

Comparison of Microwave-assisted Hydrodistillation with the Traditional Hydrodistillation Method in the Extraction of Essential Oils from *Dwarfed Cinnamomum Camphora* var. *Linaolifera Fujita* Leaves and Twigs

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Abstract: Microwave-Assisted Hydro Distillation (MAHD) and conventional Hydro Distillation (HD) are compared and evaluated in terms of extraction time, extraction yield, chemical composition, quality of the essential oil and operation consumption. Experiments results manifest that MAHD has no obvious advantage over traditional HD for the extraction of *Dwarfed Cinnamomum Camphora* var. *Linaolifera Fujita* (D-CCLF) twigs. Extraction of essential oils from D-CCLF leaves with MAHD is superior with regard to extraction time (37.5 min vs. 120 min), extraction yield (1.73 vs. 1.71%) and operation cost (0.21 k Wh/g of essential oil vs. 0.67 kWh/g of essential oil). The chemical composition and quality of the extracted essential oils by using the two methods are quite similar to each other, indicating that the utilization of microwave irradiation would not cause an adverse influence on them. Therefore, MAHD is a fast and energy-saving method for the essential oils extraction of D-CCLF leaves.

Keywords: *Dwarfed Cinnamomum Camphora* var. *Linaolifera Fujita* (D-CCLF), essential oil composition, microwave-assisted hydrodistillation, scanning electron microscopy

INTRODUCTION

Natural linalool mainly derives from fractional distillation of wild cinnamomum camphora at present, which is native to China, Japan and Taiwan. And the cinnamomum camphora which abounds in linalool, also known as Cinnamomum Camphora Var. *Linaolifera Fujita*, has been found in considerable abundance covering the provinces of Fujian, Zhejiang, Jiangxi, Hunan, Guangxi, Guizhou and Taiwan of China. In recent years, significant achievements have already been made in dwarfing Cinnamomum Camphora Var. *Linaolifera Fujita*. Dwarfed Cinnamomum Camphora Var. *Linaolifera Fujita* (D-CCLF) is rich in linalool through asexual reproduction. In addition, current methods to obtain linalool-rich essential oils from D-CCLF mainly include Hydro Distillation (HD), steam distillation, steam and water distillation.

Microwave energy, with a frequency of 2.45 GHz, is well known to have a significant effect on the rate of various processes in chemical and food industry. Much attention has been paid to application of microwave in extracting effective components from plants, namely microwave-assisted extraction. Chan *et al.* (2011) summarized the research done during the last decade on the microwave-assisted extraction of active ingredients from plants. And some new applications were also reported by others (Farhat *et al.*, 2011; Wakte *et al.*, 2011; Sahraoui *et al.*, 2011; Balasubramanian *et al.*,

2011; Geng *et al.*, 2011; Zhang *et al.*, 2011; Sui *et al.*, 2012; Liu *et al.*, 2012; Gholivand *et al.*, 2012; Sarker and Nahar, 2012; Rodrigues *et al.*, 2012; Kha *et al.*, 2012; Jiao *et al.*, 2012a, b).

However, only a few articles in the literature have focused on the Microwave-Assisted Hydrodistillation (MAHD) method for the extraction of essential oil from plants. MAHD was reported for the extraction of essential oils from *Xylopiya aromatica* (Lamarck) (Stashenko *et al.*, 2004a), *Lippia alba* (Mill.) (Stashenko *et al.*, 2004b), *Cuminum cyminum* L., *Zanthoxylum bungeanum* Maxim (Wang *et al.*, 2006), *Thymus vulgaris* L. (Golmakani and Rezaei, 2008 a, b), Rosemary (Karakaya *et al.*, 2012), *Schefflera heptaphylla* (Wang *et al.*, 2012) and Olive (Konoz *et al.*, 2012). However, to the best of author's knowledge no work has been published on the extraction of D-CCLF essential oils by MAHD and the extraction characteristics is not clear yet. Therefore MAHD is proposed to perform for the extraction of essential oils from D-CCLF leaves and twigs and the results of extraction time, extraction yield, chemical composition, quality of the essential oil and operation cost are compared with those of traditional HD.

EXPERIMENT

Materials: Leaves and twigs of asexual reproduction D-CCLF of 2~3 years old were harvested in April 2012

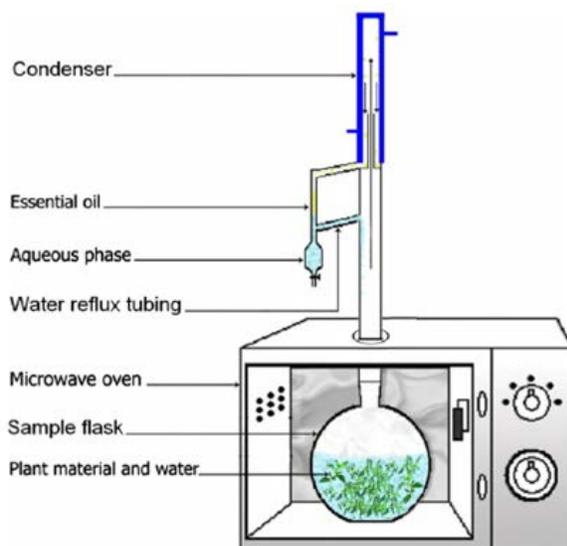


Fig. 1: Schematic representation of the microwave-assisted hydro distillation apparatus

from D-CCLF planting base of Jiangxi Spice Chemical Industrial Co., Ltd., which is located in Jinxi County, Jiangxi Province, China.

Apparatus and procedure of essential oils extraction: A MCL-3-type continuous microwave reactor (0~800 W, 2.45 GHz; Sichuan University), a modified domestic microwave oven (SANYO EM-202MS1, Panasonic, Japan), was adopted for MAHD operation. The microwave reactor was operated at 577 W power levels. One hundred grams of leaf samples or two hundred grams of twig samples were placed in a 2 L volumetric flask containing 500 mL deionized water. The flask was setup within the microwave oven cavity and a condenser was applied on the top to collect the extracted essential oils (Fig. 1, similar to the MAHD apparatus adopted by Golmakani and Rezaei (2008a)). The collected essential oils were decanted from the condensate in proper time interval. In order to remove water, the extracted essential oils were then dried over anhydrous sodium sulfate, weighed and stored in amber vials at 4°C until they were used for analysis.

HD was carried out in a similar manner as MAHD. However, the microwave oven was replaced by an electro mantle (98-I-B, Tianjin Taisite Instrument Co., Ltd, China). For ease of comparison, the electro mantle was also operated at 577 W as with microwave reactor.

Gas Chromatography-Mass Spectrometry (GC-MS): The essential oils were directly analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) (Hewlett-Packard computerized system comprising a 5890 N gas chromatograph coupled to a 59731 mass spectrometer) using a fused-silica-capillary column

with an apolar stationary phase HP5MSTM (30 m×0.25 mm×0.25 μm film thickness). GC-MS were obtained under the following conditions: carrier gas He; flow rate 1.0 mL/min; split 1:100; injection volume 0.2 μL; injection temperature 250°C; oven temperature progress included an initial hold at 60°C for 2 min, a rise to 100°C at 5°C/min and a hold at 100°C for 10 min, then a rise again to 250°C at 20°C/min and a hold at 250°C for 10 min. Mass spectrometry was carried out using electron energy of 70 eV, source temperature of 230°C, electron multiplier voltage of 1600 V and mass range of 35~500 amu. Identification of the component was based on computer matching against commercial libraries (Wiley, MassFinder 2.3 Library, NIST02). For each compound on the chromatogram, the percentage of peak area relative to the total peak areas from all compounds was determined and reported as relative amount of that compound.

Scanning Electron Micrographs (SEM): The specimens were freeze-dried, fixed on the specimen holder with aluminum tape and then sputtered with gold. All the specimens were examined by a SEM (FEI-QUANTA 200F, The Netherlands) at an accelerating voltage of 30 kV in high vacuum mode.

RESULTS AND DISCUSSION

Extraction kinetics and extraction yield: Kinetics of essential oil extraction from D-CCLF leaves using MAHD are compared with that of HD on Fig. 2. The yields obtained by the methods of both MAHD and HD are similar to each other while the differences appeared in the extraction time. The extraction time of MAHD is clearly shorter than that of conventional HD, the full extraction took 37.5 min, whilst 120 min is required by HD; With MAHD, 37.5 min of time provided equivalent yields comparable to those obtained after 120 min by HD. The ultimate yields of essential oil obtained from leaves are respectively 1.73% for MAHD and 1.71% for HD. Yields are expressed as in grams of essential oil per 100 g of leaves. These results reveal a substantial saving of time and energy.

For leaf, four phases are observed in the process of the microwave extraction as shown in Fig. 2. The first step (step 1) represents the heating phase from room temperature to boiling temperature. To reach this extraction temperature and thus to obtain the distillation of the first essential oil droplet, it is necessary to heat for only 4 min with MAHD compared with 12 min for HD. By the time the extraction of essential oils started with HD, more than 58% of total essential oils had been extracted with MAHD. This is due to the more efficient heat flow involved with microwaves. Unlike the classical conductive heating methods, microwaves can heat the entire sample almost simultaneously at a higher rate (Kaufmann and

Table 1: Chemical compositions of essential oils of leaf and twig obtained by MAHD and HD

No.	Compounds	Molecular formula	Essential oil of leaf			Essential oil of twig		
			Retention time/min	MAHD/%	HD/%	Retention time/min	MAHD/%	HD/%
1	Mesityl oxide	C ₈ H ₁₀ O	3.42	0.04	0.11	3.42	0.03	0.09
2	Leaf aldehyde	C ₈ H ₁₀ O	4.38	0.02	-	-	-	-
3	3-Thujene	C ₁₀ H ₁₆	5.97	0.09	0.08	5.97	0.07	0.13
4	α-Pinene	C ₁₀ H ₁₆	6.12	0.72	0.58	6.15	0.41	0.77
5	Camphene	C ₁₀ H ₁₆	6.55	0.06	0.05	6.55	0.05	0.11
6	β-Phellandrene	C ₁₀ H ₁₆	7.21	3.43	2.58	7.20	1.57	2.69
7	β-Myrcene	C ₁₀ H ₁₆	-	-	0.43	7.65	0.26	-
8	b-Thujene	C ₁₀ H ₁₆	7.66	0.44	-	-	-	0.46
9	α-Phellandrene	C ₁₀ H ₁₆	-	-	-	-	-	0.08
10	3-Hexen-1-ol,formate,(z)-	C ₇ H ₁₂ O ₂	8.07	0.10	0.10	-	-	-
11	α-Terpinene	C ₁₀ H ₁₆	8.43	0.15	0.18	8.42	0.15	0.23
12	Eucalyptol	C ₁₀ H ₁₈ O	8.83	14.09	11.31	8.82	10.43	12.81
13	Ocimene	C ₁₀ H ₁₆	9.32	0.76	0.65	9.32	2.31	2.99
14	g-Terpinen	C ₁₀ H ₁₆	9.64	0.37	0.43	9.64	0.41	0.55
15	2-Furanmethanol,5-etheny(tetrahydroα 5-trimethyl-cis	C ₁₀ H ₁₈ O ₂	10.21	0.25	0.19	-	-	0.31
16	D ₂ -Carene	C ₁₀ H ₁₆	-	-	-	-	-	0.19
17	Carene	C ₁₀ H ₁₆	-	-	-	10.49	0.49	-
18	cis-Linaloloxide	C ₁₀ H ₁₈ O ₂	10.71	1.02	0.84	10.83	0.75	0.61
19	Linalool	C ₁₀ H ₁₈ O	11.61	62.65	64.31	11.63	56.81	57.92
20	Camphor	C ₁₀ H ₁₆ O	-	-	-	12.72	0.88	0.88
21	Decane,2,8,8-Trimethyl-	C ₁₃ H ₂₈	13.09	3.53	3.04	13.12	4.03	2.73
22	4-Terpineol	C ₁₀ H ₁₈ O	14.80	1.07	1.07	14.87	1.67	1.64
23	α-Terpineol	C ₁₀ H ₁₈ O	15.90	3.20	2.09	16.16	2.09	3.88
24	(-)-Terpineol	C ₁₀ H ₁₈ O	-	-	1.74	16.90	2.63	-
25	Bornyl acetate	C ₁₂ H ₂₀ O ₂	-	-	-	20.72	0.13	0.10
26	Safrole	C ₁₀ H ₁₀ O ₂	-	-	-	21.21	0.19	0.18
27	D-Camphene	C ₁₀ H ₁₆	-	-	0.23	-	-	0.07
28	α-Cubebene	C ₁₅ H ₂₄	-	-	-	22.68	0.04	0.04
29	7-Oxabicyclo[4.3.0]nonane,cis-	C ₁₁ H ₁₈ O ₂	16.89	2.74	-	-	-	-
30	nyl propanoate	C ₁₂ H ₂₀ O ₂	22.98	0.04	0.04	22.98	0.05	0.04
31	Geranyl acetate	C ₁₃ H ₂₂ O ₂	-	-	0.08	-	-	-
32	Eugenol	C ₁₂ H ₂₀ O ₂	23.31	0.06	-	-	-	-
33	b-Element	C ₁₀ H ₁₂ O ₂	-	-	-	23.33	0.21	0.17
34	Methyl eugenol	C ₁₅ H ₂₄	23.49	0.04	0.15	23.48	0.13	0.12
35	1-Caryophyllene	C ₁₁ H ₁₄ O ₂	-	-	0.02	23.76	1.58	2.76
36	α-Caryophyllene	C ₁₅ H ₂₄	23.91	1.03	2.45	23.91	1.67	-
37	Germacrene D	C ₁₅ H ₂₄	24.37	0.40	0.82	24.37	0.89	0.75
38	Bicyclgermacrene	C ₁₅ H ₂₄	-	-	0.83	24.68	0.64	-
39	d-Cadinene	C ₁₅ H ₂₄	24.84	1.57	1.76	24.84	1.16	1.35
40	Cubenene	C ₁₅ H ₂₄	-	-	0.14	25.09	0.21	0.25
41	γ-Element	C ₁₅ H ₂₄	-	-	-	25.21	0.10	-
42	Nerolidol	C ₁₅ H ₂₄	25.44	0.08	0.15	-	-	0.22
43	Guaiol	C ₁₅ H ₂₆ O	25.73	0.47	0.82	-	-	-
44	d-Viridiflorol	C ₁₅ H ₂₆ O	26.29	0.92	1.26	-	-	-
45	Cyclopropanecarboxylicacid,2-methyl-,2,6-di-t-butyl-4-methylphenyl ester	C ₂₀ H ₃₀ O ₂	-	-	0.49	-	-	-
46	Eicosane	C ₂₀ H ₄₂	-	-	0.77	-	-	-
47	Eremophilene	C ₁₅ H ₂₄	-	-	0.05	-	-	-
48	2-Butynoicacid,4-[(tetrahydro-2H-pyran-2-yl)oxy]-,methyl ester	C ₁₅ H ₂₄	26.81	0.33	-	25.42	0.35	-
49	Caryophyllene oxide	C ₁₀ H ₁₄ O ₄	27.02	0.32	-	-	-	-
50	Humulene oxide	C ₁₅ H ₂₄ O	-	-	-	25.69	2.41	1.91
51	g-Gurjunene	C ₁₅ H ₂₄ O	-	-	-	25.91	0.44	0.55
52	Guaia-3,9-diene	C ₁₅ H ₂₄	-	-	-	26.20	1.92	1.31
53	But-2-ynoic acid,1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	C ₁₄ H ₂₀ O ₂	-	-	-	-	-	1.04
54	E-2-Methyl-3-tetradecen-1-ol acetate	C ₁₇ H ₃₂ O ₂	-	-	-	-	-	0.04
55	Agarospirol	C ₁₅ H ₂₆ O	-	-	-	-	-	0.07
56	10,13-Octadecadienoic acid,methyl ester	C ₁₉ H ₃₄ O ₂	-	-	-	26.78	1.63	-
57	Isoaromadendrene epoxide	C ₁₅ H ₂₄ O	-	-	-	28.73	0.06	-
58	Linoleicacid	C ₁₈ H ₃₂ O ₂	-	-	-	28.97	0.06	-
59	2-Hydroxycyclopentadecanone	C ₁₅ H ₂₈ O ₂	-	-	-	29.63	0.25	-
60	2-Methyl-Z,Z-3,13-octadecadienol	C ₁₉ H ₃₆ O	-	-	-	30.11	0.19	-
61	Extraction time (min)	-	-	45	150	-	90	90
	Yield (%)	-	-	1.73	1.71	-	0.35	0.33

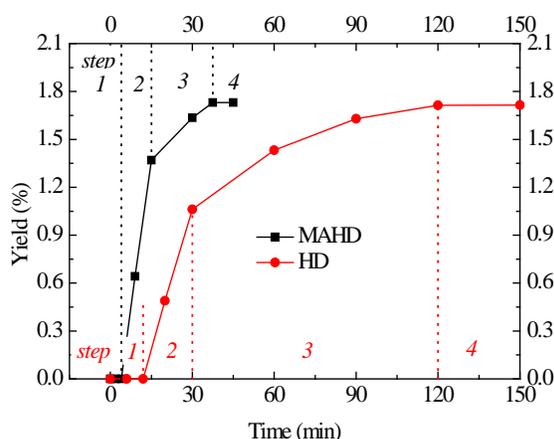


Fig. 2: Extraction yield as a function of time for the Hydro Distillation (HD) and Microwave-Assisted Hydro Distillation (MAHD) of essential oils from leaves

Christen, 2002). The second step (step 2) was represented by an increasing line which characterizes the first quantities of extracted essential oils, located at the surface of vegetable particles, accounting for approximately 79% of the yield obtained after 15 min. This phase was followed by an second increasing line (step 3) representing the internal diffusion of the essential oil from the midst of the particles towards the external medium involved by the internal warming of the water located in the plant cells. In this stage (realized into 37.5 min), the oil amount extracted represented nearly 21% of the global yield. The fourth part (step 4) corresponded to a horizontal line which marked the end of the extraction process. Bousbia *et al.* (2009) and Golmakani and Rezaei (2008a) found a yield profile similar to that described here. The profile of the conventional extraction technique HD presented four similar phases to those obtained with MAHD.

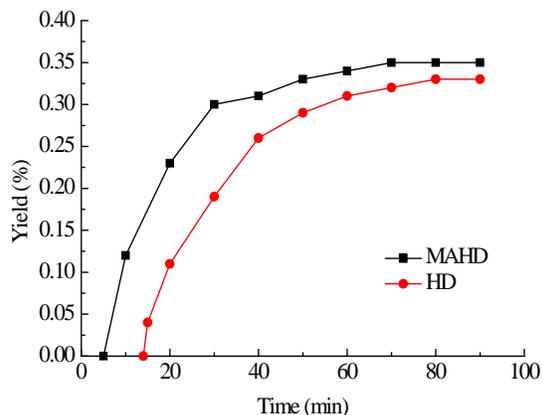


Fig. 3: Extraction yield as a function of time for the Hydro Distillation (HD) and Microwave-Assisted Hydro Distillation (MAHD) of essential oils from Twigs

The second step leads to 62% of the yield obtained into 30 min and the extraction process ended after 120 min.

Kinetics of essential oil extraction from D-CCLF twigs using MAHD is compared with that of HD in Fig. 3. The extraction temperature would reach in 5 min for MAHD, while in the case of HD it is at 14 min. Different from leaf, four obvious phases are not observed and the full extraction takes 90 min for both MAHD and HD. After 90 min of extraction, MAHD resulted in oil recovery similar to that obtained by HD (0.35% vs. 0.33%, respectively). It is may due to the structural differences between leaf and twig glandular cells. Results indicate that MAHD has no significant advantage over HD in terms of energy saving and extraction time.

Composition of essential oil: The identities of the extracted leaf and twig essential oils from both methods are shown in Table 1. Except for Camphor (compound 20), Safrole (compound 26) and Methyl eugenol (compound 35) in twig essential oils, there are no significant differences between the constituents of leaf essential oils and twig essential oils and the most abundant component Linalool (compound 19) in essential oil samples from twigs is lower than leaves. The differences could be attributed to the three following aspects:

- The selectivity of microwave heating makes some compounds easy to be extracted
- Microwave leads to pyrolysis oxidization or oxidization for some extractives
- Microwave gives rise to structural isomerism of extractives

Monoterpene compounds are the main components in the essential oils but the relative amounts differs for the two extraction methods, Linalool (compound 19) is

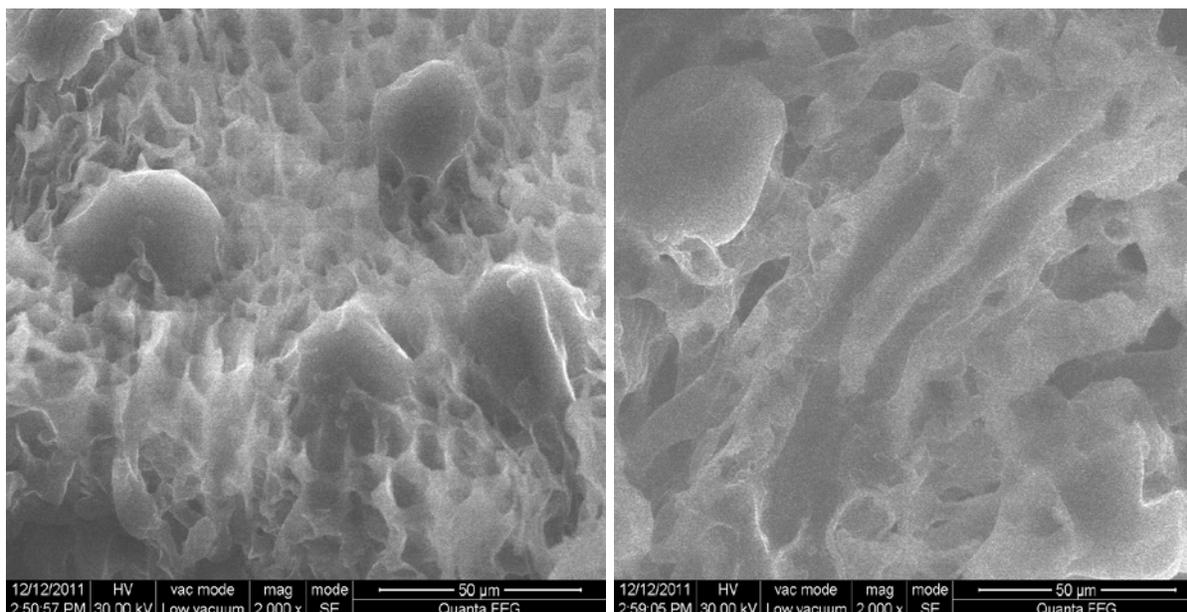
the most abundant component, Eucalyptol (compound 12) ranks the second. For leaf, Linalool presents at 62.65 and 64.31%, respectively for MAHD and HD. Eucalyptol (compound 12) are 14.09% for MAHD and 11.31% for HD. Although the relative amount of Linalool is different, the quantity of Linalool extracted from 100g of D-CCLF leaves is similar for the two extraction methods. Meanwhile, higher amounts of oxygenated compounds and lower amounts of monoterpene hydrocarbons are present in the essential oils of leaves extracted by MAHD in comparison with HD. The oxygenated fraction in leaf essential oil samples from MAHD (86.99%) is higher than HD (85.24%). Monoterpenes hydrocarbons are less valuable than oxygenated compound in terms of their contribution to the fragrance of the essential oil. Conversely, the oxygenated compounds are highly odoriferous and, hence, the most valuable. For twig, the relative amounts of Linalool, Eucalyptol and oxygenated compounds from MAHD are all slightly lower than HD; however, there are still some increase in their absolute extraction quantity. Therefore, microwave does not involve in any deterioration of the extracted components and it can be introduced as a safe method for the extraction of D-CCLF essential oils.

Evaluation of physical properties: Physical properties (specific gravity, refractive index, optical rotation in degree and appearance) of leaf and twig essential oils extracted either by MAHD and HD are shown in Table 2. No significant difference between the usual physical constants of essential oils obtained by MAHD and HD is found. The only difference is that the color of the essential oils extracted by MAHD is lighter than that obtained by HD. Therefore, taking into account physical properties of the extracted essential oils, using MAHD as a new extraction technique does not cause any problems to the essential oil extracted from the leaf and twig.

Cost, cleanliness and scale-up: For D-CCLF twig, there is no advantage for the proposed MAHD method in terms of time and energy. However, for D-CCLF leaf, MAHD resulted in significant saving in the extraction time and the energy required to perform the extraction. HD requires 120 min for the full extraction of leaf essential oils and MAHD requires only 37.5 min for the process. Considering the total period of a full recovery, the energy required to perform the two extraction methods for leaves are, respectively 1.15 kWh for HD and 0.36 kWh for MAHD. The equivalent quantities of carbon dioxide released to the atmosphere, which are estimated according to 800 g of CO₂ per kWh, are higher in case of HD (538.01 g CO₂/g of essential oil) than that of MAHD (166.47 g CO₂/g of

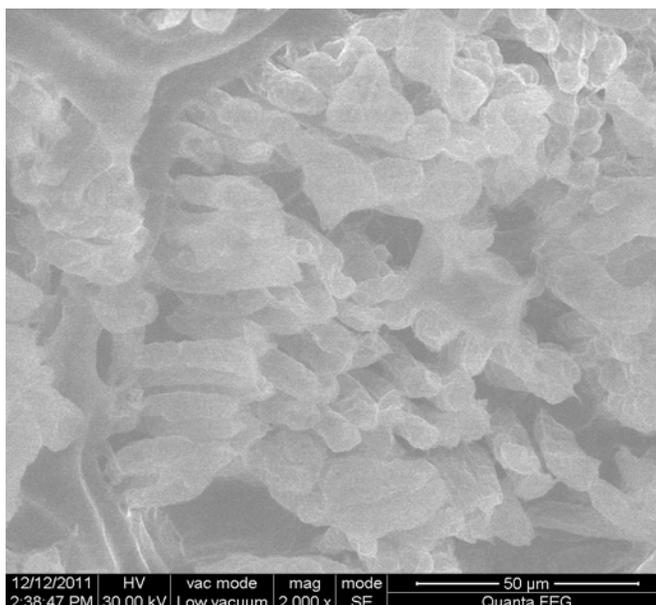
Table 2: Chemical compositions of essential oils of leaf and twig obtained by MAHD and HD

Physical properties	Essential oil of leaf		Essential oil of twig	
	MAHD	HD	MAHD	HD
Specific gravity (25°C)	0.8832	0.8818	0.8853	0.8841
Refractive index (20°C)	1.4654	1.4654	1.4631	1.4699
Optical rotation in degree	-13.2	-13.2	-6.3	-6.3
Appearance	Colorless	Pale	Pale	Yellow



(a)

(b)



(c)

Fig. 4: Scanning electron micrographs of leaves: (a) untreated, (b) after hydro distillation for 150 min and (c) after microwave-assisted hydro distillation for 45 min

essential oil). Therefore, MAHD can be suggested as an “environmentally friendly” extraction method suitable

for leaf, but is unsuitable for twig. And it could also be applied for the production of large quantities of leaf

essential oils by making use of existing large scale microwave extractors.

Structural changes after extraction: Different extraction methods (MAHD and HD) produce distinguishable physical changes in the D-CCLF leaves. Figure 4a is a micrograph of the untreated leaf, which can be compared with structures of the treated leaf in Fig. 4b (MAHD) and Fig. 4c (HD). The structures of the untreated D-CCLF leaf show glandular cells containing volatile oils. Figure 4b shows the typical structure after HD extraction, glandular cells are empty but still intact. In the case of MAHD extraction, tremendous damage on the external surface of the leaf together with dispersed cellular material is observed. This indicates that micro waves cause the glandular cells to crumble or rupture more rapidly and efficiently. Such differences can be attributed to the difference in heat transfer speed between the two extraction methods. MAHD utilizes three ways of heat transfer within the sample: irradiation, conduction and convection, while HD only covers the latter two methods. As a result, with MAHD, heat is produced more quickly from the glandular cells as well as from the outside. When the glandular cells are subjected to a severe thermal stress and localized high pressures, the pressure build-up within the cells would exceed their capacity for expansion and cause their rupture more rapidly than in conventional extraction. Similar effects were pointed out by Bousbia *et al.* (2009) for the microwave extraction of rosemary leaves.

CONCLUSION

MAHD offer important advantages over traditional HD for the extraction of leaf essential oil, but it has no obvious advantage in extracting essential oil of twig. GC-MS results indicate that there are no adverse effects on the composition of the extracted essential oils by using MAHD instead of HD. A similar extraction yield of leaf essential oils is achieved at shorter extraction time (37.5 min for MAHD vs. 120 min for HD) and lower energy consumption (0.21 kWh/g of essential oil for MAHD vs. 0.67 kWh/g of essential oil for HD). SEM images of leaves untreated or subjected to MAHD or HD emphasize the differences between the two extraction methods. Microwaves seems to cause a higher extraction efficiency of rupture for the glandular cells than in conventional HD. In a word, MAHD, an excellent alternative for HD, is a modern, green and fast method for the extraction of D-CCLF leaf essential oils.

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