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Full Length Research Paper

Chemical characterization, antioxidant and antifungal activity of essential oil from *Eucalyptus tereticornis*

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Eucalyptus tereticornis **(red gum tree, family Myrtaceae), is extensively grown throughout India under various afforestation programmes, primarily for the supply of wood, timber. Unlike, other species of eucalypt known for the presence of volatile oil in their foliage, not much information is available regarding the volatile oils of *E. tereticornis* and their bioactivity. We extracted and characterized the essential oils from its mature (adult) leaves and determined its antioxidant and antifungal activity against phytopathogenic fungi. Gas chromatography mass spectrometry (GC/GC-MS) analyses revealed that the oil, in general, was monoterpenoid in nature with 37 constituents. Among these, α-pinene was the major component (~30%) followed by 1,8-cineole (~22%). We determined the antioxidant activity of the oil (25-200 µg/ml) in terms of free radical scavenging (as DPPH activity), and H_2O_2 quenching. Further, the essential oil (0.5 – 10.0 ppm) exhibited a strong fungitoxicity against three phytopathogenic fungi viz. *Fusarium oxysporum*, *Rhizoctonia solani* and *Heminthosporium oryzae*. The antioxidant and fungitoxicity of the oil was compared to commercially used antioxidant (BHT) and fungicide mancozeb, respectively. The study concludes that essential oil from foliage of *E. tereticornis* possesses a strong antioxidant and antifungal activity worth exploitation.**

Key words: *Eucalyptus tereticornis*, volatile oil, gas chromatography mass spectrometry (GC/GC/MS), antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH), antifungal activity.

INTRODUCTION

Reactive oxygen species (ROS) including singlet oxygen ($_1O^2$), superoxide ion ($O_2^{-\bullet}$), hydroxyl ion ($OH^\bullet$) and hydrogen peroxide (H_2O_2) are highly reactive and toxic molecules produced in cells under normal metabolic processes. If not scavenged, they cause cellular damage resulting in serious health disorders. In fact, living cells possess a well organized scavenging mechanism to control ROS-induced cellular injury. However, ageing and external stresses enhance the production of ROS, thus making self-defense mechanism inefficient. Therefore, synthetic antioxidants are supplemented along with food stuffs. However, of late, due to toxicological concerns linked with synthetic compounds, the impetus has shifted to look for plant derived products as food preservatives and antioxidants (Peschel et al., 2006). In this context, essential oils from aromatic plants hold immense potential owing to their characteristic features including easy penetrability even under oxygen deficiency (Onocha et al., 2011). Inside cell, essential oils serve as powerful scavenger preventing mutations and oxidants in cells (Bakkali et al., 2008). Further, essential oils have been known to possess antifungal, antibacterial and pesticidal properties (Batish et al., 2008; Hussain et al., 2008; Pinto et al., 2007).

Eucalyptus, one of the most important genera of Myrtaceae family, is composed of large evergreen trees and shrubs of ~700 species (Batish et al., 2008; Marzoug et al., 2011). *Eucalyptus* trees have been a major source of pulp wood for high quality paper production and are grown under various afforestation/reforestation programs. Moreover, aerial parts of tree are rich source of essential oils that have been used commercially in food, flavor and perfumery and pharmaceutical industries (Batish et al., 2008; Singh et al., 2009). Earlier, studies have reported the phytotoxicity, radical scavenging and weed

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suppressing ability of oils from *Eucalyptus* species including *E. tereticornis* (Batish et al., 2008; Kaur et al., 2010). However, not much has been done to explore the antioxidant and antifungal activity of essential oils from leaves of *E. tereticornis*. The present study was therefore conducted to evaluate antioxidant and antifungal activity of *E. tereticornis* oil. The antioxidant activity was determined in terms of DPPH scavenging and H_2O_2 scavenging. The antifungal activity was evaluated against three fungal pathogens viz. *Fusarium oxysporum*, *Rhizoctonia solani* and *Helminthosporium oryzae*.

MATERIALS AND METHODS

Extraction of oil

Volatile oil was extracted using Clevenger's apparatus from freshly collected mature (adult) leaves of *E. tereticornis* growing in Panjab University Campus Chandigarh, India. Fresh leaves (250 g) were chopped and mixed with 1 L distilled water in a 2-L round bottom flask and fitted with condenser. The mixture was boiled for 3 h and oil was collected from the nozzle of the condenser, dried under sodium sulphate and stored at 4°C for further use.

Analysis of oil

The essential oil was analyzed by GC/MS on a Shimadzu QP 2010 mass spectrophotometer fitted with a flame ionization detector (FID) and coupled with a GC 2010 gas chromatograph, and equipped with SGE-BP20 polar column (30 m × 0.25 mm, i.d.; 0.25 µm film thickness). Helium (He) at a split ratio of 50:1 and a linear velocity of 38.5 cm s^{-1} was used as carrier gas. Initially, the oven temperature was 40°C held for 4 min, then programmed to 220°C at 4°C / min and held isothermally for 5 min. Mass spectral range was recorded from *m/z* 40 to 600 amu. The injector and detector temperature were 220 and 250°C, respectively.

Relative percent amount of different constituents was calculated automatically from peak area of total ion chromatograms without any correction factor. The data presented are mean percent values of three analyses. Constituents of the oil were identified by comparison of their retention indices relative to homologous *n*-alkanes (C_8–C_{32}) series and computer matching of their mass spectra with those of reference compounds in Wiley 275 and NBS 75K libraries (Adams, 2007; McLaffertty, 1989; Stein, 1998).

Procurement of test fungi

Cultures of test fungi *F. oxysporum*, *R. solani* and *H. oryzae* were procured from the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi, India and maintained on a suitable medium until used.

Antioxidant activity

DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging activity

It was evaluated by measuring the scavenging activity of essential oil and its two major monoterpenes on 2,2-diphenyl-1-picrylhydrazil (DPPH) as per Bozin et al. (2006). Briefly, sample (25 to 200 µg/ml range) solutions were mixed into 1 ml of 90 µM DPPH (Fluka) solution in methanol and final volume made to 4 ml with methanol. The absorbance of the solutions including blank (without sample) and positive control (BHT, *tert*-butylated hydroxytoluene) was read at 515 nm after 1 h incubation in the dark at room temperature on Shimadzu UV-190 spectrophotometer. Each sample assay was carried out in triplicate and data presented is mean of three. A decrease in absorbance of DPPH solution indicates increased DPPH radical scavenging activity. It was calculated in percent using the following formula:

$$\% \text{ DPPH radical scavenging} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

Hydrogen peroxide (H_2O_2) scavenging activity

H_2O_2 scavenging activity was examined as per the method of Ruch et al. (1989). A 40 mM solution of H_2O_2 was prepared in phosphate buffer (pH 7.4). The concentration of H_2O_2 was determined spectrophotometrically at 230 nm. Different concentrations (25 to 200 µg/ml) of volatile oil were added to 0.6 ml of H_2O_2 (40 mM) and absorbance of the solution was read at 230 nm after 10 min against blank containing volatile oil in phosphate buffer without H_2O_2. The percent scavenging of H_2O_2 by essential oil was calculated using the formula:

$$\% \text{ scavenging of } H_2O_2 = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Antifungal activity

The antifungal activity of essential oil (0.5, 1, 2.5, 5, and 10 ppm) was tested against the three test fungi by food poison technique (Grover and Moore, 1962). An aliquot (1 ml) of each concentration was then added to 20 ml of PDA (potato dextrose agar) for *R. solani* and CMA (corn meal agar) for *H. oryzae* and *F. oxysporum* and mixed thoroughly. Thereafter, a mycelial disc of approximately 5 mm diameter, cut from the periphery of an 8-day old culture, was inoculated in the centre of each Petri dish. Tween–80 (0.05%) mixed with distilled water instead of oil served as the control. For each treatment, five independent Petri dish replicates were maintained in a completely randomized design. These Petri plates were then incubated at 25±2°C and observations were recorded on the 8th day for *R. solani* and 9th day for *H. oryzae* and *F. oxysporum*. The activity of oil was compared against a commercial fungicide, mancozeb. Fungitoxicity was calculated as per Ramezani et al. (2002). If there was no growth, this indicated fungicidal activity (Bindu et al., 1998). The entire experiment was repeated and results are presented as means subjected to one-way ANOVA followed by separation of means at $P<0.05$.

RESULTS AND DISCUSSION

Chemical constitution of oil

The hydrodistillation of leaves of *E. tereticornis* yielded yellow colored oil (1.21%; v/w on fresh weight basis). Upon GC/MS analysis, the essential oil was found to contain 37 constituents eluted between 2 and 34 min. Of these, 35 constituents accounting for 99.54% of the essential oil were identified (Table 1). The volatile oil contained 25 monoterpenoids (~80%), 10 sesquiterpenoids (20%) and other compounds (>0.1%) (Tables 1 and 2).

The oxygenated compounds accounted for ~47% of

Table 1. Chemical composition of essential oil from fresh leaves of *E. tereticornis*.

Retention time (min)	Compound	Percentage	Retention index
	Monoterpene hydrocarbons		
2.60	α-Pinene	30.1	1038
2.88	α-Thujene	0.04	1077
2.97	Camphene	0.09	1083
3.42	β-Pinene	8.41	1114
3.63	Sabinene	0.03	1127
4.19	β-Myrcene	0.42	1163
4.25	α-Phyllandrene	1.30	1167
4.52	α-Terpinene	0.16	1172
4.87	Limonene	3.36	1204
5.88	γ-Terpinene	5.7	1245
6.50	*p*-Cymene	1.09	1272
6.78	Terpinolene	0.82	1283
	Oxygenated monoterpenes		
5.08	1,8-Cineole	21.8	1212
12.68	β-Citronellal	0.80	1480
15.17	Isoisopulegol	0.12	1559
15.44	–(-)Isopulegol	0.16	1569
15.88	*endo*-Fenchol	0.16	1584
16.41	Terpinen-4-ol	0.68	1601
17.99	*trans*-Pinocarveol	1.08	1653
18.32	Citronellyl acetate	0.08	1665
19.17	α-Terpineyl acetate	0.34	1693
19.33	α-Terpineol	2.36	1698
21.48	β-Citronellol (R)	0.31	1772
22.07	Myrtenol	0.12	1793
22.24	*trans-p*-mentha-1(7),8-dien-2-ol	0.01	1799
	Sesquiterpene hydrocarbons		
15.96	*trans*-β-Caryophyllene	0.41	1587
16.24	Aromadendrene	0.41	1595
	Oxygenated sesquiterpene		
29.46	Globulol	0.63	2070
30.12	Viridiflorol	0.09	2075
31.84	γ-Eudesmol	2.98	2166
32.03	Guaiol	2.02	2174
32.23	–(-)Aristolene	0.36	2182
32.30	Hinesol	0.62	2185
33.06	α-Eudesmol	5.77	2217
33.26	β-Eudesmol	6.71	2225

[a]Compounds presented in order of elution from the SGE-BP20 capillary column; [b]Percentage based on FID peak area normalization ($n = 3$); [c]Retention index relative to *n*-alkanes (C_{7-30}) on the SGE-BP20 capillary column.

the essential oil, whereas hydrocarbon compounds were ~52% (Table 2). The major components were α-pinene (~30%) followed by 1,8-cineole (~22%). Among monoterpenoids, 13 were oxygenated, whereas 12 were hydrocarbon in nature. On the other hand, the essential oil contained 2 hydrocarbon sesquiterpenes and 8 oxygenated sesquiterpenes. The presence of α-pinene and 1,8-cineole as major constituents of essential oil of

Table 2. Composition of the essential oil of *E. tereticornis* by chemical class.

Chemical class	% in essential oil	Total number in essential oils
Monoterpene hydrocarbons	51.52	12
Oxygenated monoterpenes	28.02	13
Total monoterpenoids	79.54	25
Sesquiterpene hydrocarbons	0.82	02
Oxygenated sesquiterpenes	19.18	08
Total sesquiterpenoids	20.00	10
Other compounds	0.05	02
Total	99.59	37

Table 3. DPPH radical and hydrogen peroxide scavenging activity of *E. tereticornis* essential oil.

Conc. (µg /ml)	Scavenging activity (%)	
	DPPH	H_2O_2
	Essential oil	
25	13.6 ± 0.89 a	7.0 ± 0.16 a
50	25.6 ± 0.62 b	10.7 ± 0.29 b
100	37.9 ± 1.15 c	16.9 ± 0.51 c
200	64.5 ± 2.99 d	42.4 ± 1.04 d
IC_{50} of oil	146.3	270.1
	BHT	
25	11.5±0.56 a	8.7±0.23 a
50	22.3±0.39 b	15.3±0.25 b
100	35.5±0.82 c	25.4±0.31 c
200	58.6±1.11 d	55.8±0.65 d
IC_{50} of BHT	163	181

Different letters within a column for a particular treatment represent significance at $P < 0.05$ applying Tukey's test.

E. tereticornis is supported by the earlier reports (Coelho-de-Souza et al., 2005; Ogunwande et al., 2003; Pino et al., 2001).

Antioxidant activity

Further, to assess the possible utilization of essential oil, we evaluated its antioxidant and scavenging activity against H_2O_2. It was observed that *E. tereticornis* essential oil strongly transformed DPPH radical into its reduced form. It increased with increasing concentration of *E. tereticornis* oil. However, essential oil exhibited a lesser scavenging activity towards H_2O_2 (IC_{50} 270.1 µg/ml) (Table 3).

The essential oil has an IC_{50} value of 146 and 270 µg/ml for DPPH and H_2O_2. DPPH scavenging activity of oil was parallel to that of BHT (163 µg/ml) thereby indicating a strong antioxidant activity. Earlier, studies have reported antioxidant activity of oil from *Eucalyptus* species (Batish et al., 2008; Marzoug et al., 2011; Singh et al., 2009). Singh et al. (2009) reported the antioxidant activity of essential oil from fresh and decaying leaves of *E. tereticornis*. Marzoug et al. (2011) demonstrated antioxidant activity of essential oils from different parts of *Eucalyptus oleosa*.

Antifungal activity

A distinct zone of inhibition was observed in all pathogenic fungi upon treatment of *E. tereticornis* oil (0.5 to 10 ppm) (Table 4). However, in the case of *H. oryzae*, an insignificant change was observed in response to lower concentrations (0.5 and 1 ppm) of *E. tereticornis* oil. Maximum effect was observed at highest concentration in the case of *F. oxysporium* and *H. oryzae*, where there was a complete suppression of radial growth. On the other hand, radial growth of

Table 4. Antifungal activity (% inhibition in radial growth) of essential oil of *E. tereticornis*.

Conc. (ppm)	*F. oxysporum*	*H. oryzae*	*R. solani*
0 (Control)	0	0	0
		Volatile oil	
0.5	3.67	10.2	22.3*
1	31.9*	14.5*	33.4*
2.5	54.1*	45.7*	45.3*
5	91.9*	67.4*	57.2*
10	100*	100*	87.3*
		Mencozeb	
10	23.5*	44.9*	35.1*
25	45.2*	64.6*	56.3*
50	56.5*	88.9*	78.6*
100	76.5*	92.3*	100*
200	88.2*	100*	100*
500	100*	100*	100*

* within a column for a particular treatment represent significance at $P < 0.05$ applying Dunnet's test.

R. solani decreased with increasing concentration of oil. A similar effect was observed when the test pathogenic fungi were treated with commercial fungicide mancozeb (Table 4). However, complete inhibition of radial growth in the case of *F. oxysporium* occurred at 500 ppm, while that of *H. oryzae* and *R. solani*, 100% inhibition was observed at 250 ppm (Table 4). It indicated that *E. tereticornis* essential oils possess strong fungitoxic activity.

Earlier, Ramezani et al. (2002) reported that essential oil of another species of *Eucalyptus*, that is, *E. citriodora* and its constituent monoterpene, citronellal inhibited radial growth and dry weight accumulation of two rice fungi – *R. solani* and *H. oryzae*. Hashem et al. (2010) reported essential oils of antifungal activity of rose geranium (*Pelargonium graveolens* L. Her.), sweet basil (*Ocimum basilicum* var. *basilicum* L.), cumin (*Cuminum cyminum* L.) against *Fusarium* sp. Nakatsu et al. (2000) opined that the antifungal activity of essential oil is due to the occurrence of mixture of compounds and not due to individual component.

Further, it has been reported that aldehydes, the main constituents of oil, irreversibly disrupt the membrane and cause leakage of electrolytes thus altering the permeability of microbial membranes leading to growth inhibition (Avissar et al., 1990).

Conclusions

The study concludes that *E. tereticornis* oil possesses a strong free radical scavenging and antifungal activity and thus could be used as a natural antioxidant / antifungal agent.

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