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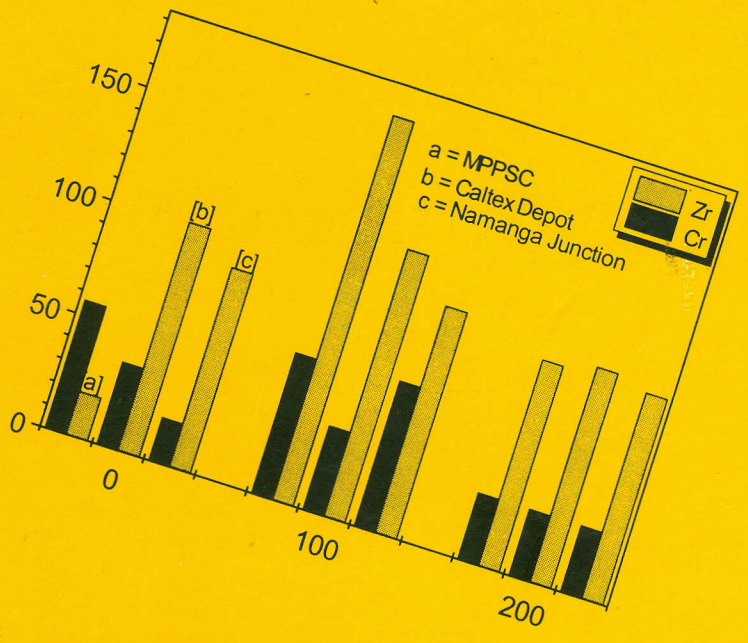

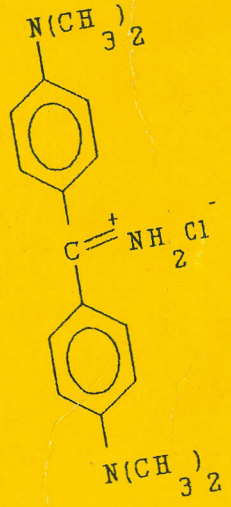

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Analysis of essential oil from Kenyan *Eucalyptus globulus* Labill.

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The essential oil of fresh leaves of *Eucalyptus globulus* Labill. (Myrtaceae) obtained from Muguga Arboretum, Kiambu District, Kenya, was extracted by steam distillation for varying times and analysed using GC and GC-MS. The optimum time was found to be 5 hours with the yield of the oil being 2.48% while the content of 1,8-cineole was found to be 55.87%. Other constituents in this medical oil include eudesmol, (±)-linalool, ß-pinene, trans-citral, carvone and α-terpineol.

Key words: Myrtaceae, *Eucalyptus globulus* Labill., 1,8-cineole, essential oil, Gas chromatography-mass spectrometry, medical oil.

INTRODUCTION

Eucalyptus globulus Labill. (Myrtaceae) is commonly known as blue gum and it is a very fast growing tree that often attains a height of 100m. It has a straight bole up to two thirds of total height and a well-developed crown. The bark breaks off continuously in strips from the trunk and branches. The juvenile bluish leaves are opposite, highly glaucous, oblong-acuminate in shape whereas the adult leaves are alternate, petiolate, non-glaucous, falcate-lanceolate in shape (Pryor, 1976). This tree is a native of Australia and is cultivated in warmer regions as a timber or decorative tree. In Kenya, this tree is grown by farmers because of its high timber yield. It is especially grown in Central, Rift Valley, Nyanza and Western Provinces (Mungai, 1990), though there is no authentic documented data on its total availability.

The crude oil of *E. globulus* is a light yellow liquid with a pronounced odour of volatile aldehydes. This oil is obtained by steam distillation of fresh leaves and terminal branches in a yield of between 0.75 and 1.25% (Guenther, 1972).

The oil of *E. globulus* was first investigated by Cloez in 1870, who isolated a substance (b.p. 175°C) which he called eucalyptol but which was evidently still accompained by terpenes and therefore not pure (Guenther, 1972). Since then various components of the volatile oil of *E. globulus* from different regions of the world have been identified by different workers and by the use of various analytical techniques (Cardoso & Cunha, 1972; Calvin & Nishimura, 1979; Allan & Peter, 1989; Agarwal *et al.*, 1990). 1,8-Cineole (eucalyptol) was found to be the major component (47-56%), other components include α-pinene, α-terpineol, globulol, τ-cadinene, α-gurjunene, eudosmol, carvone, citronellal, limonene and camphene. For this oil to be accepted by the world market the crude oil must be rectified to meet the British pharmacopoeia standards (1988) of cineole content not less than 70 per cent w/w.

In Kenya, the steam distillation trials of the leaves of *E. globulus* were performed earlier by Maitai and Talalaj (1984) and Ngunjiri *et al.* (1985). These workers did not establish the yield of the oil with respect to the cineole content. The present study was undertaken to evaluate economically the suitability of the oil obtained from the fresh leaves and terminal branches of *E. globulus* growing in Kenya mainly for pharmaceutical industry.

MATERIAL AND METHODS

Reagents and Reference Compounds

The reference compounds were obtained from Aldrich Company Limited, England. All the

solvents and other reagents used were purchased locally and were of analytical grade.

Instrumentation and Operating Conditions

The specific gravity, refractive index and optical rotation were determined at 20.0 ±0.1°C by using pycnometer, refractometer (Atago Company Limited; model 1T) and polarimeter (Instruments for Research and Industry; model 554 Bs), respectively. A 0.5% solution of the oils in n-hexane was used to determine optical rotation.

GC was performed on a Hewlett Packard 5790A gas chromatograph fitted with an FID detector and a Hewlett Packard 3393A electronic integrator. A Hewlett Packard-20M (Carbowax 20M) 50 m × 0.2 mm i.d. × 0.1 μm film thickness column with nitrogen as carrier gas at 40 ml/min was used. The temperature was programmed from 60°C (7 min) to 220°C (10 min) at 10°C/min.

GC-MS was performed on a VG Analytical 12-250 instrument equipped with a Hewlett Packard 5790A GC and a data system. The column and column temperature programme that were used for the GC analyses were used for GC-MS determinations. EIMS spectra were recorded at 70eV at a source temperature of 200°C. Injector was used in the splitless mode.

Plant Material

Fresh leaves and terminal branches of *E. globulus* were collected from Muguga Arboretum, Kiambu District, in January and April 1990 to compare the yields. They were packed in polythene bags and covered tightly to avoid loss of essential oils and then transported to Nairobi. The taxonomic identification of the plant was established by Mr. Simon Mathenge and a voucher specimen was deposited in the herbarium of the Botany Department, University of Nairobi, Nairobi, Kenya.

Extraction, Isolation and Identification

The leaves and terminal branches were cut into small pieces using a pair of scissors prior to extraction.

Four batches, each of 250g, of fresh leaves and terminal branches and 1000ml water were steam distilled using a modified Clevenger apparatus. The distillation times were 4, 5, 6 and 7 hours.

The yield of oil was expressed on moisture-free basis. The essential oils were dried over anhydrous sodium sulphate and stored in sealed ampoules at 0°C until use.

Component identity was established by retention time comparison with reference compounds and by peak enhancement using co-injection. Library MS searches and the comparison with published data and/or available mass spectra collections were also used for peak identification and correlation.

Rectification of Crude Oil

The crude oils obtained for 5 hours of steam distillation were rectified by dry distillation in an inert atmosphere and the fractions distilling in the range 170–178°C were collected. The percentage content of 1,8-cineole in the rectified oils varied between 85–90%.

RESULTS

Steam distillation of the fresh leaves and terminal branches of *E. globulus* yielded a colourless to pale yellow oil with a characteristic cineole-like odour.

Table 1 lists the percentage yields of oil from different batches of *E. globulus* fresh leaves and terminal branches (collected in January and April), refractive index, optical rotation, specific gravity and percentage yield of 1,8-cineole (of the leaves collected in April) in the oils extracted for 4, 5, 6 and 7 hours, while Table 2 lists the constituents identified with relative percentages in the oil obtained for 6 hours of steam distillation.

DISCUSSION

The yield of the oil from leaves and terminal branches picked in April 1990 is slightly higher than that of samples picked in January of the same year. This could be attributed to climatological changes. January 1990 was a comparatively hot, dry and windy month. The weather in April allows for more physiological activity in the leaves of *E. globulus* than that in January (Guenther, 1972); so

Table 1. Percentage yield of oil, 1,8-cineole and physical properties of the essential oils of *E. globulus*.

Times of Extraction (hrs)	% yield (v/w) (Jan.)	% yield (v/w) (Apr.)	Refractive Index at 20°C±0.1	Optical Rotation 20°C±0.1	Specific Gravity 20°C±0.1	% 1,8-Cineole
4	2.24	2.40	1.462	+5°	0.896	34.30
5	2.44	2.48	1.463	+5°	0.896	55.87
6	2.50	2.56	1.464	+5°	0.897	40.13
7	2.52	2.60	1.465	+8°	0.924	35.55

for better yield it is advisable to harvest the leaves in April. The physical properties of the oil of *E. globulus* arewell within the British Pharmacopoeia specifications (1988).

From Table 1 the amount of 1,8-cineole increases for the first 4 and 5 hours of extraction (from 34.30% to 55.87%) and then drops off. Since at 5 hours of extraction the yield is high (2.48% for leaves collected in April) and the 1,8-cineole content is highest, 5 hours can be considered to be the optimum time for maximum yield. This yield tallies with that reported by Guenther (1972) concerning the Australian *E. globulus*.

The high 1,8-cineole content in the oil of *E. globulus* analysed during the present study classifies it among the medicinal oils of *Eucalyptus* species (Guenther, 1972). The rectified oil with 1,8-cineole content 85–90% is phellandrene free and meets the requirement of pharmacopoeias for its use in pharmaceutical preparations, for example, cough-drops and vaporizers.

The crude oil can also be marketed for use in products where pharmaceutical standards are not required, e.g. in soaps, detergents and disinfectants. However, further investigations on the availability of this plant in different geographical regions, seasonal variations and time of harvest are required to have a conclusive information on the essential oil content of *E. globulus* trees growing in Kenya.

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Table 2. Constituents of essential oil of *E. globulus*.

Constituent	R_t (Min)	% Total	Identification method
(±)-Linalool	10.18	t	ms, pe
3-Decyn-2-ol	10.43	t	ms
4-Terpineol	11.15	t	ms
α-Terpineol	12.22	t	ms, pe
1,8-Cineole	13.13	40.13	ms, pe
Trans-Citral	13.28	t	ms
Cis-Guai-9-en-11-ol	17.22	t	ms
β-Pinene	18.01	7.63	ms, pe
β-Eudesmol	19.04	29.51	ms
α-Eudesmol	19.07		
Carone	20.01	6.48	ms
Cis(A/B)-Sclaeroxide	21.56	t	ms
2-Ethyl-5,8-dihydroxy-1,4-naphthaquinone	25.01	2.34	ms

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