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Research Article

**Determination of
Secondary Metabolites
and Antioxidant Activity
of
*Murraya koenigii***

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Abstract

This study is to quantify the secondary metabolites and the antioxidant potential of ethanolic extract of *Murraya koenigii*. The ethanolic leaves extract of *Murraya koenigii* was analysed by HPLC and GC to determine various Phytochemicals. The free radicals scavenging activity of extract was determined by using DPPH, NO and Super oxide radicals generated *in vitro*. The ethanolic leaves extract of *M. koenigii* was found to contain alkaloids (7.20mg/g), tannins (2.02mg/g), phenols (5.36mg/g) and flavonoids (3.42mg/g). The major flavonoids detected were quercetin and rutin. The ethanolic extract of *M. koenigii* was found to possess significant free radical scavenging activity against DPPH, NO and SOD anions. The medicinal property of *Murraya*

koenigii may be attributed to the presence of flavonoids and phenolic compounds with rich antioxidant potential. The therapeutic effect of this plant may be accounted for its counteracting action on free radicals *in vivo*.

Keywords: *Murraya koenigii*, Phytochemicals, free radical, scavenging activity

Introduction

Plants have been major source of medicine in all cultures from ancient times. In the traditional system, various indigenous plants are being used in the diagnosis, prevention and elimination of physical, mental or social imbalance. Phenolic compounds, ubiquitous in plants, are of considerable interest and have received more and more attention in recent years due to their bioactive functions. Polyphenols are amongst the most desirable phytochemicals because of their antioxidant activity. Natural therapy for various human ailments purified with plant products has gained much attention now a days, due to various side effects associated with allopathic medicine these can be derived from any part of the plant like bark, leaves, stem, flowers, roots, seeds, etc., [1]. Medicinal plants are believed to be an important source of chemical substances with potential therapeutic effects [2].

Free radicals play an important role in various pathological conditions such as tissue injury, inflammation, neurodegenerative diseases, cancer and aging. The Compound that can scavenge free radicals has great potential in ameliorating these diseases [3]. Inflammation is a disorder characterized by invasion of leucocytes and production of proinflammatory cytokines [4].

Murraya koenigii, commonly known as curry leaf or kari patta in Indian dialects, belonging to Family

Rutaceae which represent more than 150 genera and 1600 species^[5]. *Murraya koenigii* is a highly valued plant for its characteristic aroma and medicinal value. It is an important export commodity from India as it fetches good foreign revenue. A number of chemical constituents from every part of the plant have been extracted. The most important chemical constituents responsible for its intense characteristic aroma are P-gurjunene, P-caryophyllene, P-elemene and O-phellandrene. The plant is rich source of carbazole alkaloids^[6]. Bioactive coumarins, acridine alkaloids and carbazole alkaloids from family Rutaceae were reviewed^[7]. *M. koenigii* is widely used in Indian cookery for centuries and have a versatile role to play in traditional medicine. The plant is credited with tonic and stomachic properties. Green leaves are eaten raw for cure of dysentery, diarrhoea and for checking vomiting^[8]. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders. *M. koenigii* contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response. A number of active secondary metabolites responsible for the medicinal properties have been isolated and characterized. Therefore the present study quantifies the secondary metabolites and the antioxidant potential of ethanolic extract of *Murraya koenigii*.

Materials And Methods

Collection and Identification

Murraya koenigii was collected from Thanjavur District. The plant was authenticated by Director, Plant Anatomy & Research Center, Chennai and the voucher specimen was deposited in our laboratory.

Preparation of ethanolic extract of *Murraya koenigii*

The whole plant was shade dried and pulverized. 100g of the powder was soaked in 150ml of ethanol (w/v) for 3-5 days with intermediate shaking. This was filtered through a fine cheese cloth and the filtrate was pooled after 3 days of repeated extractions. The filtrate obtained was evaporated to dryness using rotary evaporator. The concentrate was lyophilized and used for the study.

HPLC – UV analysis (Total Phenols)

Ethanolic extract of *Murraya koenigii* was subjected to solid phase extraction using column 5mm (4.6mm), & peptides, small molecules were removed; fractionation of neutral and acidic phenolic acids was also carried out simultaneously. The resulting fraction was then subjected to reverse phase high performance liquid chromatography (RP-HPLC). The total phenolics in ethanolic extract of *M. koenigii* was detected using, Stationary phase octadecylsil silica and mobile phase (A phosphoric acid: water (0.5: 99.5v/v) B acetonitrile). The UV detector was set at 220 nm with the flow rate adjusted to 1.0ml / min. The major peaks were identified and the retention times were compared with these of standards.

Fractionation of total Alkaloids

Ethanolic extract of *Murraya koenigii* was detected using monobasic Phosphate as mobile phase (270ml. of Acetonitril). The liquid Chromatography is equipped with 235 nm detector & 4.6mm x 150 mm column. The flow rate was adjusted to 1.8ml / minute. The major peaks were identified and the total alkaloids concentration was determined.

Fractionation of total Flavonoids

HPLC Chromatography (System Name: LACKROM L-7000 MERCK, Proc Method – HITECHI) of total flavonoids. The total flavonoids in the extract was determined by using octadecylsil silica gel as stationary phase and acetonitril, sodium dihydrogen phosphate with dilute orthophosphoric acid as mobile phase. UV detector was set at 350nm with flow rate of 0.5ml/min. The major peaks in ethanolic extract of *M. koenigii* were determined in comparison to the retention time of standards run at identical conditions.

Free radical scavenging activity

1. Diphenyl – 2- Picrylhydrazyl (DPPH) radical scavenging activity.

DPPH radical scavenging assay is a commonly recommended method for assessment of antioxidant potential of plant extracts. The assay is based on the ability of DPPH, a free radical which get decolorized in the presence of antioxidants. To 200ml of ethanolic solution of DPPH (1 µg/ml) various concentration of (20mg – 100 µg/ml) in water

were added and incubated at 37°C for 30 min in dark and the absorbance was measured at 517nm. Ascorbic acid was used as the reference standard. The percentage scavenging of DPPH free radical was calculated and compared with that of the standard ascorbic acid. The IC₅₀ value also determined.

2. Superoxide anion scavenging activity

The method of Nishkimi et al.^[9] was applied for the measurement of MIT superoxide anion scavenging activity. Briefly 312µm Nitroblue tetrazolium in 120 µm phosphate buffer pH 7.4 were added to an aliquots of extract (20-100µg/ml) the reaction was started by adding 100µl of phenazine metho sulphate (120mm prepared in phosphate buffer pH 7.4) and the colour change was monitored at 560nm against water blank quercetin was used as the positive control.

3. Nitric oxide scavenging activity

The nitric oxide scavenging activity of the aqueous extract was measured by taking various concentration of extract and standard. Ascorbic acid (20-100µg/ml) dissolved in phosphate buffer (0.025m, pH 7.4) and incubated with sodium nitroprusside (5mm) in standard phosphatebuffer at 25°C for 5 hrs. After the incubation, 0.5ml of the reaction mixture was added with 0.5ml of Griess reagent (equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride in water). The absorbance of the chromophore formed was read at 540nm. The activity was compared with that of similar concentration of Ascorbic acid^[10].

Result And Discussion

Phytochemicals are naturally occurring biochemical compounds that plants developed, in order to protect themselves from oxidation, insect disease and other hazards in their environment. These phytochemicals give their characteristic colour, flavour, smell and texture. Epidemiological studies indicate that populations consuming high levels of plant derived foods have low incidence rates of various cancers (Table 1).

In this investigation, the phytochemicals in ethanolic extract of *M. koenigii* were phenols (5.36mg/g), tannins (2.02mg/g), flavonoids (3.42mg/g) and alkaloids (7.20mg/g). Flavonoids and phenols in general are highly effective in scavenging free radical and providing antioxidant defense in living cells. Quantitative analysis of ethanolic extract of *M. koenigii* was given in Figure 1.

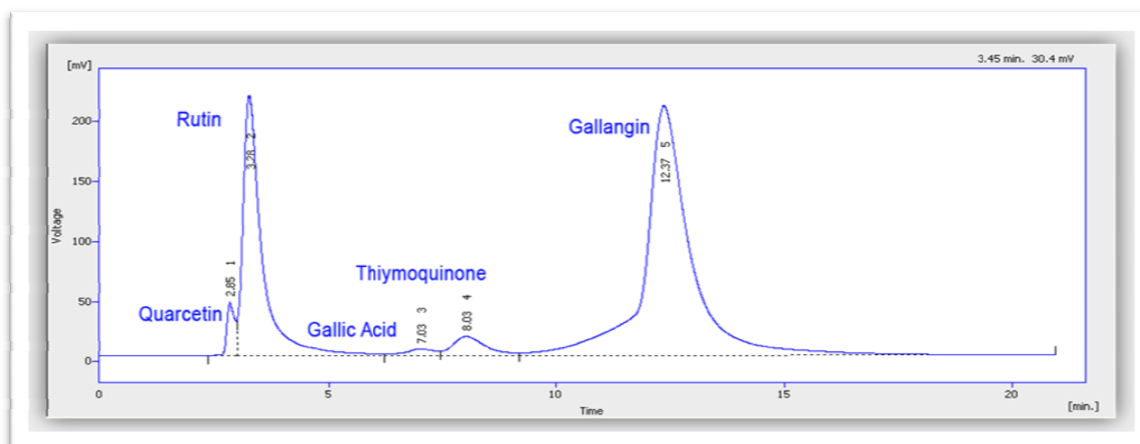
TABLE. 1. Phytochemical Analysis

S. No.	Phytochemical	Values in (mg/g)
1	Phenols (mg/g)	5.36
2	Tannins (mg/g)	2.02
3	Flavonoids (mg/g)	3.42
4	Alkaloids (mg/g)	7.20

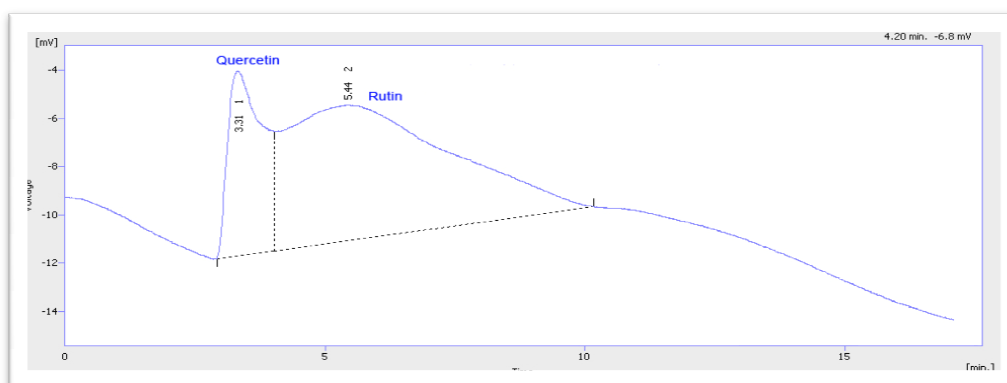
HPLC Analysis of ethanolic leaves extract of *M. koenigii*

HPLC analysis of phenols and flavonoids such as Rutin and quercetin (Fig. 1 (A) to (D)) were presented. Many reports demonstrate that antioxidant principle present in medicinal plants are responsible for their therapeutic potential^[12] flavonoic compound such as quercetin and Rutin are responsible for anti-inflammatory and anticancer properties by their terminating action of free radicals^[13]. Alkaloids have many pharmacological activities including anti cancer and anti-arhythmic effect^[14]. Alkaloids are known to reduce the inflammation level significantly. These results shows that ethanolic leaves extract of *M. koenigii* containing which could be accounted for the antioxidant and anti-inflammatory effects. In the present investigation, HPLC chromatographics pattern of the ethanolic extract of *M. koenigii* showed 2 peaks of flavonoids and 3 peaks of phenolic compounds. HPLC Analysis of ethanolic extract of *M. koenigii* was found to be rich in flavonoids such as quercetin (0.763 mg/g) and Rutin (0.244 mg/g) and phenols such as gallic acid (5.34 mg/g), cinnamic acid (0.51 mg/g), coumaric acid (0.42 mg/g).

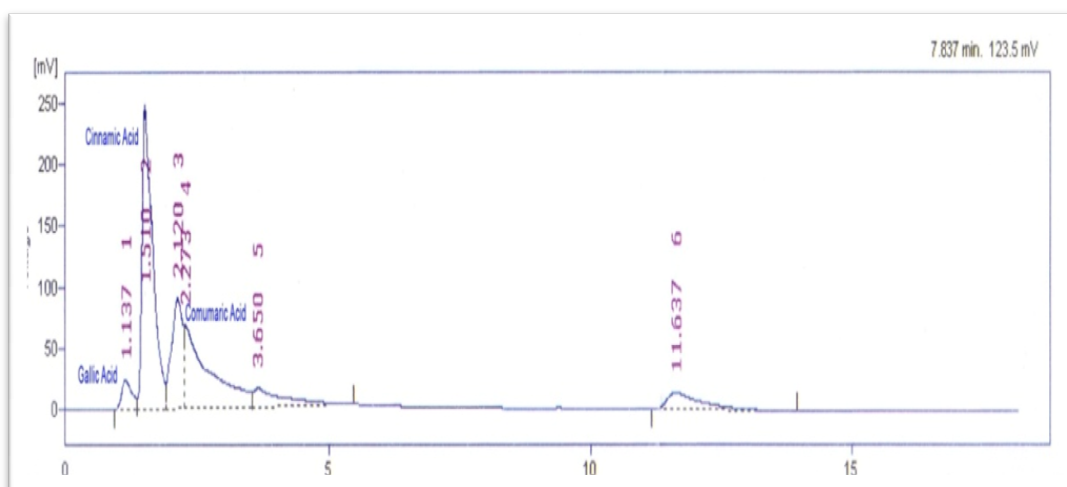
Figure 1.



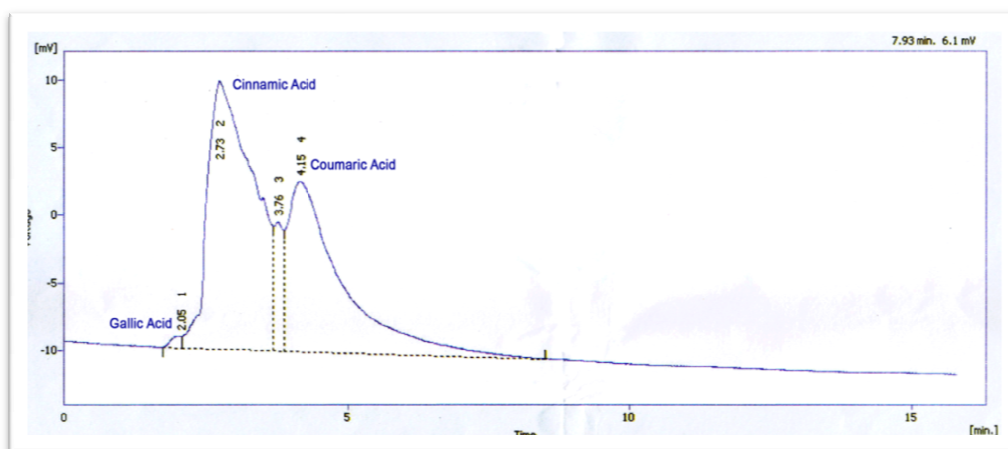
(A) HPLC Finger prints of standard flavonoids.



(B) HPLC Finger print of Flavonoids present in *M. koenigii*.



(C) HPLC Finger print of standard Phenols

(D) HPLC Finger prints of Phenols Present in *M. koenigii*.Table 2. Free Radicals scavenging activity in *M. koenigii*

Free radicals		Concentration of Standard and extract in ($\mu\text{g/ml}$)				
		20	40	60	80	100
DPPH	Ascorbic Acid	45.5	51.2	72.7	82.3	89.1
	<i>M. koenigii</i>	35.8	41.3	54.1	67.5	74.9
Nitric Acid	Ascorbic Acid	39.9	45.7	51.4	64.6	75.3
	<i>M. koenigii</i>	37.1	41.2	47.3	58.8	68.1
Superoxide	Gallic acid	38.7	40.2	58.1	62.3	89.5
	<i>M. koenigii</i>	25.2	38.4	46.1	52.9	69.3

The Natural phytonutrients presents in fruits and vegetables scavenge the free radicals and protect the cells from oxidative damages. The phytonutrients present in ethanolic leaves extract of *M. koenigii* are responsible for the traditional claim.

Invitro antioxidant assays

Antioxidants are known to exhibit their biochemical effects through numerous mechanisms, including the prevention of chain initiation, reductive capacity and radical scavenging mechanisms. Several methods have been used to measure the antioxidant activity of biological materials. It is essential to use more than one method to evaluate antioxidant capacity of plant materials simply because of the complex nature of phytochemicals present in them. Therefore, in the present study, DPPH free radical scavenging activity and nitric oxide scavenging activity assessment were done.

DPPH Scavenging Activity

The percent DPPH Scavenging activity ethanolic extract of *M. koenigii* were depicted (Table 2). As-

corbic acid was used as a positive control for comparison of plant materials. The IC_{50} value of Ascorbic acid was found to be $61.95 \mu\text{g/ml}$. The IC_{50} value of DPPH scavenging activity was found in ethanolic extract of *M. koenigii* was $47.7 \mu\text{g/ml}$. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The degree of discoloration indicated the scavenging potential of the antioxidants in the sample. The extract significantly inhibited the activities of DPPH radicals in a dose-dependent manner. The comparison of scavenging effects of ethanolic extract on DPPH radical showed consistently higher radical scavenging activity observed in seabuckthorn *Hippophae rhamnoides* seeds extracts of phenolic compounds⁽¹⁵⁻¹⁷⁾, Flavonoids⁽¹⁸⁾ and terpenes⁽¹⁹⁾ might be taken in to account.

Activity evaluation of *M. koenigii* extract showed the capacity of the plant towards nitric oxide scavenging in a dose dependent manner. Ascorbic acid, a natural antioxidant, was used as a positive control for comparison. Nitric oxide or reactive nitrogen species, formed during their reaction with

oxygen or with super oxides. These compounds are responsible for altering the structural and functional behavior of many cellular components. Incubation of solutions of sodium nitroprusside in PBS at 25°C for 2 hours resulted in linear time dependent nitrite production, which is reduced by the ECH. The IC₅₀ of ascorbic acid was found to be 48.55 µg/ml. The order of the Scavenging activity was found in ethanolic extract of *M. koenigii* was IC₅₀ 44.25 µg/ml. Nitric oxide scavenging activity of ethanolic leaves extract of *M. koenigii* is an important chemical mediator generated by endothelial cells, macrophages, neuron and it is involved in the regulation of various physiological process like control of arthritis, cytotoxic effects alzheimer's disease^[20]. Higher nitric oxide scavenging activity was reported by Athiperumalsami ⁽²¹⁾ in the methanol and water extracts of *H. ovalis* but the IC₅₀ values were much lower than that of standard tocopherol.

Superoxide anion is a free radical created from the normal process of energy generation in the human body. Superoxide anion is toxic to cells and tissues and plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or singlet oxygen. The concentration of standard gallic acid to inhibit 50% of superoxide formation was found to be 49.15 µg/ml. The scavenging activity of *M. koenigii* extract was found to be IC₅₀ 44.25 µg/ml. The superoxide anions are toxic intermediates formed during inflammatory process and found to enhance the risk of inflammation related disorders such as arthritis and atherosclerosis. Super oxide anion is a free radical that plays an important role in the formation of reactive oxygen species such as hydrogen peroxide, hydroxyl / radicals, or singlet oxygen in living organism. The therapeutic activity of medicinal plants can be determined by superoxide activity ^[22].

Conclusion

The result of preliminary phytochemical screening shows the presence of flavonoids such as quercetin and rutin. The phenolic compounds and Alkaloids were also analysed in the *M. koenigii*. A large number of these compounds are known to possess strong antioxidant properties. Although crude extract from various parts of *M. koenigii* have numerous medical applications,

modern drugs can be developed after extensive investigation on its bioactivity, mechanism of action, pharmacotherapeutics, toxicity and after proper standardization and clinical trials. The available literature and wide spread availability of *M. koenigii* in India thus makes it an attractive candidate for further pre-clinical and clinical research. The free radical scavenging activity of *M. koenigii* revealed that they can be used for the Prevention or treatment of human diseases such as cancer, arthritis, diabetes mellitus which are associated with oxidative stress.

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