation and Antibacterial and Antifungal Properties)

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Abstract: *Murraya koenigii is a valuable medicinal plant which has been used in traditional system of medicine since long time. The extraction of active ingredients such as alkaloids, flavonoids, triterpenes, steroids etc. from the plant facilitates pharmacological studies. The present investigation is to evaluate the proper taxomic status and preliminary phyto-chemical characters as well as physicochemical standards and thin layer chromatographic analysis of the leaves of Murraya koenigii*
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 (Alcaligenesfecalis, Enterobacteraerogenes, Escherichia coli, Klebsiellapneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas pseudoalcaligenes and Salmonella typhimurium) and two fungal strains (Aspergillusbrasiliensis, candida albicans). The most susceptible bacterial strains were Bacillus subtilis and Staphylococcus aureus whereas showing antifungal activity against Aspergillusbrasiliensis. The leaf extracts in organic solvents (methanol) showed better antimicrobial activity as compared to aqueous extracts. Results of present study show that leaves of Murraya koenigii having antimicrobial activity and can be used as natural antimicrobial agent.

Keywords: *curry leaves, murraya koenigii, phytochemical, physicochemical parameters, antibacterial, antimicrobial, antifungal.*

I. Introduction

Knowledge of plants and healing has been closely linked from the time of man's earliest social and culture grouping. Besides medicine, mankind is almost completely dependent on plants for requirements such as food, shelter and clothing. Therefore, one of the biggest myths attending the use of herbs today is the idea that because something is "natural", it is completely safe. This is dangerous notion because some of the drugs that are available from plants are very toxic in natural state. So, the use of herbal medicine however, can be made relevant and popular after evaluating them for their quality, safety and efficacy (WHO, 1991). The therapeutic activity of herbs is because of various constituents present in them. The therapeutic effects of herbal products is inconsistent and varies because the chemical constituents vary; they depend on various factors and one of them is the source. In some plants toxic constituents are also present therefore it is essential to evaluate their quality, safety and efficacy. Correct Identification and quality assurance of the starting material is, therefore an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy (Joshi et al., 2004). The present work is aimed at the pharmacological and phytochemical analysis of murrayakoenigii(Curry leave).In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances 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 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 meter in height found throughout India up to an altitude of 1500 meter 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 curries 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 sesqui terpenoids 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*

Experimental

Plant Collection:

Fresh leaves of *Murraya koenigii* were collected in the month of March 2015, from Himalaya's/ Herbal Research and Development Institute (HRDI), Selaqui, Dehradun, Uttarakhand, India. The authenticity of the plant was confirmed by comparing their morphological characters. Besides, the identity of the plant was also confirmed by Dr. Manoj Kumar Tyagi (Associate Professor) chemistry for further confirmation, the microscopic characteristics of this plant was studied and compared with available literature. The fresh plant material collected was thoroughly cleaned by washing under running tap water and air-dried in shade for seven days. It was then homogenized to fine powder and stored in air-tight bottles for further studies.

Preparation of Plant Extracts*:*

Aqueous Extraction:

Ten grams of dried plant material was extracted in distilled water for 6 hour at slow heat. After every 2 hours it was filtered through eight layers of muslin cloth and centrifuged at 2000 round/minutes for 25 minutes. The supernatant was collected. This procedure was repeated twice and after 8 hour, 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 methanol kept on a rotary shaker for 20 hours at room temperature. Thereafter, it was filtered and centrifuged at 2000 round/minutes for 20 minutes. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume. It was stored at 5 °C in airtight bottles for further studies. Same procedure followed for the extraction of *Murraya* koenigii in petroleum ether and acetone.

Determination of loss on drying

The loss on drying was determined by weighing 3 gram of crude powder of Murraya koenigii in an evaporating dish and then dried in an oven at 104 ºC till constant weight was obtained. The weight after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of sample taken initially.

Determination of total ash

The total ash value of crude powder of *Murraya koenigii* (MCR) was determined by incinerating 1.5 gram of accurately weighed crude powder in a tarred silica crucible. It was incinerated in a muffle furnace at a temperature not exceeding 455 ºC until free from carbon, then cooled and weighed.

Determination of acid insoluble ash

The total ash obtained was boiled for 6 minute with 25 mL dilute hydrochloric acid. The insoluble matter was collected on the filter paper placed in a Gooch crucible, washed with water and heated till the constant weight was obtained. The percentage of acid insoluble ash was calculated with reference to the sample taken initially.

Determination of solubility

The qualitative solubility test of the different extracts of *Murraya koenigii* crude: MCR in Petroleum ether: MPE, in Acetone: MAC, and in Methanol: MME was determined for different solvents with different polarities.

Determination of melting point

The melting point of the crude powder and different extracts of *Murraya koenigii* were done at Department of Applied Chemistry, Meerut by open capillary method (Apparaoetal., 1971; Sukhwal et al., 1995).

Determination of pH

The crude powder of *Murraya koenigii* and its different extracts were dissolved in distilled water and were kept in water bath for 20 minute. It was then filtered and then pH of the filtrate was noted down with the help of a Systronic pH meter (pH system 361).

Determination of heavy metals

Contamination of medicinal plant materials with heavy metals can be attributed to many cases including environmental pollution and traces of pesticides. Therefore, detection of heavy metals is important for herbal drugs. The analysis for heavy metals like arsenic, chromium, cobalt, lead, mercury and nickel for crude powder and different extracts of *Murraya koenigii* were performed.

Antibacterial and antifungal properties of *Murraya koenigii*

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 Alcaligenesfecalis ATCC8750, Enterobacteraerogenes ATCC13048, Escherichia coli ATCC25922, Klebsiellapneumoniae NCIM2719, Proteus mirabilis NCIM224, Proteus vulgaris NCTC8313, Pseudomonas aeruginosa ATCC27853, Pseudomonas pseudoalcaligenes ATCC17440 and Salmonella abony NCTC6017 and fungal cultures Aspergillusbrasiliensis 16404, candida albicans 10231.

Culture media and inoculums

Sabouraud Dextrose (SD) and soyabean Casein Digest (SCD) media (Hi media) were used for fungal and bacterial cultures, respectively. Bacterial cultures, freshly grown at 30-35 °C for 24 hours and fungal cultures at 20-25 °C for 48 hours were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 10^5cfu mL $^{-1.}$

All the microbial cultures were maintained at 4 °C on soyabean casein digest agar slants (for bacteria) and Sabouraud dextrose agar slants (for yeast).

Preparation of test compound

The extracts of *Murraya koenigii* were diluted in 100% dimethylsulphoxide (DMSO) and stock prepared of 25 mg mL $^{-1.}$

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 (10^6cfu mL $^{-1}$) of the bacterial and fungal strains respectively. The sterilized Whatman no. 1 filter paper disc of 6 mm. were impregnated with 1000 µg mL $^{-1}$ 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 µgmL $^{-1}$) and chloramphenicol (2 µg mL $^{-1.}$) were used as control. The plates were incubated at 30-35 °C for 24 hours and at 20-25 °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 method8. For broth dilution, 1 mL of the standardized suspension of the strain (106 cfu mL $^{-1}$) was added to each tube containing extracts at various concentrations in soyabean casein digest medium. The tubes were incubated at 30-35 °C for 24 hours and at 20-25 °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 is no turbidity after incubation.

Results and Discussion

The results of melting point and pH of the crude powder and different extracts of curry leaves (murraya koinigii) are shown in **Table 1**. The melting point of crude powder (MCR), acetone extract (MAC) and methanol extract (MME) was < 300 °C and that of petroleum ether extract (MPE) was 206-210 °C. All the samples were acidic in nature. The methanol extract (MME) was the most acidic in nature. Determination of heavy metals in crude powder and different extracts of Curry Leaves *(murraya koinigii)* MCR: Crude powder, MPE: Petroleum ether extract, MAC: Acetone extract, MME: Methanol extract, NDT: Not detected. The presence of heavy metals in the medicinal plants exceeding certain limit tends to cause a health hazard. Thus, the crude leave powder of Curry leaves and its extracts (MPE, MAC and MME) were analyzed for the presence of heavy metals. The results showed that heavy metals were not present in any of the sample drugs. Therefore, the sample drugs investigated were free from heavy metal contamination. The antimicrobial efficacy of the leaves extracts of *Murraya koenigii* was determined on the basis of zone of inhibition **Table 2** and minimum inhibitory concentration **Table 3**. 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.

Table 1. Quality control parameters of *Murraya koenigii.*

Sr. No.	Particulars	Values
1	pH	6.3 -6.4
2	Ash values(%)	
i	Total ash	4.06 ± 0.05
ii	Water soluble ash	1.0 ± 0.05
ii	Acid insoluble ash	1.24 ± 0.11
3	Extractive values(%)	
i	Petroleum ether (60-80%)	5.60 ± 0.15
ii	Acetone (80%)	4.80 ± 0.05
iii	Methanol (90%)	21.36 ± 0.15
iv	Aqueous	16.05 ± 0.15
4	Loss on drying (%)	10.06 ± 0.15
5	Crude fiber content (%)	70.25 ± 0.15

Table 2. Antimicrobial activity of leaves extracts of *murraya koinigii.*

Test organism	Methanol Extract	Aqueous Extract	Control	
			Nystatin (2 µg mL $^{-1}$)	Chloramphenicol (2 µg mL $^{-1}$)
Bacillus cereus	9.22	7.25	..	18.67
Bacillus megaterium	10.37	8.22	..	18.08
Bacillus subtilis	14.39	11.67	..	17.96
Staphylococcus aureus	16.64	12.09	..	20.22
Staphylococcus epidermidis	12.29	10.61	..	19.87
Staphylococcus subfava	9.33	6.19	..	19.00
Alcaligenesfecalis	5.55	..	..	18.76
Enterobacteraerogenes	..	..	..	20.19
Escherichia coli	8.05	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
Aspergillusbrasiliensis	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 3. Minimum inhibitory concentration (MIC) of leaves extracts of M*urraya koenigii.*

Test organism	Methanol Extract (mg mL^{-1})	Aqueous Extract (mg mL^{-1})
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	..
Alcaligenesfecalis	2.50	..
Enterobacteraerogenes	..	..
Escherichia coli	2.50	..
Klebsiella pneumonia	2.50	..
Proteus mirabilis	..	..
Proteus vulgaris	..	..
Pseudomonas aeruginosa	1.25	2.50
Pseudomonas pseudoalcaligenes	..	..
Salmonella abony	1.25	..
Aspergillusbrasiliensis	0.312	0.625
candida albicans	0.625	2.50

Values are average of three replicates

Conclusions

From the present study, it can be concluded that crude powder of Curry Leaves (murraya koinigii) leave was dark green in color with characteristic odour and tasteless. The extractive yield of MPE, MAC and MME was 5.6%, 4.8% and 21.36% respectively. The crude powder and different extracts of curry leaves (Murraya koenigii) leave (MPE, MAC and MME) were free from heavy metal and microbial contamination. All the sample drugs were acidic in nature. MPE, MAC and MME were maximally soluble in polar solvents.

The present investigation concludes that leaves extracts of M*urraya 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 *Aspergillusbrasilliensis*. This qualitative and quantitative approach to understand the plant *murraya koenigii*, does help in better identification, taxonomical position and medicinal importance of this unused multi valuable traditional herb in depth. The adulterants in drug obtained from *Murraya koenigii* can be identified by this investigation and ensure the proper use of this plant in pharmaceutical field.

Acknowledgements

The authors are highly thankful to the management of Herbal Research and Development Institute (HRDI), Selaqui, Dehradun, Uttarakhand, India and Management of Samarth Analytical Laboratory, Nasik, Maharashtra, India, for providing necessary facilities.

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