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ARTICLE

Characterization and Antioxidant Activity of Essential Oils from Fresh and Decaying Leaves of *Eucalyptus tereticornis*

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The composition of essential oils hydrodistilled from fresh and decaying leaves of *Eucalyptus tereticornis* was analyzed by means of gas chromatography and mass spectrometry, and a total of 68 constituents were identified. The essential oils were assayed for antioxidant activity in terms of scavenging of 2,2-diphenyl-1-picrylhydrazil (DPPH) and hydroxyl ($OH^{\bullet}$) radical, and superoxide anion ($O_2^{-\bullet}$).The major constituents of the fresh leaf oil were α-pinene (28.53%) and 1,8-cineole (19.48%), whereas in the decaying leaf oil, β-citronellal (14.15%), (−)-isopulegol (13.35%), and (+)-β-citronellol (10.73%) were the major components. Both essential oils exhibited a strong radical scavenging activity against DPPH radical with IC_{50} values of 110 and 139.8 μg/mL for fresh and decaying leaf oil, respectively (IC_{50} of BHT = 164.2 μg/mL). Further, the essential oils (at 400 μg/mL) also exhibited $OH^{\bullet}$ (56−62%) and $O_2^{-\bullet}$ (65−69%) scavenging activity parallel to the commercial antioxidant BHT/ascorbic acid. However, unlike the essential oils, the major monoterpene constituents exhibited significantly less scavenging activity (<35% DPPH or $OH^{\bullet}$; at 400 μg/mL). The study concluded that fresh and decaying leaves of *E. tereticornis* are a source of monoterpenoid rich oil exhibiting antioxidant activity.

KEYWORDS: ***Eucalyptus tereticornis*** **(forest red gum); fresh and decaying leaf oil; GC−MS analyses; monoterpenoids; antioxidant activity**

INTRODUCTION

Plant essential oils consist of a complex mixture of a variety of compounds, including mono- and sesquiterpenes, alcohols, esters, aldehydes, ketones, etc. Within the plant, they act as signal molecules and also provide defense against a variety of pests, including herbivores, fungi, and bacteria (*1*). In fact, essential oils and aromatic plants have been known for centuries for their uses in flavor and fragrance, as preservatives, and as antimicrobials (*1*, *2*). Of late, because of the toxicological implications of synthetic products, there has been a renewed interest in the use and exploration of essential oils as natural antioxidants and preservatives in the food and flavor industry (*2*−*4*).

Under normal conditions, living cells protect themselves from the highly reactive oxygen species (ROS) [singlet oxygen ($_1O^2$), superoxide ion ($O_2^{-\bullet}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2)] that are generated within the cells as byproducts of normal metabolism. If not scavenged, these ROS damage proteins, lipids, enzymes, and nucleic acids, result in oxidative injury and disease induction, and eventually lead to death (*5*). However, with aging and in response to external factors such as pollutants, ionizing radiations, tobacco smoke, pesticides, and pathogens, the generation of ROS and the risk of ROS-induced injury increase manyfold (*5*). To overcome these negative effects, consumption of a diet rich in antioxidant compounds or dietary supplements of synthetic antioxidants is required (*6*).

Eucalyptus L′ Herit is a large genus with ∼700 species of tall evergreen trees distributed throughout the world (*7*). In fact, *Eucalyptus* species are one of the most extensively planted pulpwood species. The trees are planted largely for their leaves that are rich in essential oils and are exploited commercially for their use in food, flavor, pharmaceutical, and perfumery industries (*7*). The major oil-yielding *Eucalyptus* species include *Eucalyptus citriodora* (lemon-scented gum), *Eucalyptus globulus* (Tasmanian blue gum), *Eucalyptus camaldulensis* (river red gum), *Eucalyptus polybractea* (blue mallee), and *Eucalyptus staigeriana* (lemon-scented ironbark). The essential oil from *Eucalyptus* is a primary oil in the world's major oil trade industry (*8*). However, there are certain other species of *Eucalyptus* that have been planted extensively under various afforestation/reforestation programs and for paper/pulp industry in various parts of the world (*9*). These include *Eucalyptus saligna*, *Eucalyptus grandis*, and *Eucalyptus tereticornis*. Among these, *E. tereticornis* (forest red gum) is the most commonly planted species under various forestry programs in India (*9*). It is planted for its heartwood because it is strong and hard and is used for timber and railway sleepers (*10*).

The leaves from a number of *Eucalyptus* species are a rich source of essential oils that possess a wide range of biological properties, including antimicrobial, fungicidal, insect repellent, herbicidal, and acaricidal activity (*1*). However, no attempt has been made to characterize the antioxidant activity of essential oils

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extracted from fresh and decaying leaves (left in the field as waste upon tree harvest) of *E. tereticornis*. Thus, the aim of this study was (a) to investigate the chemical composition of the essential oils isolated from fresh and decaying leaves of *E. tereticornis* and (b) to determine the antioxidant and DPPH radical scavenging activities of essential oils and their major constituents, in vitro.

MATERIALS AND METHODS

Materials. In April 2007, fresh leaves were plucked and decaying leaves were collected from the floor of ~22-year-old trees of *E. tereticornis* Sm. growing on the campus of Panjab University. These were placed in polythene bags, brought to the laboratory, cleaned of adhered materials and soil particles, and used for extraction of oil. The identification of the trees was authenticated by the herbarium, Department of Botany, Panjab University, and the voucher specimens of the plant material have been deposited there.

The pure reference compounds, viz., limonene, α-pinene, and β-pinene (Lancaster, Morecambe, England); (−)-linalool, β-citronellal, β-eudesmol, *cis*-jasmone, (+)-β-citronellol, (−)-β-citronellol, α-terpineol, α-terpinyl acetate, and (−)-isopulegol (Sigma-Aldrich Co., St. Louis, MO); β-myrcene and citronellyl acetate (AlfaAesar); 1,8-cineole, *p*-cymene, terpinen-4-ol, and α-terpinene (Acros Organics, Geel, Belgium); and *trans*-β-caryophyllene and caryophyllene oxide (TCI, Tokyo, Japan), were purchased. All other chemicals and reagents used in the study for biochemical estimations were of technical grade and purchased from Sisco Research Laboratory Pvt., Ltd., Sigma-Aldrich Co., Merck Ltd., or Loba-Chemie Pvt., Ltd.

Essential Oil Extraction. Essential oil was extracted from fresh and decaying leaves of *E. tereticornis* by hydrodistillation using a Clevenger's apparatus (*11*, *12*). Fresh or decaying leaves (~500 g) were boiled in 3 L of distilled water for 5 h in a round-bottom flask fitted with a condenser. The oils were collected from the nozzle of the condenser, dried over sodium sulfate, and stored at 4 °C for further identification by gas chromatography (GC) and gas chromatography−mass spectroscopy (GC−MS) and antioxidant studies.

GC and GC−MS Identification. The essential oils were analyzed by GC and GC−MS as described by Singh et al. (*11*, *12*). GC was conducted on a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector (FID) and a DB-5 column [60 m × 0.25 mm (inside diameter), film thickness of 0.25 μm]. Helium (He) was used as the carrier gas at a split ratio 1:20, and the column flow rate was 1 mL/min. The injector and ion source temperatures were set at 250 and 280 °C, respectively. The oven temperature was programmed from 50 °C (held isothermally for 2 min) to 260 °C with a ramp of 4 °C/min and held at 260 °C for 3 min. Relative amounts of different components were determined by computerized peak area normalization without any correction factor and were based upon three injections of the oil.

All the oil samples were then analyzed by GC−MS using a Shimadzu QP 2010 mass spectrophotometer equipped with fused silica (SGE BP 20) capillary columns [30 m × 0.25 mm (inside diameter), 25 μm film thickness] using helium (He) as a carrier gas at a split ratio of 1:50 and a linear velocity of 38.5 cm/s. The injector and ion detector temperatures were set at 220 and 250 °C, respectively. The oven temperature was programmed from 70 °C (held for 4 min) with a ramp of 4 °C/min to 220 °C (held for 5 min). The mass spectra were scanned in the range of m/z 40−600. Different constituents were identified on the basis of (i) coelution and comparison of retention times with those of pure reference samples, (ii) comparison of their retention indices (RI) relative to homologus series of *n*-alkanes (C_7−C_{30}; Supelco, Bellefonte, PA), (iii) computer matching of mass spectra with the HP-5872 library (Hewlett-Packard), and (iv) consulting libraries of Wiley 275 and NBS 75K (*13*), NIST 98 (*14*), and the compilation by Adams (*15*).

Antioxidative Capacity. The antioxidant capacity of fresh and decaying leaf oil and their major constituents [α-pinene, 1,8-cineole, β-citronellal, (−)-isopulegol, and (+)-β-citronellol] was evaluated in terms of DPPH (2,2-diphenyl-1-picrylhydrazil) free radical, hydroxyl radical, and superoxide anion scavenging activity.

Free Radical Scavenging Capacity (RSC). RSC was evaluated by measuring scavenging activity against DPPH as described by Bozin et al. (*16*). Briefly, samples (essential oil and major components in the 25−400 μg/mL range) were mixed with 1 mL of a 90 μM DPPH (Fluka, Buchs, Switzerland) solution in methanol, and the final volume was made 4 mL with methanol. A parallel blank (without sample) and positive control (BHT, *tert*-butylated hydroxytoluene) were also maintained. The solutions were incubated for 1 h in the dark at 25 °C, and the absorbance was read at 515 nm on a Shimadzu UV-190 spectrophotometer. Three replicates were maintained for each sample. A decrease in the absorbance of the DPPH solution indicates an increased RSC. It was calculated in percent using the following formula:

$$\text{RSC}\ (\%) = 100(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}})$$

The concentration of the essential oils and major monoterpenes that caused 50% neutralization and/or scavenging (IC_{50} values) was determined using regression analysis.

Hydroxyl Radical ($OH^{\bullet}$) Scavenging Activity. It was assessed in terms of competition between 2-deoxyribose and samples (essential oil and major components) for $OH^{\bullet}$ generated via the Fenton reaction. $OH^{\bullet}$ degrades 2-deoxyribose to form thiobarbituric acid reactive substance (TBARS) that could be measured at 532 nm (*12*). The reaction mixture (3 mL) contained 3 mM 2-deoxyribose, 0.1 mM $FeCl_3$, 1 mM H_2O_2, 0.1 mM EDTA, 0.1 mM ascorbic acid, 0.02 M phosphate buffer (pH 7.4), and a sample solution (25−400 μg/mL). It was incubated at 37 °C for 1 h, followed by addition of 1 mL each of 1% TBA and 2.8% trichloroacetic acid, and heated at 100 °C for 20 min. The test tubes were cooled, and the absorbance was read at 532 nm against a blank containing phosphate buffer and 2-deoxyribose. A parallel control with BHT was also maintained. The percent inhibition (I) of 2-deoxyribose decomposition was calculated using the formula

$$I\ (\%) = 100(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}})$$

Superoxide Anion ($O_2^{-\bullet}$) Scavenging Activity. $O_2^{-\bullet}$ was generated by the phenazine-methosulfate (PMS)-NADH method and assayed in terms of inhibition of nitroblue tetrazolium (NBT) reduction (*17*) with a slight modification (*18*). The reaction mixture contained 16 mM Tris-HCl buffer (pH 8), 50 μM (NBT), 80 μM NADH, and sample solutions (25−400 μg/mL). The reaction was started by adding 0.5 mL of 10 μM PMS, and the reaction mixture was incubated at 25 °C for 5 min. Ascorbic acid was used as a parallel control. $O_2^{-\bullet}$ scavenging activity was calculated by measuring the decrease in absorbance at 560 nm against a blank without sample solutions, using the following formula:

$$I\ (\%) = 100(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}})$$

RESULTS AND DISCUSSION

Chemical Composition of the Volatile Oil. The essential oil obtained upon hydrodistillation of fresh leaves of *E. tereticornis* was pale yellow in color with a yield of 1.18% (v/w, on a fresh weight basis). In contrast, the oil hydrodistilled from decaying leaves was dark yellowish to light brown in color with a yield of 0.13% (v/w, fresh weight). The GC−MS analyses of fresh and decaying leaves revealed the presence of 43 and 48 constituents, respectively, eluted between 2 and 35 min. Of these, 42 and 47 components constituting 99.87 and 99.88%, respectively, were identified in fresh and decaying leaf oil (**Table 1**). In general, the oils were monoterpenoid in nature. The fresh leaf oil contained 50.67% oxygenated compounds, 23 monoterpenes (~71%) and 19 sesquiterpenes (28.85%) (**Table 1**). In contrast, the decaying leaf oil contained 85.50% oxygenated compounds, 29 monoterpenes (~83.6%) and 14 sesquiterpenes (15.39%) (**Table 1**). The major constituents of the fresh leaf oil were α-pinene (28.53%) and 1,8-cineole (19.48%). In contrast, in the decaying leaf oil, β-citronellal (14.15%), (−)-isopulegol (13.35%), and (+)-β-citronellol (10.73%) were the major components (**Table 1**). All three major monoterpenoid constituents of decaying leaf oil were absent in fresh leaf oil. Likewise, the isopulegols that constituted ~21% of the decaying leaf oil were absent in the fresh leaf oil

Table 1. Chemical Characterization of the Essential Oil Extracted from Fresh and Decaying Leaves of *E. tereticornis*

RI[a]	component[b]	% age[c] fresh leaf oil	% age[c] decaying leaf oil	identification method[d]
	Monoterpene Hydrocarbons			
1038	α-pinene	28.53	6.03	co-GC, MS, RI
1077	α-thujene	0.01	–	MS, RI
1083	camphene	0.04	–	MS, RI
1114	β-pinene	5.33	1.41	co-GC, MS, RI
1127	sabinene	0.04	–	MS, RI
1163	β-myrcene	0.54	–	co-GC, MS, RI
1167	α-phellandrene	1.32	–	MS, RI
1172	α-terpinene	0.20	–	co-GC, MS, RI
1204	limonene	3.29	0.84	co-GC, MS, RI
1245	γ-terpinene	6.58	0.12	co-GC, MS, RI
1272	*p*-cymene	1.01	1.24	co-GC, MS, RI
1283	terpinolene	1.08	–	MS, RI
	Oxygenated Monoterpenes			
1212	1,8-cineole	19.48	6.58	co-GC, MS, RI
1353	*cis*-rose oxide	–	1.46	MS, RI
1367	*trans*-rose oxide	–	0.62	MS, RI
1427	dihydromyrcenol	–	0.27	MS, RI
1480	β-citronellal	–	14.15	co-GC, MS, RI
1537	*iso*-pinocamphone	–	0.19	MS, RI
1553	(−)-linalool	0.04	–	co-GC, MS, RI
1559	isoisopulegol	–	7.39	MS, RI
1569	(−)-isopulegol	–	13.35	co-GC, MS, RI
1584	*endo*-fenchol	0.01	0.60	MS, RI
1601	terpinen-4-ol	0.84	–	co-GC, MS, RI
1609	*p*-menth-3-en-8-ol	–	0.14	MS, RI
1624	isopulegol isomers	–	0.60	MS, RI
1653	*trans*-pinocarveol	0.04	2.23	MS, RI
1660	(−)-β-citronellol	–	0.54	co-GC, MS, RI
1665	citronellyl acetate	–	8.98	co-GC, MS, RI
1672	(+)-carvotanacetone	0.02	–	MS, RI
1688	limonen-4-ol	0.13	–	MS, RI
1693	α-terpinyl acetate	–	0.56	co-GC, MS, RI
1698	α-terpineol	2.38	0.57	co-GC, MS, RI
1700	borneol	–	0.21	MS, RI
1723	2,3-pinanediol	0.03	–	MS, RI
1772	(+)-β-citronellol	–	10.73	co-GC, MS, RI
1793	myrtenol	–	0.18	MS, RI
1799	*trans*-*p*-mentha-1(7),8-dien-2-ol	–	0.12	MS, RI
1853	*p*-cymen-8-ol	0.05	–	MS, RI
1938	*cis*-jasmone	–	0.16	co-GC, MS, RI
2102	*p*-menthane-3,8-diol	0.03	2.44	MS, RI
2136	tetrahydrogeranylacetone	–	0.11	MS, RI
2261	citronellic acid	–	1.78	MS, RI
	Sesquiterpene Hydrocarbons			
1483	α-cubebene	0.05	–	MS, RI
1496	α-copaene	0.09	–	MS, RI
1587	*trans*-β-caryophyllene	0.43	2.15	co-GC, MS, RI
1595	aromadendrene	0.46	1.57	MS, RI
1633	*allo*-aromadendrene	0.05	0.13	MS, RI
1658	α-humulene	0.07	–	MS, RI
1706	α-selinene	0.03	–	MS, RI
1750	germacrene D	0.05	–	MS, RI
	Oxygenated Sesquiterpenes			
1969	caryophyllene oxide	–	2.37	co-GC, MS, RI
2006	*epi*-globulol	0.06	0.50	MS, RI
2070	globulol	0.70	3.37	MS, RI
2075	viridiflorol	0.14	0.29	MS, RI
2110	rosifoliol	0.02	–	MS, RI
2120	spathulenol	–	0.38	MS, RI
2166	γ-eudesmol	3.80	0.24	MS, RI
2174	guaiol	2.90	0.19	MS, RI
2182	(−)-aristolene	0.47	–	MS, RI
2185	hinesol	0.72	0.84	MS, RI
2212	7-epi-α-cadinol	0.16	–	MS, RI
2217	α-eudesmol	8.43	0.40	MS, RI
2225	β-eudesmol	10.22	2.71	co-GC, MS, RI
2294	caryophylla-4(12), 8(13)-dien-5β-ol	–	0.25	MS, RI
	Other Compounds			
1355	melonal	–	0.32	MS, RI
1337	unidentified	–	0.11	–
1342	neohexane	–	0.12	MS, RI
1422	*tert*-butanol	–	0.15	MS, RI
1994	γ-nonanoic lactone	–	0.30	MS, RI
2117	unidentified	0.04	–	–
total monoterpenoids		71.02	83.60	
monoterpenoid hydrocarbons		47.97	9.64	
oxygenated monoterpenoids		23.05	73.96	
total sesquiterpenes		28.85	15.39	
sequiterpene hydrocarbons		1.23	3.85	
oxygenated sesquiterpenes		27.62	11.54	
others		0.04	1.0	
total identified		**99.87**	**99.88**	

[a] Retention index relative to *n*-alkanes (C_{7-30}) on the SGE-BP20 capillary column. [b] Compounds presented in order of elution from the SGE-BP20 capillary column. [c] Percentage based on FID peak area normalization (n = 3). [d] Methods: co-GC, identification based on retention times of authentic reference compounds on the DB-5 column; MS, tentatively identified on the basis of computer matching of mass spectra of peaks with HP-5872, Wiley 275, NBS 75K, and NIST 98 libraries; RI, tentatively identified on the basis of matching of the retention index with published literature.

(**Table 1**). α-Pinene and 1,8-cineole that were the major monoterpenoids in fresh leaf oil constituted only 6.03 and 6.58%, respectively, of decaying leaf oil. Among the sesquiterpenes, β-eudesmol and globulol were the major oxygenated sesquiterpenes in fresh and decaying leaf oil, respectively (**Table 1**). The presence of 1,8-cineole and α-pinene as the major components in fresh leaf oil of *E. tereticornis* is in agreement with earlier reports (*19*−*22*). However, to the best of our knowledge, this is the first time an attempt has been made to characterize the essential oil from decaying leaves of *E. tereticornis*.

Antioxidant Activity. Since the oil was rich in monoterpenoids, we evaluated antioxidant activity of crude young and decaying leaf oils and their major constituents [α-pinene, 1,8-cineole, β-citronellal, (−)-isopulegol, and (+)-β-citronellol] and in terms of DPPH free radical, hydroxyl radical ($OH^{\bullet}$), superoxide anion ($O_2^{-\bullet}$) scavenging activity. The oil from fresh and decaying leaves of *E. tereticornis* exhibited a strong DPPH radical scavenging capacity (RSC) and converted DPPH radical into its reduced form (**Table 2**). At 100 μg/mL, fresh and decaying leaf oils showed RSC of ∼64 and ∼54%, respectively. RSC increased further with the concentration of crude oil, and at 400 μg/mL, fresh and decaying leaf oil exhibited RSC of ∼98% (**Table 2**).The activity of fresh leaf and decaying leaf oil (with IC_{50} values of 110 and 139.8 μg/mL, respectively) was even greater than that of commercially used antioxidant BHT (IC_{50} = 164.2 μg/mL). However, the RSC of all five major constituents was much lower compared to that of crude oil (**Table 2**). Their activity was in the range of 12−20 and 20−35% at 200 and 400 μg/mL, respectively. In general, the

Table 2. DPPH Free Radical Scavenging Activity of Essential Oil Extracted from Fresh and Decaying Leaves of *E. tereticornis*, α-Pinene, 1,8-Cineole, β-Citronellal, (−)-Isopulegol, (+)-β-Citronellol, and BHT[a]

concn (μg/mL)	fresh leaf oil	decaying leaf oil	α-pinene	1,8-cineole	β-citronellal	(−)-isopulegol	(+)-β-citronellol	BHT
25	17.20 ± 0.45 a	15.52 ± 0.54 a	2.19 ± 0.32 a	1.76 ± 0.15 a	2.57 ± 0.21 a	6.68 ± 0.30 a	1.96 ± 0.13 a	13.31 ± 0.12 a
50	32.78 ± 1.36 b	31.98 ± 1.69 b	7.74 ± 0.09 b	6.81 ± 0.28 b	5.15 ± 0.25 a	11.99 ± 0.08 b	4.41 ± 0.21 a	23.63 ± 0.45 b
100	63.85 ± 2.09 c	53.98 ± 0.36 c	13.12 ± 0.31 c	11.93 ± 0.29 c	7.84 ± 0.12 b	17.11 ± 0.30 c	8.45 ± 0.56 b	40.29 ± 0.65 c
200	82.80 ± 2.90 d	65.59 ± 2.70 d	16.2 ± 0.71 d	16.08 ± 0.30 d	11.77 ± 0.77 c	20.07 ± 0.68 d	13.35 ± 0.86 c	66.67 ± 0.13 d
400	98.48 ± 1.28 e	98.12 ± 1.80 e	35.63 ± 0.41 e	31.33 ± 1.31 e	20.03 ± 0.75 d	34.52 ± 1.41 e	23.67 ± 0.80 d	96.23 ± 1.56 e
IC_{50} (μg/mL)	110.0	139.8	581.4	653.1	1067.4	626.8	861.5	164.2

[a] Data presented as means ± the standard error. Different letters within a column for a particular treatment represent significance at $P < 0.01$ applying Tukey's test.

Table 3. Hydroxyl Radical (OH•) Scavenging Activity of Essential Oil Extracted from Fresh and Decaying Leaves of *E. tereticornis*, α-Pinene, 1,8-Cineole, β-Citronellal, (−)-Isopulegol, (+)-β-Citronellol, and BHT[a]

concn (μg/mL)	fresh leaf oil	decaying leaf oil	α-pinene	1,8-cineole	β-citronellal	(−)-isopulegol	(+)-β-citronellol	BHT
25	9.23 ± 0.08 a,A	7.69 ± 0.20 a,A	1.06 ± 0.06 a,A	1.95 ± 0.24 a,A	6.67 ± 0.70 a,A	0.95 ± 0.18 a,A	8.76 ± 0.78 a,A	15.93 ± 0.90 a,A
50	17.43 ± 1.05 b,B	16.03 ± 1.28 b,B	2.77 ± 0.33 b,B	3.22 ± 0.30 a,AB	10.5 ± 1.23 b,A	6.07 ± 0.34 b,B	16.12 ± 0.70 b,B	35.73 ± 1.24 b,B
100	30.91 ± 1.44 c,C	26.28 ± 0.64 c,C	5.82 ± 0.37 c,C	5.86 ± 0.73 b,B	15.35 ± 0.35 c,B	10.10 ± 0.40 c,C	20.65 ± 0.56 c,B	73.23 ± 1.93 c,C
200	42.89 ± 1.80 d,D	34.62 ± 0.56 d,D	9.46 ± 0.24 d,D	10.63 ± 0.65 c,C	18.59 ± 0.53 d,B	18.71 ± 0.43 d,D	32.53 ± 1.50 d,C	87.74 ± 2.11 d,D
400	56.37 ± 1.24 e,E	62.71 ± 2.48 e,E	16.06 ± 0.43 e,E	17.01 ± 0.44 d,D	28.81 ± 1.02 e,C	31.03 ± 1.45 e,E	48.23 ± 0.64 e,D	100 e,E
IC_{50} (μg/mL)	312.4	304.3	1261.5	1208.2	777.5	632.9	402.1	85.78

[a] Data presented as means ± the standard error. Different lowercase and uppercase letters within a column for a particular treatment represent significance at $P < 0.05$ and 0.01, respectively, applying Tukey's test.

Table 4. Superoxide Anion ($O_2^{-\bullet}$) Scavenging Activity of Essential Oil Extracted from Fresh and Decaying Leaves of *E. tereticornis*, α-Pinene, 1,8-Cineole, β-Citronellal, (−)-Isopulegol, (+)-β-Citronellol, and Ascorbic Acid[a]

concn (μg/mL)	fresh leaf oil	decaying leaf oil	α-pinene	1,8-cineole	β-citronellal	(−)-isopulegol	(+)-β-citronellol	ascorbic acid
25	8.97 ± 0.11 a,A	7.30 ± 0.62 a,A	6.70 ± 0.72 a,A	5.23 ± 0.26 a,A	6.51 ± 0.47 a,A	8.64 ± 0.59 a,A	7.40 ± 0.19 a,A	6.04 ± 0.57 a,A
50	19.86 ± 0.77 b,B	17.53 ± 0.63 b,B	12.10 ± 0.96 b,A	8.50 ± 0.15 b,A	12.50 ± 0.55 b,A	15.48 ± 0.77 b,B	12.98 ± 0.21 b,A	24.47 ± 0.58 b,B
100	38.75 ± 0.39 c,C	32.40 ± 1.28 c,C	21.67 ± 1.45 c,B	14.73 ± 0.52 c,B	16.03 ± 0.64 c,B	20.95 ± 0.56 c,C	14.83 ± 0.33 c,B	43.21 ± 0.42 c,C
200	53.91 ± 2.03 d,D	41.42 ± 0.70 d,D	34.50 ± 1.56 d,C	24.09 ± 1.15 d,C	19.69 ± 0.45 d,C	26.54 ± 1.29 d,D	26.56 ± 0.24 d,C	54.98 ± 1.29 d,D
400	69.12 ± 1.15 e,E	65.51 ± 1.39 e,E	48.23 ± 0.77 e,D	37.90 ± 0.67 e,D	29.30 ± 0.76 e,D	40.37 ± 0.55 e,E	43.64 ± 0.85 e,D	76.00 ± 1.50 e,E
IC_{50} (μg/mL)	234.5	274.7	390.0	526.6	769.5	512.3	462.5	207.5

[a] Data presented as means ± the standard error. Different lowercase and uppercase letters within a column for a particular treatment represent significance at $P < 0.05$ and 0.01, respectively, applying Tukey's test.

activity of major monoterpenoids decreased in the following order: (−)-isopulegol > α-pinene > 1,8-cineole > (+)-β-citronellol > β-citronellal (**Table 2**). The RSC of fresh and decaying leaf oils was greater than the sum total of their major constituents. It indicated that different monoterpenes are acting in synergism to determine the overall RSC of crude oil.

Further, we evaluated the scavenging activity of decaying leaf oil against OH• generated via Fenton reaction using 2-deoxyribose. Unlike RSC, the OH• scavenging activity of fresh and decaying leaf oils and major components was lower. At 400 μg/mL, fresh and decaying leaf oils showed OH• scavenging activity of 56.4 and 62.7%, respectively (**Table 3**). Among the five major monoterpenes, the OH• scavenging activity of (+)-β-citronellol was the maximum followed in decreasing order by (−)-isopulegol > β-citronellal > 1,8-cineole > α-pinene (**Table 3**). The OH• scavenging activity of decaying and fresh leaf oil had IC_{50} values of 304.3 and 312.4 μg/mL, respectively, that was much higher than the value of 85.78 μg/mL for commercial antioxidant BHT.

The crude oil hydrodistilled from fresh and decaying leaves of *E. tereticornis* also exhibited $O_2^{-\bullet}$ scavenging activity with IC_{50} values of 234.5 and 274.7 μg/mL, respectively, whereas that of the antioxidant ascorbic acid was 207.5 μg/mL (**Table 4**). In general, the activity of crude oil from both fresh and decaying leaves was greater than that of five major constituents that were tested for their scavenging activity (**Table 4**). The $O_2^{-\bullet}$ scavenging activity of the major constituents was in the range of 29−48% at a concentration of 400 μg/mL. Among these, α-pinene showed the maximum activity, and it was followed by (+)-β-citronellol and then (−)-isopulegol (**Table 4**).

Though it is the first time that antioxidant activity of essential oil of *E. tereticornis* has been reported, earlier studies reported antifungal, antimicrobial, larvicidal, and herbicidal activities of essential oil from *E. tereticornis* (*1*, *19*, *23*−*25*). Though the eucalyptus oil has been widely used in various food, fragrance, and pharmaceutical industries, very few studies have been undertaken to evaluate the antioxidant and radical scavenging properties. The observed antioxidant activity of essential oil from fresh and decaying leaves of *E. tereticornis* parallels other studies reporting antioxidant activity of essential oils from other *Eucalyptus* species [*E. globulus* (*26*), *Eucalyptus polyanthemos*, and *Eucalyptus perriniana* (*27*)].

The study concludes that not only the fresh green leaves of *E. tereticornis* but also the decaying leaves present on the floor could serve as an important bioresource for the extraction of monoterpenoid (~81%) rich oil. The radical scavenging and antioxidant activity further implies its use in oxidative disease protection and as a natural antioxidant in the food industry.

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