

ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL FROM THE LEAVES AND STEMS OF *MURRAYA KOENIGII*

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Article Received on
03 May 2017,

Revised on 24 May 2017,
Accepted on 14 June 2017

DOI: 10.20959/wjpr20177-8566

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ABSTRACT

Leaves and stems of *murraya koengii* were collected from the PCSIR locality and their essentials oils were extracted through hydro-distillation using Dean Stark apparatus. Extracted both oils were dried over anhydrous sodium sulfate and 0.17% and 0.06% yields were obtained for leaves and stems respectively. Their GC-MS analysis was performed for chemical constituents. Major constituents in the essential oil of leaves were α -pinene 2.7%, spathulenol 19.10%, caryophyllene 18.4%, caryophyllene oxide 17.6%, α -caryophyllene 10.2%, β -elemine 9.1%, and germacerene 4.3% and while in the stem essential oil major constituents were α -pinene 4.0%, β -terpineol

2.9%, α -caryophyllene 7.2%, epiglobolol 5.4%, spathulenol 6.6%, clarene oxide 1.2%, heptatrioctanol 2.3% and phytol 1.9% respectively. Their antioxidant activity of both essential oils was measured by using DPPH and it was concentration dependant. Maximum activity was found at 100 μ l was 78% and 59% for essential oil of leaves and stem respectively as compared to ascorbic acid.

KEYWORDS: *Murraya Koenigii*, Essential oil, Hydro-distillation, GC-MS analysis, Caryophyllene, Spathulenol.

1. INTRODUCTION

Murraya koenigii belongs to family *Rutaceae*, which have 150 genera and over 1600 species. It is an aromatic, pubescent, deciduous shrub or small tree and widely distributed in south -

east Asia, Australia and the Pacific islands.^[1] It can grow up to 6 m in height and diameter is about 15-40 cm with short trunk. The leaves are pinnate and 15-30 cm long, with 11-25 leaflets, each leaflet length is about 2–4 cm and breadth is of 1–2 cm. Margins irregularly serrate, petioles 2-3 mm long. Flowers of this tree are bisexual, complete, sweetly scented, white, stalked, regular with average diameter of fully opened flower being in average 1.12 cm inflorescence, terminal cymes each bearing 60-90 flowers.^[2] Curry leaves possess strong spicy and seasoning type flavor. Scented and flavorful curry leaves is a popular spice and is widely cultivated for well recognized medicinal and culinary purpose. Culinary value of curry leaf is related to the organoleptic properties e.g. color, odor and flavor to its carbohydrates richness including sugars and minerals. The useful parts of this plant are the leaves, root and the bark. *Murraya koenigii* is being used as stimulant, anti-dysenteric and for the management of cholesterol and diabetes mellitus. Owing to its beneficial aspects it is utilized as traditional functional food and nutraceuticals as well as for anatomical applications such as antioxidant, anticancer, hyperglycemic, hypercholesterolemic, anticancer, antibacterial, antiulcer and anthelmintic agent.^[3] The major constituent responsible for the aroma and flavor has been reported as pinene, sabinene, caryophyllene, cadinol and cadinene. The leaves have a slightly pungent, bitter and feebly acidic taste, its chemical constituents have been studied by various authors.^[4,5] In addition, leaves are also rich in fibers, minerals and vitamins such as calcium, carotene, nicotinic acid and vitamin A in curry leaves and phosphorous, calcium, iron, vitamin B2, niacin and vitamin C in curry leaves.^[6] *Murraya koenigii* leaves contain different phyto-compounds including alkaloids, flavonoids, furocoumarins, terpenoids and tannins. A few bioactive compounds such as mahanimbilyl acetate, girinimbilyl acetate and bicyclomahanimbiline have been isolated and reported to possess antimicrobial and antioxidant activity.^[6] Leaves are rich in many bioactive compounds like polyphenols, alkaloids and flavonoids which showed multiple bioactive functions like antioxidant, anticancer, antimicrobial, antidiabetic and hepatoprotective. The two carbazole alkaloids namely mahanimbine and koenigine found in these leaves showed higher antioxidant activities.^[7,8]

Antioxidants reduce the risk of heart disease and enhance immunity; therefore it is imperative that it should be supplied to body through external sources.^[9] A research was conducted to determine total antioxidant activity of curry leaf extract that showed 16.20g/100 g of extraction yield from water and 10.95g/100 g from ethanol while 21.25 microg GAE from water and 90.00 µgGAE from ethanol. Water and ethanolic extract of curry leaf

showed 91.6 and 98.6% lipid peroxidation inhibition while alpha tocopherol, BHA and BHT exhibited 36.9, 94 and 976% inhibition of lipid eroxidation respectively. Free radicals reactive oxygen species and reactive nitrogen species generated in our bodies can create oxidative stress. A balance between free radicals and antioxidants is necessary for proper physiological function. Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases. Hence application of external source of antioxidants can assist in coping this oxidative stress. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have recently been reported to be dangerous for human health. Curry leaves essential oil have been studied in detail by various authors but from the best of our knowledge this is the first report to study the essential oil of stem and its antioxidant activity. Thus, the current research was designed to investigate the essential oil of stem and leaves of *murraya koengii* for their chemical constituents and antioxidant activity.

2. MATERIALS AND METHODS

2.1 Extraction of essential oil

Fresh green leaves and stems of *murraya koengii* 1 Kg each were collected from the local market and after that chopped into small pieces and subjected to hydro-distillation by using Linkersson apparatus.^[10] for 10 hrs. The steam distillate was extracted twice with petroleum ether (2×150 ml). The organic layer was dried over anhydrous sodium sulfate, which on removal of solvent afforded pale colored oil. Dried oils were stored in an air tight amber colored bottle at 4 °C in refrigerator for further studies.

2.2 GC-MS studies of the essential oil of the fresh green leaves and stems of *murraya koenigii*

Fresh green leaves and stems of *murraya koenigii* were chopped and essential oil was extracted through hydro-distillation by using linkersson apparatus. Their chemical constituents were analyzed by GC-MS. Agilent 5973-6890 gas chromatograph–mass spectrometry system, operating in EI mode at 70 eV equipped with a split-splitless injector was used. Helium was used as a carrier gas at the flow rate of 1 ml/min, while HP-5MS (30 m, 0.25 mm, 0.25 μ m) capillary column was used. The initial temperature was programmed at 50-100°C at the rate of 5°C/min and then 100-250°C at the rate of 3°C/min followed by a constant temperature at 260°C for a period of 20 minutes. Sample (2 μ l) was injected to the column programmed at 200°C and resolution of components was attained. The identification

of possible components was performed by matching their retention indices and mass spectra with those obtained the library of National Institute of Standard and Technology (NIST).

2.3 Antioxidant activity of essential oil of *allium cepa* mature bulb

Antioxidant activity of the essential oil of the fresh green leaves and stems of *murraya koenigii* was evaluated by using 2,2-diphenyl-1-picrylhydrazyle (DPPH) radical. The DPPH assay was performed by following the method of.^[11] Briefly, the samples of different concentration of 20 ul, 40 ul, 60 ul, 80 ul and 100 ul were mixed with 3 ml of methanol of DPPH solution. The absorbance of the resulting solution and the blank (with only DPPH) were recorded at λ 517 nm by UV-Vis spectrophotometer, after an incubation time of 30 minutes at ambient temperature against ascorbic acid as a positive control. For each samples three replicates were recorded. The percentage of radical scavenging activity was calculated using the following equation.

$$\text{DPPH scavenging effect (\%)} = (A_0 - A_1)/A_0 \times 100$$

Where A_0 is the absorption of the control at 30 minutes and A_1 is the absorbance of the sample at 30 minutes.

3. RESULTS AND DISCUSSION (Times New Roman, 12, Bold)

The essential oil extracted from the green fresh leaves and stems of *murraya koenigii* were extracted through hydro-distillation and 0.17% and 0.06% yields of the leaves and stem oils was obtained. This yield of essential oil of fresh leaves was higher as compared to stems, similar findings has also been reported.^[12] Yang et al, 2000 and Yan 2009,^[13,14] has also compared the essential oil in different parts of the same plant and found that oil was low in the stem as compared to leaves. Their GC-MS analysis was performed for their chemical constituents. Major constituents in the *murraya koenigii* leaves essential oil were α -pinene 2.7%, spathulenol 19.10%, caryophyllene 18.4%, caryophyllene oxide 17.6%, α -caryophyllene 10.2%, β -elemenene 9.1%, and germacerene 4.3% and while in the stem essential oil major constituents were α -pinene 4.0%, β -terpineol 2.9%, α -caryophyllene 7.2%, epiglobolol 5.4%, spathulenol 6.6%, clarene oxide 1.2%, heptatrioctanol 2.3% and phytol 1.9% respectively. The results of essential oil of leaves of *murraya koenigii* are more or less similar to previous reports.^[15,16] The contents of essential oil of stems of *murraya koenigii* are different from the contents of leaf which may be due to difference in morphology and physiology of the parts. The contents in the stem are also lower as compared to leaves due to different metabolic function of the parts of the plant.

Their antioxidant activity of the essential oils from the leaves and stem of the murraya koenigii was also performed by using DPPH as scavenging radical. Five concentrations 20, 40, 60, 80 and 100 μ l of each oil were used for antioxidant activity and 33%, 46%, 62%, 69%, and 78% for leaves and 27%, 35%, 44%, 51% and 59% for stem oil were measured respectively. The activity reported earlier was better than our results, it may be due to difference in the contents.^[17,18] The antioxidant activity is mainly dependent upon the phenolic contents of the oil, so lower efficiency of the stem essential oil might be due to that reason.

4. CONCLUSIONS (Times New Roman, 12, Bold)

The essential oil from the fresh green leaves and stem of murraya koenigii was extracted through hydro-distillation and its chemical constituents were determined by GC-MS while its antioxidant study was carried out with DPPH. Their GC-MS analysis was performed for their chemical constituents. Major constituents in the murraya koenigii leaves essential oil were α -pinene 2.7%, β -pinene 1.6%, spathulenol 19.10%, caryophyllene 18.4%, caryophyllene oxide 17.6%, α -caryophyllene 10.2%, β -elemenene 9.1%, and germacerene 4.3% and while in the stem essential oil major constituents were α -pinene 4.0%, β -terpineol 2.9%, α -caryophyllene 7.2%, epiglobolol 5.4%, spathulenol 6.6%, clarene oxide 1.2%, heptatrioctanol 2.3% and phytol 1.9% respectively. Antioxidant activity of the essential oil was determined by using DPPH and ascorbic acid as standard. Their activity at 100 μ l concentration were 78% and 59% for essential oil of the leaves and stems respectively. Moderate antioxidant activity was observed in the essential oils from both the parts of murraya koenigii. It is concluded that stems and leaves of the murraya koenigii both possess essential oil with specific aroma and these can be utilized as flavouring agent for food purpose. But for confirmation of this fact further studies are required.

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