Variation in Composition and Yield of Foliage Oil of *Eucalyptus Polybractea*

ZAFAR IQBAL*, MUHAMMAD AKHTAR, TARIQ MAHMOOD QURESHI,
JAVED AKHTER AND RASHID AHMAD
Nuclear Institute for Agriculture and Biology (NIAB), Jhang Road, Faisalabad, Pakistan.

(Received on 31st May 2010, accepted in revised form 30th July 2010)

Summary: *Eucalyptus polybractea* (blue mallee) is the essential oil rich species used in the commercial production of pharmaceutical-grade *Eucalyptus* oil in Australia. This species was grown at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan during 2004-08 to investigate the quantity and quality of its foliage oil. The oils were extracted by hydro-distillation method, from the leaves of four year aged ten *E. polybractea* plants. The data showed a significant intra-species variation in their oil contents (29.3 to 41.8 mg g^{-1} fresh weight of leaves). Out of ten plants eight contained oil >30 mg g^{-1} fresh weight of leaves. The components of the extracted oils varied from 12-26 as detected by GC/FID on Carbowax 20 M packed glass column. Among all the oil components, 1, 8-cineole was the major compound (91.7–94.2 %), while the other identified compounds were α-pinene (0–1.2 %), β-pinene (0.4–2.3 %), limonene (0.2–1.3 %), *p*-cymene (1.23–2.75 %), and terpinene-4-ol (0.6–0.92 %). The extracted oils from all the *Eucalyptus polybractea* plants contained high amount of 1, 8-cineole (>90 %), therefore, classified as species of high quality medicinal oil.

Introduction

Eucalyptus (family Myrtaceae), known as mallee in Australia [1], has become the most widely planted tree in the world which occupies more than 18 million hectares [2]. Its leaves contain oil glands capable to produce oils of different composition [3], hence is categorized according to their composition as medicinal, perfumery, and industrial. The medicinal value of *Eucalyptus* oil is based largely on its 1, 8- cineole content [4]. According to international standards, the minimum cineole content should be 70 % in pharmaceutical grade *Eucalyptus* oil [5]. Medicinal *Eucalyptus* oil is largely employed in the preparation of liniments, inhalants, cough syrups, ointments, toothpaste, and pharmaceutical flavorings. The oil of *Eucalyptus* species has also antioxidant properties [6] and anti-inflammatory effects [7]. There is also a potential to capture new markets as an alternative natural industrial solvent to replace tri-chloromethane which has recently been banned for further production [8, 9]. In addition, cineol has been found to enhance the stability of petrol-ethanol fuel mixture when added in small quantities [10].

Eucalyptus polybractea R. T. Baker, (blue mallee) is the key species used for commercial production of pharmaceutical-grade oil in Australia [4]. It is a small multi-trunked sclerophyll tree that grows naturally in western New South Wales and Victoria, Australia. Its bark is smooth and fibrous near the trunk base, leaves are disjunct and linear to narrow-lanceolate. Its Juvenile leaves are glaucous and adult leaves grey-green [11]. Many researchers has reported that oil yield from blue mallee foliage is generally higher than other commercial *Eucalyptus* (as high as 65 mg g^{-1} fresh weight) and the oil comprises of up to 95 % (V/V) cineole [12].

Introduction of *Eucalyptus* in Pakistan is not new but the earlier scientists focused their research only on commercial *Eucalyptus* species usually grown for wood production. In this regard Qadri [13] recommended a large scale plantation of *Eucalyptus camaldulensis*, *E. citriodora*, *E. melanophloia*, *E. microtheca*, *E. robusta*, *and E. tereticornis*, and Pryor [14] recommended all the above-mentioned species with the exception of, *E. robusta.* In Pakistan some work has been reported on the leaf oil of commercial *Eucalyptus* species, grown for wood purpose [15, 16] but no study has been found on any oil rich *Eucalyptus* species. Keeping in view the importance of medicinal value of *Eucalyptus* oil, *E. polybractea* plants were grown to explore its oil potential in our environment with respect to content and chemical composition.

Results and Discussion

Essential oils extracted from all the *Eucalyptus polybractea* plants under study were clear colorless mobile liquids having camphor like smell predominantly of 1, 8-cineole. Grieve [17] also reported similar characteristics regarding colour and smell of *Eucalyptus polybractea* oil. The amount of extracted oils from the foliage of all the plants under study varied significantly ($F = 126.68$, $df = 9$, $p = 0.0000$) [18] and ranged between $29.3 \pm 0.36 – 41.8 \pm 0.60$ mg g^{-1} fresh weight (fw) (Table-1). The

*To whom all correspondence should be addressed.

maximum amount of oil (41.8 mg g^{-1} fw) was found in the leaves of plant No. 2 followed by 4, 3, 7, 9, 10, 6, 1, 8 and 5 (Table-1). Leaves of the two plants (5 and 8) produced <30 mg g^{-1} oil while the remaining eight plants produced >30 mg g^{-1} oil (Table-2). Goodger *et al*., [12] reported higher oil yield from the foliage of blue mallee (up to 65 mg oil g^{-1} fw) than other commercial *Eucalyptus* species. Whereas, Wildy *et al.*, [19] and other [20] found average leaf oil content of 21.2 mg g^{-1} fw in the same species grown at 12 different locations of Western Australia. The present study found comparable results to those reported by Goodger, and Woodrow [21]; they reported essential oil 30.8-61.1 mg g^{-1} fw in the leaves of same species. The foliage oil of *E. polybractea* grown at Faisalabad, was higher (29.3 – 41.8 mg g^{-1} fw.) as compared to the oil content produced by commercial *Eucalyptus* species *i.e. Eucalyptus camaldulensis* (3.0 - 11.7 mg g^{-1} fw.) [15] *and Eucalyptus globules* (5.3 - 10.6 mg g^{-1} fw.) [16] grown in the same environment. Variation in oil content/composition in different trees of the same species at same location may be due to ontogeny, phenology, and stage of leaves (juvenile, intermediate, adult, and mature) [21, 22].

Table-1: Leaf oil yield by hydro-distillation from different *Eucalyptus polybractea* plants.

Plant No	Mean leaf oil yield (mg g^{-1} fresh weight of leaves)	Standard deviation
1	30.10 e	0.40
2	41.80 a	0.60
3	35.10 c	0.46
4	38.53 b	0.87
5	29.30 e	0.36
6	32.17 d	0.71
7	34.93 c	0.75
8	29.70 e	0.60
9	34.57 c	0.75
10	32.40 d	0.46

Figures in a column sharing the same letter(s) do not differ significantly at $p < 0.01$

Table-2: Oil yield range in different *Eucalyptus polybractea* plants.

Oil yield range (mg g^{-1} fw)	Plant number	Percent plants
> 40	2	10.0
35-40	3, 4	20.0
30-35	1, 6, 7, 9, 10	50.0
< 30	5, 8	20.0

Chemical Composition of Eucalyptus Polybractea Oils

Components of leaf oils extracted from 10 different *Eucalyptus polybractea* plants were determined by gas chromatography. Total number of detected compounds in the oils of these plants ranged between 12-26 (Table-3). The retention time of standard compounds is shown in (Table-4). The identified components in all the extracted oils were 5 to 6. Based on the peak area/peak height, concentration of all the detected/identified compounds was calculated by the C-R4A Chromatopac (Shimadzu) software. The major identified compounds in these oils were α-pinene, β-pinene, limonone, *p*-cymene, terpinene-4-ol and 1, 8-cineole which ranged between 0 – 1.2 %, 0.4 – 2.3 %, 0.2 – 1.3 %, 1.23 – 2.77 %, 0.60 – 0.92 % and 91.7 – 94.18 % respectively. The detected compounds varied significantly ($p < 0.01$), except 1, 8 cineol which varied non-significantly ($F = 1.67$, df = 9, $p = 0.1616$) [18] among all the extracted oils (Table-3). The DMR test showed highly significant variation ($F = 153.76$, df = 9, $p = 0.0000$) [18] in the concentration of α-pinene in all the extracted oils which was maximum (1.2 %) in plant No 5 while not detectable in plant No 2, 3 and 9. The concentration of β-pinene (second identified compound) also varied significantly ($F = 112.36$, df = 9, $p = 0.0000$) [18] in different plants. The maximum concentration of β-pinene was 2.3 % and the minimum was 0.4 % in the oil of tree No 5 and 2, respectively. The 70 percent plants contained β-pinene below 2 % while the 30 percent contained above 2 % (Table-3). Our findings for α and β-pinene are higher than those reported by Goodger, and Woodrow [21] they reported α-pinene, 0.32 to 0.6 % and β-pinene, 0.09 to 0.17 % in the oil of same species, but close to as reported by Wildy *et. al.*, [19] they estimated α-pinene, 1.51 % and β-pinene, 0.7 % in the oil of blue mallee. Limonine concentration in these extracted oils was significantly variable ($F = 57.61$, df = 9, $p = 0.0000$) [18] and ranged between 0.2 – 1.3 % (Table-3). Highest amount of limonine (1.3 %) was found in the oil of plant No 5 while lowest (0.2 %) in plant No. 2. Goodger, and Woodrow [21] and Wildy *et. al.*, [19] reported limonine in the oil of same species from 1.11 to 1.94 % and 2.12 %. Wildy *et. al.*, [19] reported para cymene (1.36 %) in the oil of *E. polybractea* which lies within the range (1.23-2.75 %), as determined in our oil samples but higher to the findings of Goodger, and Woodrow [21], they estimated para cymene 0.26 to 1.04 % in the oil of same species. Concentration of terpinene-4-ol, varied between 0.60-0.92 % in the oils of blue mallee grown at NIAB. Our findings of terpinene-4-ol are lesser than as reported by Goodger, and Woodrow [21] and close to Wildy *et. al.*, [19] they reported it 1.01 to 1.54 % and 0.81 %, respectively. The major compound in all the oils was 1, 8-cineole which showed remarkably narrow range between 91.7 – 94.18 %, suggesting strong genetic control of essential oil composition, its amount was lowest (91.7 %) in the oil of tree No 5 and highest (94.18 %) in tree No 2. All the plants of *E. polybractea* were found containing 1, 8-cineole above 90 % (Table-3).

Table-3: Detected / identified compounds and concentration of identified compounds on 15 % Carbowax 20 M packed glass column in the oil of different *Eucalyptus polybractea* plants.

Plant No	Compounds		α- Pinine (%)	β-Pinene (%)	Limonine (%)	*p*-Cymene (%)	Terpinene-4-ol (%)	1,8 Cineol (%)
	Detected	Identified						
1	13	5	1.1ab	0.93 d	0.65 b	1.78 d	0.68 bc	92.08 N.S
2	17	5	0.0 d	0.40 e	0.20 d	1.23 e	0.60 c	94.18 N.S
3	20	4	0.0 d	0.97 d	0.74 b	2.14 bc	0.81 ab	92.52 N.S
4	26	5	0.7 c	0.77 d	0.62 b	1.57 d	0.74 abc	93.45 N.S
5	19	6	1.2 a	2.30 a	1.30 a	2.30 b	0.92 a	91.70 N.S
6	15	5	0.75 c	0.85 d	0.59 b	1.87 cd	0.67 bc	93.10 N.S
7	20	5	0.71 c	1.73 c	0.78 b	2.31 b	0.73 abc	92.25 N.S
8	12	6	1.12 ab	2.16 ab	1.27 a	2.77 a	0.88 a	91.76 N.S
9	18	4	0.0 d	1.96 bc	0.69 b	2.28 b	0.67 bc	92.33 N.S
10	20	6	0.96 b	2.05 ab	1.24 a	2.75 a	0.84 ab	91.93 N.S

Figures in a column sharing the same letter(s) do not differ significantly at $p < 0.01$
N.S. = Non significant

Table-4: Retention time of different standard compounds on 15 % Carbowax packed glass column.

Compound	Retention time (min.)
α – Pinine	0.95
β-Pinene	1.26
p-Cymene	1.78
α – Phalendrene	2.17
Limonine	2.58
1, 8 cineol	3.13
γ – Terpinene	3.63
Terpinene-4-ol	8.34
Citronellal	14.27

Most of the literature indicated the presence of 1, 8-cineole up to 95 % in the oil of *Eucalyptus polybractea* [12] which resembles with our findings for 1, 8-cineole in the oil of same species. Goodger, and Woodrow [21] reported 1, 8-cineole, 87.0-90.8 % and Wildy *et. al.*, [19] reported it 87.32 %in the oil of *E. polybractea* which is also not much lower as compared to our findings. Goodger, and Woodrow [4] reported 90-92 % and 92-94 % 1, 8-cineole in the essential oils extracted from two micropropagated clones of *E. polybractea* which is almost similar to our findings. The quantity of 1, 8-cineole in the leaf oil of *Eucalyptus camaldulensis* and *Eucalyptus globules* grown at Faisalabad was 41.6 - 85.67 % and 17.46 - 51.62 %, respectively, [15, 16] which clearly indicated that different trees of both the commercial species produced oil having 1, 8-cineole in much wider range and mostly unsuitable for medicinal use as compared to *E. polybractea* oil which produced high quantity/quality of medicinal oil having 1, 8-cineole in narrow range (91.7-94.18 %). Some earlier studies have indicated that citronellal and phalendrene, which can be found in some *Eucalyptus* species, are weak mutagenic and carcinogenic, respectively, [23] and both these compounds were found absent in all the extracted oils under study, which is clear from the chromatogram of the *E. polybractea* oil (Fig. 1) that no peak of α-phalendrene and citronellal appeared at retention time 2.17 and 14.27 minutes, respectively, whereas both these peaks were present in the chromatogram of standard compounds (Fig. 2). Therefore, leaf oil of *E. polybractea* species grown at NIAB, Faisalabad could be most suitable oil for medicinal uses as it contained highest percentage of 1, 8-cineole (>90 %) and is free from undesirable compounds.

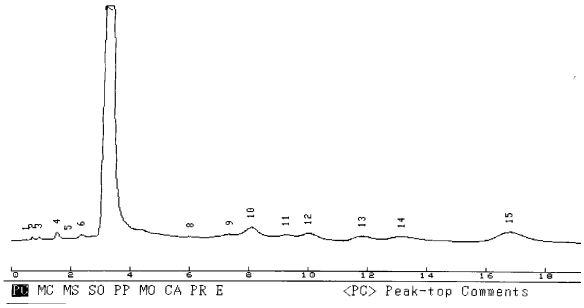

Fig. 1: Chromatogram of leaf oil of *Eucalyptus polybractea* on 15 % Carbowax 20M packed glass column.

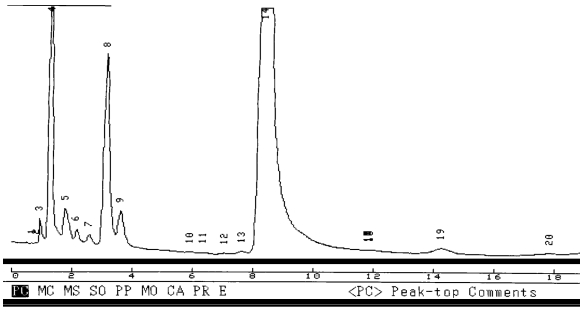

Fig. 2: Chromatogram of different standard compounds on 15 % Carbowax 20M packed glass column.

Experimental

Seeds of *Eucalyptus polybractea* (blue mallee) were provided by Australian Tree Seed Center, CSIRO, Forestry and Forest Products PO Box E4008, Kingston ACT 2604, Australia. The seed germination was carried out in oval shaped plastic pots containing sand. After two months of germination, seedlings were transferred into plastic bags containing soil. The plants of six month age were transferred in the field area of Nuclear Institute

for Agriculture and Biology (NIAB), Faisalabad having normal soil with clay loam texture. Faisalabad is situated between longitude 73°74' East and latitude 31°-25' North, with an elevation of 184 meters (604 ft) above sea level.

Collection of Leaf Samples and Extraction of Oil

Leaf samples were collected from uncut saplings of four year old *Eucalyptus polybractea* species and were retained temporarily in cool conditions (12-18 °C) and subjected to hydro-distillation at normal pressure [19] within 24 h of harvest. The oil extracted from each sample was found containing fraction of water, which was removed by adding anhydrous sodium sulphate (75 mg mL^{-1}) and stored at -20 °C until analyzed.

Chemical Composition of the Extracted Oils

Determination of the chemical composition of the extracted *Eucalyptus polybractea* oil from each plant was carried out by Perkin-Elmer gas chromatograph (Model 3920), equipped with flame ionization detector (FID) and Shimadzu C-R4A chromatopac. The column used was made of glass (2 m x 2 mm i.d.) packed with 15 % Carbowax 20 M on chromosorb W AW (80-100 mesh). Identification of the compounds was carried out by comparing their retention time with the retention time of standard compounds. Concentration of the detected/identified compounds was determined by the C-R4A Chromatopac (Shimadzu) software considering the peak area/peak height.

Oil Analysis

Instrumental Conditions

Column temperature programming: 80 °C (1 min.), increase @ 16 °C/min (80-160 °C), stay at 160 °C (8 min.).

Injector temperature:	150 °C
Detector temperature:	200 °C
Nitrogen flow rate:	25 mL/min
Hydrogen pressure:	20 psi
Air pressure:	50 psi

Conclusion

The essential oils extracted from all the *E. polybractea* plants under study were almost close to each other for their oil content and chemical composition. Oil of all the plants contained 1, 8-cineole more than 90 % and free from undesirable components like phalendrene and citronallal, therefore, our present study had clearly suggested that the oils of the *Eucalyptus polybractea* species grown at NIAB, Faisalabad is most suitable for medicinal uses.

Acknowledgements

The authors are grateful to P. M. Potto for the provision of *Eucalyptus polybractea* seeds from Australian Tree Seed Center, CSIRO Forestry and Forest Products PO Box E4008, Kingston ACT 2604, Australia.

References

1. D. Bown, In *Encyclopedia of Herbs and Their Uses*, DK Publishing Inc., New York, pp. 280 (1995).
2. D. Grattapaglia, In *Genomics of Tropical Crop Plants Volume 1*, *Genomics of Eucalyptus*, *a Global Tree for Energy*, *paper and Wood*, Springer Publisher New York. pp. 259 (2008).
3. M. Grieve, In *A modern herbal*, Tiger Books International London. Dorset Press, pp. 287 (1992).
4. J. Q. D. Goodger and I. E. Woodrow, *Journal of Forest Ecology and Management*, **255**, 3652 (2008).
5. J. Coppen and C. Chandrasekharan, In *Flavours and fregrances of plant origin*, *Eucalyptus oil*, FAO-Food and Agriculture Organization of the United Nations, Rome (1995).
6. J. Grafsmann, S. Hippeli, K. Dornisch, Urohnert, N. Beuscher and E. F. Elstner, *Arzneim Forsch/Drug Research*, **50**, 135 (2000).
7. U. R. Juergens, U. Dethlefsen, G. Steinkamp, A. Gillissen, R. Repges and H. Vetter, *Respiratory Medicine*, **97**, 250 (2003).
8. A. F. M. Barton, J. Tjandra and P. G. Nicholas, *Journal of Agriculture and Food Chemicals*, **37**, 1253 (1989).
9. A. F. M. Barton, P. P. Cotterhill and M. I. H. Brooker, *Silvae Genetica*, **40**, 37 (1991).
10. J. Tjandra., Ph. D. *Thesis*, *Eucalyptus oil: Sources*, *Analysis and Chemical Properties*. Murdoch University, Perth, Western Australia, (1986).
11. G. J. Harden, In *Flora of New South Wales - Volume 2*. New South Wales University Press, Kensington, NSW. ISBN 0-86840-164-1 (1991).
12. J. Q. D. Goodger, C. A. Connolly and I. E. Woodrow, *Journal of Forest Ecology and Management*, **250**, 196 (2007).
13. S. M. A. Qadiri, Selection of *Eucalyptus* species for afforstation in West Pakistan, in Proceedings

of the Second Pakistan Silvicultrual Conference, pp. 145 (1966).
14. L. D. Pryor, *Pakistan Journal of Forestry*, **18**, 45 (1968).
15. Z. Iqbal, H. N. Bhatti and M. Naeem, *Journal of the Chemical Society of Pakistan*, **30**, 468 (2008).
16. Z. Iqbal, H. N. Bhatti, I. N. Ahmad and S. A. S. Chatha, *Journal of the Chemical Society of Pakistan*, **28**, 397 (2006).
17. M. Grieve, "*A modern Herbal*" Tiger books international Londen. P. 287 (1992).
18. R. G. D. Steel and J. H. Torrie, Principals and procedures of statistics. Mc Graw-Hill Book Company, Inc. New York. (1980).
19. D. T. Wildy, J. S. Pate and J. R. Bartle, *Journal of Forest Ecology and Management*, **134**, 206 (2000).
20. M. A. Zaidi, G. Shaheen, N. Jehan et al., *Journal of the Chemical Society of Pakistan*, **32**, 77 (2010).
21. J. Q. D. Goodger and I. E. Woodrow, *Journal of Forest Ecology and Management*, **258**, 650 (2009).
22. A. F. M. Barton, P. P. Cotterill and M. I. H. Brooker, *Silvae Genetica*, **40**, 37 (1991).
23. J. C. Doran, In *Eucalyptus leaf oils: Use*, *Chemistry*, *Distillation and Marketing*, ACIAR/CSIRO, Inkata press, Melbourne, pp. 11-25 (1991).
24. N. Pages, G. Fournier, F. L. Luyer and M. C. Marques, *Planets Medicinales et. Phytotherapie*, **24**, 21 (1990).