Comparative Account of the Antioxidant Property between Mature and Young Leaves of *Murraya Koenigii*

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Abstract- In this study, a comparative analysis of antioxidant content and radical scavenging activity between young and mature leaves of *Murraya koenigii* was conducted. The findings clearly indicate that maturity significantly influences the phyto-chemical profile of the leaves. Mature leaves were found to contain a higher total antioxidant content compared to young leaves, suggesting a more developed secondary metabolite profile with age. Furthermore, radical scavenging assays demonstrated that mature leaves exhibited superior free radical neutralizing ability, confirming their potential as a more effective natural antioxidant source. These results highlight the importance of leaf maturity in determining the medicinal and nutraceutical value of *Murraya koenigii*. The enhanced antioxidant properties in mature leaves could be leveraged in the development of plant-based health supplements and functional foods. Future studies may further explore the specific compounds responsible for this variation and assess the bioavailability and efficacy of extracts in vivo.

Key words: Young, Mature, Antioxidant, Neutraceuticals and Scavenging.

Introduction

Murraya koenigii, commonly known as **curry leaf** or **karipatta**, belongs to the family **Rutaceae**, is a small, tropical to sub-tropical tree or shrub that typically grows to 6-15' feet tall and is noted for its pungent, aromatic, curry leaves which are an important flavoring used in Indian/Asian cuisine. Yellow curry powder (developed by the British during the time of their colonial rule in India) is a blend of many different Indian spices, one of which is sometimes (but not always) curry leaf[1-4]. People generally use the fresh leaves, dried leaf powder and essential oil for flavouring soups, curries, fish and meat dishes, egg dishes, traditional curry powder blends etc. The aromatherapy industry uses the essential oil in the making of soaps and cosmetics[5-7]. For natural hair tone and hair growth, one can use the blanked residue of boiled curry leaves along with coconut oil[8-10]. It can be used as antihelmetics, it also acts as febrifuge, blood purifier, antifungal, depressant, anti-inflammatory, body aches, for kidney pain and vomiting [11-15]. *Murraya koenigii* is used as a stimulant and anti-dysenteric. It is also effective against diabetes Mellitus[16-19]. Leaves are applied externally to bruises and eruption[20].

The leaves and roots are bitter in taste analgesic, cure inflammation and itching[21-22]. It is also useful in leucoderma and blood disorders and also cures diseases like piles[23-24]. It can be also used to stop vomiting[25-26] by infusion of the toasted leaves. If someone is bitten by poisonous animals, local application of the leave paste is effective[27-29]. The essential oil from *M. koenigii* leaves showed antibacterial effect against *B. subtilis, Staph. aureus, C. pyogenes, P. vulgaris* and *Pasteurella multicida*[30-31]. Acetone extract of *M. koenigii* is active against *Aspergillus niger*, benzene extract is most active against *Penicillum notatum*. The literature showed the antioxidative properties of the extract of *M. koenigii* leaves were done using different solvents. Alkaloid Koenoline isolated from the root bark of *M.koenigii* is found to exibit cytotoxic activity against KB cell culture system. The alcohol extract of stem bark (1 gm/kg body weight) is effective against carrageenan-induced inflammation. Crude root extract also showed anti-inflammatory activity[32]. Bioactive alkaloids, kurryam and koenimbine obtained from fractionated n-hexane extract of the seeds of *M. koenigii* were found to exhibit inhibitory activity against castor oil-induced diarrhoea and prostaglandin[33, 34].

Aim and Objectives

To do the comparatively analyse the antioxidant levels in mature and young leaves of the curry plant (*Murraya koenigii*), thereby determining the variations in antioxidant content during different stages of leaf development.

Objectives include: (i)To extract and quantify the antioxidant compounds present in both mature and young leaves of the curry plant.

(ii) Successive extraction and qualitative analysis.

(iii) **Characterization**: Evaluation of the influence of leaf maturity on the antioxidant potential will be processed using in vitro biochemical assays such as DPPH.

Material and Methodology

Collection of Plant Material

Two different types of leaves i.e., mature and young leaves of *Murraya koenigi* were collected from the locality of Balawala, Dehradun, Uttarakhand in the month of January 2025 and were identified and authenticated by the Botanical Survey of India, Dehradun. The leaves were then shade dried for few days until there is no moisture left in them.

Chemicals

1,1-diphenyl-2-picrylhhydrazyl (DPPH), Ascorbic acid (standard), Petroleum Ether, Chloroform, Methanol and Water. All the reagent and solvent sused were of AR grade.

Extraction

Curry leaves (50g) were crushed and were extracted again and again via Soxhlet method using 250 ml of Petroleum Ether, Chloroform, and Methanol. The leaves were then macerated in 500ml water for 48 hours and then sample was put into orbital shaker at room temperature and 150 RPM for 48 hours. Afterwards the sample was extracted in Rotary Vacuum Evaporator. The extracts after removal of solvents were stored at 4°C until used for antioxidant assay. The efficacy of

extracts was quantified based on the dry weight of the whole extract per volume of assay solution.

Fig 1 : Soxhlet Apparatus

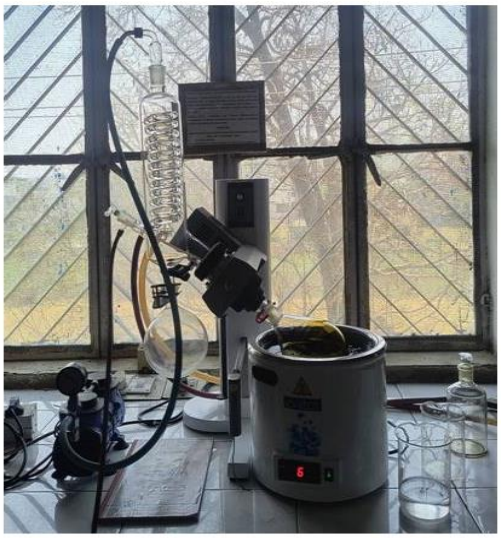

Fig 2 : Rota evaporator

Distillation

The Solvents Petroleum Ether, Chloroform and Methanol for extraction of Curry Leaves were recycled using Distillation unit for further laboratory use.

Fig 3 :Distillation Unit

Fig 4 : Orbital Shaker

DPPH Spectrophotometric

Each extract stock solution (1mg/10ml) was diluted to final concentration ranging from (20-100µg/ml) in methanol 2 ml of DPPH solution was added to 1ml of extract solutions of different concentrations and allowed to

react at room temperature in dark condition. After 30 min., the absorbance was measured at 517 nm and the percentage scavenging capacity was calculated[34].

Fig 5 : DPPH Stock Solution and Micropipette

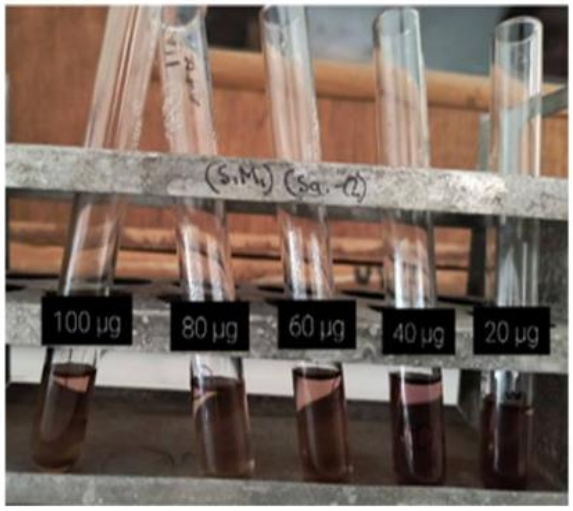

Fig 6 : DPPH Plant Extracts

Estimation of free radical scavenging activity

The ability to scavenge 1, 1-diphenyl 1-2- picrylhydrazyl (DPPH) radical by curry leaves extracts was estimated by the method Negi and Jayaprakasha[35- 36].

Yield- Maximum yield was obtained by Soxhlet extraction with Methanol and lowest yield was with Petroleum Ether in both mature and young leaves.

Result and Discussion

Table-1 Yield of Extracts

Extract	Yield Percentage	
	Mature	Young
Petroleum Ether	0.716%	4.9%
Chloroform	1.8%	5.6%
Methanol	13.4%	25%
Water	10.13%	14.27%

Table-2 In Vitro Anti-oxidation Activity

Ascorbic acid (Dilution)	% RSA
1µg/ml	2.86
5 µg/ml	12.86
10 µg/ml	25.71
15 µg/ml	40.00
20 µg/ml	50.00

Table-3 % RSA of crude Extract of Mature leaves

Dilution	%RSA of Extracts			
	Petroleum Ether	Chloroform	Methanol	Water
20 µg/ml	6.40	12..0	49.60	35.24
40 µg/ml	11.60	18.5	55.34	42.45
60 µg/ml	14.20	27.0	61.73	50.84
80 µg/ml	22.00	35.5	67.89	59.23
100 µg/ml	29.00	47.0	73.44	66.4
150 µg/ml	37.00	62.0	88.66	82.04
200 µg/ml	46.00	82.5	104.03	97.85
250 µg/ml	58.00	93.0	119.9	113.09

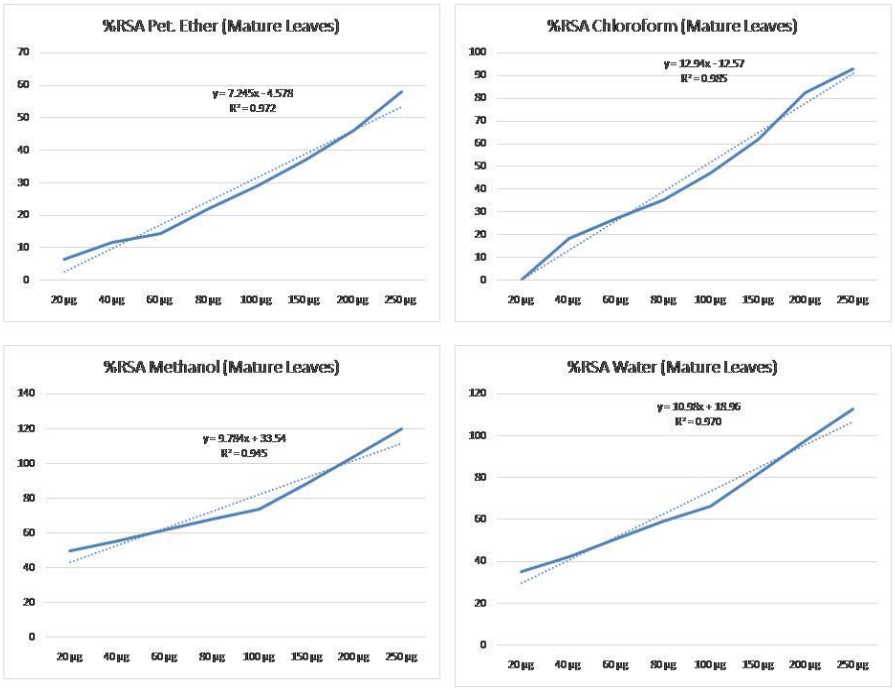

Figure-7% RSA Graphs of Various Extract of Mature Leaves of *Murraya koenigii*

Table-4 Absorbance of Crude Extracts of Young Leaves

Dilution	% RSA of Extracts			
	Petroleum Ether	Chloroform	Methanol	Water
20 µg/ml;	2.50	10.075	26.849	25.659
40 µg/ml	6.80	14.845	32.887	30.456
60 µg/ml	10.50	22.04	38.925	36.443
80 µg/ml	15.60	30.859	46.052	44.390
100 µg/ml	20.30	42.06	59.179	55.63
150 µg/ml	28.00	53.048	68.529	64.52
200 µg/ml	35.00	65.997	83.549	80.21
250 µg/ml	42.00	79.157	99.179	95.63
300 µg/ml	50.20	94.377	114.599	111.05

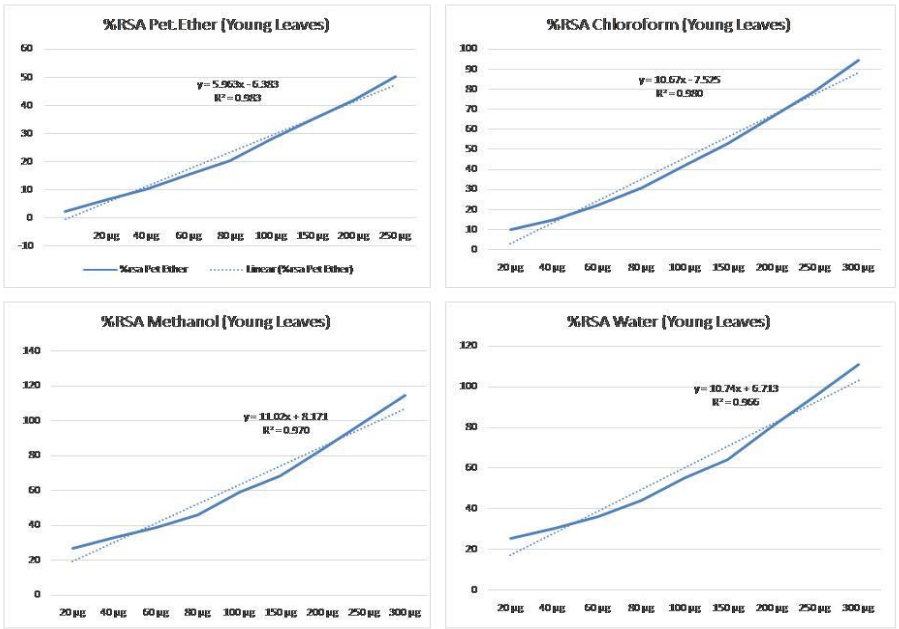

Figure- 8% RSA Graphs of Various Extract of Young Leaves of *Murraya koenigii*

Scavenging Activity

The DPPH radical scavenging activity of both young and mature *Murraya koenigii* leaves increased in a dose-dependent manner. Mature leaf extracts showed significantly higher DPPH scavenging activity at all tested concentrations compared to young leaf extracts.

At a concentration of 100 µg/mL, the mature leaves exhibited a maximum scavenging activity in all four solvents, whereas young leaves showed Y% (insert your value). The IC value for mature leaves were found to be **216.67 µg/mL** , **138.6 µg/mL, 47.08 µg/mL** and **58.01 µg/mL** which was lower than that of young leaves which were **298.78 µg/mL**,**153.274 µg/mL** , **87.64µg/mL** and **101.042 µg/mL** for **Petroleum Ether, Chloroform , Methanol and Water** respectively indicating stronger antioxidant potential in mature leaves. These results suggest that leaves of *Murraya koenigii* possess higher free radical scavenging activity, possibly due to increased accumulation of antioxidant phytochemicals such as phenolics and flavonoids with age.

Phytochemical Analysis

Table-5 Phytochemical analysis of old leaves in different solvents:

Solvent	Saponin	Tannins	Steroid	Alkaloids	Flavonoids
Petroleum Ether	-	+	+	-	-
Chloroform	-	-	+	+	+
Methanol	-	+	+	-	-
Distilled Water	-	-	+	-	+

*__Note__: **(+)** : Present , **(-)** Absent

Table-6 Phytochemical analysis of young leaves in different solvents

Solvent	Saponin	Tannins	Steroids	Alkaloids	Flavonoids
Petroleum Ether	-	+	+	-	+
Chloroform	-	-	+	+	-
Methanol	-	+	+	-	-
Distilled Water	-	-	+	-	+

*__Note__: **(+)** : Present , **(-)** Absent

Discussion

The present study compared the antioxidant activity of young and mature leaves of *Murraya koenigii*. It was observed that leaves showed higher antioxidant activity compared to young leaves based on the DPPH assay. The increased antioxidant activity in leaves may be due to the higher concentration of phenolic compounds and flavonoids. As the leaf matures, it is exposed to more environmental stressors, which may trigger increased biosynthesis of protective antioxidant molecules. These results align with a previous study[37], who reported higher antioxidant content in mature leaves of medicinal plants. However, another study[38] found higher antioxidant activity in younger neem leaves, indicating that age-related trends may vary by species. The findings suggest that mature curry leaves may be a more effective source of antioxidants for use in food and pharmaceutical applications. One limitation of the study is that it only used in vitro assays and did not consider seasonal variations. Also, detailed compound-specific analysis was not performed.

Conclusion

In this study, a comparative analysis of antioxidant content and radical scavenging activity between young and mature leaves of *Murraya koenigii* was conducted. The findings clearly indicate that maturity significantly influences the phytochemical profile of the leaves. Mature leaves were found to contain a higher total antioxidant content compared to young leaves, suggesting a more developed secondary metabolite profile with age. Furthermore, radical scavenging assays demonstrated that mature leaves exhibited superior free radical neutralizing ability, confirming their potential as a more effective natural antioxidant source. These results highlight the importance of leaf maturity in determining the medicinal and nutraceutical value of *Murraya koenigii*. The enhanced antioxidant properties in mature leaves could be leveraged in the development of plant-based health supplements and functional foods. Future studies may further explore the specific compounds responsible for this variation and assess the bioavailability and efficacy of extracts in vivo.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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