Chemical Composition and Inhibitory Activity of Essential Oil from Decaying Leaves of *Eucalyptus citriodora*

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A study was undertaken to explore the content and composition of volatile oil from decaying leaves of lemon-scented eucalypt (*Eucalyptus citriodora* Hook.) not analyzed earlier. GC and GC-MS analysis of the oil (yield 0.6%) revealed the monoterpenoid nature with citronellal (52.2%), citronellol (12.3%) and isoisopulegol (11.9%) as the major constituents. Overall, 17 components were identified that accounted for over 94% of the decaying leaf oil. Surprisingly, the decaying leaf oil contained nearly 1.8% of *trans*-rose oxide, which is generally absent in eucalypt essential oil. Decaying leaf oil and its major 2 components (citronellal and citronellol) inhibited the germination and root elongation of two weeds – *Cassia occidentalis* (broad-leaved) and *Echinochloa crus-galli* (grassy weed). Based on the dose-response studies, I_{50} values were determined for decaying leaf oil and the effect was more on germination only of broad-leaved weed (*C. occidentalis*), whereas that of citronellal and citronellol were on germination as well as root length of *E. crus-galli* (grassy weed). Based on I_{50} values it was observed that citronellal was more phytotoxic and germination inhibiting in nature, whereas citronellol was a more potent root inhibitor, thereby indicating a possible different mode of action. The study concludes that decaying leaf oil hold a good commercial value for exploitation as weed management agent.

Key words: Decaying Leaf Essential Oils, I_{50} Values, Germination Inhibitor

Introduction

Eucalyptus L'Herit (Myrtaceae), a native plant of Australia, is a unique genus of tall trees and shrubs comprising of around 800 species widely cultivated in various parts of the world (Brooker and Kleinig, 2004). These are commonly called gum trees as these exude a gum and are known world over for insect-repellant properties. The eucalypt trees are characterized by evergreen foliage that is variably fragrant due to the presence of volatile essential oils. These find an extensive use in perfumery and pharmaceutical industry and their amount and fragrance varies with the species. In India, eucalypt was first introduced in 1792 and is now one of the major trees of the over 32.6 Mha area under the forest plantations (FAO, 2001). Besides, these are also cultivated along roadsides, parks, and gardens mainly for aesthetic value and along agricultural fields as windbreaks and shelterbelts. Some of the species commonly grown in India are red gum (*E. camaldulensis*), lemon-scented gum (*E. citriodora*), Tasmanian blue gum (*E. globulus*) and Cider gum (E. *tereticornis*). Among these, *E. citriodora* is a large, quick-growing tree with smooth and white bark and lemon-scented leaves. It is extensively planted and coppiced for the extraction of essential oil that is rich in citronellal and used in perfumery and as flavouring agent. The oil is known to possess a wide spectrum of biological activities including fungicidal (Ramezani *et al.*, 2002), insecticidal (Isman, 2000), nematicidal (Pandey *et al.*, 2000) and allelopathic properties (Kohli, 1990). For various industrial uses the oil is generally extracted from the juvenile and adult foliage. However, no attempt has ever been made to explore the essential oil from the decaying leaves that form a matrix on the floor of its plantations owing to the evergreen nature of the tree and their slow decomposition rate. Further, there are reports that the floor of *E. citriodora* lacks any vegetation (Kohli, 1990); this is generally

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correlated with the emission of volatile oils from the foliage. However, nothing is known regarding the role of decaying leaves/litter fallen on the floor with reference to the amount of essential oil, its chemical constitution and role as germination inhibitor. This information can serve as an important resource for exploring its commercial utilization. With this objective, a study was therefore planned to determine the content, composition and phytotoxicity of essential oil in the decaying leaves of *E. citriodora.*

Materials and Methods

Plant material and oil extraction

Decaying leaves were collected from the floor of the nearly 25-year-old trees of lemon-scented eucalypt (*Eucalyptus citriodora* Hook.) growing on Panjab University campus, Chandigarh, India in April 2004.

The essential oil was extracted by steam-distillation using Clevenger's apparatus. Nearly 250 g of decaying leaves were mixed into 1 l of distilled water in a 2 l round bottom flask fitted with a condenser. After boiling contents for 2 h, the oil was collected from the nozzle of the condenser, dried over sodium sulphate and its amount was measured to determine yield. It was stored at 4 °C for further identification and bioassay.

Analysis of oil

The essential oil was analyzed by GC and GC-MS. GC was performed on a Shimadzu GC-14B gas chromatograph with a flame ionization detector and using a Supelco wax column (60 m × 0.25 mm i.d., film thickness 0.25 μm) and N_2 as carrier gas. The oven temperature was programmed from 40 °C, initially held isothermal for 4 min, to 220 °C at the rate of 4 °C/min and held for 5 min. Relative amounts of different constituents were determined by computer based calculation of peak area normalization without any correction factor. Peaks obtained were compared with data obtained from GC-MS.

GC-MS was done on a Q Mass 910 Perkin-Elmer Mass Spectrophotometer equipped with fused silica (BP 21) capillary columns. The analysis was carried out using a BP-21 column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The injector and detector temperature were fixed as 230 °C and 250 °C, respectively, and He was used as carrier gas. The oven temperature was programmed from 40 °C, initially held isothermal for 7 min, to 190 °C at the rate of 5 °C/min and finally held isothermally for 20 min.

Identification of components

The compounds were identified on the basis of computer matching of mass spectra using the library search system HP-5872 (Hewlett-Packard), consulting data bases *viz.* Wiley 275 and NBS 75K libraries (McLafferty, 1989), NIST 98 (Stein, 1990), and compilation by Adams (1995).

Dose-response study and inhibitory activity

The role of the essential oil from decaying leaves and its two major monoterpenes (citronellal and citronellol) as germination inhibitor was explored against two weedy species *viz. Cassia occidentalis* (broad-leaved) and *Echinochloa crus-galli* (grassy weed). For this, seeds of each test weed were divided into 22 groups (7 each for essential oil, citronellal, and citronellol, and one for control) of 100 each and dipped in distilled water for 24 h for imbibition prior to germination. These were then equidistantly placed in 15 cm diameter petri dishes (20 seeds each in 5 petri dishes as replicates), lined with a single layer Whatman no. 1 filter circle moistened with 7 ml distilled water. The filter paper was treated with eucalypt oil or citronellol or citronellal so as to have a concentration of 0.06, 0.12, 0.24, 0.60, 1.2, 2.4 and 6.0 mg/ml. The concentrations used were in mg/ml instead of mM or μM since the molecular weight of oil was not known. After the treatment, petri dishes were sealed with a paraffin film to avoid loss of oil on vaporization. Treatment without oil or pure monoterpenes in a similar manner served as control. Petri dishes were placed in a growth chamber at (24 ± 3) °C and a 16 h/8 h light/dark photoperiod, a photon flux density of approximately 150 μmol m^{-2} s^{-1} and relative humidity of around 75%. After a week, the number of seeds that germinated was counted and the root length of emerged seedlings was measured.

The significance of observed values in response to treatment was determined over control by 2-sample *t*-test at $p < 0.05$ whereas differences within treatments were analyzed by one-way ANOVA followed by separation of means using the SPSS package (version 10).

Results and Discussion

The yield of volatile essential oil from the decaying leaves of *E. citriodora* was 0.60% (v/w). GC and GC-MS analysis of this oil revealed its monoterpenoid nature including 16 monoterpenes and 1 sesquiterpene (Table I). These were isolated at retention times between 5 to 35 min, and 17 constituents were identified accounting for 94.21% of the oil. The monoterpenes included four hydrocarbons (2 cyclic – α-pinene and isolimonene; 2 acyclic – myrcene, β-citronellene), seven alcohols (3 acyclic – linalool, citronellol, geraniol; 4 cyclic – isopulegol, isoisopulegol, α-terpineol, *trans*-pulegol), one of each of acyclic aldehydes (citronellal), cyclic ethers (*trans*-rose oxide) and allylphenyl monoterpenes (eugenol), and two acyclic monoterpene esters (citronellyl and linalool esters). The only sesquiterpene identified was β-caryophyllene – a cyclic one (Table I). Among the constituents, five major ones were monoterpenes *viz.* citronellal (52.2%), citronellol (12.3%), isoisopulegol (11.9%), β-citronellene (6.1%) and isopulegol (5.0%), and accounted for over 87% oil content. The rest of the 12 constituents were nearly or less than 7%. Though, the composition of oil is in conformity with that reported hitherto in fresh leaves of *E. citriodora* (Dethier *et al.*, 1994; Chalchat *et al.*, 2000), yet the relative amount of individual constituents differed. Surprisingly, the decaying leaf oil contained nearly 1.8% of *trans*-rose oxide (an important compound in flavour fragrant industry), which is generally absent or present in very low amount in eucalypt essential oils. Citronellal though observed was a major constituent in decaying leaf oil, but its amount (52%) was not sufficient to make it a potential resource for perfumery industry that normally requires 65–80%. Nevertheless, other monoterpenes like *trans*-rose oxide, citronellol and pulegol isomers are commercially important and find extensive use in flavour and fragrance industry. Furthermore, the decaying leaf oil can be explored for weed management, as the essential oils and their constituent monoterpenes are presently being viewed as excellent weed suppressants (Singh *et al.*, 2003). However, the decaying leaf oil has not been assessed for such an activity and its phytotoxicity is unknown. Studies in this regard reveal that decaying leaf oil and its two major constituents, citronellal and citronellol, inhibited the germination and initial growth of test weeds (Figs. 1, 2).

Table I. Composition of the decaying eucalypt leaf oils as revealed by GC and GC-MS analysis.

Constituent	Retention time [min]	Amount
α-Pinene	5.9	0.19
Myrcene	6.0	0.02
Linalool	11.22	0.04
trans-Rose oxide	14.95	1.77
trans-Pulegol	15.53	0.99
Citronellal	19.44	52.23
Isopulegol	21.50	5.02
Isoisopulegol	21.72	11.9
α-Terpineol	22.47	1.04
Isolimonene	23.08	0.51
β-Citronellene	24.10	6.12
Linalool acetate	25.17	0.49
Citronellol	26.60	12.31
Geraniol	27.36	0.36
Eugenol	30.29	0.35
Citronellyl acetate	30.82	0.17
β-Caryophyllene	31.76	1.51
Total		*94.21*

A significant reduction in germination and root length of test weeds was observed with eucalypt oil, citronellal and citronellol, though the magnitude of inhibition differed (Figs. 1, 2). Based on the dose-response curves, I_{50} values were calculated (Table II). In general, the effect of decaying leaf oil was more on the germination of broad-leaved weed *C. occidentalis* compared to grassy weed *E. crus-galli* indicating the species-specificity. However, the effect was similar on the root length of both the weeds. In contrast the effect of both citronellol and citronellal was on germination as

Table II. I_{50} values (in mg/ml) of *E. citriodora* decaying leaf oil and its two major components for germination and root elongation in *C. occidentalis* and *E. crus-galli*. Values in parenthesis indicate I_{50} in mM.

Treatment	Germination		Root elongation	
	C. occidentalis	*E. crus-galli*	*C. occidentalis*	*E. crus-galli*
Decaying leaf oil	1.09	1.45	0.31	0.35
Citronellal	0.55 (3.56)	0.14 (0.91)	0.27 (1.75)	0.13 (0.84)
Citronellol	1.67 (10.64)	0.24 (1.54)	0.13 (0.83)	0.09 (0.58)

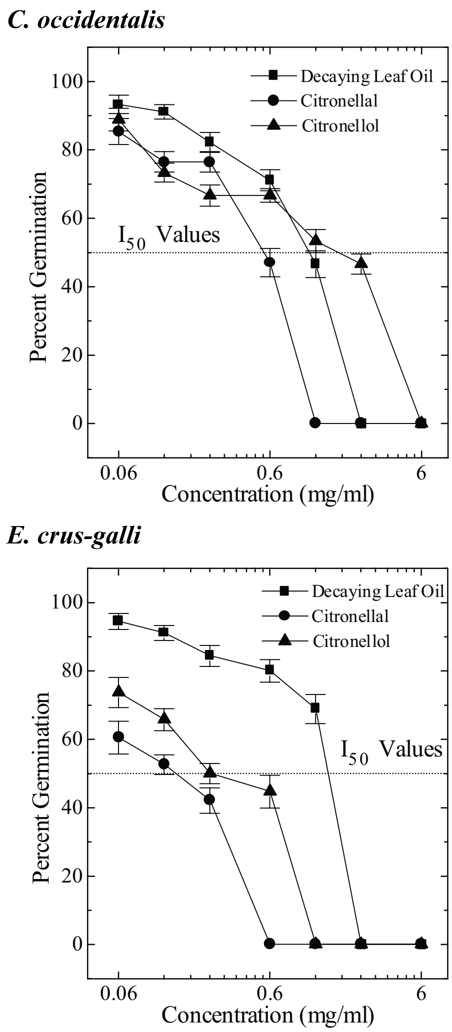

Fig. 1. Effect of decaying eucalypt leaf oil and its two major constituent monoterpenes on the percent germination of test weeds measured after one week. Data present mean ± SE.

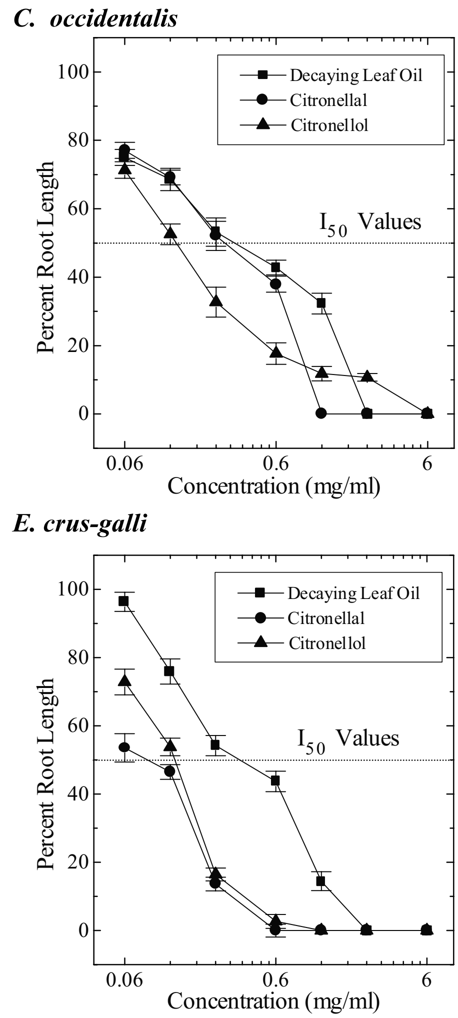

Fig. 2. Effect of decaying eucalypt leaf oil and its two major constituent monoterpenes on the percent root length of test weeds measured after one week. Data present mean ± SE.

well as root length of *E. crus-galli.* Based on I_{50} values it was observed that citronellal was more phytotoxic and germination inhibiting in nature, whereas citronellol was a more potent root inhibitor (Table II), thereby indicating a possible different mode of action. The variability in the effect of crude oils compared to the two major monoterpenes is probably due to the fact that oil is a composite mixture of a number of monoterpenes and these two compounds could not account for the observed inhibitory pattern of crude oils. Probably some other components, though present in lesser amount, are also responsible for the observed inhibition. Such an inhibitory effect of essential volatile oils is not surprising as the essential oils and their monoterpenoid constituents are known allelochemicals (Asplund, 1968; Vaughn and Spencer, 1993). At present, due to their germination inhibitory effects, these essential oils are being screened as possible candidates for the control of weed species (Singh *et al.*, 2003) since they offer a number of novel characteristics such as biodegradability, unique modes of action and very little or no mammalian toxicity (Isman, 2000). Based on the present study, it is thus concluded that decaying eucalypt leaf oil suppresses the germination and root growth of weed species and thus hold a good promise for future exploitation for weed management purposes.

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