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Phytochemical Analysis and Antimicrobial Activity of Four Different Extracts from the Leaves of *Murraya koenigii*

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A B S T R A C T

Four different extracts of *Murraya Koenigii*(Rutaceae) leaves were screened for their phytochemical composition and antimicrobial activity. The present study investigates the qualitative and quantitative analysis of the major phytochemicals in four different extracts of Murraya Koenigii. The constituents screened for were tannins, saponins, flavanols, flavonoids, total phenolic compounds, proanthocyanidins, carotenoids, alkaloids, glycosides, reducing sugars and aminoacids. The antimicrobial activity of the extracts against *Methicillin Resistant Staphylococcus aureus, Micrococcus luteus, Bacillus subtilus, Psedomonas aeruginosa, Escherichia coli, Candida albicans and Aspergillus Niger* were screened. The study shows that all the extracts posses' remarkable antimicrobial and antifungal activity.

Introduction

Phytochemicals are natural bioactive compounds found in plants and these are divided into two groups; primary and secondary compounds. These classes perform functions in plant metabolism. Amino acids, sugars, proteins and chlorophyll are known as primary compounds while secondary compounds consists of alkaloids, terpenoids, phenolic compounds etc.(Krishnaiah *et al.,* 2009) Since human's existence on planet, man has been dependent on nature for curing various diseases. Herbal medicine has proved efficacious and potent in the treatment of many chronic diseases that orthodox medicine cannot cure**.** Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to

effectively treat human diseases. The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals.

Murraya Koenigii (Rutaceae) is a tropicalto sub-tropical tree which is native to India and Sri-Lanka.The family Rutaceae has more than150 genera and 1600 species (Krishnaiah *et al.,* 2009). The plant is a rich source of carbazole alkaloids (Sathyavati *et al.,* 1978).Its leaves are used in many dishes as curry leaves. The leaves are pinnate, with 11-21 leaflets, each leaflet 2-4cm long and 1-2cm broad. They are highly aromatic. Curry leaves have many medicinal properties. It stimulates digestive enzymes and helps in breakdown of food more easily, good remedy for nausea and indigestion. It also improves eye-sight and prevents cataract. Eating curry leaves lowers blood glucose level. The benefits of curry leaves also owe to its flavour and taste and are added to dishes to enhance the smell, taste and its value.

Phytochemical studies on the leaves, stem, bark and root of this plant have shown the presence of large concentration of alkaloids, phenolic compounds and very high radical scavenging activity (Kumar *et al.,* 1999; Sharif *et al.,* 2007). The antioxidant activity of curry leaf is attributed to mahanimbine, murrayanol and mahanine (Sharif *et al.,* 2007; Tachibana *et al.,* 2001).

Health benefits of Murraya Koenigii leaves are anti-diabetic (Ningappa *et al.,* 2008), antioxidant (Kesari *et al.,* 2005), antimicrobial (Vandana *et al.,* 2012), anti-inflammatory (Ningappaa *et al.,* 2008), hepatoprotective (Darvekar *et al.,* 2011), anti-hypocholestremic & hypolipidemic (Gupta *et al.,* 2007), anti-diaarhoel(Iyer *et al.,* 1990), cytotoxic (Mandal *et al.,* 2010) antidementia (Nutan *et al.,* 1998) etc.

The present study investigate the comparision of qualitative and quantitative analysis of the different constituents & antimicrobial activity of four different extracts of Murraya Koenigii.

Materials and Methods

Plant materials

Fresh leaves of Murraya Koenigii were collected from Ponneri, Thiruvallur district, Tamilnadu, India during the month of September. The collected leaves were identified taxonomically and authenticated by Dr. G. Jeya Jothi, Taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai. A voucher specimen (No: LCH 401) was deposited at Herbarium, Department of Plant Biology & Biotechnology, Loyola College, Chennai. The leaves were washed thoroughly 2-3 times with running tap water and once with sterile water, air dried, powdered and used for extraction.

Preparation of plant extract

50 g of air dried and coarsely powdered plant material was extracted successively with 100ml of methanol, water, acetone and hexane using a soxhlet's apparatus for 48 hrs each. The four crude extracts were stored at 5degrees in labelled sterile screw-capped bottles for further use.

Preliminary phytochemical analysis

Qualitative analysis of Phytochemicals

Initial screening test of the four extracts were performed to ascertain the presence or absence of phytoconstituents such as flavonoids, flavonols, tannins (Ferric chloride test & lead acetate test) saponins (Frothing test), anthraquinones (Brontrager's

test), steroids (Salkowski test and Libarman-Burchard's test), glycosides, sugars, amino acids and alkaloids (Dragendroff's test, Meyer's test, Hager's test, and Wagner's test) using standard procedure (Mani *et al.,* 2012; Sofowora *et al.,* 1993; Somolenski *et al.,* 1974).

Quantitative determination of Phytochemicals

Determination of total phenolic content

Total phenol contents in the various extracts were determined by the modified Folin-Ciocalteu method of Zovko *et al* (Zovko *et al.,* 1973). An aliquot of 0.5 ml of each extract (1 mg/ml) was mixed with 2.5 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 2 ml (75% w/v) of sodium carbonate (Na_2CO_3). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for colour development. Absorbance was then measured at 765 nm using Hewlett Packard, UV/visible light spectrophotometer. Samples of extract were evaluated at a final concentration of 1 mg/ml. Total phenolics content were expressed as mg/g tannic acid equivalent using the following equation from the calibration curve: $Y = 0.1216\times$, $R^2 = 0.936512$, where × is the absorbance and Y is the tannic acid equivalent in mg/g.

Determination of total flavonoids

The method is based on the formation of the flavonoids - aluminium complex which has an absorptivity maximum at 420nmdescribed by Ordonez *et al* (Ordonez *et al.,* 2010).Half a ml of various solvent extracts (1 mg/ml) was mixed with 0.5 ml of 2% aluminium chloride ($AlCl_3$) prepared in ethanol. The resultant mixture was incubated for 60 min at room temperature for yellow colour development which indicated the presence of flavonoid. The absorbance was measured at 420 nm using UV–VIS spectrophotometer. Extract samples were evaluated at a final concentration of 1 mg/ml. Total flavonoid content was calculated as quercetin equivalent (mg/g) using the following equation based on the calibration curve: $Y = 0.255x$, $R^2 = 0.9812$, where × is the absorbance and Y is the quercetin equivalent.

Estimation of total flavonols

Total flavonol content was determined by adopting the procedure described by Karunakaran and Kumaran (Karunakaran *et al.,* 2007). The reaction mixture consisting of 2 ml of the sample, 2 ml of $AlCl_3$ prepared in ethanol and 3 ml of (50 g/l) sodium acetate solution was allowed to incubate for 2.5 h at 20°C.

Absorbance at 440 nm was measured. Total flavonol content was calculated as mg/g of quercetin equivalent from the calibration curve using the equation: Y $Y = 0.0255\times$, $R^2 = 0.9812$ where × is the absorbance and Y is the quercetin equivalent.

Estimation of proanthocyanidins

Proanthocyanidins contents was estimated by the procedure done by Asafa *et al* (Ashafa *et al.,* 2011).A volume of 0.5 ml of each extract solution was mixed with 3 ml of 4% v/v vanillin prepared in methanol and 1.5 ml of hydrochloric acid and then vortexed. The resulting mixture was allowed to stand for 15 min at room temperature followed by the measurement of the absorbance at 500 nm. Total proanthocyanid in content was expressed as catechin (mg/g) using the following equation of the curve: $Y = 0.5825\times$, $R^2 = 0.9277$, where × is the absorbance and Y is the catechin equivalent.

Tannin determination

Tannin content of the samples was determined according to the modified vanillin-HCl methanol method as determined by Noha *et al* (Noha *et al.*, 2011).The vanillin-HCL reagent was prepared by mixing equal volume of 8% HCl and 1% vanillin in methanol. The reagent was mixed just prior to use. About 0.2 g of the ground sample was placed in a small conical flask. Then 10 ml of 1% concentrated HCL in methanol was added. The flask was capped and continuously shaken for 20 min and the content was further centrifuged at 2500 rpm for 5 min. About 1.0 ml of the supernatant was pippetted into a test tube containing 5 ml of vanillin-HCL reagent. Absorbance at 450 nm was read on spectrophotometer after 20 min of incubation at 30°C. A standard curve was prepared expressing the result as catechin equivalent as follows: Tannin (%) = C × 10 × 100/200. Where: C = Concentration corresponding to the optical density; 10 = volume of the extract (ml); 200= Sample weight (mg).

Saponin determination

Five grams of plant sample was dispersed in 50 ml of 20% v/v ethanol prepared in distilled water. The suspension was heated over hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 50 ml of 20% ethanol. The combined extracts were reduced to 20 ml over hot water bath at about 9°C. The concentrated solution obtained was shaken vigorously with 10 ml of diethyl ether in a 250ml separating funnel; the aqueous layer was collected while the ether layer was discarded. The purification process and repeated. Twenty millilitre of but-1-ol was added to the filtrate and then washed twice with 10 ml of 5% w/v aqueous sodium chloride. The whole mixture was heated to evaporation on hot water bath and later oven dried at 40°C to a constant weight. The percentage saponin content of the sample was calculated using the formula described by Okwu and Josiah (Okwu *et al.*, 2006)

Alkaloid determination

Alkaloid content of the plant sample was determined using the method described by Onyilagha and Islam (Onyilagha *et al.*, 2009). Five gram of the powdered sample was weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. The alkaloid content was determined using this formula;

%Alkaloid=final weight of sample
Initial weight of extract×100.

Sources and maintenance of organisms

Stock cultures of *Methicillin Resistant Staphylococcus aureus, Micrococcus luteus, Bacillus subtilus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans and Aspergillus Niger* were obtained from Department of Microbiology, Dr.MGR Janaki College, Chennai. They were maintained on Nutrient Agar at 4^{o}C and sub-cultured into nutrient broth. Twenty four hour old cultures were prepared freshly for every study.

Antibacterial assay

Antibacterial assay of four crude extracts were performed using Mueller Hilton medium for bacterial strains using disc diffusion method following the National Committee for Clinical Laboratory Standards methods (Trease, 1989; NCCLS, 1993).

Disc Diffusion Assay

The sterile petri dishes containing solid and sterile Mueller Hilton agar medium (HiMedia) were used. Sterile paper disc (6 mm) was saturated with 100μland 200μl of four different extracts of Murraya Koenigii at a concentration of 1mg and 2mg/disc respectively and allowed to dry for 24 h. The dried surface of the Mueller Hilton agar plate was streaked with the bacterial strain and dried discs were placed per Petri dish in triplicates. The plates were then incubated at 35°C for 24 h. Positive and negative controls were performed for every test. For each plate, negative controls were maintained where pure solvents were used instead of extract. Ampicillin and streptomycin were used as positive control. Microbial growth was determined by measuring the diameter of the zone of inhibition with a caliper. The inhibition zones would thus include disc size of 6 mm.

Determination of antifungal activity

The agar well diffusion method (King *et al.*, 2001) was modified. Potato dextrose agar (PDA) was used for fungal cultures. The culture medium was inoculated with the fungal strain *Aspergillus niger* and *Candida albicans* separately suspended in potato dextrose broth. A total of 6 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at28oC for 48 h. The diameters of zone of inhibition observed were measured. Antifungal activities were determined by measuring diameter of inhibition zone (DIZ) in mm.

Results and Discussion

Preliminary phytochemical analysis

Phytochemical analysis of the four different extracts of murraya Koenigii revealed the presence of phenolic compounds, tannins, flavonols, flavonoids, proanthocyanidins, carotenoids, alkaloids, glycosides, sugars & aminoacids.

Quantification of phytochemicals

Due to the vast differences in the nature of the phytochemical constituents found in a plant, there is no particular solvent that is known to extract all the compounds. Therefore in this study solvents like ethanol, water, acetone and hexane are used for extraction to accommodate the range of polarities of the compounds present in Murraya Koenigii leaves. The results showed that the choice of these various solvents play a crucial role in the quantitative analysis of different phytochemicals extracted from the leaves of *Murraya Koenigii.*

Quantification of compounds obtained from the extract varies greatly among the four solvents, which is an indication that solvents have different extracting capacity for phytochemicals.

Table.1 Preliminary Phytochemical analysis of the four different extracts of Murraya Koenigii

Sample	Phenolic compounds	Tannins	Flavonols	Flavonoids	Proantho cyanidins	Carotenoids	Glycosides	Sugars	Aminoacid
Ethanol extract	++	+++	+++	+++	++	++	++	+	++
Aqueous extract	++	++	++	++	++	++	++	++	++
Acetone extract	+++	++	++	++	+++	+++	++	++	++
Hexane extract	++	++	++	++	++	++	++	++	++

Table.2 Quantitative Phytochemicals evaluated of the four different extracts of *Murraya koenigii*

Sample	Total Phenol (mg/g)	Tannins (mg/g)	Flavonols (mg/g)	Flavonoids (mg/g)	Proantho-cyanidins (mg/g)	Saponins (mg/g)	Alkaloids (mg/g)
Ethanol extract	142.32	73.95	91.13	45.76	774.72	62.33	49.09
Aqueous extract	139.26	114.76	61.97	97.12	168.91	20.46	36.73
Acetone extract	459.63	45.39	40.83	19.69	541.77	35.13	23.21
Hexane extract	169.36	65.43	71.43	17.38	244.65	70.68	29.58

Table.3 Antimicrobial activity of various extracts of *Murraya koenigii*

Microorganisms	Methanol Extract		Aqueous extract		Acetone Extract		Hexane Extract		Positive Control-Ampicillin/ streptomycin/ Flucanazole
	Test (mm)	Solvent Control	Test (mm)	Solvent Control	Test (mm)	Solvent Control	Test (mm)	Solvent Control	
Methicillin Resistant Staphylococcus aureus	14	2	11	0	13	1	14	1	18
Micrococcus luteus	8	2	9	0	7	1	8	1	18
Bacillus subtilus	10	2	10	0	9	1	11	1	18
Psedomonas aeruginosa	14	2	9	0	12	1	13	1	18
Escherichia coli	16	2	14	0	12	1	14	1	18
Candida albicans	13	2	11	0	10	1	10	1	17
Aspergillus Niger	11	2	10	0	9	1	10	1	17

The antimicrobial activity of the extracts of *Murraya koenigii* leaves extracted using four different solvents was evaluated on the basis of inhibition zone in mm. In the present investigation the extract was found to be effective against all the microorganisms.

All the extracts show antimicrobial activity and hence support the medicinal usage of the extracts of *Murraya koenigii* as drugs for the therapy of infectious diseases.

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