

Chemical composition, antifungal and antioxidant properties of seasonal variation in *Eucalyptus tereticornis* leaves of essential oil

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Research Article

Keywords: Eucalyptus tereticornis, Essential oil, Seasonal variation, Antioxidant activity and Antifungal properties

Posted Date: April 22nd, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4291069/v1>

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Additional Declarations: The authors declare no competing interests.

Abstract

The study aims to evaluate the chemical composition and anti-microbial activities of essential oils (EOs) extracted from fresh and shed dried leaves of *Eucalyptus tereticornis* clone C-83 during three consecutive seasons - winter, summer and rainy to determine the best harvesting time for obtaining the highest essential oil yield. The chemical composition was analyzed using a Gas Chromatography–Mass Spectrophotometry (GC-MS) and major compounds in fresh leaves oil were Eucalyptol (54.88%), Camphene (30.40%), α -Eudesmol (22.44%) and 3-Carene (19.50%). Dry leaf oil predominantly comprised Isopulegol (53.53%), 3-Isopropenyl-6-methyl-1-cyclohexene (17.29%) and Cis-Sabinene hydrate (13.55%). Isoterpinolene held a substantial presence at 5.61%, while Guaiol notably contributed at 2.49%. 2-isopropyl-4-methyl-2,5-dihydrooxazole registered at 2.32%, indicating its significant presence in the analyzed composition. Additionally, Bicyclogermacrene and Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-, exhibited noticeable percentages at 1.43% and 1.32%, respectively, underscoring their relative abundance within the sample. It revealed a concentration dependent rise in antioxidant effectiveness, with summer consistently exhibiting the highest radical scavenging potential of 93.58% at 4% concentration. Additionally, the oil showed antifungal properties against four plant-damaging fungi- *Magnaporthe grisea*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Fusarium oxysporum*- signifying potent antifungal properties in eucalyptus oils. The radical scavenging activity with increasing concentration across all seasons, showcasing higher efficacy as the concentration of essential oil rises, with the summer season consistently displaying the highest antioxidant potential across all concentrations. The study enhances comprehension of EOs and offers a pharmacological analysis, potentially advancing its exploration as a functional drug in agricultural and cosmetic sectors.

Introduction

Eucalyptus plants, known for their rapid growth and impressive sizes reaching up to 80–90 meters, are extensively cultivated worldwide (Kaur et al. 2011). These plants are significantly contributing to afforestation or reforestation programmes in tropical and temperate regions and also major supply for pulp and paper industries efforts (Trabado and Wilstermann, 2008). The introduction of Eucalyptus in Europe traces back to 1792 in France, spreading its seeds across European nations and eventually worldwide, becoming one of the most prevalent species by the 19th century (Potts et al., 2004). As per a survey across 65 countries renowned for extensive Eucalyptus plantations estimates that the collective expanse of plantations globally has surpassed 22.57 million hectares (Zhang and Wang, 2021).

Eucalyptus species, belonging to the Myrtaceae family, is classified as aromatic and medicinal plants. Their essential oil exhibits antioxidant and antimicrobial properties, widely used in pharmaceuticals and cosmetics (Almas et al., 2021), recognized for their diverse essential oils containing over 50 components, have found extensive applications. Their safety and non-toxic nature have been confirmed by evaluations conducted by the United States Food and Drug Administration and the Council of Europe (Batish et al., 2008). Eucalyptus species, renowned since ancient times for their multifaceted components (wood, fibers, cellulose, dyes, pulp, gum, resin, essential oils), holds diverse applications across sectors such as construction, medicine production and plant protection (Salehi et al., 2019).

The plants with substantial biomass and rich essential oils, like Eucalyptus, serve as valuable sources of natural compounds with interesting and useful bioactive properties and varied uses extend beyond human health and medicine, making them valuable in different industries (Ait-Ouazzou et al., 2011; Santos et al., 2012; Mediavilla et al., 2021; Tit and Bungau, 2023). The EOs usually derived from the aerial parts of the plants containing oxygenated compounds and has activities against different bacterial and fungal strains due to presence of high percentages of

monoterpenes, thymol, cinnamic, eugenol and polyphenols (Roby et al., 2002; Quinn et al., 2004; Husnu et al., 2007; Ghaffar et al., 2015). The EOs derived from Eucalyptus leaves, among the most significant among woody and non-woody trees, offer a wide range of plant essential oils, containing diverse chemicals like esters, monoterpenes, aldehydes, ketones, sesquiterpenes, play crucial roles in plant defence mechanisms against pests and microorganisms (Batish et al., 2008). Among plant pathogens, fungi cause various diseases, leading to significant losses in crop production and post-harvest stages (Fletcher et al., 2006). Particularly, *Rhizoctonia* and *Fusarium* root rot are extensively researched and problematic, causing immeasurable yield losses in agriculture and horticulture, even impacting the wood industry (Williamson-Benavides and Dhingra, 2021). Furthermore, the eco-friendly nature of EOs, their biodegradability and low toxicity to vertebrates make them easily isolable and environmentally safe (Isman, 2006; Isman and Machial, 2006; Koul et al., 2008; Isman and Miresmaili, 2011).

Antioxidants, present in a variety of sources such as plants, microorganisms and some animal tissues, serve a crucial role in preventing reactions that lead to food spoilage, encompassing disruption, sourness and color changes. The efficacy of these natural antioxidants, including those within Eucalyptus oils, may vary depending on factors such as plant species, extraction methods and environmental conditions (Can et al. 2005; Shebis et al. 2013). These compounds actively neutralize free radicals and Reactive Oxygen Species (ROS) within cells, offering protection against chronic diseases such as cardiovascular ailments, aging, heart disease, anemia, cancer and inflammation (Vaibhav et al. 2011). The study aimed to analyse the antioxidant potential using DPPH and examined the anti-fungal activity against four fungus strains- *Rhizoctonia solani*, *Rhizoctonia bataticola*, *Fusarium oxysporum* and *Magnaporthe grisea*- from both fresh and shed leaf extracted oil across three consecutive seasons: winter, summer and rainy periods, offering multifaceted advantages across industries and promoting human well-being.

Materials and Methods

2.1. Essential Oil Extraction and Chemical Analysis

Fresh leaves of *E. tereticornis* clone C-83 were gathered from the Research Farm at CCS Haryana Agricultural University, situated in Hisar, Haryana, India (latitude 29° 09' N and longitude 75° 43' E) over the experimental duration from December 2021 to August 2022. Sampling was conducted thrice, corresponding to winter (December), summer (April) and rainy (August) seasons. The fresh and healthy leaf samples were procured from various trees of the same clone. A portion of these leaves was immediately utilized for oil extraction, while others were set aside for drying under shade. The verification of the plant specimen's authenticity was confirmed by the Forestry Department of CCS HAU.

The essential oils under scrutiny in this investigation were obtained via hydro-distillation of 200g of fresh *E. tereticornis* leaves, employing a Clevenger-type apparatus for duration of 3 hours. The extraction of essential oils was conducted in triplicate for each oil sample. Following extraction, the oil was dried utilizing anhydrous Na₂SO₄. The yield of extracted oil from both fresh and shed dried leaves, for both fresh and dry oils, was computed as a percentage relative to the respective weight of the oil and the weight of the Eucalyptus leaves, employing the prescribed formula.

$$\text{Oilyield (\%)} = \frac{\text{Weight of oil (ml)}}{\text{weight of Eucalyptus leaves (g)}} \times 100$$

The obtained samples were relocated to opaque glass containers and preserved at 4°C throughout the experimental duration. The *E. tereticornis* essential oil was thinned down using Dichloromethane to facilitate gas

chromatography-mass spectrometry analysis (GC-MS).

2.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The *E. tereticornis* essential oil underwent analysis via GC-MS using an Agilent GC/MS system (Agilent Technologies). The setup featured a DB-5 fused silica column measuring 30 m × 0.25 mm i.d. The temperature in the oven initiated at 40°C and gradually increased to 250°C at a rate of 4°C. The transfer line temperature was maintained at 260°C. Helium gas served as the carrier gas, operating at a linear velocity of 31.5 cm/s. The split ratio stood at 1/60, while the ionization energy was set at 70 eV. A 35-minute scan time was employed, covering a mass range from 40 to 300 atomic mass units (1 Atomic mass unit = 1.66×10^{-27}). To quantify the compounds in the oils, the area normalization method was used, disregarding response factors. Identification of the oil components relied on comparing their mass spectra to those in a computer NIST library.

2.3. In Vitro Antioxidant Assay (DPPH Activity)

The assessment of *E. tereticornis* essential oil's capability to neutralize the free-radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was conducted following the methodology outlined by Trigui et al. (2013), with minor adaptations. Samples of Eucalyptus essential oil sourced from clone C-83 across three seasons (winter, summer and rainy) were diluted to various concentrations (0.5, 1, 2 and 4%) using ethanol. These solutions were then left in darkness for an incubation period of 30 minutes. Subsequently, the absorbance was measured at 517 nm. The percentage of DPPH scavenging effect was computed using the following formulae:

$$\text{DPPH Radicals scavenging rate (\%)} = 1 - \frac{A_1}{A_0} \times 100$$

Here, A1 represents the absorbance of the sample and DPPH solution and A0 represents the absorbance of the blank control.

2.4. In Vitro Antifungal Activity

Different fungal strains including *Magnaporthe grisea*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Fusarium oxysporum* were utilized to test the antifungal activity of *E. tereticornis* essential oil. The poisoned food technique (Adjou et al., 2012) was employed for this purpose. Specific initial concentrations (0.5, 1, 2 and 4%) of *E. tereticornis* essential oil derived from four distinct clone C-83 collected across winter, summer and rainy seasons were prepared. These concentrations were mixed with cooled molten PDA (45°C) that contained 0.5% (v/v) of Tween 80 and were dispersed in sterile Erlenmeyer flasks by manual rotation. The resulting mixture was poured into sterile petri dishes and left to solidify. Agar discs (6 mm in diameter) containing mycelia from 7-day-old pure cultures were centrally placed on each plate, alongside control plates that lacked the essential oil. Each treatment was replicated three times and the plates were incubated at a temperature of $28 \pm 2^\circ\text{C}$. After seven and twenty-one days of incubation, the diameter of fungal colonies was measured. All experiments were conducted in triplicate and the percentage inhibition of mycelial growth was calculated using the formula developed by Philippe et al. (2012):

$$\text{Inhibition of mycelial growth (\%)} = \frac{(d_c - d_t)}{d_c} \times 100$$

Where, d_c is the mean diameter of the colony in the control sample (6mm) and d_t is the mean diameter of the colony in the treated sample (6mm).

2.5. Statistical Analysis

Statistical analysis of the data was conducted using OPSTAT. A factorial randomized block design was applied to field experiments, while laboratory experiments utilized a factorial complete randomized design. Differences were deemed statistically significant at a level of $P < 0.05$.

Results and Discussion

3.1. Seasonal Variation in *E. tereticornis* Essential Oil

The results represent the seasonal variations in oil yield obtained from Eucalyptus leaves, distinguishing between fresh and dry leaf extraction (Fig. 1). In winter, the yield of fresh leaf oil is 0.65%, rising to 0.95% in summer and reaching 0.79% during the rainy season. Comparatively, the yield of oil from dry leaves is notably higher, measuring at 1.5% in winter, escalating to 3% in summer and then stabilizing at 1.7% during the rainy season. These values signify fluctuations in oil production influenced by seasonal changes, emphasizing the differential impact on oil extraction from Eucalyptus leaves across varying seasons and leaf conditions. The climatic factors variability such as temperature, total duration of exposure to sun and precipitation could directly influence the oil yield during the two harvesting seasons (Valmorbida et al. 2006 and Figueiredo et al., 2008). In the extraction of EOs from eucalyptus, hydrodistillation commonly serves as the preferred method. Extraction yields typically span between 0.06–7%, while the chemical makeup of the resulting EOs is contingent upon the plant's species and varieties (Bignell et al., 1997).

The essential oils obtained from fresh leaves in winter, summer and rainy seasons exhibited notable quantitative distinctions in their chemical compositions. In the winter season's leaf oil, the key components comprised camphene (30.40%), 3-carene (19.50%), p-cymene (12.31%), cis-sabinene hydrate (7.37%), D-limonene (7.12%), α -Eudesmol (5.33%) and trans-ocimenol (3.53%). Whereas, the summer season featured Eucalyptol as the primary compound, constituting 54.88% of the analyzed sample. Following closely, 3-Carene accounted for 18.92%, while α -Phellandrene held a significant presence at 9.06%. p-Cymene followed at 5.02%, showcasing its relative importance in the composition. Other compounds contributing to the makeup included Terpinen-4-ol at 1.9%, trans-Sabinol and trans-Ocimenol, both at 1.53%, along with α -Eudesmol at 1.34%. Additionally, Borneol registered at 0.48%, while Aromandendrene stood at 0.42%, indicating their lower yet discernible presence in the analyzed composition. These compounds collectively represent the major constituents, delineating their varying degrees of prevalence within the sample analyzed.

On the other hand, in the rainy season's oil, α -Eudesmol emerged as the most abundant compound, accounting for a significant portion at 22.44%, followed by Camphene, another prevalent compound, at 13.11%. 3-Carene registered a substantial presence at 8.47%, while α -Phellandrene and D-Limonene made notable contributions at 7.62% and 5.42%, respectively. Additional constituents included (-)-Globulol at 4.72%, Aromandendrene at 3.39%, Eucalyptol at 3.30% and Terpinen-4-ol at 1.37%, emphasizing their relative prominence in the sample composition. Collectively, these chemicals underscore the major constituents within the sample analyzed, showcasing their varying degrees of abundance and significance. Moreover, the tested *E. tereticornis* essential oils exhibited notable amounts of several minor constituents, as depicted in Table 1 and Fig. 2.

The oils obtained from shed dry leaves during winter, summer and rainy seasons displayed notable quantitative disparities. In the winter season's leaf oil, 3-Isopropenyl-6-methyl-1-cyclohexene stood out prominently, comprising a significant portion at 13.86%, followed by Cis-Sabinene hydrate at 13.55%. Notably, 3-Carene, another prevalent compound, contributed significantly at 10.14%. Additionally, α -Eudesmol registered at 9.87%, while 3-Carene maintained a substantial presence at 4.3%. Isoterpinolene closely followed at 6.05%, with (-)-Globulol at 4.3%,

emphasizing their relative importance in the composition. Further, 5-Azulenemethanol and α -Phellandrene exhibited notable percentages at 3.93% and 2.74%, respectively, highlighting their significant contributions to the overall composition.

Whereas, the major constituents in the summer season were primarily dominated by Isopulegol, significantly constituting 53.53% of the composition, followed by 3-Carene at 13.72%. Isoterpinolene held a substantial presence at 5.61%, while Guaiol notably contributed at 2.49%. 2-isopropyl-4-methyl-2,5-dihydrooxazole registered at 2.32%, indicating its significant presence in the analyzed composition. Additionally, Bicyclogermacrene and Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-, exhibited noticeable percentages at 1.43% and 1.32%, respectively, underscoring their relative abundance within the sample. These compounds collectively represented the major constituents, outlining their substantial roles in shaping the chemical composition.

Moreover, the primary constituents in the rainy season's oil were led by 3-Isopropenyl-6-methyl-1-cyclohexene, emerging as the predominant component at 17.29%. Following closely, 3-Carene exhibited notable presence at 10.63%, signifying its significance in the sample. Substantial contributions were noted from 4-Ethyl-o-xylene at 8.18% and Eucalyptol at 7.6%, underlining their relative abundance. Additionally, α -Eudesmol and D-Limonene contributed significantly at 6.4% and 6.22%, respectively, while α -Phellandrene registered at 5.35%, highlighting its relative prominence. These compounds, along with trans-Ocimenol and trans-Sabinol at 5.05% and 3.08%, collectively represented the major constituents, delineating their varying degrees of abundance within the analyzed sample. Moreover, the tested *E. tereticornis* essential oils contained significant amounts of several minor constituents, as seen in Table 1.

Table 1
Seasonal variation in chemical composition and area (%) of essential oils from fresh and dry leaves of *Eucalyptus tereticornis*

S. No.	Name	Formula	RT	Area (%)					
				FW	FS	FR	DW	DS	DR
1.	2-isopropyl-4-methyl-2,5-dihydrooxazole	C ₇ H ₁₃ NO	1.772	-	-	1.32	1.09	2.32	0.85
2.	2'-Methyl-6'-(3-(trimethylsilyl)propyl)actophenone	C ₁₅ H ₂₄ OSi	1.933	1.23	1.1	-	-	-	-
3.	(R)-7-Benzenesulfonyl-2-methoxy-bicyclo[4.1.0]heptane	C ₁₄ H ₁₈ O ₃ S	5.802	0.35	0.72	1.12	-	-	0.78
4.	3-Carene	C ₁₀ H ₁₆	5.991	19.5	18.92	8.47	10.14	13.72	10.63
5.	Spiro[2.4]heptane, 1,5-dimethyl-6-methylene-	C ₁₀ H ₁₆	6.406	0.84	-	0.24	1.47	-	0.93
6.	3-Isopropenyl-6-methyl-1-cyclohexene	C ₁₀ H ₁₆	7.115	-	-	-	13.86	-	17.29
7.	Camphene	C ₁₀ H ₁₆	7.228	30.4	-	13.11	-	-	-
8.	4(10)-Thujene	C ₁₀ H ₁₆	7.667	0.33	0.54	1.22	-	-	1.2
9.	α-Phellandrene	C ₁₀ H ₁₆	8.086	-	9.06	7.62	2.74	-	5.35
10.	4-Ethyl-o-xylene	C ₁₀ H ₁₄	8.573	-	-	-	-	-	8.18
11.	p-Cymene	C ₁₀ H ₁₄	8.746	12.31	5.02	3.65	-	-	-
12.	Isopulegol	C ₁₀ H ₁₈ O	8.839	-	-	-	-	53.53	-
13.	D-Limonene	C ₁₀ H ₁₆	8.878	7.12	-	5.42	-	-	6.22
14.	Cis-Sabinene hydrate	C ₁₀ H ₁₈ O	8.943	7.37	-	-	13.55	-	-
15.	Eucalyptol	C ₁₀ H ₁₈ O	8.964	-	54.88	3.3	-	-	7.6
16.	Isoterpinolene	C ₁₀ H ₁₆	10.666	-	-	-	-	5.61	-
17.	γ-Terpinene	C ₁₀ H ₁₆	10.908	0.39	0.37	0.73	0.73	-	0.45
18.	Fenchol	C ₁₀ H ₁₈ O	11.83	1.06	0.33	0.27	2.18	0.17	1.52
19.	Myrtenol	C ₁₀ H ₁₆ O	12.116	0.23	-	-	2.64	-	2.83
20.	trans-Verbenol	C ₁₀ H ₁₆ O	12.587	0.23	-	-	-	-	-
21.	trans-Sabinol	C ₁₀ H ₁₆ O	12.68	1.44	1.53	-	2.41	0.75	3.08

S. No.	Name	Formula	RT	Area (%)					
				FW	FS	FR	DW	DS	DR
22.	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	C ₁₀ H ₁₈ O	13.018	0.27	-	-	0.59	-	0.32
23.	Trans-4-Thujanol	C ₁₀ H ₁₈ O	13.026	-	0.08	0.24	-	-	0.33
24.	Umbellulone	C ₁₀ H ₁₄ O	13.473	0.25	-	-	-	-	-
25.	Sabinone	C ₁₀ H ₁₄ O	13.473	-	0.27	-	-	-	0.57
26.	Borneol	C ₁₀ H ₁₈ O	13.65	1.44	0.48	-	2.18	0.6	1.49
27.	Terpinen-4-ol	C ₁₀ H ₁₈ O	14.053	1.78	1.9	1.37	3.25	-	2.79
28.	(1S)-1,3,3-trimethylnorbornan-2-ol	C ₁₀ H ₁₈ O	14.279	-	-	-	6.05	-	-
29.	Carveol	C ₁₀ H ₁₆ O	14.456	-	0.13	-	-	0.54	-
30.	trans-Ocimenol	C ₁₀ H ₁₈ O	14.56	3.53	1.53	-	-	-	5.05
31.	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8- trimethyl-4-vinyl-	C ₁₅ H ₂₄	22.086	0.18	-	0.41	-	1.32	-
32.	Aromandendrene	C ₁₅ H ₂₄	22.702	0.6	0.42	3.39	1.83	1.25	1.06
33.	Bicyclogermacrene	C ₁₅ H ₂₄	24.478	0.37	-	-	-	1.43	0.35
34.	Epiglobulol	C ₁₅ H ₂₆ O	26.059	-	-	0.87	0.7	0.29	0.56
35.	Viridiflorol	C ₁₅ H ₂₆ O	26.423	0.13	0.08	0.96	-	-	-
36.	Spatulenol	C ₁₅ H ₂₄ O	26.946	0.09	-	-	0.42	1.07	2.08
37.	(-)-Globulol	C ₁₅ H ₂₆ O	27.131	1.54	0.04	4.72	4.3	-	4.96
38.	Guaiol	C ₁₅ H ₂₆ O	27.357	-	0.15	-	0.77	2.49	0.99
39.	β-Eudesmol	C ₁₅ H ₂₆ O	28.251	-	0.05	0.37	-	-	-
40.	5-Azulenemethanol	C ₁₇ H ₂₈ O ₂	28.496	0.73	-	8.7	3.93	-	2.56
41.	α-Eudesmol	C ₁₅ H ₂₆ O	29.113	5.33	1.34	22.44	9.87	-	6.4

RT-Retention Time, FW- Fresh Winter, FS- Fresh Summer, FR-Fresh Rainy, DW- Dry Winter, DS-Dry Summer and DR-Dry Rainy

The composition of *E. tereticornis* essential oils examined in this study exhibits considerable variability. There's notable fluctuation in compound percentages across seasons, indicating shifts in composition. For instance, 3-Carene ranges notably, varying from 8.47–19.5%, showing significant abundance differences. α-Eudesmol, a compound with high variability, records 22.44% in fresh rainy season leaves and 9.87% in dry winter season leaves.

Fenchol, (-)-Globulol, Terpinen-4-ol, Borneol and γ -Terpinene exhibit fluctuating percentages, varying between seasons (ranging from 0.17–2.18%, 0.04–4.96%, 1.37–3.25%, 0.48–1.49% and 0.37–0.73%, respectively). p-Cymene demonstrates substantial variance, fluctuating between 3.65% and 12.31% across different seasonal extracts. Moreover, 4(10)-Thujene ranges from 0.33% in fresh winter to 1.22% in fresh rainy extracts, showcasing changes in abundance. Conversely, Viridiflorol notably fluctuates, presenting at 0.13% in fresh winter, 0.08% in fresh summer and a substantial increase to 0.96% in fresh rainy extracts, highlighting significant differences in its occurrence across seasonal extractions. These compounds exemplify the diverse chemical makeup within Eucalyptus leaf extracts, reflecting distinct variations linked to seasonal factors.

The chemical compounds extracted from shed dry Eucalyptus leaves exhibit noteworthy variations in their percentage areas across different seasons. Epiglobulol showcases varying presence, from 0.7% in dry winter to 0.29% in dry summer, rising to 0.56% in dry rainy extracts, indicating marked differences based on seasonal extraction. Spatulanol demonstrates significant variations, starting at 0.42% in dry winter, escalating notably to 1.07% in dry summer and further increasing to 2.08% in dry rainy extracts, signaling substantial fluctuations across seasons. Similarly, Guaiol exhibits notable differences, ranging from 0.77% in dry winter to 2.49% in dry summer, before decreasing to 0.99% in dry rainy extracts, highlighting significant variations in its abundance across different seasonal extractions. These compounds emphasize the diverse chemical compositions within Eucalyptus leaf extracts, illustrating distinct variations influenced by seasonal factors. The data underscores the variability in compound percentages, showcasing the diverse chemical makeup in Eucalyptus leaf extracts obtained from different seasons and types of leaves.

Our results align with previous research conducted on four Eucalyptus species cultivated in Tunisia, which detailed the chemical compositions of *E. salubris* (27 compounds; 99.2%), *E. salmonophloia* (31 compounds; 99.2%), *E. oleosa* (32 compounds; 97.6%) and *E. gracilis* (18 compounds; 97.7%) (Marzoug et al., 2010). Lucia et al. (2007) highlighted that the primary constituents of *E. grandis* in Argentina were α -pinene (52.71%) and 1,8-cineole (18.38%). Liu et al. (2008), focusing on Eucalyptus essential oil (EEO) collected in Hainan (China), identified alloocimene (43.22%) and α -pinene (13.63%) as the major components. However, our investigation found varying content of eucalyptol (also known as 1,8-cineole) in EEO, ranging between 54.88–3.30% and it was undetectable in certain oil types. This fluctuation might be elucidated by the transformation of 1,8-cineole, α -pinene, limonene and α -terpineol from the same source material (Keszei et al., 2008). Diverse extraction methods potentially led to the conversion of one substrate into the other three compositions. Hence, dissimilarities between our study and prior research could be attributed to variations in plant variety, genetic differences resulting from generations and hybridizations, growth conditions, extraction techniques and even the years and seasons of harvest. Variations in essential oil (EO) compositions can arise from the method and drying procedures applied to the botanical material before extraction, as well as from storage conditions (Lemos et al., 2012; Pimentel et al., 2013).

Therefore, considering these findings alongside prior studies, it's evident that the components within *E. tereticornis* essential oil exhibit variability in their composition based on leaf type (fresh or dry) as well as environmental factors and harvesting time. The plant's adaptable metabolism significantly impacts the essential oil's quality, quantity and chemical makeup, potentially leading to the creation of a distinctive and specific chemical composition.

3.2. Evaluation of Antioxidant Activity: DPPH

The evaluation involved the impact of *E. tereticornis* essential oil, extracted from fresh leaves during winter, summer and rainy seasons on DPPH radical scavenging capacity was evaluated by the determination of the radical

scavenging activity (%). The radical scavenging activity (%) of essential oils from Eucalyptus observed at varying concentrations exhibited distinct trends in different seasons. At a concentration of 0.5%, the activity ranged from 51.32% in winter to 63.15% in summer, with a mean value of 59.13%. Moving to a concentration of 1%, a similar trend was observed, with values spanning from 58.09% in winter to 73.24% in summer, averaging at 68.14%. As the concentration doubled to 2%, a significant increase in radical scavenging activity was noticeable, ranging from 62.55% in winter to 84.16% in summer, reaching a mean value of 76.90%. At the highest concentration of 4%, the activity spiked further, with values ranging between 76.11% in winter to 93.58% in summer and a mean of 87.39%. Overall, there's a clear ascending pattern in radical scavenging activity with increasing concentration across all seasons, showcasing higher efficacy as the concentration of essential oil rises, with the summer season consistently displaying the highest antioxidant potential across all concentrations (Table 2). α -pinene (Lado et al., 2004) and p-cymene (Lee and Shibamoto, 2001) were previously studied and didn't exhibit considerable antioxidant activity. Conversely, γ -terpinene was found to display high activity in prior reports (Ruberto and Baratta, 2000). The antioxidant properties of limonene were also previously documented (Lado et al., 2004). Also, Singh et al. (2009) documented the antioxidant properties found in the essential oil obtained from both fresh and decomposing leaves of *E. tereticornis*.

Table 2 Radical scavenging activity (%) of essential oils obtained from leaves of <i>E. tereticornis</i> harvested during the winter, summer and rainy seasons				
Concentration (%)	Winter	Summer	Rainy	Mean
0.5	51.32	63.15	62.91	59.13
1	58.09	73.24	73.10	68.14
2	62.55	84.16	83.99	76.90
4	76.11	93.58	92.47	87.39
Mean	62.02	78.53	78.11	

3.3. Antifungal Activity

The investigation delved into the antifungal activity of Essential Oils (EOs) against four phytopathogenic fungal strains: *Magnaporthe grisea*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Fusarium oxysporum*. The data has showcasing the impact of escalating EO concentrations on the mycelium growth of these fungal strains (Table 3). Across the winter, summer and rainy seasons, all three EOs exhibited a dose-dependent inhibition of the fungal strains growth. Statistical analysis revealed significant variations in mycelial growth inhibition percentage among the studied fungal strains. *F. oxysporum* and *R. solani* displayed more sensitivity to most EOs, with complete growth inhibition observed at doses ranging from 2 to 4%. Particularly, the EO derived from winter season leaves showcased remarkable effectiveness, resulting in a complete 100% mycelial growth inhibition across all fungal species at various concentrations. Similarly, *M. grisea* was significantly inhibited by EOs from all three seasons, achieving 100% growth inhibition within the 2 to 4% concentration range. In contrast, *R. bataticola* displayed total inhibition at a concentration of 4%. The most potent EO in terms of inhibition was sourced from the winter season, followed by rainy and summer seasons, suggesting diverse chemical components within these oils, necessitating further in-depth exploration to comprehend their varying properties. These findings align with prior research exploring the antifungal activity of Eucalyptus essential oils (EOs) against diverse phytopathogenic fungi, revealing variations in

chemical compositions among Eucalyptus species. In a recent study (Amri et al., 2023), it was observed that Eucalyptus citriodora Hook completely halted the growth of seven Fusarium strains at a concentration of 4 µL/mL. Similarly, another investigation (Kouki et al., 2023) noted that EOs from Eucalyptus oleosa F. Muell. ex Miq. exhibited inhibitory effects on five Fusarium strains with minimum inhibitory concentrations (MICs) of 6 µL/mL.

Moreover, studies like Kim et al. (2012) indicated the antifungal potential of key compounds found in Eucalyptus, including 1,8-cineole, isopulegol, D-limonene and aromadendrene. These compounds, prominent in the Eucalyptus oils studied here, displayed significant antifungal properties, aligning with the present study's outcomes. According to Nakatsu et al. (2000), the antifungal effectiveness of essential oil is attributed to the collective mixture of compounds rather than individual components. Additionally, research suggests that aldehydes, prominent constituents of the oil, bring about irreversible disruption of membranes, inducing electrolyte leakage and altering microbial membrane permeability, thereby inhibiting growth (Avisar et al., 1990).

Table 3
Seasonal variation in growth inhibition percentage of *Eucalyptus tereticornis* essential oils against the fungus strains tested

Microorganisms	Concentration	Winter	Summer	Rainy
Magnaporthe grisea	0.5%	100.00	77.78	80.00
	1%	100.00	86.67	93.33
	2%	100.00	100.00	100.00
	4%	100.00	100.00	100.00
	Control	0.00	0.00	0.00
Rhizoctonia solani	0.5%	91.11	57.78	100.00
	1%	100.00	91.11	100.00
	2%	100.00	100.00	100.00
	4%	100.00	100.00	100.00
	Control	0.00	0.00	0.00
Rhizoctonia bataticola	0.5%	66.67	13.33	35.56
	1%	73.33	40.00	62.22
	2%	80.00	60.00	82.22
	4%	86.67	100.00	100.00
	Control	0.00	0.00	0.00
Fusarium oxysporum	0.5%	100.00	68.89	86.67
	1%	100.00	93.33	100.00
	2%	100.00	100.00	100.00
	4%	100.00	100.00	100.00
	Control	0.00	0.00	0.00

In vitro assessments showcased a dose-dependent inhibition of fungal growth by all EOs, corroborating numerous literature findings on the concentration-dependent antifungal activity of essential oils (Ud-Daula et al., 2016; Su et al., 2006). Insights into the antifungal mechanism reveal that apolar terpenes, characteristic of EOs, can permeate the fungal membrane, disrupting its structure by increasing permeability (Cristani et al., 2007).

Conclusion

The study highlighted the effect of the harvesting time on the essential oils composition and biological activities of *Eucalyptus tereticornis*. The study comprehensively examined *E. tereticornis* essential oils, delineating seasonal variations, chemical compositions and functional attributes. The study identified summer as the optimal harvesting time, yielding the highest oil quantities from fresh and dry leaves, showcasing distinct compositions. This exploration unravelled major compounds in these oils, showcasing distinct compositions between fresh and dry leaves, emphasizing the prevalence of key elements like Eucalyptol, Camphene, α -Eudesmol, Isopulegol, 3-Isopropenyl-6-methyl-1-cyclohexene and Cis-Sabinene hydrate. Notably, the oil displayed concentration-dependent antioxidant efficacy, with summer consistently exhibiting superior radical scavenging potential. Additionally, its potent antifungal activity against key plant-damaging fungi (*Magnaporthe grisea*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Fusarium oxysporum*) underscores its potential in combating fungal threats. The essential oils extracted from the fresh leaves were characterized by their richness in Further studies are still recommended to accurately identify the bioactive molecules in this extract for the exploitation of this plant in various pharmaceutical fields and the promotion of the desired biological activities.

Declarations

Acknowledgements

This work form part of the Ph.D. programme of the first author. The authors acknowledge the laboratory and other facilities provided by different institutions such as CCS HAU, Hisar (Haryana); Vivekananda Global University, Jaipur and ANDUAT, Kumarganj (Ayodhya). We would like to thank R.K. Jha (Professor, DRPCAUI, Pusa) for his thoughtful comments and reviews of an early version of this manuscript.

Author Contribution

Mamta Khaiper and P.K. Poonia have conducted the field experiment and collected sampling of Eucalyptus leaves for extraction of essential oils; Ishu Redhuand Preety Vermacritically done the result of analysis; R.N. Sheokand and Mohammed Nasirfinalized the extraction methods based on availability of instrument as well as resources; Anjali Tiwari and Vikas Kumar wrote the critically manuscript along with arrange journal format for submission. All authors reviewed properly before the submission.

Funding

Not applicable

Availability of data and materials

The raw data were submitted to Depart of Forestry, College of Agriculture, CCS Haryana Agricultural University, Hisar (Haryana).

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interest

Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Conflicts of Interest

The authors have declared that there are no conflicts of interest.

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Figures

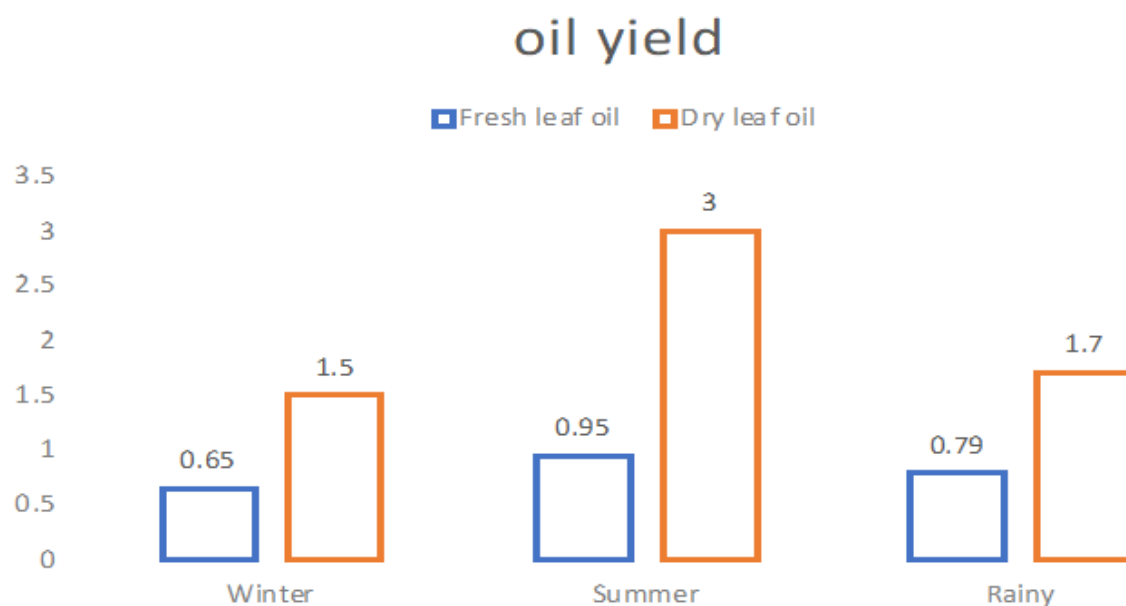


Figure 1

Yield of *E. tereticornis* leaf oils from fresh and dried leaves across winter, summer and rainy seasons

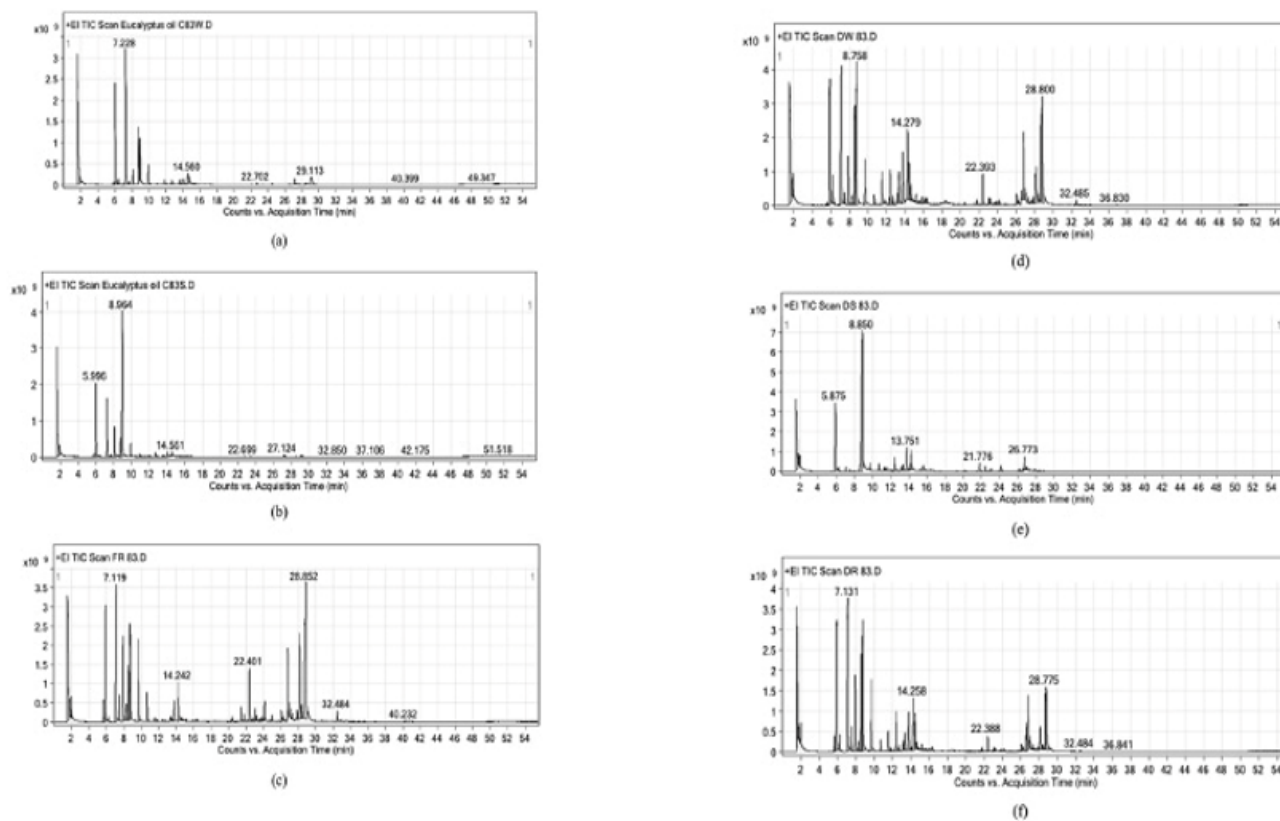


Figure 2

Gas Chromatography–Mass Spectrophotometry (GC-MS) of *E. tereticornis* oil from fresh and dry leaves during winter, summer and rainy season (a) Fresh winter, (b) Fresh summer, (c) Fresh rainy, (d) Dry winter, (e) Dry summer and (f) Dry rainy