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FACTORS INFLUENCING THE YIELD AND THE QUALITY OF THE OBTAINING ESSENTIAL OIL FROM THE LEAVES OF EUCALYPTUS CITRIODORA HOOK. GROWING IN CRETE

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ABSTRACT

Yield and composition of essential oil of *Eucalyptus citriodora Hook.* (growing in Crete, Greece) were evaluated, using different dryness procedures (open air and oven dryness) and different distillation parameters (the degree of plant comminution and the duration of distillation). The chemical composition of the essential oil was determined by GC and GC/MS. More than eighty components were detected; thirty-six of them, representing the 96.53% of the total oil, were identified. The main resulted constituents were *citronellal* (68.91-81.22%), *β-citronellol* (6.48-12.81%), *isopulegol* (3.85-9.27%), and *citronellyl acetate* (0.40-3.75%). The dryness and the winning parameters have highly significant effects on the essential oil yield (1.77-4.50%). Most of the studied essential oil components have undergone significant simultaneous effects of the dryness and the distillation parameters on their yields. *Citronellal* yield was highest with the comminuted leaves for 1 hour of distillation under the partial dryness; the hydrocarbon monoterpenes with the integral leaves for 2 hours after the 12-days of dryness; whereas the sesquiterpenes with the comminuted leaves for 1 hour after the 3-weeks of dryness and the total oxygenated compounds with the integral leaves for 1 hour under the partial dryness.

Keywords: Eucalyptus citriodora Hook., Essential oil, Yield, Dryness, Comminution, Duration, Composition

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INTRODUCTION

Eucalyptus citriodora Hook., a native of Queens land Australia, belongs to the family *Myrtaceae* and grows in Greece too. Commonly known as "Spotted Gum" "Citron Scented Gum" or "Lemon Scented Gum", it is a tall graceful tree, easily recognised by the drooping habit of its branches. The leaves are well known for their exquisite lemon scent when crushed. It is readily identified by its characteristic fruit and by the deliciously fragrant *Citronellal* odour of the crushed leaves. The eucalyptus oil is used as an antiseptic and deodorant. It is applied externally in ointments and liniments as a counterirritant. For catarrhal conditions of the respiratory tract it has been given internally, either on sugar or mixed with olive oil. The *Eucalyptus citriodora* leaves essential oil present a continuous and increasing interest in the perfumery industry.

The dryness process is of great importance since it has to allow the retention of flavour, colour and aroma of the dried plants, it reduces the growth of micro-organisms and prevents some biochemical reactions[1]. However, the dryness induces negative physical and chemical modification of the quality of the dried plant. Despite its position in the drug processing, only few investigations are known about the effects of the temperature and the duration of the dryness process on the quality and the quantity of the essential oil. Decreases of the essential oil content and slight changes of the essential oil quality, induced by the dryness, were reported by very few studies[2].

The techniques employed in the extraction of the essential oil from a plant have significant effects on the final composition of the product obtained[3,4,5,6]. Preliminary studies, carried out on different plant materials, confirmed these effects on the essential oil yield and composition and especially on *E. globulus* where the essential oil has changed by the different distillation durations[7].

As far it concerns our knowledge, there was no methodical work reported on the effects of the dryness processes and the extraction parameters on the essential oil of *E. citriodora* leaves. Consequently, the aims of our work were to evaluate the yield and the composition of the essential oil from *E. citriodora Hook.* (growing in Crete, Greece) under different dryness methods (oven and open-air dryness) and different extraction parameters (comminution of the plant materials and the distillation duration) and also to find the best combinations corresponding to the highest yields of the different essential oil constituents.

RESULTS AND DISCUSSION

Winning effect

The significant effects of the extraction type, and the duration of the extraction found on the quality and quantity of the *E. citriodora* essential oil composition are in the agreement with the conclusions of several other authors[3,4,5,6,7,8,9,10] confirming that the essential oil yield and quality are significantly affected by the different extraction procedures (Table 1).

As found by Renedo et al.[9] for the *E. globulus*, the essential oil's yield of the integral leaves of *E. citriodora* depends also on the duration of the extraction. The yields obtained by 2 and 3 hours of hydrodistillation were not significantly different from each other, but substantially higher than that of 1 hour of hydrodistillation, confirming the postulation that, the longer the duration, the higher the

essential oil yield. Thereby, the hydrodistillation for 2 hours was sufficient for the complete oil extraction from the *E. citriodora* integral leaves (Fig. 1).

Table 1. The essential oil yield (%) for the different dryness and extraction combinations.

	Partial	12days	3weeks	Oven
Hydrodistillation of integral leaves for 1 hr	4.26	2.98	1.77	4.07
Hydrodistillation of integral leaves for 2 hrs	4.5	3.28	2.22	4.18
Hydrodistillation of integral leaves for 3 hrs	4.5	3.25	2.27	4.27
Hydrodistillation of comminuted leaves for 1 hr	3.96	2.95	1.78	3.23

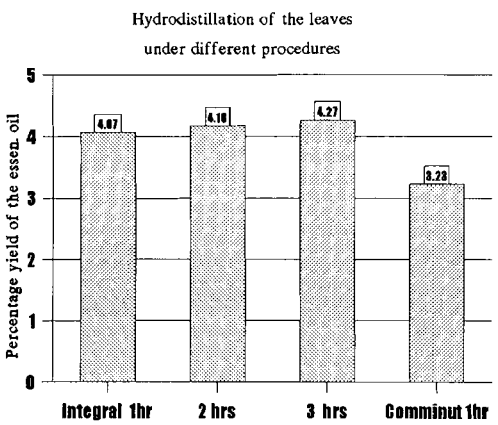

Fig. 1: Percentage yield of the essential oil from *E. citriodora* leaves (integral distilled for 1, 2, 3 hrs and comminuted for1 hr) after dryness in the oven

Table 2. Percentage chemical composition of the essential oil from the E. citriodora leaves obtained under different winning procedures. (* = Distill. of 1hr, Comm. Leav., Partial dryn.)

	Components	**Minimum**	**Maximum**	*
1	*a-Pinene*	0.01	0.10	0.07
2	*a-Thujene*	0.01	0.03	0.01
3	*Sabinene*	0.14	0.76	0.33
4	*a-Phellandrene*	0.01	0.07	0.02
5	*β-Pinene*	0.02	0.15	0.05
6	*Myrcene*	0.01	0.04	0.01
7	*γ-Carene*	0.02	0.16	0.02
8	*Limonene*	0.03	0.31	0.05
9	*Δ_3-Carene*	0.01	0.06	0.02
10	*γ-Terpinene*	0.02	0.23	0.07
11	*p-Cymene*	0.02	0.08	0.08
12	*α-Terpinolene*	0.02	0.34	0.18
13	*Cycloenane*	0.04	0.45	0.07
	Hydrocarbons	0.41	2.00	0.98
14	*1,8-Cineole*	0.05	0.42	0.03
15	*Rose oxide*	0.01	0.09	0.01
16	*Isopulegol*	3.85	9.27	3.95
17	*α-Terpineol*	0.06	0.46	0.23
18	*Citronellyl acetate*	0.40	3.75	1.71
19	*Neryl acetate*	0.01	0.10	0.01
20	*Geranyl acetate*	0.01	0.03	0.01
21	*β-Citronellol*	6.48	12.81	6.96
22	*Nerol*	0.06	0.15	0.06
23	*Linalool*	0.02	0.07	0.01
24	*cis-Jasmone*	0.21	0.60	0.21
partial Oxygenated compounds		13.03	25.17	13.25
25	*Citronellal*	68.91	81.22	81.22
Total Oxygenated compounds		93.27	98.31	94.47
26	*trans-Caryophyllene*	0.14	2.81	0.65
27	*α-Humulene*	0.01	0.19	0.05
28	*β-Patchoulene*	0.01	0.09	0.03
29	*γ-Elemene*	0.06	1.33	0.08
30	*allo-Aromadendrene*	0.03	0.05	0.03
31	*Veridiflorol*	0.02	0.18	0.02
32	*Palustrol*	0.03	0.15	0.03
33	*β-Eudesmol*	0.01	0.06	0.01
34	*Globulol*	0.03	0.32	0.06
35	*Juniper camphor*	0.01	0.11	0.02
36	*Spathulenol*	0.07	0.61	0.10
	Sesquiterpenoids	0.45	5.47	1.08

The comminution effect

The comminution of the *E. citriodora* leaves affected their oil yield by decreasing it in comparison to the integral ones (Table 2), which is in perfect agreement with other works[4,5,8] done on similar plant materials and assigning the decrease of the essential oil yield after comminution and with the postulation of Guenther[11]. Since the comminution was done using the liquid Nitrogen and the distillation was performed directly after the leaves' comminution, the losses of the essential oil by the evaporation and the enzymatic deterioration were negligible. The main reason of essential oil losses, probably, were due to the hard tissue of the *E. citriodora* leaves, which are considered to be hard tissues[12]. With the comminution these tissues keep strongly the minuscule droplets of oil liberated by the comminution from the internal tissues of the grounded leaves avoiding in this way their extraction by the Hydrodiffusion. Our results for the comminution join the formulation of Svoboda and Deans[13], that the loss of volatile compounds resulting from the grinding may account for this as glands holding the oil have been raptured.

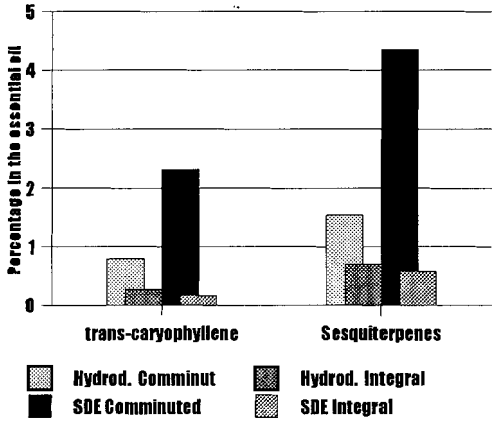

Fig. 2: Comminution effect on the sesquiterpenoids and trans-caryophyllene after hydrodistillation and SDE of the E.citriodora leaves

The comminution for the two winning methods (hydrodistillation and SDE - Simultaneous Distillation Extraction), affected the pattern of the *E. citriodora* essential oil composition[4,5,6,7,8]. Generally, without consideration of the extraction types, the comminution increased the yields of the sesquiterpenoids' component (Fig. 2) -the increases by comminution of sesquiterpenes and *trans-caryophyllene* (main sesquiterpene) were respectively 318.45% and 577.14% - and of almost all of the constituents of the partial oxygenated compounds group.The hydrocarbon monoterpenes and the total oxygenated compounds, including the *citronellal* yields decreased with the comminution. The yield of *citronellal* depended on both the leaves quality and the winning method, since the interaction was highly

significant up to 1%; thus the best combination for the highest yield of *citronellal* was the - Hydrodistillation of the grounded leaves.

Dryness effect

The yields obtained with the open air dryness processes for the three durations were in perfect agreement with the results obtained by Miranda and Zayas (1985)[14] and the quotation of Guenther (1950)[11] on *E. citriodora* and by Renedo et al. (1990)[9] on *E. globulus* for the dryness durations effect on the loss of the essential oil content; in the way that the essential oil content of the *E. citriodora* has shown decreases with the dryness duration which could be counted mainly as evaporation losses as postulated by Baritaux et al, (1992)[15].

The oven dryness at 40°C has produced a decrease by 8.37% of essential oil content in relation to the partial drying, that gave the highest yield in the open air dryness, and it was higher than the 12-days and the 3-weeks dryness (fig. 3). Thus the oven dryness could be considered as the most advisable drying process that can be used properly for the *E. citriodora* leaves dryness without any considerable losses and that can be achieved in a shorter period of time than the open air one.

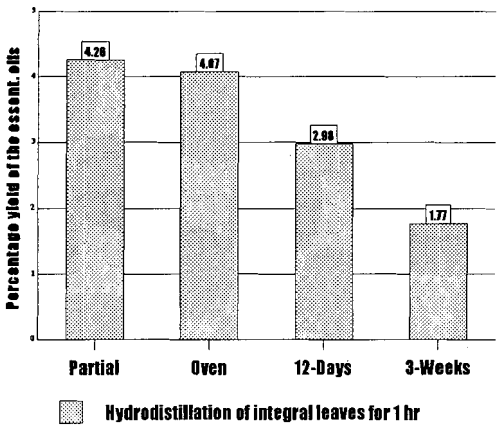

Fig. 3: Dryness effect on the yield of Eucalyptus leaves essential oil

The losses of essential oil yield incited by the oven drying of the *E. citriodora* leaves was found to be inferior than the ones done at 55°C[16] and at 50°C[17] for the chamomile producing respectively 33% and 25% of losses. Thereby, the oven dryness at 40°C for the *E. citriodora* leaves is an interesting dryness process with the gain of time and the minimum losses of oil content (compared to the partial, 12-days and 3-weeks dryness) and confirming in this way the interest of the oven dryness use as found by Chatzopoulou et al[10] for the *Juniperus communis*. At 40°C, the water elimination is much enhanced than the oil evaporation.

Significant effects of the type and of the duration of the winning method, as far as of the degree of comminution of the plant materials were found also on the essential oil composition (fig. 4), which are in the agreement with the conclusions of different authors[3,4,5,6,7,9] confirming that the extraction processes affect significantly the final product obtained.

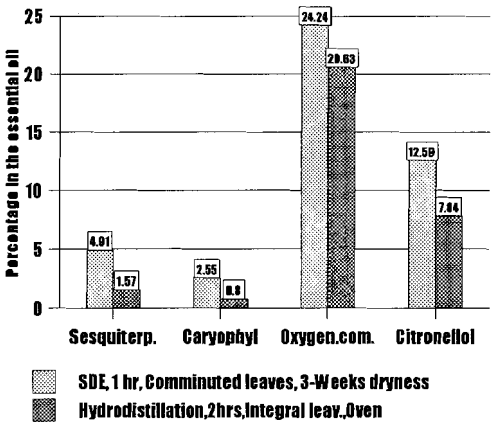

Fig. 4: Influence of different parameters on the percentage yield of certain group of constituens and components of the Eucalyptus essential oil

The duration effect

Since the chronology of appearance of the different constituents of an oil throughout the distillation are not the same - depending among the other reasons on their boiling point -, the pattern of the *E. citriodora* essential oil composition has varied through the three different durations of hydrodistillation. Thus, the total oxygenated compounds and the *citronellal* had their highest yields with the shorter duration (1hr) but declined by increasing the duration, because the higher boiling oxygenated compounds are present first in the distillation[3,4,5,6]. The hydrocarbons yield followed the reverse trend than the oxygenated compounds that increased by increasing the hydrodistillation duration, since they appear much later in the extraction process[3]. The sesquiterpenoids component and constituents and also the traces elements need longer extention of distillation to reach their higher yields (Fig. 5). However, they increase in the detriment of the oxygenated compounds and mainly the *citronellal*. Another explanation for the duration effects is the increase of the solubility of the essential oil constituents during distillation, that's why the oxygenated compounds decreased and the hydrocarbons and sesquiterpenoids increased because of the higher solubility of the oxygenated compounds in the distillation water than the other essential oil constituents.

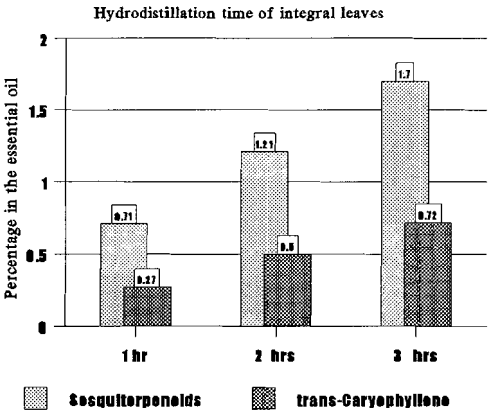

Fig. 5: Hydrodistillation duration effect on the *Sesquiterpenoids* and *trans-caryophyllene*

Extracting procedure effect

For the Simultaneous Distillation Extraction, the same results were obtained for the total oxygenated compounds and the *citronellal* which were highest after 1hr of SDE and the hydrocarbons, the esters and the sesquiterpenoids that were higher after 2hrs of SDE due to the same reasons of the chronological appearance of the different constituents depending on the extraction duration and also the difference in the solubility of the essential oil compounds.

The oxygenated compounds had the highest yield (21.66%) by the SDE for 1hr. The β-*citronellol* had 11.32% (for 1 hr) as highest yield with the SDE.The *isopulegol* and the *citronellyl acetate* had their respective highest yields (5.59%) and (3.62%) with the SDE of 1 hr (Fig. 6). However, the *citronellal* presented the highest yield 84.53% by the 1hr hydrodistillation.

In the 1hr of hydrodistillation the yields of the above constiuents have undergone a decrease of 38.82% for the oxygenated compounds, 22.30% for β-*citronellol* and 12.82% for *citronellal* in the SDE.

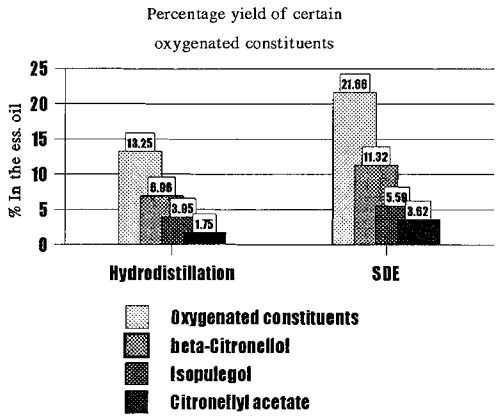

Fig. 6: Extraction effect on oxygenated constituents after 1 hr of hydrodistillation and SDE of the comminuted Eucalyptus *citriodora* leaves

For both the hydrodistillation and the SDE, the total oxygenated compounds and its main component (*citronellal*) had their highest yields with the shorter duration (1hr), whereas the reverse trend was observed for the hydrocarbon monoterpenes, the sesquiterpenes and the trace elements (less than 1% yield) depending on their chronology of appearance through the distillation that vary on their boiling points[3] and also on their different solubilities in the water. For the sesquiterpenes, the SDE for 1 and 2 hours (Fig. 7) were found to be more efficient than the hydrodistillation ones, while the latter were more efficient for the total oxygenated compounds and the *citronellal*. However the hydrocarbon monoterpenes changed their pattern, depending on the degree of the plant materials' comminution, since the interaction between the extraction types and the comminution degree of the plant materials was found to be highly significant for them; thus the best combination was SDE for 2hrs of integral leaves.

CONCLUSIONS

- The Cretan *Eucalyptus citriodora* leaves present an interesting source of essential oil that could be easily exploited in industry.
- The 2hrs of hydrodistillation is sufficient time for the complete oil winning from the integral leaves of *E. citriodora*.
- The essential oil yield and quality were significantly affected by the different extraction procedures and the different dryness methods.

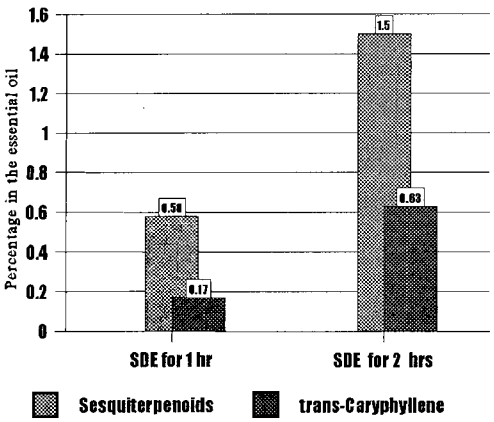

Fig. 7: SDE duration effect on the sesquiterpenoid*s and trans-caryophyllene.*

- The comminution of leaves produces a significant decrease of the essential oil yield; thus the comminution of the *E. citriodora* leaves does not present any interest to increase the yield, by strongly holding of the essential oil droplets by the small particles size.
- The *E. citriodora* essential oil composition presents a high yield of *citronellal* which permits to this oil to be a commercial one.
- The dryness and the parameters of the winning methods were found to affect significantly the chemical composition from the point view of the percentage and not the constituent composition of the *E. citriodora* essential oils.
- The partial dryness and the hydrodistillation of the comminuted leaves for 1hr present the best combination to maximise the *citronellal* yield.

EXPERIMENTAL PART

Plant materials collection

Several leaf samples of *Eucalyptus citriodora* were collected in the locality of Nerokouros, situated at 4.5km of Chania (Crete), between the end of December 1994 and March 1995. The samples included leaves of different morphological forms and physiological stages. Specimens of the *E. citriodora* were stored in the Herbarium of Mediterranean Agronomic Institute of Chania. After the preliminary studies on the leave's samples, one was chosen for the entire investigation of the present study.

Dryness of plant materials

Open-air dryness

After taking the initial weight of the samples, the leaves were shared out on several filter papers. They were put in a dark room without air circulation. The ambient temperature was among 20° and 23°C. A daily measurement for the decreasing of the weight was done. Three durations were considered; a) *the partial dryness*, where the dryness was stopped between 14 and 20% of the initial water content b) *the 12-days* and finally c) *the 3-weeks* dryness process stopped at the free water content. In addition leaves were stored in the oven at 105°C for two days till obtaining the dry weight and the initial water content of the leaves was determined consequently. The water content of all the studied samples was varied between 48% and 50% of their total initial weights.

Oven dryness

After several tentative experiments the temperature of 40°C was performed for the oven dryness, which was stopped after attaining the stable weight of the leaves. After the end of the dryness period, the plant materials were kept separately in plastic bags and stored at - 20°C till to be distilled or extracted.

Preparation of plant materials

The plant materials were of two types, integral and comminuted. The integral leaves were used as they are, unless they are so long where only one central cut was permitted to facilitate their entrance to the hydrodistillation or/and the extraction flasks.

The comminution of the plant material was carried out as follows: the leaves were cut in small pieces, put in a big porcelain mortar and submerged in liquid Nitrogen for 15 to 20min and then ground to powder in a blender for half to one minute. The comminuted plant materials passed through a sieve of 850µm. The comminuted leaves were distilled directly after their comminution in order to minimise the loss of essential oil by the evaporation.

Hydrodistillation

The essential oil determination was performed using the Clevenger-type apparatus suggested by the European Pharmacopoeia. The weight of 20gr of *E. citriodora* integral or comminuted leaves were used. The deionized water was added 17 times the weight of the plant materials; the distillation rate was 3.5ml/min[4].

The average essential oil yield (performed after five repetitions with a SDev. ±0.85%) was expressed on the free moisture basis percentage. The isolated oil samples were dried over anhydrous sodium sulphate and stored approximately at 4°C in vials of 2ml.

Simultaneous Distillation Extraction (SDE)

Using the Micro-Steam distillation extraction apparatus (produced by CHROMPACK, The Netherlands), the weight of 3gr of leaves (integral or comminuted) has undergone the Simultaneous

Distillation Extraction. After finishing the procedure, the flask containing the extract of volatiles was concentrated using an ice bath to reach 1.5ml of volume. Then, sealed in small vial of 2ml.

Quantitative and qualitative analysis of essential oils

For the GC and the GC-MS analysis the essential oil samples were diluted 1:10 (100 µl of essential oil to 1 ml of n-pentane), whereas the SDE extracts were used as they are without any dilution. Quantitative analysis was carried out using a gas chromatograph (Hewlett Packard, model 5890 series II, controlled by the HP 3365 series II Chemstation software) equipped with FID. A capillary column HP-FFAP (50 m x 0.2 mm i.d.) coated with cross-linked polyethylene glycol-TPA phase with 0.33 µm film thickness was used and programmed as following: 55°C (for 2 min) $\xrightarrow{25°C/min}$ 210°C (20 min). Carrier gas was Helium at a velocity of 22 cm/sec (flow rate = 0.8 ml/min). The percentage compositions of the obtained essential oils resulted after three chromatographic runs of each sample with a s.d. of ±0.25%. Qualitative analysis was performed by a gas chromatograph similar to the one mentioned above, coupled to a VG TRIO 2000 Mass Spectrometer. Chromatographic conditions were identical to that described above. Mass spectra were taken at 70eV at a scanning speed of 1scan/sec from 40 to 240m/z. The identification of peaks was carried out by computerised MASS LYNX software from the Wiley Library.

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