Antioxidant Activity of *Murraya Koenigii* Linn Leaves

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Summary

The different extracts of *Murraya koenigii* Linn (Rutaceae) were evaluated for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Ascorbic acid was used as reference standard. The extracts exhibited strong antioxidant radical scavenging activity with IC_{50} value of 4.72 µg/ml, 4.10 µg/ml, 4.46 µg/ml and 2.69 µg/ml for acetone, alcohol, aqueous extract and ascorbic acid respectively. The phytochemical screening suggests that phenolic and flavonoids present in these extracts of the leaves might provide considerable antioxidant activity.

Key words: *Murraya koenigii* Linn; antioxidant activity; DPPH; different extracts; ascorbic acid; IC_{50} value.

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Introduction

Antioxidants are the substances used by the body to protect itself from the damage caused by oxidation by producing free radicals, which starts chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins that damage cells[1]. Hence the research has been focused on use of antioxidants, with particular emphasis on naturally derived antioxidants, which may inhibit reactive oxygen species and may display protective effects. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants[2]. In this context, one such plant is *Murraya koenigii*.

Murraya koenigii Linn (Rutaceae) commonly known as Meethi 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[3].

In Traditional System of Medicine, it is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavoring agent in curries and chetneys. The oil is used externally for bruises, eruption, in soap and perfume industry[4]. The phytoconstituents isolated so far from the leaves are alkaloids viz., mahanine[5], koenine, koenigine, koenidine[6], girinimbiol, girinimibine[7], koenimbine, O-methyl murrayamine A, O-methyl mahanine, isomahanine, bismahanine, bispyrayafoline[8] and other phytoconstituents such as coumarin glycoside viz., scopotin, murrayanine, calcium, phosphorus, iron, thiamine, riboflavin, niacin, vitamin C, carotene and oxalic acid[9]. The essential oil from leaves yielded di-α-phellandrene, D-sabinene, D-α-pinene, dipentene, D-α-terpinol and caryophyllene[10]. It is reported to possess antibacterial, antifungal, larvicidal, anticarcinogenic, hypoglycemic, anti-lipid peroxidative, hypolipidemic and anti-hypertensive activity[11]. Our aim in the present work was to evaluate its *in vitro* antioxidant DPPH free radical scavenging activity.

Materials and methods

Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid was purchased from Loba Chemie Pvt Ltd., Mumbai. All the chemicals and reagents used were of analytical grade.

Plant material:

Murraya koenigii Linn leaves were collected and authenticated by Central Council for Research in Ayurveda and Siddha, Bangalore. A voucher specimen (RRI/BNG/SMP/Drug Authentication/2008-09/267) has been preserved in our Department of Pharmacognosy for the future reference.

Extraction procedure

Shade dried leaves (470 g) were coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (soxhlet). The extraction was done with different solvents in their increasing order of polarity such as petroleum ether (60-80^{0}C), chloroform, acetone, alcohol and water. Each time the marc was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The yield was found to be 3.01, 1.77, 3.37, 3.26 and 15.5 % w/w with reference to the air dried plant.

Preliminary phytochemical screening

The coarse powder of leaves of *Murraya koenigii* (25 g) was subjected to successive extraction with different solvents in their increasing order of polarity from petroleum ether (60^0-80^{0}C), chloroform, acetone, alcohol and water. The extracts were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents[12].

Evaluation of Antioxidant Activity:

The free radical scavenging activity of the different extracts of Murraya *koenigii* were measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging method[13]. Briefly, 0.1 mM solution of DPPH in methanol was prepared. 1 ml of the solution was added to 3

ml each of acetone, alcohol and aqueous solution in methanol at different concentration (500 – 1.95 µg/ml).

The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a spectrophotometer (UV- VIS Shimadzu). Reference standard compound used was ascorbic acid (100 – 1.95 µg/ml). The IC_{50} value is the concentration of sample required to inhibit 50 % of the DPPH free radical. The IC_{50} value for the sample was calculated using log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%) = $100 \times A_1 / A_0$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in presence of the standard sample or extracts.

Results and Discussion

Preliminary phytochemical screening

Preliminary phytochemical screening of different extracts showed the presence of alkaloids, tannins, phenol, saponin, flavonoids, steroids, coumarins and sugars. Acetone, alcohol and aqueous extracts were chosen as it contains flavonoids and phenols which are generally potent inhibitors of free radicals[14].

DPPH radical scavenging activity

As shown in Table 1 & 2, acetone, alcohol and aqueous extracts have shown potent DPPH radical scavenging activity with an IC_{50} value of 4.72 µg/ml, 4.10 µg/ml and 4.46 µg/ml. IC_{50} value of ascorbic acid was found to be 2.69 µg/ml.

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents then losing color stoichiometrically with the number of electrons consumed which is measured spectrometrically at 517 nm[15, 16, 17]. Ascorbic acid is a potent free radical scavenging. So when compared to such pure compound, IC_{50} value of the different crude extract is quite good proving that they are potent DPPH free radical scavenger. This can be attributed to flavonoids and phenols present in the extract. Further research is therefore needed for the isolation and identification of the antioxidant compounds.

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Table No. 1: DPPH free radical scavenging activity of different concentrations of acetone, alcohol and aqueous extract of *Murraya koenigii*

Sl No.	Extract used	Concentration [µg/ml]	% Inhibition
1.	Acetone extract	500	98.34
		250	97.24
		125	97.43
		62.5	96.17
		31.25	93.89
		15.6	93.54
		7.8	81.32
		3.9	43.65
		1.95	28.45
2.	Alcohol extract	500	97.23
		250	96.54
		125	94.96
		62.5	92.96
		31.25	92.76
		15.6	93.45
		7.8	79.54
		3.9	47.45
		1.95	23.34
3.	Aqueous extract	500	77.34
		250	75.88
		125	74.83
		62.5	74.23
		31.25	70.59
		15.6	65.32
		7.8	60.87
		3.9	41.25
		1.95	25.08

Table No. 2: IC_{50} values of DPPH free radical scavenging activity of ascorbic acid, acetone, alcohol and aqueous extract of *Murraya koenigii*

Sl. No.	Name of compound/ extract	% Inhibition [mean ± SEM]	IC_{50} value (µg/ml)
1.	Standard (Ascorbic acid)	97.13 ± 12.64	2.69
2.	Acetone extract	81.81 ± 19.92	4.72
3.	Alcohol extract	79.80 ± 18.68	4.10
4.	Aqueous extract	62.82 ± 13.62	4.46

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