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Antioxidant Activity of *Cymbopogon martini* (Roxb.) Wats. Essential Oil from Different Ecotypes in Karnataka, India

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***Abstract*:** Palmarosa essential oil, derived from the *Cymbopogon martini* of the Poaceae family, has long been used in traditional medicine, particularly in India. Despite its widespread use, the antioxidant properties of Palmarosa oil remain underexplored. This study investigates its potential as an antioxidant, focusing on oil extracted from plants cultivated in the plains of South India. We employed two common methods: the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the Nitric Oxide (NO) scavenging assay. Results indicated that Palmarosa oil displayed significant antioxidant effects, efficiently scavenging DPPH and NO free radicals. The IC50 values from the DPPH and NO assays suggest potent antioxidant activity, comparable to well-known antioxidants. These findings support the therapeutic value of Palmarosa oil, emphasizing its potential as a natural source of antioxidants for various health applications.

***Keywords*:** Antioxidant, DPPH assay, Nitric Oxide scavenging, Palmarosa essential oil, and Therapeutic applications.

1. INTRODUCTION

Medicinal plants have been integral to traditional medicine, offering remedies for ailments caused by microorganisms such as bacteria, fungi, and viruses (Ashraf et al., 2023; Breijyeh & Karaman, 2024). Among these plants, essential oils, particularly those derived from leaves, have attracted significant attention for their therapeutic properties (Bolouri et al., 2022). Palma Rosa oil, extracted from *Cymbopogon martinii*, is an essential oil known for its floral aroma, golden color (Figure. 1A and B), and medicinal benefits. Despite its traditional use in treating various conditions (Walters, 2022). The *Cymbopogon* genus, known for its fragrant oils, has been widely studied for its antibacterial, anti-fungal, and insecticidal properties, with compounds such as geraniol and citronellol identified as key bioactive agents (Dangol et al., 2023). However, the antioxidant activity of Palma Rosa oil, particularly its ecotype variations, remains poorly understood. Current studies on synthetic antioxidants have raised concerns over their side effects, making plant-based alternatives like Palma Rosa oil an area of growing interest. This research aims to address the gap by exploring the antioxidant potential of Palma Rosa oil from various ecotypes of Karnataka, South India. The study will employ established assays such as DPPH and Nitric Oxide scavenging to assess the oil's ability to neutralize free radicals and mitigate oxidative stress. The findings will contribute to a deeper understanding of the variations in antioxidant activity across different ecotypes, potentially influencing the therapeutic applications of Palma Rosa oil. The objective is to identify the most potent ecotype, paving the way for future research into its use as a natural antioxidant in medicine and related industries.

2. MATERIALS AND METHODS

2.1. Plant Collection and Documentation

The study was carried out in 2021 at the Department of Biotechnology and Genetics, M S Ramaiah College of Arts, Science, and Commerce, Bengaluru. Wild ecotypes of *Cymbopogon martinii* were collected and comprehensively documented. This documentation process included key details such as the collection date, exact location, and a reference map for easy access. In addition, habitat characteristics were recorded to capture the natural environment of the plants. Environmental factors such as temperature, altitude, longitude, humidity, rainfall, wind speed, and topography (latitude and altitude) were also noted to provide a clearer understanding of the growing conditions. Six distinct ecotypes of *C. martinii* were selected from different ecological regions in Karnataka, chosen

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based on variations in their environmental conditions. These ecotypes were labelled as Ecotype 1 (E1), Ecotype 2 (E2), Ecotype 3 (E3), Ecotype 4 (E4), Ecotype 5 (E5), and Ecotype 6 (E6) (Table 1). Each of these ecotypes was authenticated by submitting plant samples to the Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD) in Uttarahalli, Bangalore, India where voucher numbers were assigned for proper identification (Authentication/SMPU/RARIMD/BNG/2019-20/432, 433, 444, 445, 446, and 447). The collected samples of these medicinal and aromatic plants were studied and conserved at the National Bureau of Plant Genetic Resources (NBPGR), Delhi

2.2. Extraction of Essential Oil

The essential oil of *Cymbopogon martinii* (Palmarosa) was extracted using the steam distillation method by Koul et al. (2004). This method ensures that the volatile compounds, including the desired aroma and therapeutic properties, are preserved during extraction.

A B

Figure 1A and 1B. Habitat and spikelet's of *Cymbopogon martini* (Roxb.) Wat

Preparation of plant material: Fresh leaves and stems of *Cymbopogon martinii* were harvested, washed thoroughly to remove any dirt or debris, and air-dried for a short period (1-2 hours). The plant material was cut into smaller pieces to facilitate better steam penetration during the distillation.

Steam Distillation: The prepared plant material was placed in the distillation chamber of a steam distillation apparatus with enough water to generate steam. The steam was passed through the plant chamber material, causing the essential oil to evaporate (Koul et al., 2004).

Collection: The essential oil was now cooled through a cooling system back into a liquid. The mixture of water and essential oil was collected in a receiving flask. Because essential oils are typically immiscible with water, they separate naturally, with the oil floating on top of the water.

Separation of oil: From the hydrosol (water phase) using a separator funnel. The hydrosol, often known as Palmarosa water or *Cymbopogon* hydrosol, can also be used in cosmetic formulations but is discarded or stored separately.

Storage: The essential oil was stored in airtight amber glass bottles to shield it from light and oxygen, which could potentially reduce its quality. These bottles were placed in a cool, dark environment to preserve the oil's efficacy.

2.3. Antioxidant Activity

The antioxidant activity of essential oils was assessed by two distinct methods: the DPPH assay (Khalaf et al., 2008), and the NO scavenging assay (Ghosh et al., 2010), For the DPPH radical scavenging assay, the ability of essential oils, and standard (Gallic acid) to donate hydrogen atoms or electrons was evaluated spectrophotometrically as per the method of Khalaf et al. (2008) with slight modifications. The stable radical DPPH was used as the reagent. Essential oil

Table 1. Environmental factors influencing plant habitat

Parameter	E1 Himavadgopala hill	E2 Siddarabetta	E3 Chikkaballapur	E4 Siddharuda mutt	E5 Karanji mantapa	E6 Koppa
Altitude	11454m	1200m	925m	720m	606m	565m
Latitude	11' 25' 53.12"N	14"37'12.00"N	12'58'20.7912"N	14'48'21.9712" N	13'20'17.7468" N	13'25'22.5874" N
Longitude	77'14'4.36"E	74050'60.00"E	77'34'50.3148" E	11'91'19.1297" E	77'6'5.0760" E	12'66'23.9852" E
Temperature (^{o}C)	18-34	21-28	21-31	20-30	18-35	18-35
Humidity (%)	71-85	68-82	68-80	70-80	70-80	70-82
Rainfall (mm)	791-800	700-860	680-900	680-880	600-700	1400-1500
Wind (Km/h)	8.41-14.1	13.80-14.50	12.3	12.38	6.8-9.2	6.8-9.2
Soil type	Red, loamy soil	Lateritic soil	Red loamy and black soils	Red sandy and black soils	lateritic gravelly soil	Red sandy & black soil

Source: Ministry of Environment, Forest and Climate Change, New Delhi, Government of India (E1 and E3); Tumkur Forest Department (E2); IIT Dharwad, Karnataka (E4); Karnataka Biodiversity Board (E5 & E6)

was diluted in methanol to obtain concentrations of 2.000, 1.000, 0.500, 0.250, 0.125, and 0.0625 mg mL^{-1}. A 0.0002% DPPH solution was prepared, and 2 mL was added to each test container containing varied amounts of essential oil. After thorough mixing the solutions were incubated in the dark for 30 min. The optical density was measured at 517 nm using a UV-visible spectrometer (Shimadzu UV-1280 wavelength scanning from 190-1100nm) with DPPH as a control. Inhibition of DPPH activity was calculated as (Eq. 1):

% inhibition of DPPH activity = A – B/A x 100

Where: A = Optical density of control; B = Optical density of sample

2.4. Nitric Oxide (NO) Scavenging Activity

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and measured using the Griess reagent, as described by Ghosh et al. (2010). At physiological pH, SNP undergoes a spontaneous reaction in aqueous solutions to release NO, which then reacts with oxygen to form nitrite ions. These nitrite ions can be quantified by responding with the Griess reagent. A solution containing 5 mM SNP in phosphate-buffered saline (PBS) was prepared and incubated at 25°C for 180 minutes. After incubation, the solution was mixed with the Griess reagent, and the absorbance of the resulting chromophore was measured at 546 nm using a spectrophotometer. Gallic acid was used as a standard reference. This method was adapted from Ghosh et al. (2010). The scavenging activity was calculated using the following formula (Eq. 2):

Nitric Oxide scavenged (%)= A – B/A x100

Where: A = Optical density of the control; B = Optical density of the essential oil sample

2.5. Calculation of Half-maximal Inhibitory Concentration (IC_{50})

The IC50 values were determined to evaluate the required to scavenge 50% of the radicals. A lower IC50 value indicates stronger antioxidant activity. To calculate the IC50, the percentage of inhibition was plotted against the sample concentration, and a regression analysis was performed to obtain the best-fit line. The IC50 was then determined from the curve where the percentage inhibition reached 50%.

2.6. Statistical Analysis,

The data were subjected to a two-way ANOVA (SPSS version) followed by Duncan's multiple range test(Duncan 1955) to identify significant differences between groups. Statistical analysis was conducted using SPSS version 30, and a p-value of less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

The current study aimed to evaluate the *in vitro* antioxidant activity of *Cymbopogon martinii* (Palmarosa) essential oil, derived from different ecotypes in Karnataka, India. The antioxidant potential was assessed using two different assays: DPPH radical scavenging and NO scavenging methods.

3.1. DPPH Assay

The inhibition of DPPH radicals increased with the concentration of Palmarosa essential oil. Ecotype S6 exhibited the highest percentage of inhibition across all concentrations, reaching 70.70% at 500 µg (Table 2, Figure 7), indicating its potent antioxidant activity. This was followed by ecotypes S4 and S5 (Table 2, Figure 5 & 6) which also demonstrated relatively high inhibition percentages, especially at the higher concentrations.

The results suggest that the antioxidant activity of the essential oil increases with concentration, which is consistent with findings from other studies investigating the antioxidant properties of essential oils. For instance, *Rosmarinus officinalis* (rosemary) and *Lavandula angustifolia* (lavender) have also shown increased DPPH radical scavenging activity at higher concentrations (Hendel et al., 2024). Furthermore, the variation in activity among the ecotypes implies that environmental factors or genetic diversity might play a role in the antioxidant efficacy of

Table 2. Antioxidant potential of various *C. martinii* ecotype samples at different concentrations

Sample conc. (µg)	Percentage inhibition (DPPH assay)					
	S1	S2	S3	S4	S5	S6
100	1.7±0.20	9.3±0.20	31.1±0.20	23.9±1.00	16±0.09	38.2±0.24
200	2.6±0.30	16.6±0.50	33.8±0.60	43.2±0.32	27.7±0.60	50.2±0.24
300	2.8±0.10	21.6±1.10	41.3±0.31	57.9±0.70	40.9±0.92	50.9±0.80
400	3.2±0.20	26.76±0.20	57.2±0.32	69.7±0.60	52.2±0.28	59.9±0.70
500	4.9±0.20	32.6±0.70	64±0.12	71.2±0.21	56.6±0.40	70.7±0.60

Palmarosa oil (Jamwal et al., 2024).

3.2. Nitric Oxide Scavenging Assay

The NO scavenging assay is another crucial method for assessing antioxidant activity, as nitric oxide is a free radical involved in various physiological and pathological processes. The percentage inhibition of NO radicals also increased at 500 µg, sample S6 demonstrated the highest inhibition at 57.60% (Table 3, Figure 13), followed closely by S5 (57.30%, Figure 12). These findings are in line with the results from the DPPH assay, further supporting the strong antioxidant properties of the Palmarosa oil.

Sample S6 not only exhibited the highest DPPH radical scavenging activity but also showed a remarkably low IC50 value for the NO scavenging assay (8.125 µg, Table 4, Figure 13), indicating a higher potency of this ecotype in scavenging NO compared to others (Table 3). The IC50 values for the NO assay ranged from 319.50 µg for S1 to 8.125 µg for S6, with S6 demonstrating superior activity . Kim et al. (2022) also reported that essential oils from *Cymbopogon citratus* exhibited a natural source of readily available, low-cost extracts rich in antioxidants. The high potency of S6 suggests that this ecotype may be particularly valuable for therapeutic applications targeting oxidative stress and related conditions.

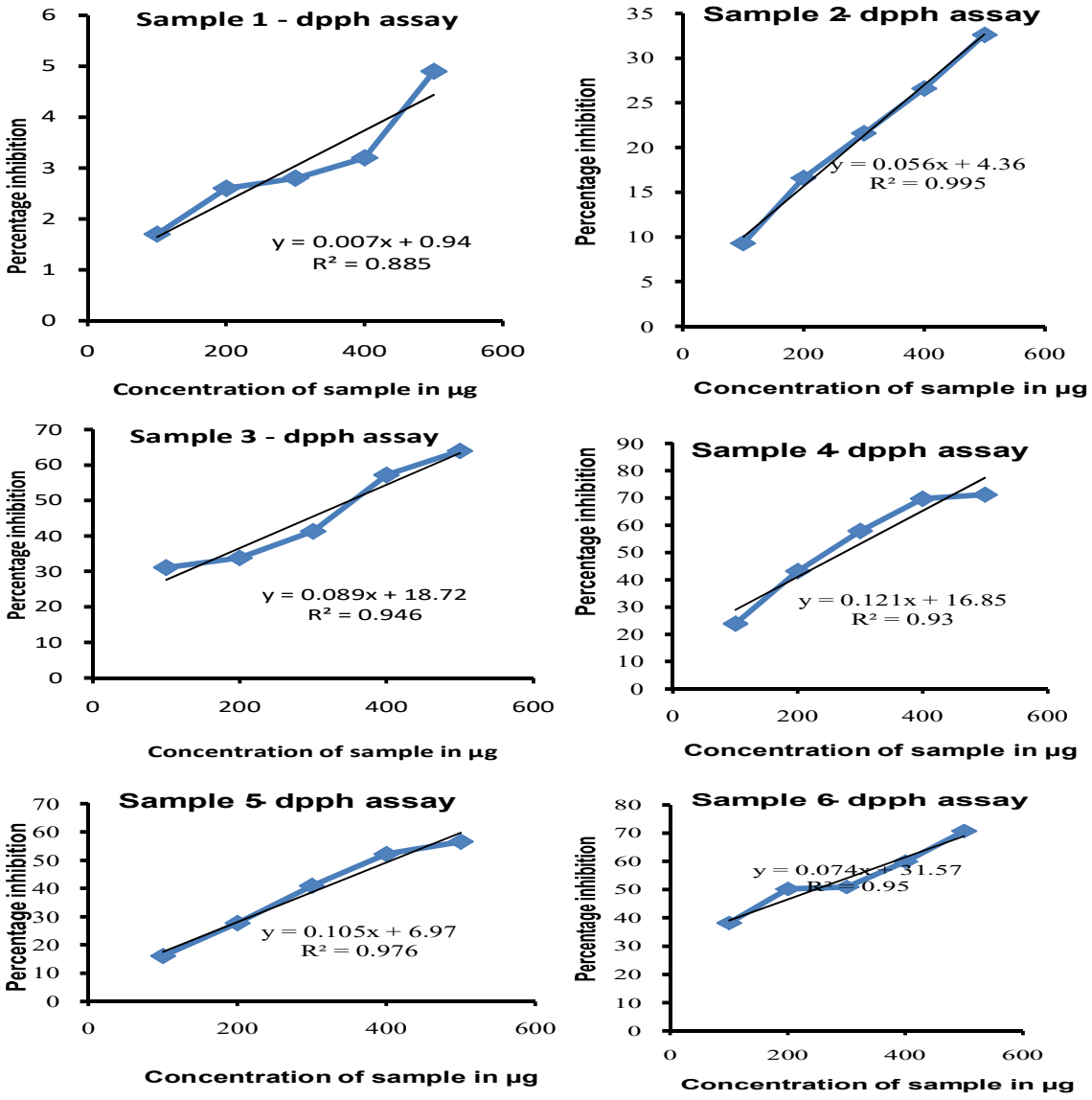

Figure 2-7. DPPH scavenging activity of *C. martini* ecotypes (E1-E6) at different concentrations

Table 3. Inhibition in Nitric Oxide (NO) by scavenging assay (%)

Sample conc. (µg)	Percentage inhibition					
	S1	S2	S3	S4	S5	S6
100	40.30±0.44	4.10±0.20	34.80±0.90	18.00±0.86	42.30±0.45	51.00±1.14
200	46.60±0.48	20.40±0.60	41.10±0.14	24.50±0.54	47.50±0.40	53.36±0.44
300	49.10±0.54	42.20±0.60	47.00±0.20	33.20±0.24	50.00±0.18	55.10±0.15
400	53.00±2.00	58.46±0.35	52.83±2.06	42.90±0.79	51.70±0.60	56.40±0.58
500	57.10±0.30	66.50±0.90	55.40±0.44	52.20±0.28	57.30±0.45	57.60±0.43

The values represent mean±standard error, with Duncan's multiple range test used to determine significant differences between groups based on 'F' ratios

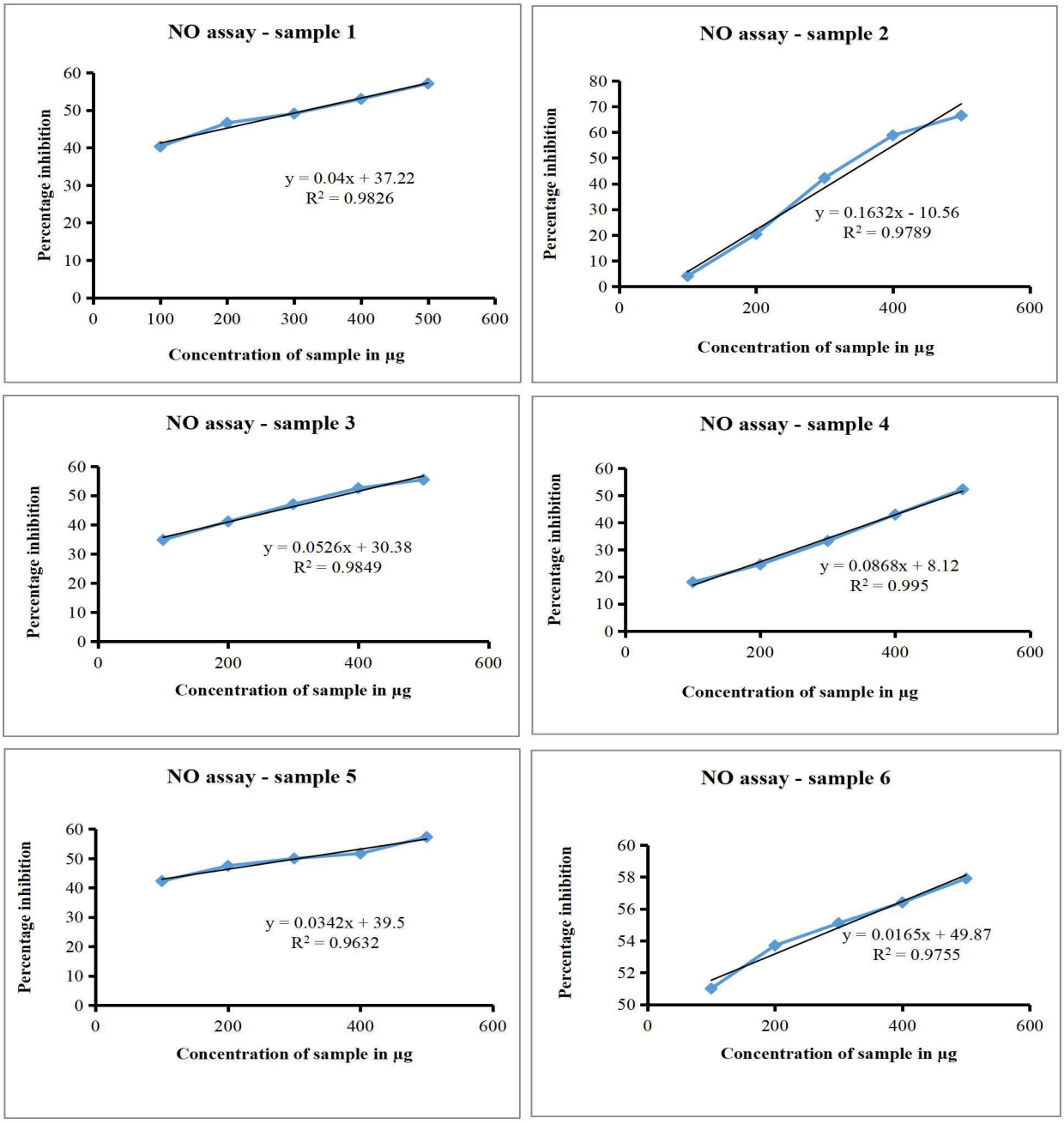

Figure 8-13. Bvaluation of nitric oxide scavenging activity in *C. martini Ecotypes* (E1-E6) across various concentrations

Table 4. IC50 values from DPPH and nitric oxide (NO) scavenging assays

Sample	DPPH IC50 (µg)	NO IC50 (µg)
S1	708.57±6.08	319.50±7.5
S2	815.00±14	371.53±3.07
S3	356.48±9.10	377.31±5.18
S4	273.32±4.03	488.66±9.83
S5	409.81±7.2	308.82±6.04
S6	249.05±7.05	8.125±0.04

3.3. IC50 Values

The IC50 values, which represent the concentration required to inhibit 50% of the free radicals, were calculated for both the DPPH and NO scavenging assays (Table 4). The lower the IC50 value, the stronger the antioxidant activity. For the DPPH assay, ecotype S6 had the lowest IC50 value at 249.05 µg (Table 4), indicating its superior ability to scavenge free radicals compared to the other samples. This was also true for the NO scavenging assay, where S6 demonstrated the lowest IC50 value (8.125 µg), a highly significant finding. In comparison, other ecotypes showed much higher IC50 values, with S1 and S2 showing the least efficient scavenging of both DPPH and NO radicals (Table 4). These findings align with previous research on the antioxidant activities of essential oils., *Cymbopogon citratus* (lemongrass) essential oil has shown similar results, with lower IC50 values correlating with higher antioxidant activity (Aldawsari et al., 2023). The results suggest that Palmarosa oil, particularly from ecotype S6, has strong potential as a natural antioxidant, possibly due to its high content of bioactive compounds such as terpenoids and phenolic compounds.

4. CONCLUSION

This study highlights the significant antioxidant activity of *Cymbopogon martinii* (Palmarosa) essential oil, particularly from ecotype S6, which exhibited superior radical scavenging activity and reduced power across multiple assays. The consistent findings across the DPPH and NO scavenging assays, and the low IC50 values, suggest that this essential oil could be a potent natural antioxidant. Given its potential to neutralize free radicals, Palmarosa essential oil may find applications in therapeutic fields of oxidative stress-related diseases. Further investigations into the chemical composition of the oil, particularly the identification and quantification of specific active compounds, could provide deeper insights into its medicinal potential.

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Conflict of Interest:

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Authors's Contributions

Dr. Vijayalakshmi: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing (90%)

S. Uma shivanna: Conceptualization (10%)

M.I. Farzana Tasneem: Investigation (10%)

Data Availability:

Authentication from Regional Ayurveda Research Institute for Metabolic Disorders (RARIMD) in Uttarahalli, Bangalore.

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