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Analysis of the essential oil of *Juniperus procera Endl.* growing in Kenya

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Summary

The essential oil of the wood of *Juniperus procera Endl. (Cupressaceae)* from the Aberdares mountains, Central Province, Kenya has been extracted by steam distillation for varying times and analyzed using GC and GC-MS. The optimum time has been found to be 8 hours with the yield of the oil and cedrol as 0.48 and 79.10%, respectively. Other constituents include 3,4-dimethylbiphenyl, a-cedrene, camphor, trans-geraniol, eugenol, and a-terpineol.

Résumé

L'huile essentielle du bois de *Juniperus procera Endl. (Crupressaceae)* des montagnes Aberdares, de la province Centrale du Kenya, a été extraite par distillation à vapeur à des temps varies et analysé en utulisant le GC et GC-MS. Le temps optimum a été de 8 heures avec une production de l'huile et le cedrol à valeur de 0.48% et 79.10% respectivement. Les autres constituants includent le 3,4-dimethyl-biphenyl, l'α -cedrene, le camphor, le transgeraniol, l'eugenol et l'α -terpineol.

Introduction

Juniperus procera Endl. (Cupressaceae) is commonly known as pencil cedar (Kiswahili-mwangati). It is an evergreen dioescious timber tree attaining up to 36 m height. Its crown is pyramidal when young and spreads with age. The bark is pale brown, fibrous, cracking and peeling in long narrow strips. It has a foliage of two types - (juvenile and adult) - leaves on young trees are needle-like while leaves on adult trees are usually grey, paired triangular and sharp-pointed. This tree is found distributed from Arabia across Zambia through Ethiopia, Kenya, and Tanzania [1]. In Kenya, *J. procera* is found growing in highland and mountainous areas above 1750 m altitude. It dominates in the higher parts of the mist forests especially on shallow rocky soils, though there are no statistics to show the number of *J. procera* trees in Kenya [2].

Cedarwood oil is produced by steam distillation of *J. procera* wood shavings; Schimmel *et al.* [3] distilled shavings and pencil slats derived from wood of East African cedar tree and obtained essential oils in yields of 3.20 and 3.24%, respectively. Macculoch [4] reported a yield of 2% oil from fresh wood shavings. The older the shavings, the lower was the yield of oil. Fresh wood shavings contained 1.42%, two year-old shavings 0.28%, and seven year-old 0.02%. Gildmeister and Hoffmann [5] reported the following ranges for the physical properties of cedarwood oil; optical rotation - 16° 0' to - 38° 30', refractive index (at 20°C) 1.480 to 1.509, and specific gravity (at 15°C) 0.968 to 0.988.

By 1952, the only constituent identified in the oil of *J. procera* was cedrol. Beckly [6] found that the wood of young trees yielded oil with the largest amounts of cedrol (23-76%). Cunha and Roque [7] analyzed cedarwood oil from East Africa by GLC and TLC techniques. They found cedrol to be the major compound; other components were cedrene, a-piene, &-phellandrene, limonene, caryophellene, p-cymene, 1,8-cineole, camphor and, aromadendrene. This observation contrasts that of Maitai and Talalaj [8] that East African cedarwood yield oil (2-3%) which contains cedrene (up to 80%) as the major component. This calls for detailed analysis of cedarwood oil from *J. procera* growing in Kenya, hence the present work.

Cedarwood oil is used in perfumery as an odour fixant, mainly in soap industry, in sprays, floor polishes, and lubricating greases [9].

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Experimental

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°C ± 0.1 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 x 0.2 mm i.d. x 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

The wet wood of *J. procera* was collected in January 1990 from the Aberdares mountains, Central province, Kenya. The taxonomic identification of the plant was established and a voucher specimen no. 90/04 (T.A.R. Akeng'a and S.G. mathenge) was deposited in the herbarium of the Botany Department, University of Nairobi, Nairobi. The wood was turned into sawdust using a Planer machine (SCM, Italy; model 2250).

Extraction, isolation and identification

Five batches each of 250 g of sawdust and 1000 ml water were hydrodistilled using a modified Clevenger apparatus. The distillation time was varied in multiples of four hours up to a maximum of twenty hours.

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

Compounds were identified by their EI (electron impact) mass spectral data, order of elution and relative GC retention times, and by comparison of their mass spectra and GC retention times to those of authentic samples. The identity was further confirmed by peak enhancement by co-injection of the essential oil with available known compounds.

Results and discussion

Steam distillation of the fresh wood shavings yielded a yellow thick viscous oil with a sweet balsamic odour, which on standing gave a large amount of long needle-like crystalline material.

The percentage yields of oil from different batches of *J. procera* fresh wood shavings, refractive index, specific gravity, optical rotation, and percentage of cedrol in the oils extracted for 4,8,12,16, and 20 hours are listed in Table 1.

Table 2 lists the constituents identified with relative percentages in the oil obtained for 12 hours of extraction.

The percentage yield by wood shavings ranges from 0.32 to 0.80%, this yield is not strictly comparable with that reported by Maitai and Talalaj [8] from trees collected from Nyahururu. The difference in the yield could be due to seasonal variations that exist between the Aberdares mountains region and Nyahururu (though these regions are within the same geographical classification, Rift Valley), and the difference in the age of the trees used (though the ages are not known). The yield is in agreement with that reported by MacCuloch [4] and although the age of the tree is unknown it can be concluded that the trees were at most seven years old. The physical properties of the oil extracted from *J. procera* closely agree with those determined by Gildmeister and Hoffman [5] for East Africa cedarwood oil.

Table 1: Percentage yield of oil, cedrol and physical properties of the essential oils of *J. procera*

Time of extraction (hr)	% yield (w/w)	Refractive index at 20° C ± 0.1	Optical rotation 20° ± 0.1	Specific gravity 20° ± 0.1	% Cedrol
4	0.32	1.494	-10°	0.996	84.13
8	0.48	1.494	-11°	0.996	97.10
12	0.64	1.495	-11°	0.998	24.05
16	0.76	1.496	-12°	0.999	26.52
20	0.80	1.496	-12°	0.999	31.93

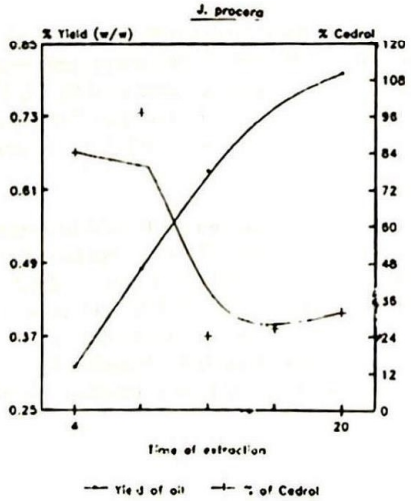

Fig. 1: Graphical representation of the relationship between (a) yield of oil, (b) percentage of cedrol with distillation time for *J. procera* wood shavings

Cedrol is the major component of the oil extracted for 4 and 8 hours (see Table 1). There is a sharp increase in the amount of cedrol from 84.13% for 4 hours of extraction to 97.10% for 8 hours of extraction after which the amount drops off randomly. This random drop is due to the increase in the number of components extracted with increase in time, for example for 20 hours of extraction 33 compounds were eluted, while 8 compounds were eluted for 8 hours of extraction. The increase in the number of compounds could also be due to decomposition products as a result of prolonged heating. Eight hours is the optimum time when a high percentage of cedrol (most valuable component) is obtained, although the percentage yield is relatively low (0.48%) compared to the yields for 12, 16, and 20 hours (0.04, 0.76, and 0.80% respectively). The fact that cedrol content determined by the present work is up to 97.10% contrasts the report by Maitai and Talalaj [8] that East African cedarwood oil contains cedrene up to 80%. In fact the percentage of α-cedrene was found to be 13.69% (12 hours of steam distillation). Very few compounds in the oil of *J. procera* can be identified from the oil for 8 hours (8 compounds); for instance, α-cedrene was not found in the oil of *J. procera* obtained for 4 and 8 hours of steam distillation within the limits of detection of the present methods used for analysis. Hence the chromatograms of oil obtained for 12 hours were used to identify the compounds in the oil of *J. procera* since they were observed to have a representative number of peaks.

Table 2: Constituents of essential oil of *J. procera*

Constituents	R_t (min)	% Total	Identification method
Camphor	9.47	13.45	ms, pe
a-cedrene	10.33	13.69	ms, pe
4-Terpineol	11.22	t	ms
a-Terpineol	12.07	0.23	ms, pe
Geranyl isobutyrate	13.42	t	ms
trans-Geraniol	15.02	1.58	ms, pe
Cedrol	17.42	24.05	ms, pe
Eugenol	18.08	0.44	ms, pe
3,4-diethylbiphenyl	18.57	4.01	ms
1, 1 Diphenyltetradecane	19.12	t	ms

t = trace amount, less than 1% of oil
ms = EIMS matching with library spectrum
pe = peak enhancement by co-injection with an authentic sample

The other major components in the oil of *J. procera* is 3,4-diethylbiphenyl (1.08-28.48%), this compound has not earlier been reported in the essential oil of *J. procera*. The amounts of camphor and α-cedrene extracted for 4 and 8 hours of steam distillation are negligible. The highest amount of camphor (13.70%) is obtained for 16 hours while the highest amount of α-cedrene (13.69%) is obtained for 12 hours of steam distillation. For 3,4-diethylbiphenyl, the highest amount (28.48%) is obtained for 12 hours. Since these compounds are not available in significant amounts (>50%) then it can be considered uneconomical to obtain them from the essential oil of *J. procera*.

The quality of cedrol in this oil can be improved by refining the oil, this removes all the impurities thus making the oil valuable. Good quality cedrol can be obtained by fractional distillation of the oil, cedrol distills off between 200 and 292°C and then freezes out. Due to its high boiling point and faint pleasant odour cedrol is used mainly as a fixative in perfumes, cosmetics, and soaps [9].

Conclusion

The analysis of the essential oil of *J. procera* shows that the oil contains a high percentage of cedrol, 3,4-diethylbiphenyl which has not earlier been reported in the oil of *J. procera*. This oil should be exploited for the production of cedrol as this is of great importance in many industries. However, further investigations on the availability of this plant in different geographical regions, seasonal variations, age of plant are required in order to have conclusive information on the essential oil content.

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