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Variations in Oil Potential and Chemical Composition of *Eucalyptus crebra* Among Different Districts of Punjab–Pakistan

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ABSTRACT

The present research work was aimed to study the intraprovenance variation for content and chemical composition of essential oil obtained from leaves of *Eucalyptus crebra.* For this purpose, leave samples of mature *Eucalyptus crebra* plants were collected from different districts of Punjab and oil was extracted and purified after pretreatment. Oil potential was calculated and samples were characterized for their chemical composition by Gas Liquid Chromatography. Significant variations were observed regarding oil potential (range 0.29 - 1.33%) of *Eucalyptus crebra* leaves among different districts of Punjab. *Eucalyptus crebra* oil was found to be principally comprised of 1, 8 - cineole (11.87 - 43.80%) and α -pinene (1.6 - 18.23%), whereas β - pinene (0.54 - 2.13%), α - phellandrene (0.55 - 7.98%), ∇^3limonene (2.7%; only in district Jhang) and ∇^3 carene (1.32 - 5.63%; in four districts) were present as minor constituents. Present studies show that *Eucalyptus crebra* is a potential source of essential oil and should be further characterized regarding its constituents for various commodities of cosmetics, medicinal and pharmacological attributes.

Key Words: *Eucalyptus crebra;* Essential oil; 1, 8 - cineole; α -pinene; β - pinene; α - phellandrene; ∇^3 carene; Characterization

INTRODUCTION

Much research has been conducted on medicinal properties of *Eucalyptus* species. *Eucalyptus* oil and its fresh leaves are used in steam inhalation treatments, consumed in leas and in bathing. *Eucalyptus* species are well known for their tolerance to a wide range of soil types and climates (Adhikari *et al.*, 1992).

Eucalyptus crebra is one of the most widely distributed *Eucalyptus* species. *Eucalyptus* species are used as an important source of timber, firewood, shelterbelt and as a honey tree (Boland *et al.*, 1984). It is also reported to be used as an anesthetic, antiseptic and astringent. *Eucalyptus* leaves are a traditional aboriginal herbal remedy (Chevallier, 1996). The leave oil is reported to be a powerful antiseptic and used all over the world for treatment of cough and cold, sore throat and other infections (Farah *et al.*, 2002).

All species of *Eucalyptus* contain *Eucalyptus* oil, less than 20% that is enough with the commercial point of view and only 10% of these accounts for the entire world production of essential oil (Peter, 2000). The aromatic oils are used as fragrance components in soap, detergents and toiletries etc. *Eucalyptus* oil is being extracted in many countries like China, India, South Africa, Portugal, Brazil and Tasmania on commercial scale. In 1992, the world *Eucalyptus* oil production was estimated to be 4000 tons of which 60 - 70% was consumed in medicinal market (Research Bureau of Industrial Advisory Centre, 2002). As in Pakistan, there is no facility for the extraction of essential oils, so country has to depend upon import, which is over 200 tons per annum. Essential oils normally imported in Pakistan are Camphor oil, Cedar oil, Cinnamon oil, Clove oil, *Eucalyptus* oil, Lavender oil, Lemon oil, Orange oil, Peppermint oil, Rose oil, Sandal oil, Galancal oil (Research Bureau of Industrial Advisory Centre, 2002). The percentage and composition of *Eucalyptus* oil vary significantly from species to species as well region to region due to different agro climatic environment. Cineole rich *Eucalyptus* species are becoming popular and *Eucalyptus crebra* is one of those species. It is reported that oil yield in *Eucalyptus crebra* varies up to 1.47% (Zafar *et al.*, 2003).

This paper describes variations in oil potential and chemical composition of *Eucalyptus crebra* among different districts of Punjab-Pakistan.

MATERIALS AND METHODS

Collection of samples. Fresh leaves samples from mature *Eucalyptus crebra* were collected from different regions of Punjab, Pakistan during the month of May and June 2004. The leaves were immediately preserved in polyethylene bags under refrigerator and transferred to experimental Laboratory.

Chemicals and reagents. Pure standards of α -pinene, β - pinene, α - phellandrene, 1, 8 - cineole, p - cymene, ∇^3limonene, γ - terpinene, camphene, linalool, 4 - terpineol, α - terpineol, geraniole and ∇^3 carene were obtained from Sigma Chemical company Co. (St. Louis, MO). All other chemicals and reagents used in the present research work were purchased from Sigma-Aldrich Chemical.

Oil isolation. Freshly collected 300 g leaves were weighed and hydrodistilled for three hours for complete extraction of essential oil, using a commercial Clevenger-type apparatus. The oil samples obtained from ten separate hydrodistillations were freed from moisture by adding anhydrous sodium sulfate and absolute oil samples were obtained.

Percentage yield of oil. The amount of extracted oil was determined and percentage yield of the extracted oil from each sample was calculated on the basis of *Eucalyptus crebra* leaves by using following formula:

$$\% \text{ age yield of oil} = \frac{\text{Weight of Oil}}{\text{Weight of Eucalyptus leaves}}$$

Colour and aroma. Colour and aroma of *Eucalyptus crebra* oil sample was noted carefully.

Gas liquid chromatographic analysis. The composition of essential oil was determined by gas liquid chromatography using Perkin Elmer Gas Chromatograph model 3920 equipped with flame ionization detector (FID) Shimadzu Chromatopac model C-R4A, fitted with hydrogen generator model HG-501 (GE USA). Glass column (2 m × 2 mm id) packed with 15% carbowax 20 m on chromosorb WAW was use and following conditions were adjusted:

Column temperature 80 - 160^{0}C, Ramp Rate 16^{0}C/min., Analysis Time 8 min., Injector temp. 150^{0}C, Detector temp. 200^{0}C, Nitrogen as Mobile gas with 25 mL/min flow rate, Hydrogen pressure 20 psi, Air pressure 50 psi and 0.06 µL vol. injected each time (Zafar *et al.*, 2003).

RESULTS AND DISCUSSION

The essential oil content from the leave samples of *Eucalyptus crebra* from 15 districts of province Punjab, Pakistan showed significant variations in the oil potential as well as in the chemical composition of essential oils (Table I). All the extracted oils were of light yellow colour having camphor like smell.

The results revealed highest (1.33%) and lowest (0.29%) oil content in the leaves of *Eucalyptus crebra* collected from Shekhupura and Bahawalnagar, respectively. The soil of district Shekhupura and Lahore was found to be very fertile regarding the essential oil production from *Eucalyptus crebra* leaves. It is obvious from the results that 20% districts of Punjab had above 1.0%, 40% in the range of 0.8 - 1.0%, 13% in the range of 0.6 - 0.8%, 20% in the range of 0.4 - 0.6 % and 6.0% in the range of 0.2 - 0.4% essential oil yield from *Eucalyptus crebra* leaves. Zafar *et al.* (2003) also reported similar results regarding oil potential for the different *Eucalyptus* species from Faisalabab i.e. 0.58 - 1.47%. Wildy *et al.* (2000) investigated four promising *Eucalyptus* species from Western Australia at six locations and reported 0.01 - 13.0% oil production. These variations might be attributed to different agro-climatic regions and soil composition in the districts of Punjab.

Seven to 28 compounds were detected in essential oils, of which 3 to 4 were well identified as the major compounds in *Eucalyptus crebra* leaves oil (Table I). The major components were α -pinene (15.91%), β - pinene (0.54%), α - phellandrene (0.59%) and 1, 8 - cineole

Table I. Variation in oil potential and chemical composition of *Eucalyptus crebra* leave oil from different districts of Punjab, Pakistan

Sample	Oil Potential (% age)	Possible Compounds with concentration (% age)					
		α -pinene	β - pinene	α - phellandrene	1, 8 – cineole	∇ limonene	∇^3 carene
FSD	0.97± 0.04	15.91± 1.11	0.54 ± 0.04	0.59 ± 0.05	43.80 ± 2.62	---	---
SGD	0.94± 0.05	14.36 ± 1.20	2.13 ± 0.87	1.81 ± 0.10	30.19 ± 2.01	---	---
JHG	0.95± 0.04	1.66 ± 0.08	---	1.51 ± 0.13	30.69 ± 2.11	2.37 ± 0.09	---
SWL	0.63± 0.02	13.81 ± 1.10	---	---	38.12 ± 2.38	---	5.63 ± 0.60
SKP	1.33± 0.06	18.23 ± 1.99	0.58 ± 0.02	0.55 ± 0.03	39.0 ± 2.30	---	---
LHR	1.12± 0.05	14.17 ± 1.30	---	1.49 ± 0.11	38.76 ± 1.99	---	---
RYK	0.88± 0.04	7.49 ± 0.49	---	---	25.79 ± 1.78	---	4.96 ± 0.44
BWP	0.96± 0.03	13.27 ± 1.12	---	1.26 ± 0.21	29.96 ± 2.06	---	1.32 ± 0.21
BWN	0.2 ± 0.03	8.24 ± 0.38	---	1.11 ± 0.17	11.87 ± 1.76	---	---
VHR	0.8 ± 0.03	14.3 ± 1.30	0.20 ± 0.01	---	21.21 ± 1.29	---	---
MTN	0.5 ± 0.03	6.61 ± 0.31	---	7.98 ± 0.47	20.37 ± 1.09	---	---
LYA	0.64± 0.02	11.06 ± 1.05	1.71 ± 0.05	---	23.79 ± 1.77	---	---
CKL	1.00± 0.06	12.96 ± 1.06	---	0.79 ± 0.04	26.79 ± 1.28	---	---
NKA	0.45± 0.02	12.29 ± 1.09	---	1.77 ± 0.18	25.03 ± 1.06	---	3.84 ±
GRW	0.59± 0.03	6.06 ± 0.29	---	2.76 ± 0.91	14.94 ± 1.12	---	---

Values (mean ± SD) are average of duplicate samples analyzed in triplicate; FSD = Faisalabad, SGD = Sargodha, JHG = Jhang, SWL = Sahiwal, SKP = Shekhupura, LHR = Lahore, RYK = Rahim Yar Khan, BWP = Bahawalpur; BWN = Bahawalnagar, VHR = Vehari, MTN = Multan, LYA = Layyah, CKL = Chakwal, NKA = Nankana, GRW = Gujranwala

(43.8%). It is evident from results (Table I) that the chemical composition of essential oils varied in leaves collected from different areas. Similar variations in chemical composition in essential oil have been reported by previous workers within Pakistan and also across the world (Sheih, 1996; Bignell *et al.*, 1997; Oyedeji *et al*., 2000; Pegula *et al.*, 2000; Tsiri *et al.*, 2003; Zafar *et al.*, 2003).

It could be concluded from the present studies that *Eucalyptus crebra* is a potential source of essential oil and it can grow in diverse type of climatic conditions. It should be further characterized for various commodities of cosmetics, medicinal and pharmacological attributes.

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