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Composition of Leaf Essential Oil of *Eucalyptus camaldulensis*

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Variation in the yield and chemical composition of essential oil obtained from the leaves of *Eucalyptus camaldulensis* from saline and non-saline provenances of Pakistan is presented. *Eucalyptus camaldulensis* leaves contained 0.98 and 0.96 % essential oils from saline and non-saline areas, respectively. GC-MS analysis of the *E. camaldulensis* leaves essential oil revealed the presence of 24 and 27 compounds from the saline and non saline samples, respectively. The principal constituent in the essential oil from saline and non-saline provenances was 1,8-cineole 34.42 and 40.05 %, respectively. Other major constituents were: α-pinene (14.68 and 12.43 %), γ-terpinene (9.42 and 7.48 %), ledol (7.42 and 7.67 %) and *t*-pinocarveol (8.36 and 3.32 %), respectively. Statistical analysis of the data showed that there were no significant differences in the yield of essential oil of *E. camaldulensis* from saline or non-saline habitats of Pakistan. However, the concentration of most of the chemical components varied significantly ($p < 0.05$) due to salt stress.

Key Words: Essential oils, GC-MS, Salinity, 1,8-Cineole, β-Pinene, *p*-Cymene.

INTRODUCTION

Considerable research has been conducted to uncover the medicinal properties of *Eucalyptus* spp. *Eucalyptus* oils and its fresh leaves are used in steam inhalation and for treatment of cough and cold, sore throat and other infections[1]. *Eucalyptus* spp. are well known for their tolerance to a wide range of soil types and climates[2]. Over 750 species of this potential medicinal tree have so far been discovered on the globe[3]. However, *Eucalyptus camaldulensis* is one of the most widely distributed *Eucalyptus* species. It is also regarded as one of the most widely planted trees in the world (*ca*. 5000,000 ha planted)[4,5]. *Eucalyptus camaldulensis* is used as a rich source of timber, firewood, shelterbelt and as a honey tree[5]. It is also reported to be used as an anesthetic, antiseptic and astringent. *Eucalyptus camaldulensis* leaves are a traditional aboriginal herbal remedy. The leaf oil is reported to be a powerful antiseptic and used widely for treatment of cough and cold, sore throat and other infections[1].

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Literature reported a considerable variation in the yield of leaf essential oil from *E. camaldulensis*[5-8]. The variation in the chemical composition of eucalyptus essential oil with respect to seasons has also been reported in the literature[9]. The major components in the eucalyptus essential oil reported were 1,8-cineole, β-pinene, γ-terpinene. Essential oils with high cineole contents demonstrate good antimicrobial[10] and nematicidal activity[11].

Soil salinity is a key ecological stress that severely influences plant productivity. Due to continuous build up of salts in the soil, millions of hectares of arable land have now become unfit for cultivation. Soil salinity causes modification in different morphological, physiological and biochemical as well as anatomical characteristics[12-13].

Eucalyptus camaldulensis grows well in slightly alkaline soils, where it can withstand both salinity and waterlogging. The demarcation of the phenomenon of its tolerance to salinity and waterlogging is not explicit. This may be because of considerable variation among different provenances[14]. However, high salt content of the soil or water has significant adverse effects on the growth of eucalyptus plants. For example, in a sand culture experiment, plant height and stem diameter decreased by 36 and 55 %, respectively, when water with an electrical conductivity (EC) of 9-10 dS/m was used (compared to the control plants irrigated with water of EC 1.6 dS/m[15]. A confused picture also emerged from experiments in the field, on a saline and waterlogged site in Australia. A 50 % decrease in canopy volume was observed with an increase in electrical conductivity in the upper soil profile to 5 dS/m[16]. In one adaptation trial near Tandojam, Pakistan, only 13 % *E. camaldulensis* of plants survived for 24 months. This performance however was eclipsed by every other genotype in the trial[17].

Although there are several reports in the literature which shows that salinity of the soil or water could adversely affect the growth of *E. camaldulensis*. The effects of salinity on the content and composition of the oil of *E. camaldulensis* from sub-continental region have not yet been reported. Thus, this experiment was performed to determine whether or not salinity could alter the content and composition of *E. camaldulensis* oil.

EXPERIMENTAL

Fresh fully matured green leaves were excised from the seven year old plants of a *E. camaldulensis* population cultivated at Pakka Anna (a saline area); district Toba Tek Singh, Punjab, Pakistan as well as from the plants growing in the University of Agriculture, Faisalabad, Pakistan (a non-saline area) during April-May. The leaves were immediately preserved in polyethylene bags under refrigerator and transferred to the experimental laboratory.

Isolation of the essential oils: The freshly collected leaves (300 g) were weighed, washed with distilled water and hydrodistilled for 3 h using 1 L distilled water/sample for complete extraction of essential oil, using a commercial Clevenger-type apparatus. Distillates of essential oils were dried using anhydrous sodium sulfate as moisture absorbent, filtered and stored at -4 ºC until analyzed.

Essential oils analysis

Physical analysis: Determination of oil specifications such as refractive index (RI) (40 °C), density (25 °C), solubility (25 °C) and specific gravity (25 °C) were determined following the standard methods[18]. A refractometer model No. 922313 (Bellingham and Stanley Ltd., London) was used for the determination of refractive index (40 °C) of the eucalyptus oil. Density and specific gravity were determined with a pycnometer at 25 °C.

Chromatographic analysis

Gas chromatography: The essential oils were analyzed using a Perkin-Elmer gas chromatograph model 8700, equipped with flame ionization detector (FID) and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220-290 ºC, respectively. Column oven temperature was programmed from 80-220 ºC at the rate of 4 ºC min^{-1}, initial and final temperatures were held for 3 and 10 min, respectively. Helium was used as carrier gas with flow of 1.5 mL min^{-1}. A sample of 1.0 μL was injected, using slit mode (split ratio, 1:100). All quantification was done by a built-in data-handling program provided by the manufacturer of the gas chromatograph (Perkin-Elmer, Norwalk, CT, USA). The composition was reported as a relative percentage of the total peak area.

Gas chromatography/mass spectrometry: GC-MS analysis of the essential oils were performed using an Agilent-Techno-logies (Little Falls, California, USA) 6890N Network gas chromatographic (GC) system, equipped with an Agilent-Technologies 5975 inert XL mass selective detector and Agilent-Technologies 7683B series auto injector. Compounds were separated on HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm; Little Falls, CA, USA). A sample of 1.0 μL was injected in the split mode with split ratio 1:100. For GC/MS detection, an electron ionization system, with ionization energy of 70 eV, was used. Column oven temperature program was the same as in GC analysis. Helium was used as a carrier gas at a flow rate of 1.5 mL min^{-1}. Mass range was 50-550 m/z while injector and MS transfer line temperatures were set at 220 and 290 ºC, respectively.

Compounds identification: The identification of the oil constituents was based on a comparison of their retention indices relative to (C9-C24) *n*-alkanes either with those of published data or with authentic compounds. Compounds were also identified using their MS data compared to those from the NIST mass spectral library and published mass spectra[19].

Soil sampling and pretreatment: Ten soil samples from each location were collected during leaf sampling at the depth of 15 cm from the soil surface and 3 feet away from the main tree trunk during the course of the experiment. A sub sample of soil (200 g) was taken from each sample and dried in an oven (EYELA, VOC-300 SD, Tokyo, Japan) at 90 ºC for 5 days. An oven-dried soil sub-sample (100 g) was taken to make saturation paste with distilled water. The extraction was obtained using a vacuum pump.

Nutrient analysis of soil: The determination of potassium, sodium and calcium in the soil saturation extract was carried out using a flame photometer (Jenway PFP-7). The concentration of magnesium in the soil extract was determined using a atomic absorption spectrometer (Perkin-Elmer Analyst-300). Chloride content in the extracts was determined by a chloride analyzer (Sherwood Chloride Analyzer 920). Electrical conductivity (EC) and pH of the soil extract was measured by pH/Cond (Inolab), level 1 m.

Statistical analysis: The data for all variables were subjected to analysis of variance using a COSTAT computer package (Cohort Software, Berkeley, California). The mean values were compared with the least significance difference test following Snedecor and Cochran[20].

RESULTS AND DISCUSSION

Physico-chemical properties of soil samples from saline and non-saline areas have been presented in Table-1. The values of electrical conductivity, sodium absorption ratio (SAR) and exchangeable sodium percentage (ESP) and the contents of Na^+ and Cl^- were found to be drastically higher in saline areas as compared with those of non-saline area. Furthermore, the concentrations of Ca^{2+} and K^+ were also quite higher in the soil samples from the saline areas as compared with those of its counterpart. All these determinations clearly show that the Pakka Anna site is highly salt-affected.

TABLE-1
PHYSICO-CHEMICAL CHARACTERISTICS OF SOIL
FROM SALINE AND NON-SALINE AREAS

Characteristics	Saline area	Non-saline area	p^a
Saturation percentage	30.0 ± 1.30	28.8 ± 1.90	0.41
pH	9.90 ± 0.30	8.40 ± 0.60	0.26
EC[b]	19.0 ± 2.90	1.70 ± 0.10	0.00
Ca^{2+} meq/L	6.00 ± 0.70	2.50 ± 0.10	0.00
Mg^{2+} meq/L	0.23 ± 0.01	0.18 ± 0.01	0.00
K^+ meq/L	5.00 ± 0.18	1.70 ± 0.01	0.00
Na^+ meq/L	141.2 ± 9.30	4.00 ± 0.10	0.00
Cl^- meq/L	107.6 ± 7.90	12.2 ± 1.70	0.00
SAR[c]	88.5 ± 3.90	3.00 ± 0.20	0.00
ESP[d]	54.0 ± 4.90	2.80 ± 0.20	0.00

Values are means ± SD for ten soil samples from each provenance, analyzed individually in triplicate; [a]Probability value of $p \leq 0.05$ was considered to denote a statistical significance difference; [b]EC = Electrical conductivity of soil extract in mS/cm; [c]SAR = Sodium adsorption ratio in $(mmol/L)^{1/2}$; [d]ESP = Exchangeable sodium percentage.

Table-2 shows the important specifications of *E. camaldulensis* essential oil from saline and non-saline areas of Pakistan. As far as the concentration of essential oil of *Eucalyptus camaldulensis* (*E. camaldulensis*) leaves is concerned, there was no significant variation in the essential oil content of samples collected from saline or

non-saline areas (Table-2). The mean essential oil contents were 0.98 and 0.96 % obtained from the samples collected from saline and non-saline areas, respectively.

TABLE-2
PHYSICAL CHARACTERISTICS OF ESSENTIAL OIL OF *E. camaldulensis* FROM EACH PROVENANCE

Parameters	Essential oils		p[a]
	Saline	Non-saline	
Oil content (% fresh matter basis)	0.98 ± 0.10	0.96 ± 0.12	0.83
Refractive index (40 °C)	1.4620 ± 0.01	1.4580 ± 0.02	0.77
Solubility (mL/5mL of 70 % ethanol)	0.93 ± 0.10	1.05 ± 0.16	0.33
Density (25 °C)	0.938 ± 0.04	0.936 ± 0.04	0.95
Specific gravity (25 °C)	0.920 ± 0.04	0.919 ± 0.04	0.97
Physical appearance	Colorless – dark yellow	Off white – dark yellow	–

Values are means ± SD for three *E. camaldulensis* essential oils from each provenance, analyzed individually in triplicate; [a]Probability value of $p \leq 0.05$ was considered to denote a statistical significance difference.

The values of density (25 °C), specific gravity (25 °C), solubility (25 °C), refractive index (40 °C) and appearance of the investigated essential oil as given in Table-2, were in close agreement with those already reported[21]. The results from both the regions indicated that these physical properties of the oil were not significantly affected due to high salt content in soil. No earlier reports available regarding the effect of salt stress on the physical properties of eucalyptus essential oils.

Table-3 shows the components of essential oil of *E. camaldulensis* as analyzed by GC and GC-MS. A total of 24 and 27 components were detected, representing 97.69 and 95.27 % of the oil from saline and non-saline samples, respectively. The principal component of *E. camaldulensis* essential oil was 1,8-cineole. The mean values of 1,8-cineole content of *E. camaldulensis* essential oil of from saline and non-saline provenances of Pakistan were 34.42 and 40.05 %, respectively. Other major components of *E. camaldulensis* essential oils from saline and non-saline areas were α-pinene (14.68 and 12.43 %), γ-terpinene (9.42 and 7.48 %), *t*-pinocarveol (8.36-3.32 %), ledol (7.42 and 7.67 %) and aromadendrene (2.63 and 2.78 %), respectively. β-pinene and *t*-pinocarveol contents were strongly affected by salt stress and their concentration increased significantly ($p < 0.05$) from 2.33 and 3.32 % (non-saline samples) to 6.66 and 8.36 % (saline samples), respectively. The variations in the contents of most of the investigated essential oils constituents, with respect to salt stress, were statistically significant ($p < 0.05$).

As far as is concerned about the groups of chemical constituents, the analyzed essential oils mainly consisted of oxygenated monoterpenes. *Eucalyptus camaldulensis* essential oils collected from saline and non-saline populations consisted of 48.63 and 49.57 % oxygenated monoterpenes, repectively. Eucalyptol was the major oxygenated monoterpenes present in both the oils. Eucalyptus camaldulensis essential oils from saline and non-saline crops also contained considerable quantity of monoterpene

TABLE- 3
COMPOSITION (%) OF DIFFERENT COMPONENTS OF ESSENTIAL OIL
OF *E. camaldulensis* FROM TWO DIVERSE PROVENANCES

Components[a]	Essential Oil		p[b]	Mode of Identification[c]
	Saline	Non-Saline		
α-Pinene	14.68 ± 0.42	12.43 ± 0.37	0.00	RT, RI, MS
Camphene	0.87 ± 0.07	2.96 ± 0.12	0.00	RT, RI, MS
β-Pinene	6.66 ± 0.17	2.33 ± 0.19	0.00	RT, RI, MS
Eucalyptol	34.42 ± 1.21	40.05 ± 1.20	0.00	RT, RI, MS
γ-Terpinene	9.42 ± 0.36	7.48 ± 0.29	0.00	RT, RI, MS
δ-Terpinene	1.11 ± 0.06	1.17 ± 0.05	0.25	RT, RI, MS
α-Iinene epoxide	0.27 ± 0.02	0.28 ± 0.02	0.57	RT, MS
Isoamyl isovalerate	1.07 ± 0.09	1.10 ± 0.07	0.67	RT, MS
Fenchyl alcohol	0.79 ± 0.03	0.89 ± 0.05	0.04	RT, MS
α-Camphdenic Aldehyde	0.66 ± 0.03	0.67 ± 0.02	0.65	RT, MS
t-Pinocarveol	8.36 ± 0.40	3.32 ± 0.11	0.00	RT, RI, MS
Myrtenal	0.94 ± 0.03	0.97 ± 0.06	0.48	RT, RI, MS
Z-Carveol	1.15 ± 0.04	1.25 ± 0.07	0.09	RT, RI, MS
d-Carvone	0.51 ± 0.03	0.36 ± 0.02	0.00	RT, RI, MS
o-Cymene 5-ol	0.46 ± 0.03	0.54 ± 0.02	0.00	RT, MS
Benzyl valerate	–	0.14 ± 0.01	–	RT, MS
α-Gurjunene	–	0.26 ± 0.02	–	RT, RI, MS
β-gurjunene	–	0.22 ± 0.03	–	RT, RI, MS
Aromadendrene	2.63 ± 0.16	2.78 ± 0.20	0.36	RT, RI, MS
Alloaromadendrene	0.89 ± 0.05	0.97 ± 0.07	0.18	RT, RI, MS
Phenethyl Isovalerate	0.90 ± 0.03	1.01 ± 0.06	0.05	RT, MS
Ledene	0.45 ± 0.05	0.52 ± 0.03	0.11	RT, MS
Epiglobulol	1.83 ± 0.09	1.96 ± 0.03	0.07	RT, RI, MS
Ledol	7.42 ± 0.05	7.67 ± 0.08	0.01	RT, RI, MS
Viridiflorol	1.13 ± 0.04	2.76 ± 0.06	0.00	RT, RI, MS
Eremophilene	0.78 ± 0.03	0.85 ± 0.02	0.03	RT, RI, MS
γ-Cadinene	0.29 ± 0.01	0.33 ± 0.02	0.04	RT, RI, MS
Total	97.69	95.27	–	–

[a]Values are means ± SD for three *E. camaldulensis* essential oils from each provenance, analyzed individually in triplicate; [b]Probability value of $p \leq 0.05$ was considered to denote a statistical significance difference; [a]Compound listed in order of elution from a HP-5MS column; [c]RT, identification based on retention time; RI, Identification based on retention index; MS, identification based on comparison of mass spectra.

hydrocarbons, 32.74 and 26.37 %, respectively. The *E. camaldulensis* essential oil from saline area contains 4.59 and 11.73 % whereas, *E. camaldulensis* essential oils from non-saline contains 5.41 and 13.92 % of sesquiterpene hydrocarbon and oxygenated sesquiterpene contents, respectively.

The investigated essential oil concentration in the present analysis of *E. camaldulensis* leaves was comparable to those reported by Farah *et al.*[8] and Bastailer, *et al.*[22] from Morocco and Turkey, respectively. Where as, Moudachirou *et al.*[7] reported a variable oil content of 0.6-1.4 % from different locations of Benin. Chalchat *et al.*[23]

reported the oil yield of *E. camaldulensis* to be 0.50 % from Jerusalem. A significantly higher oil yield (2.3-3.0 %) of *E. camaldulensis* has also been reported by Shieh[6] from Taiwan with respect to different seasons. This variation of essential oil content of *E. camaldulensis* from different countries might be attributed to the diverse agroclimatic conditions of those regions.

It could be projected that NaCl induced altered pattern of N and C metabolism in *E. camaldulensis* might have changed the pattern of ion accumulation, which in turn may have resulted in changes in composition of some components of essential oil. There are no earlier reports on the effect of salinity of the composition of eucalyptus essential oil to compare present results. However, the values of β-pinene and *p*-cymene content in the present analysis were in good agreement to those of earlier studies from different agroclimatic regions of the world[6,24-27]. The values of 1,8-cineole were also within the range of those reported by Tsiri *et al*.[9] (Greece; 25.3-44.2 %), Pegula *et al*.[24] (Mozambique; 37.1-40.0 %), Oyedeji *et al*.[25] (Nigeria; 32.8-70.4 %) and Shieh[6] (Taiwan; 34.0-68.2).

Conclusion

In conclusion, soil salinity did not affect the essential oil content of *E. camaldulensis* leaves. However, it had a significant effect on the per cent compositions of some components of essential oil. Therefore, this species can be grown in the saline areas of Pakistan and sub-continent to benefit from its potential essential oil for its various medicinal and pharmacological uses.

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