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Chemical Compositional and Intra Provenance Variation for Content of Essential Oil in *Eucalyptus crebra*

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Abstract: Variation in the oil yield and composition of essential oils from fifteen differently *Eucalyptus crebra (E. crebra)* trees adopted of Faisalabad was presented. Hydro-distilled leaves of *Eucalyptus crebra* ranged 0.29-1.33 % of essential oil. Extracted oils were resolved and identified by GC/FID (Gas chromatography) on Carbowex-20 M packed glass column. Maximum components (33) were detected in the oil of two different trees (i.e. tree No. 6 and 10). A total of 33 components were detected, out of which 6 amounting to approximately 60% of the oil were identified. In *Eucalyptus crebra* oils six components (α-pinene, β-pinene, Δ^3 carene, α-phallendrine, Limonene and 1, 8-cineole), were positively identified. The principal constituent 1, 8-cineole, found in the fifteen oils of different trees, was ranged (11.87-43.80%). The other major components were ranged: β-pinene (0.20-12.13%), α-pinene (1.68-18.23%), Δ^3 carene (1.32-5.63%) and α-phallendrine (0.55-7.98%). Only in the oil of one tree limonene (2.37%) was found. Chemical composition of oils from tree to tree varied significantly which may be due to the differences in their genetic make up (e.g., age, height) and may also be the effect of salinity. *Eucalyptus crebra* trees oil shown 1, 8-cinole percentage more than 70%, for that reason oils of *Eucalyptus crebra* trees under study were found suitable for medicinal purposes.

Key words: Essential oil, *Eucalyptus crebra,* Gas Chromatography, 1, 8-cineole, α-pinene, medicinal purposes

INTRODUCTION

Essential oils are volatile organic compounds found in various plant tissues such as fruits, leaves, flowers, bark, stem, seeds, wood and roots. The quality of essential oils depends on the several factors including the part of the plant used, the plant variety and its country of origin, the method of extraction and the refining process[1].

Eucalyptus is a diverse genus of trees (rarely shrubs), the members of which dominate the tree flora of Australia. *Eucalyptus* oils and its fresh leaves are used in steam inhalation treatments, consumed in lease and used in bathing. *Eucalyptus* sp. is well known for their tolerance to a wide range of soil types and climates[2]. There are almost 600 species of *Eucalyptus,* mostly native to Australia, with a very small number found in adjacent parts of New Guinea and Indonesia. *Eucalyptus* can be found in almost every part of the continent, adapted to all of Australia's climatic conditions. Many, but far from all, are known as gum trees; other names for various species include mallee, box, ironbark, stringybark and ash[3]. Leaves of all species contain less amount of *Eucalyptus* oil and less than twenty have enough oil of commercial value and of these only ten accounts for the entire world production of essential oils[4].

Eucalyptus is regarded as one of the most widely planted in the world (Ca 5000,000 ha planted)[5]. *Eucalyptus* is typically an Australian tree, well known for its tolerance to wide range of soil types and climates. It is now growing in North and South Africa, Pakistan, India, Sri Lanka and Southern Europe. Genus *Eucalyptus* belongs to family Myrtaceae. *Eucalyptus* trees are characterized as ever green, leathery, whitish leaves that hang vertically, their edges facing the sun, their ragged bark and peculiar aroma. Leaves contain oil glands which produce oils of different composition[6].

The *eucalyptus* species are the most difficult to distinguish from one another because of their physical resemblance with other oils. For most *eucalyptus* species mild climate is the best where there are warm summers, temperate winters, moderate rainfall, dry atmosphere and plenty of sunlight. Temperature tolerance ranges generally between 15 to 100°F. Quick changes in temperatures, however, are stressful for the *eucalyptus* especially in the weaker trees. The perfect soil for most *eucalyptus* species is deep, well-drained loamy soil. The soils moisture content is too important because most *eucalyptus* species are dependent upon massive amounts of water for their quick growth[6].

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Eucalypts crebra belongs to Kingdom, Plantae-Plants. Leung[7] described that the *Eucalyptus* are generally not eaten by the animals due to their tallness, un-attractive leaves and their essential oil content. Only a few species are grazed and eaten by animals, these include *E. populnea, E. ochriloia, E. papuana, E. pilligaeilis* and *E. cladocylax*. The oil percentage and its composition in different *Eucalyptus* sp. vary significant. Cineole rich *Eucalyptus* species are becoming popular and *Eucalyptus crebra* is one of those species. It is documented that oil yield in *Eucalyptus crebra* varies from 0.40- 1.47%[8] and its cineole varies from 0.4-63.2%[9].*Eucalyptus* oil is obtained by steam distillation from the fresh leaves. Seasonal variation in the content and composition of the oil has also been reported in the literature[10]. It contains mainly 1, 8-cineole (eucalyptol); plus triterpenes (ursolic acid derivatives), mono-terpenes and pinene, D-limonene, p-cymene; sesquiterpenes (aroadendrene, alloaromadendrene, globulol); aldehydes (myrtenal) and kenos (carvone)[11,12]. *Eucalyptus* oil has numerous uses, but it is currently most widely used in non-prescription pharmaceuticals. *Eucalyptus* oil based products have been used as a traditional non-ingestive treatment for coughs and colds[13], a topically applied medication for relief of muscular pain[14] and as a solvent/sealer in root canal dentistry[11]. It uses as fragrance reagent in soaps, detergents and perfumes and as a flavoring agent in food[15]. The oil has household uses as well, where it is used in spot and stain removers and as a component of wool wash formulations made for washing woolen garments. It has also been used as a flotation agent in the mining industry[16]. It was becoming more evident that the *Eucalyptus* has healing powers and should be used medicinally. *Eucalyptus* oil is official in the Indian Pharmacopoeia as a counter-irritant and wild expectorant[17] and official in the Chinese Pharmacopoeia as a skin irritant used in nerve pain[18]. The present Ayurevedic Pharmacopoeia indicates its topical application for headache due to colds[19].

Eucalyptus is planted primarily for ground water and salinity control. In Pakistan 6.2 million hectares area has been affected by salinity at different levels. The oil, percentage and chemical composition may vary from tree to tree within a species. Until now, the intra provenance variation for content and chemical composition of essential oil in *Eucalyptus crebra* has not yet been reported. It is, therefore, needed to explore this species from oil point of view. Growing *Eucalyptus* in saline areas for essential oils may help the poor farmers to solve their socio-economic problems and be a source of foreign exchange savings being spent on its import.

MATERIALS AND METHODS

Material: Fresh leaves of fifteen *E. crebra* trees were collected from Punjab Forestry Research Institute, Faisalabad during the month of May and June 2004. The leaves were immediately preserved in polyethylene bags under refrigerator and transferred to experimental lab. Pure standards of α-pinene, β-pinene, Δ^3 Carene, α-phallendrine, Limonene and 1, 8-cineole was obtained from Sigma Chemical Co. (St. Louis, MO).

Extraction of essential oil: Fresh and clean *Eucalyptus* leaves were weighed and taken (about 500 g) in 3 L round bottom flask. The leaves were soaked in water, heated the flask on electro thermal and this distillate collected in the condensate collector was automatically re-circulated into the distillation flask and the oil started accumulating in the condensate collector. This process was continued for 2-2.5 hours for maximum extraction of oil, using a commercial Clevenger-type apparatus[20]. The oil was allowed to stand for sufficient time, to be clear and then it was collected out carefully after draining out condensed water. The oil extracted from different *Eucalyptus crebra* trees by hydro distillation contained fraction of water which was removed by adding small amount of anhydrous Na_2SO_4 in it and the absolute oil was obtained.

Analysis of oil: The amount of extracted oil was determined and the %age yield of the extracted oil from each *E. crebra* trees was calculated on the basis of the weight of *Eucalyptus crebra* leaves. Color and aroma of the oil obtained from each *E. crebra* trees was noted carefully.

Gas liquid chromatographic analysis: The analysis of the chemical composition of essential oil was made by high-resolution gas liquid chromatography (HR-GLC) using Perkin-Elmer gas chromatograph model 3920, fitted with Glass column (2 m x 2 mm id) packed with 15% Carbowax 20 M on Chromosorb WAW and a flame ionization detector (FID). For the supplementation of hydrogen to flame ionization detector (FID), hydrogen generator model HG-501 (GE USA) was used. Oxygen free nitrogen was used as a carrier gas at a flow rate of 25 mL/min. Other conditions was as follows: initial oven temperature, 80°C; ramp rate, 1°C/min; final temperature, 160°C; injector temperature, 150°C; detector temperature, 200°C and temperature hold, 1 min before the run and 10 min. after the run. A sample volume of 0.06 μL was injected and total run time was 30 min. Essential oil components was identified by comparing their relative and absolute retention times to those of authentic standards of Sigma-

Aldrich. A Chromatographic Station (CSW32) data handling program did all of the evaluation and quantification. The composition of the essential oil constituents was reported as a relative percentage of the total peak area.

RESULTS AND DISCUSSION

The volatile leaf oils form fifteen different *Eucalyptus crebra* tree were extracted by hydro distillation during May-June 2004. All the extracted oils were of light yellow in color having camphor like smell. These results were agreeable with the results of Nicolle[21] and Grieve[6].

Table 1 shows that the oil percentage of the fifteen different *Eucalyptus crebra* trees. The maximum oil was obtained from tree No. 5 (1.33%) and the minimum oil was obtained from tree No. 9 (0.29%). The over all range of fifteen *Eucalyptus crebra* ranged 0.29-1.33% of essential oil (Table 1). The percentage of oil was quite comparable with the reported *Eucalyptus crebra* oil yield; 1.47%[8]. Tree to tree variation in the oil yield of different *Eucalyptus* species was reported by various researchers. This tree to tree variation in oil yield may be due to the difference in the genetic character, because of their cross pollination properties.

Table 2 shows the oil yield range in different *Eucalyptus crebra* trees. The oil yield was above 1% in 20% trees and bellow 1% in 80% of trees. Only three trees were found containing above 1% essential oil, six trees were found containing oil between 0.6-0.8% where as in the remaining four trees, the oil percentage ranged between 0.2-0.6% (Table 2).

Table 3 shows the composition of components of essential oil of *Eucalyptus crebra* as analyzed and identified by HR-GLC. A total of 33 components were detected, out of which six components amounting to approximately 60% of the oil were identified. Of the six identified compounds, the oil comprised principally of 1, 8-cineole. *Eucalyptus crebra* oil extracted from the leaf samples of tree No. 1 indicated the presence of 24 different components from which four were the major components. The identified compounds in the oil of tree No. 1 were α-pinene, β-pinene, α-phallendrine, 1, 8-cineole. The concentration of α-pinene and 1, 8-cineole were within the range of those reported by Iqbal *et al.*[8] (19.82%) and Bignell *et al.*[9] (0.4-63.2%), respectively. β-pinene content was found to be 0.54% were also within the range of reported content (0 to 20%)[9]. Fourth major content was α-phallendrine (0.59%) (Table 3). No reported concentration of the α-phallendrine in oil of *E. crebra* was found.

Table 1: Maximum essential oil yield of different *Eucalyptus crebra* trees

Tree No.	Oil percentage
1	0.97±0.11
2	0.94±0.12
3	0.95±0.12
4	0.63±0.08
5	1.33±0.15
6	1.12±0.14
7	0.88±0.10
8	0.96±0.12
9	0.29±0.04
10	0.89±0.10
11	0.55±0.07
12	0.64±0.08
13	1.00±0.13
14	0.45±0.05
15	0.59±0.06

Values are means±SD of *E. crebra* essential oils from Pakistan, analyzed individually in triplicate

Table 2: Oil yield range in different *Eucalyptus crebra* trees

Oil yield range	Tree No.	Total tree	Percent trees
Above 1.0%	5, 6, 13	3	20
0.8-1.0%	1, 2, 3, 7, 8, 10	6	40
0.6-0.8%	4, 12	2	13.33
0.4-0.6%	11, 14, 15	3	20
0.2-0.4%	9	1	6.66

Table 3: Composition (%) of different components of essential oil of *Eucalyptus crebra* trees

	Components (%)					
	α-pinene	β-pinene	Δ^3 Carene	α-phallendrine	Limonene	1, 8-cineole
Tree 1	15.91±2.10	0.54±0.06	----	0.59±0.06	----	43.80±0.70
Tree 2	14.36±1.83	2.31±0.28	----	1.81±0.19	----	30.19±0.90
Tree 3	1.66±0.20	----	----	1.51±0.15	2.37±0.29	30.69±0.90
Tree 4	13.81±1.50	----	5.63±0.69	----	----	38.12±0.80
Tree 5	18.23±2.37	0.58±0.06	----	0.55±0.06	----	39.00±0.78
Tree 6	14.17±1.80	----	----	1.49±0.15	----	38.76±0.79
Tree 7	7.49±0.37	----	4.96±0.55	----	----	25.79±1.20
Tree 8	13.27±1.49	----	1.32±0.17	1.26±0.12	----	29.96±1.30
Tree 9	8.24±0.50	----	----	1.11±0.10	----	11.87±1.00
Tree 10	14.03±1.79	0.20±0.01	----	----	----	21.21±1.10
Tree 11	6.61±0.80	----	----	7.98±0.35	----	20.37±1.10
Tree 12	11.06±1.15	1.71±0.18	----	----	----	23.79±1.20
Tree 13	12.96±1.45	----	----	0.79±0.05	----	26.79±1.30
Tree 14	12.29±1.40	----	3.84±0.49	1.77±0.18	----	25.03±1.20
Tree 15	6.06±0.73	----	----	2.76±0.25	----	14.94±0.90

Values are means±SD of *E. crebra* essential oils from Pakistan, analyzed individually in triplicate

E. crebra oil of tree No. 2 showed 27 peaks of different compounds from which four components were identified. The identified compounds in the oil of tree No. 2 were α-pinene, β-pinene, α-phallendrine, 1, 8-cineole. The contents of α-pinene, β-pinene, α-phallendrine, 1, 8-cineole of essential oil from Pakistan was found to be 14.36, 2.33, 1.81 and 30.19%, respectively (Table 3). The values of all the content were quite comparable to the reported literature. *Eucalyptus crebra* oil of tree No. 3 showed 28 peaks of different compounds. The concentration of α-pinene were quite comparable to those of Iqbal *et al.*[8]. The concentration of 1, 8-cineole and Limonene were 30.69 and 2.37%, respectively. These concentrations were also with in the range of the finding of Bignell *et al.*[9]. The contents of α-phallendrine of essential oil from *Eucalyptus crebra* of tree No. 2 and 3 were not found to compare from the literature.

E. crebra oil of tree No. 4 showed 23 peaks of different compounds from which three compounds were identified. The concentration of α-pinene was found, 13.81% which is near the results reported by Iqbal *et al.*[8]. They reported it 19.82% in the oil of same species. It also within the range (0.1-28.3%) reported by Bignell *et al.*[9]. The concentration of 1, 8-cinole was found 38.12% with in the range 0.4-62.2% reported by Bignell *et al.*[9] in the oil of same species. The concentration of Δ^3-carene was found 5.63%. *E. crebra* oil from tree No. 5 showed 26 peaks of different compounds from which four compounds were identified. The concentration of α-pinene 18.23% and β-pinene 0.58% which is much near to the results reported by Iqbal *et al.*[8]. They reported that α-pinene (19.82%) and β-pinene (0.40%) in oil of same species, respectively.

E. crebra oil of tree No. 6 showed 33 peaks of different compounds from which three compounds calibrated. The amount of α-pinene was found 14.17% which is closed agreement to the results reported by Iqbal *et al.*[8]. The amount of 1, 8-cinole was found 38.76% with in the range 0.4-62.2% reported by Bignell *et al.*[9]. The amount of α-phallendrine was found 1.49%. No reference about the concentration of α-phallendrine in the oil of *E. crebra* was found. However most of the researchers have shown its presence in varying amounts in the oil of other *Eucalyptus* species. The values of 1-8, cineole of the leaves of *E. camaldulensis* were (25.3-44.2%) those reported by Tsiri *et al.*[22]. *E. crebra* oil of tree No. 7 showed 31 peaks of different compounds from which three compounds were identified by comparing the retention time with the retention time of the standards. The amount of α-pinene was found 7.49% which is much less as reported by Iqbal *et al.*[8]. They reported it 19.82% in the oil of same species but it also within the range (0.1-28.3%) reported by Bignell *et al.*[9]. The amount of 1, 8-cineole was 25.79% with in the range 0.4-62.2% reported by the Bignell *et al.*[9] in the oil of same species. The amount of Δ^3 carene was also found 4.96% in the *E. crebra* oil.

E. crebra oil of tree No.8 showed 26 peaks of different compounds from which four compounds were recognized. The concentration of α-pinene was found 13.27% which is near the results (19.82%) reported by the Iqbal *et al.*[8]. The concentration of α-phallendrine and Δ^3 carene were found 1.26 and 1.32%, respectively. The concentration of 1, 8-cinole was found 29.96% with in the range 0.4-62.2% reported by Bignell *et al.*[9] in the oil of *E. crebra*. *E. crebra* oil of tree No. 9 showed 29 peaks of different compounds from which three compounds were identified. The concentration of α-pinene was present 8.24% which was much less as reported by Iqbal *et al.*[8], 19.82% in the oil of *E. crebra*. The concentration of 1, 8-cineole was found 11.87% with in the range 0.4-62.2% reported by Bignell *et al.*[9] in the oil of same species. The concentration of α-phallendrine was 1.11%.

E. crebra oil of tree No. 10 showed 22 peaks of different components from which three components were identified by comparing the retention time of the standards. The component α-pinene was found 14.03% which is near the results reported by Iqbal *et al.*[8], 19.82% in the oil of same species. The components β-pinene and 1, 8-cineole was found 0.2 and 21.21%, respectively. These components were with in line when compared to the literature. Bignell *et al.*[9] reported that β-pinene and 1, 8-cineole 0-20% and 0.4-62.2% was present in the *E. crebra* oil. *E. crebra* oil of tree No. 11 showed 28 peaks of different compounds from three compounds were identified. The concentration of α-pinene was found 6.61% which is much less as compared to literature[8], (19.82%). The concentration of 1, 8-cineole was found 20.37% with in the range 0.4-62.2% reported by Bignell *et al.*[9] in the oil of same species. The concentration of α-phallendrine was 7.89%. Literature has shown its presence in varying amounts in the oil of other *Eucalyptus* species[22].

E. crebra oil of tree No. 12 showed 25 peaks of different compounds from which three major compounds were identified. The value of α-pinene was found 11.06% which was near the results reported by Iqbal *et al.*[8] (19.82%). The value of β-pinene and 1, 8-cineole was found 1.71%, 23.79% respectively. The value of β-pinene and 1, 8-cineole was with in the range 0-20%, 0.4-62.2% respectively reported by Bignell *et al.*[9] in the oil of *E. crebra*. *E. crebra* oil of tree No.13 showed 26 peaks of different compounds from which also three compounds were identified. The value of α-pinene was found 12.96%

which was near with the results reported by Iqbal *et al.*[8]. And also within the range (0.1-28.3%) reported by Bignell *et al.*[8]. The value of α-phallendrine was found 0.79%. The value of 1, 8-cineole was found 26.79% with in the range 0.4-62.2% reported by Bignell *et al.*[9].

E. crebra oil of tree No. 14 showed 27 peaks of different compounds from which four compounds were identified by comparing the retention time with the retention time of the standards. The amount of α-pinene was found 12.29% which was with in the range to the results reported by Bignell *et al.*[9]. The amount of α-phallendrine and Δ^3-carene was found 1.77 and 3.84%, respectively. The amount of 1, 8-cineole was found 25.03% with in the range 0.4-62.2% reported by Bignell *et al.*[9]. *E. crebra* oil of tree No. 15 showed 26 peaks of different components from which three components were identified. The amount of α-pinene was found 6.06% which is much lesser as reported by Iqbal *et al.*[8]. The amount of 1, 8-cineole was found 14.94% with in the range 0.4-62.2% reported by Bignell *et al.*[9] in the oil of same species. The amount of α-phallendrine in the oil of *E. crebra* was also found (2.79%).

Thus, it could be concluded from the present studies the oil percentage and chemical composition may vary from tree to tree within a species. It is, therefore, needed to explore this species from oil point of view Thus, it could be concluded from the present studies, the highest concentration of 1, 8-cineole in *E. crebra* may make it prominent than the other species for medicinal purpose. Therefore, this specie can be benefit from its potential essential oil for various commodities of medicinal and pharmacological attributes.

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