Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 939-942
Received: 14-05-2017
Accepted: 15-06-2017

Aju BY
Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India

Rajalakshmi R
Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India

Mini S
Department of Biochemistry, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India

Correspondence
Aju BY
Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India

Evaluation of antioxidant activity of *Murraya koenigii* (L.) Spreng using different *in vitro* methods

Aju BY, Rajalakshmi R and Mini S

Abstract
The present study investigates the phytochemical composition and antioxidant potential of three different extracts of *Murraya koenigii. Murraya koenigii* (Linn.) Spreng, locally known as "curry veppila" (curry leaf tree) belonging to the Rutaceae family, is commonly used as raw material for traditional medicinal formulation in India. Evaluating the phytochemical composition and antioxidant potential becomes essential if it is to be consumed as a drug. Three different extracts (Methanol, Hydro alcohol and Aqueous) of *Murraya koenigii* leaves were screened for their phytochemical composition and antioxidant activity using standard protocols. The results of phytochemical analysis reveals the presence of tannins, saponins, flavonoids, phenolic compounds, alkaloids, cardiac glycosides, phytosterols and triterpenoids in various extracts. The total phenolics and flavonoids were rich in hydroalcoholic extracts. Hydroalcoholic extract shows strong antioxidant activity. Result of the present study shows that hydroalcoholic extract of *Murraya koenigii* was rich in phenolics and flavonoids and have strong antioxidant potential. The strong antioxidant potential of the plant may be due to its high phenolics and flavonoids content.

Keywords: *Murraya koenigii*, Extracts, Antioxidant potential, *in vitro* methods

Introduction
Murraya koenigii, commonly known as curry leaf or curry veppila in Indian dialects, belonging to family Rutaceae. It is a strong-smelling umbrageous small tree or shrub 4-6m height. *Murraya koenigii* is native to tropical Asia and is distributed from the Himalayan foothills of India. It is cultivated throughout India and elsewhere in the world. The leaf is used in South India as a natural flavoring agent in various curries. The leaves of this plant are used traditionally in the Indian Ayurvedic system to treat diabetes. It is acrid, analgesic, bitter, cooling, alexiteric, anthelmintic, carminative, purgative and stimulant and used to allay heat of the body, blood disorders, diarrhea, dysentery, eruption, inflammation, itching, kidneypain, leukoderma, piles, snakebite, thirst and vomiting. In traditional system of Medicine, it is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavouring agent in curries and chutneys [1, 2]. The plant based medicine has been the strong hold of traditional societies in dealing with health problems. Plants are a natural source of biologically active compounds known as phytoconstituents [3]. The World Health Organization has estimated that 80% of the populations rely upon traditional medicine for their primary health care needs [4, 5]. The phytoconstituents have been found to act as antioxidants by scavenging free radicals and many have therapeutic potential for free radical associated diseases. Reactive oxygen species (ROS) including hydroxyl radicals, singlet oxygen, hydrogen peroxide and superoxide radicals are frequently generated as byproducts of biological reaction [6]. *Murraya koenigii* is rich in various phytochemicals and have strong antioxidant potential. However there is no more studies concerning the antioxidant potential of murraya koenigii using various *in vitro* methods. The present study deals with the antioxidant power of various extracts of *Murraya koenigii* using different *in vitro* methods.

Materials and Methods
The leaves of *Murraya koenigii* were collected from Trivandrum. The collected plant materials were washed with distilled water and air dried in shade for three weeks at room temperature. The dried samples were milled into powder using an electric blender. The powder was stored in a sample tube and placed in a refrigerator prior to analysis.

Dried leaf powder (5g) was extracted with methanol (63 ^{0}C), hydro alcohol (80 ^{0}C) and water (100 ^{0}C) using soxhlet apparatus. Each extract obtained was filtered using Whatman No.1 filter paper, dried to a semisolid mass and the yield of each extract was recorded and stored in a refrigerator at 4 ^{0}C till further use.

i) Phytochemical analysis
A stock concentration of 1% (W/ V) of each successive extract obtained using methanol, hydro alcohol and water was prepared using the respective solvent. These extracts along with positive and negative controls were tested for the presence of active phytochemicals viz: tannins, alkaloids, phytosterols, triterpenoids, flavonoids, cardiac glycosides and saponins following standard methods [7, 8].

a) Determination of total phenolic and flavonoid content
Aluminium chloride colorimetric technique was used for flavonoids estimation [9]. Total phenolic content of different fractions were determined by the Folin-Ciocalteu method as described by Singleton *et al.*, [10].

ii) Determination of antioxidant capacity
Antioxidant activity was determined using standard protocols of total antioxidant activity assay [11], DPPH radical scavenging assay [12], reducing power assay [13], nitric oxide radical scavenging activity [14], hydrogen peroxide scavenging activity assay [15], hydroxyl radical scavenging assay [16], superoxide radical scavenging activity assay [17].

Results
The results of the phytochemical composition and antioxidant activities of *Murraya koenigii* are recorded (Table 1-8, Fig 1).

Table 1: Levels of phytochemicals in various extracts.

Phytochemicals	Methanol	Hydro alcohol	Water
Tannin	-	+	+
Alkaloids	+	+	-
Phytosterols	+	+	-
Triterpenoids	-	+	-
Flavonoids	+	+	+
Saponins	_	-	-
Cardiac glycosides	+	+	-

+ = Presence; - = Absence

Table 2: Total phenolic and flavonoid content of various extracts of *Murraya koenigii*

Extract	TPC* (as percent gallic acid equivalents (GAE).	TFC* (as percentage of quercetin.)
Water	30.97 ± 0.04%	4.53± 0.01%
Hydro alcohol	58.32 ± 3.79%	19.92 ±0.05%
Methanol	39.20 ±0.06%	6.96± .01%

*Data is presented as the mean ± standard deviation SD
TPC*-Total phenolic content
TFC*-Total flavonoid conent

Table 3: Results showing total antioxidant activity of various extracts of *Murraya koenigii.*

Extract	TAC(µg of ascorbic acid per mg of extract)
Water	17.28±0.31
Hydro alcohol	22.94±0.01
Methanol	8.61±0.01

*Data is presented as the mean ± standard deviation SD

Table 4: DPPH radical scavenging activity of various extracts of *Murraya koenigii*

Extract	IC 50 (µg /ml)
Water	540
Hydro alcohol	210
Methanol	440

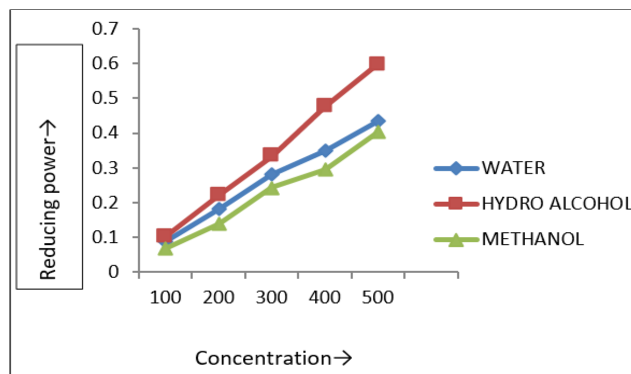

Fig 1: Reducing power of various extracts of *Murraya koenigii*

Table 5: Nitric oxide radical scavenging acivity of (IC_{50}) *Murraya koenigii* extracts.

Extract	IC 50 (µg/ml)
Water	2122
Hydro alcohol	1194
Methanol	1571

Table 6: Hydrogen peroxide scavenging activity of (IC_{50}) *Murraya koenigii* extracts

Extract	IC 50 (µg/ml)
Water	1183
Hydro alcohol	525
Methanol	834

Table 7: Hydroxyl radical scavenging activity (IC_{50}) of *Murraya koenigii* extracts

Extract	IC 50 (µg/ml)
Water	3340
Hydro alcohol	971
Methanol	2130

Table 8: Superoxide anion (O_2.-) radical-scavenging activity (IC 50) of *M.koenigii* extracts

Extract	IC_{50} (µg/ml)
Water	184.93
Hydro alcohol	155.45
Methanol	212.25

Discussion
Preliminary phytochemical screening of the various extracts of *Murraya koenigii* leaves revealed the presence of different kinds of chemical groups such as tannins, saponins, flavonoids, phenolic compounds, alkaloids, cardiac glycosides, phytosterols and triterpenoids [Table 1]. Total phenolic content is expressed as percent gallic acid equivalents. Hydro alcoholic extract of *Murraya koenigii* shows higher amount of phenolics (58.32 ± 3.79%). Total flavonoid content is expressed as percent quercetin equivalents. Total flavonoid content was maximum in hydro alcoholic extract of *Murraya koenigii* (19.92 ±0.05%) [Table 2].
Antioxidant activities of various extracts of *Murraya koenigii* was analysed by different antioxidant assays. The total antioxidant capacity (TAC) was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. It evaluates both

water-soluble and fat-soluble antioxidants (total antioxidant capacity). The results [Table 3] indicate higher total antioxidant capacity (expressed as ascorbic acid equivalent) in the hydro alcoholic extract (22.94±0.01 μg of ascorbic acid per mg of extract).

The DPPH radical scavenging assay is based on the ability of antioxidants, to decolorize DPPH. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm. Antioxidants donate an electron to DPPH and decolorize it, which can be quantitatively measured from the changes in absorbance. All of the assessed extracts of *Murraya koenigii* were able to reduce the stable, purple-colored radical DPPH to the yellow-colored DPPH-H form with IC_{50} (50% of reduction) values as follows: 540 μg/ml for water, 210 μg/ml for hydro alcohol, 440 μg/ml for Metahnol extracts [Table 4].

Various concentrations (100-1000 μl) of leaf extracts of *Murraya koenigii* were found to have significant reducing power. All extracts exhibited a dose dependent increase in reducing power (Fig: 1). Hydro alcoholic extract of *Murraya koenigii* showed more reducing power. The reducing power of the extracts increased with increasing concentration, which suggests that the electron donating ability of the extracts is concentration dependent.

Nitric oxide scavenging activity is estimated by the use of Griess IlIosvoy reaction [18]. The compound sodium nitroprusside decompose in aqueous solution at physiological pH (7.2) producing NO_. Under aerobic conditions, NO_ reacts with oxygen to produce stable products (nitrate and nitrite). The quantities of which can be determined using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. NO scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidants. The hydro alcoholic extract of *Murraya koenigii* effectively reduced the generation of nitric oxide from sodium nitroprusside. IC_{50} value of *Murraya koenigii hydroalcoholic* extract showed nitric oxide radical scavenging activity at the concentration of 1194 μg/ml [Table 5].

H_2O_2 is highly important because of its ability to penetrate into biological membranes. H_2O_2 itself is not very reactive, but it is toxic to cell because it may give rise to hydroxyl radicals in the cells [19]. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water [20]. The results show that all the extracts had potent H_2O_2 scavenging activity which may be due to the antioxidant compounds. As the antioxidant components present in the extracts are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O. Hydro alcoholic extract of *Murraya koenigii* exhibits greater scavenging activity compared to all other extracts [Table 6].

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. Hydroxyl radical scavenging assay is used to find the scavenging activity of free hydroxyl radicals in the presence of different concentrations of plant samples. The model used is ascorbic acid-iron-EDTA model of hydroxyl radical generating system. This is a totally aqueous system in which ascorbic acid, iron and EDTA conspire with each other to generate hydroxyl radicals. In the present study the hydroalcoholic extract of *Murraya koenigii* showed potent hydroxyl radical scavenging activity. The IC_{50} value of hydroalcoholic extract of *Murraya koenigii* was found at the concentration of 971μg/ml [Table 7].

Superoxide dismutase (SOD) is a metalloenzyme that catalyze the dismutation of superoxide radical into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) and consequently provide an important defense mechanism against superoxide radical toxicity [21]. The principle involved in this assay is the conversion of Nitroblue Tetrazolium (NBT) into NBT diformazan via superoxide radical. SOD utilizes the highly water-soluble tetrazolium salt and that produces a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with O2 is linearly related to the xanthine oxidase (XO) activity and is inhibited by SOD. The result of superoxide radical (SO) scavenging activity obtained for the extract of *M.koenigii* showed dose dependent free radical scavenging activity and the percentage of inhibition [Table 8]. In the present study, *M.koenigii* hydro alcoholic extract was found to be a superior scavenger of superoxide radicals.

Conclusion

The antioxidant capacity, total phenolic and flavonoid content of different extracts of *Murraya koenigii* were evaluated. Hydro alcoholic extract of *Murraya koenigii* is rich in flavonoids and phenolics and have greater antioxidant capacity compared to other two extracts. A significant correlation was obtained between antioxidant activity and phenolic content indicating that phenolic compounds contribute significantly to antioxidant activity of the investigated plant.

Acknowledgements

The financial assistance from DST/ INSPIRE, New Delhi, India and the facilities provided by University of Kerala are greatly acknowledged.

References

1. Anonymous. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. Publication & Information Directorate, New Delhi: CSIR, 1998; 446-448.
2. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A Handbook of Medicinal Plants. Jodhpur: Agrobios, 2003; 352-353.
3. Farnsworth NR: Ethnopharmacology and Drug Development.In: Ethnobotany and the search for new drugs, Wiley, Chichester (Ciba Foundation Symposium 185); 1994.
4. Sindhu RK, Arora S. Phytochemical and Pharmacognostical studies on *Murraya koenigii* L. spreng roots. Drug Invention Today. 2012; 4:325-336.
5. Kurain JC: In: Plants That Heal, Oriental Watchman Publishing House, Pune, India. 1995; 296.
6. Kikuzaki H, Nakatani N. Antioxidant effects of some ginger constituents. Journal of Food Science. 1993; 58(6):1407-1410.
7. Harborne JB, Phytochemical method, A Guide to Modern technique of Plant Analysis. 3rd Edition, Chapman and Hall. New York. 1998; 198.
8. Kokate CK. A text book of Practical pharmacognosy. 5th Edition, Vallabh Prakashan New Delhi, 2005; 107-111.
9. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 2002; 10:178-182.
10. Singleton V, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates

and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol 299 (Oxidants and Antioxidants Part A). 1999; 152-178.

11. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999; 269:337-341
12. Blois MS. Antioxidant determination by the use of a stable free radical, Nature, Song. 1958; 181:1199-1200.
13. Oyaizu M. Studies on products of browning reaction prepared from glycosamine. Japanese journal of nutrition. 1986; 44:307-314.
14. Ilavarasan R, Mallika M, Venkataraman S. Anti inflammatory and antioxidant activites of Linn bark extract. Afr. J. Trad. 2005; 2(1):70-85.
15. Ruch RJ, Cheng SJ, Klaunig JE. *Carcinogenesis*, 1989; 10:1003.
16. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple "test tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem. 1987; 165:215-219.
17. Fontana M, Mosca L, Rosei MA. Interaction of enkephalines with oxyradicals. Biochem. Pharmacol. 2001; 61:1253-1257.
18. Garrat DC. The Quantitative analysis of Drugs. Chapman and Hall Ltd., Japan. 1964; 3:456-458.
19. Gulcin I, Huyut Z, Elmastas M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. Arab J Chem. 1964; 30:43-53.
20. Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxidant activities of methanol extract of Sambucus ebulus L. flower. Pak J Biol Sci. 2009; 12:447-450.
21. Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 1972; 46(2):849-54.