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Composition comparison of essential oils extracted by hydrodistillation and microwave-assisted hydrodistillation from *Piper nigrum* L.

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Abstract
The essential oils of black pepper (*Piper nigrum* L.) were isolated by classical hydrodistillation (CHD) and microwave-assisted hydrodistillation (MHD) in yields of 1.24% and 1.45%, respectively. This study compared the chemical composition of the essential oils obtained by the two methods of hydrodistillation. We controlled also the effect of the volume of hydrodistillation solvent and the effect of microwave power on the oils chemical composition and yields. The optimum conditions for MHD were: microwave delivered power 700 W, microwave radiation time 30 min. and a 1:2 ratio of spice to water. The obtained oils were analyzed by GC and GC-MS. The main components from MHD oil were β-caryophyllene (8.25-52.68%), caryophyllene oxide (4.79-63.13%), sabinene (2.04-11.73%), α-copaene (5.95-9.28%) and cubenol (3.85-5.10 %). While the most abundant constituents identified in the CHD oil were β-caryophyllene (47.14-50.88 %), α-copaene (7.79-8.02 %), sabinene (5.52-6.92 %) and cubenol (3.97-5.20 %). No significant effect of the volume of CHD hydrodistillation solvent was found concerning the chemical composition oil. In contrary, the quantity of solvent and the microwave power showed several variations in the chemical composition of MHD oil.

Keywords: P. nigrum L., essential oils, microwave hydrodistillation, classical hydrodistillation, β-caryophyllene, caryophyllene oxide.

1. Introduction:
Piperaceae family has four genera and more than 2000 species. They grow in warm, mild, or cold climates, according to García et al. [1]. Many of which are widely used in folk medicine for the antibacterial, antifungal and antiprotozoan properties. These effects could be due to the presence of essential oils. In fact, the essential oils of some Piper species were investigated and antibacterial [2], antifungal [3], antileishmanial [4] and anti-Trichomonas [5] activities were detected.
The techniques used for extraction since decades have the limitations of requiring longer extraction times, large solvent volumes and cause degradation of thermo labile components. In order to increase the productivity, modern methods of extraction have been developed like ultrasonic waves, supercritical fluids or microwaves to shorten the extraction time, decrease the solvent consumption, increase the extraction yield, and enhance the quality of extracts [6-10].
The use of microwave energy for the extraction of active substances from plant materials results in more effective heating, faster energy transfer, reduced thermal gradients, selective heating, reduced equipment size, faster response to process heating control, faster start-up and increased production. During absorption, the microwave energy is converted into kinetic energy, thus enabling the selective heating of the microwave-absorbent parts of the plant material.
The analysis of the volatile constituents of some Piperaceae family species has been the subject of diverse studies [10-13] which have revealed the presence of monoterpenes and sesquiterpenes hydrocarbons. β-caryophyllene, 3-carene, limonene, β-pinene and α-phellandrene have been found to be the main compounds in P. nigrum.
The essential oils of black pepper (*Piper nigrum* L.) from China [14] isolated by hydrodistillation (CHD) and microwave-assisted hydrodistillation (MHD) were found to be similar including 3- α -carene (31.9% in HD oil and 33.2% in MHD), limonene (19.3%, 20.2%), caryophyllene (18.4%, 16.0%), β-pinene (13.0%, 14.0%), α-

pinene (5.8%, 6.7%), δ-elemene (1.8%, 1.3%) and α-copaene (1.9%, 1.6%). While the oil from the fruits of *P. nigrum* grown in Cameroon [15] contained sabinene (11.2%), δ-3-carene (18.5%), limonene (14.7%) and β-caryophyllene (12.8%). In the study developed by Martins et al. [16], the essential oils of *P. nigrum* fruit collected in the west of Africa, presented the major constituents: limonene (18.8%), trans-β-caryophyllene (15.4%), sabinene (16.5%), and β-pinene (15.4%). in the essential oil fraction of *Piper nigrum* fresh fruits from Malaysia [17] obtained by (CHD) method, limonene was the major compound present with 35.06% of total oil followed by beta-pinene (12.95%) and linalool (9.55%).
This study compared the chemical composition of the essential oils of *P. nigrum* fruit obtained by classical hydrodistillation (CHD) and microwave hydrodistillation (MHD). Two factors are investigated: volume of solvent and the microwave power.

2. Material and Methods

2.1 Classical extraction of essential oils (CHD)

25 g of pepper seeds were powdered and subjected to hydrodistillation in 400 mL of water using a Clevenger-type apparatus for 1.5 h. The yield (v/w) of the oil (1.24%) was calculated on the basis of dry weight of the plant material. The obtained essential oils were dried over anhydrous sodium sulfate and stored at 4°C until analysis.

2.2 Microwave extraction of essential oils (MHD)

Microwave-assisted hydrodistillation (MHD) was used by a sort of Clevenger-type distillation device with a Dean-Stark distillation reservoir. This adapted to global warming by microwave radiation through a conventional samsung microwave MS-23F301EFS model set at 2450 MHz, 700 W. 50 mL of distilled water were added to 25 g of the plant material. The yield obtained after 35 min of extraction was 1.45%.

3. Gas chromatography-mass spectrometry analysis

Essential oil composition was determined by gas chromatography coupled to mass spectrometry (GC-MS) analysis on a *Trace GC ULTRA* gas chromatograph coupled to a *Polaris Q MS* ion trap mass spectrometer. The column was VB-5 (Methylpolysiloxane, 5% phenyl), 30 m x 0.25 mm x 0.25 μm film thickness with helium as carrier gas. Injection was performed at 220˚C in the split mode; 1μL of sample was injected. GC oven temperature was kept at 40°C for 2min and programmed to 180˚C at a rate of 4˚C/min and increased to 300°C at a rate of 20°C/min then kept constant at 300˚C for 2min.
The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 200°C, resolution, 1000. Mass units were monitored from 30 to 450 m/z. The components of the oils were identified by comparison of the mass spectra fragmentation patterns with those found in databases or libraries (NIST02 [18], Adams [19], Wiley [20]).

4. Results and discussions

Esssential oils obtained by microwave hydrodistillation (MHD) and classical hydrodistillation (CHD) were compared in terms of yield and chemical composition. Two factors are investigated: volume of solvent and the microwave power. The variation of yields of both the two oils was given in Figure 1.
Results showed a better yield of oil (1.5%) in MHD extraction with less solvent consumption 50 mL. The ratio of matrix to solvent was 0.5. While, CHD method presented a lower yield 1.24% and required a high quantity of solvent 400 mL. The ratio of matrix to solvent plays an important role in extraction [21]. In conventional extraction method, a higher ratio of solvent volume to solid matrix was necessary to obtain better extraction yields. Whereas, in case of MHD, a higher ratio of solvent to matrix ratio may give a lower yield due to non uniform distribution and exposure to microwaves. In conventional heating, heat is transferred from the heating medium to the interior of the sample, while in microwave heating; heat is dissipated volumetrically inside the irradiated medium. In MHD extraction, the solvent volume should be sufficient enough to immerse the plant matrix completely in the solvent throughout the entire microwave irradiation process. The absorption efficiency is largely related to the moisture contents of the material; the water molecules convert the microwave energy into heat and the result is a sudden rise in temperature inside the material. According to Paré [22], when the plant cells are subjected to severe thermal stress and localized high pressures, the pressure build-up within the cells exceeds their capacity for expansion, and causes their dislocation more rapidly than in conventional extraction and leads the release of their contents in the middle of extraction. The polar solvent (water) absorbs all the microwave energy; heats up until it reaches the boiling point, diffuses into the sample matrix and

solubilizes the analytes. The heat transfer in the solid matrix is made by conduction from the high quantity of solvent. In this case, the mechanism of extraction assisted by microwaves is not fundamentally different from that of the classical solid-liquid extraction.

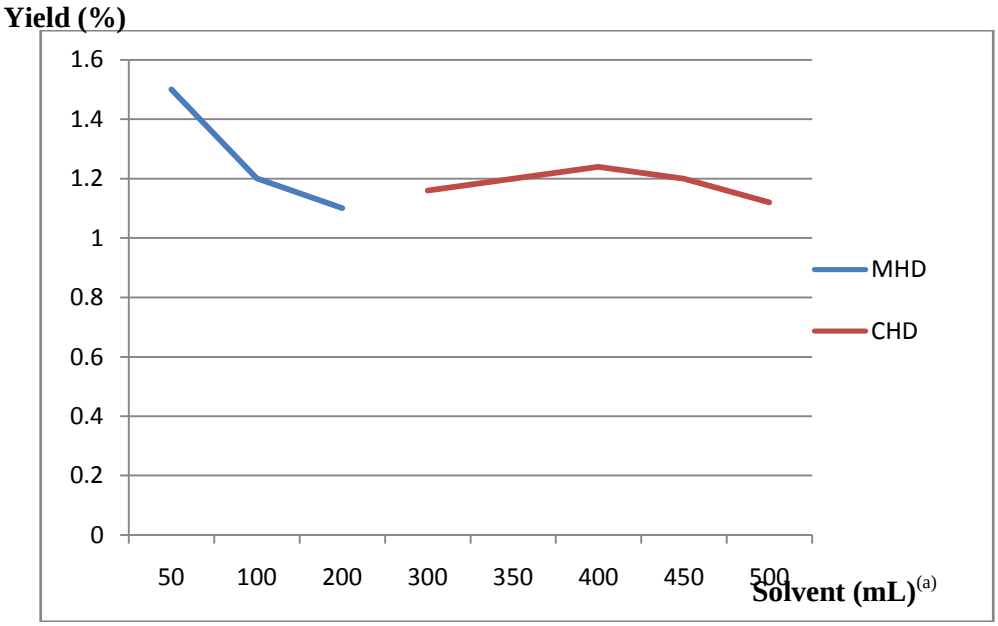

Figure 1: Influence of the solvent volume on the essential oils yields of CHD and CHD extractions.
(a) Volume of water in the extraction oil.

Also, in this study, the variation of microwave power from 280W to 700W revealed significant effects on the yield of MHD oil. Results are presented in Figure 2. The yield increased from 0.5% to 1.2%. Ying et al. [14] presented a high yield of piper oil (3.8%) at 800W in microwave extraction.

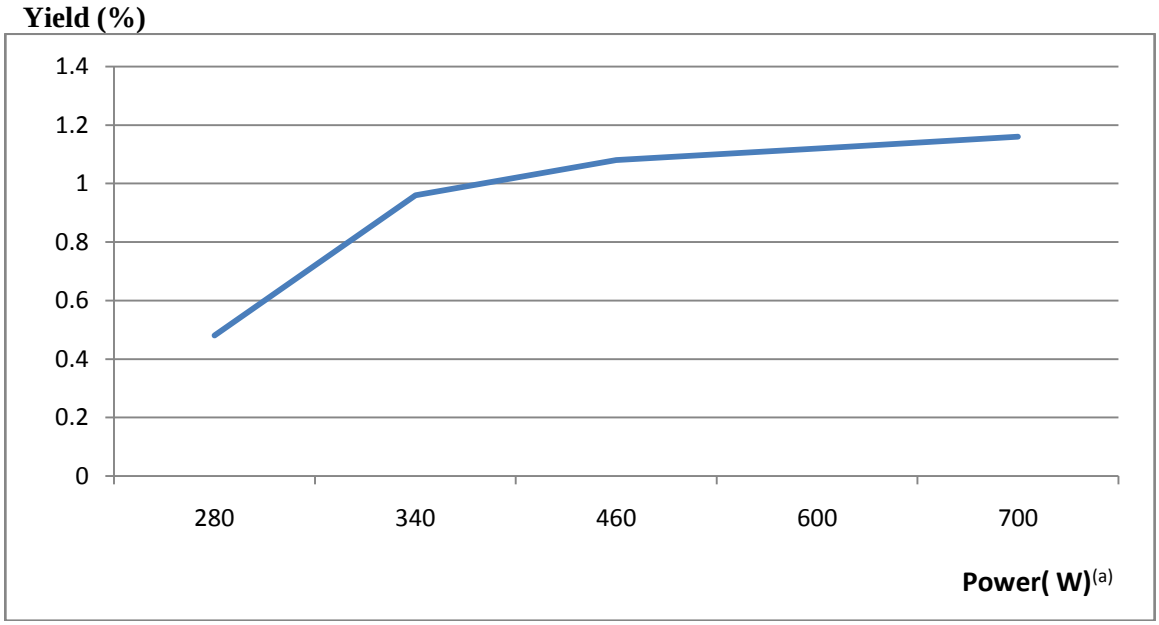

Figure 2: Influence of the microwave power on the essential oils yields of MHD extraction.
(a) Microwave power used in microwave hydrodistillation of essential oils.

Moreover, in the present work, the effect of solvent volume, and microwave power on the chemical composition of essential oils was investigated. Results presented in Figure 3 showed that CHD extraction had no significant qualitative and quantitative effects of solvent volume on the chemical composition of oil.

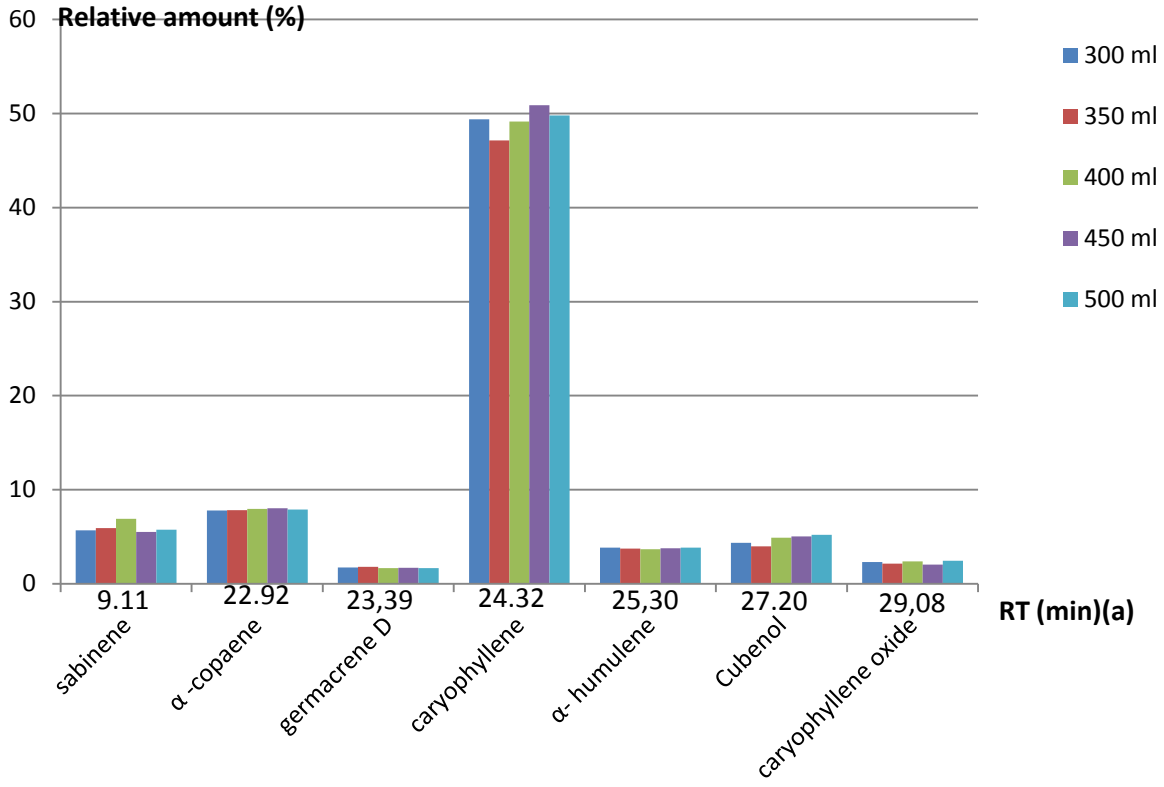

Figure 3: Influence of the solvent volume on the percentage of CHD essential oils compounds.
(a) Retention time in GC analysis.

Also, in the CHD oil, β-caryophyllene as sesquiterpene hydrocarbon, was the major compound present with (47.14-50.88 %). It has shown anti-inflammatory [23] and anesthetic [24] effects. This compound was followed by α-copaene (7.79-8.02 %), sabinene (5.52-6.92 %), cubenol (3.97-5.20 %), humulene (3.67-3.86 %), caryophyllene oxide (2.03-2.46 %), and germacrene (1.68-1.80 %). The caryophyllene oxide oxygenated sesquiterpene well known as preservative in food, drugs and cosmetics, has been tested in vitro as an antifungal against dermatophytes [25] and smooth-muscle-relaxant activity [26]. Conventional extraction oil was highly rich in the sesquiterpene hydrocarbons compounds which consisted of approximately 69.76% of the total oil. Monoterpenes hydrocarbons and oxygenated sesquiterpenes were present in relatively lower amounts, representing 6.92 % and 2.46%, respectively.

The literature on the genus *Piper* shows a great variability in chemical composition among different species. These results are different from the earlier published data reported by Jirovetz et al. [13] on *P. nigrum* dominated by germacrene D (11.01%) and b-pinene (10.02%). Ying et al. [15] from China presented a different oil chemical composition: 3-Δ-carene (31.9%), limonene (19.3%), caryophyllene (18.4%), β-pinene (13.0%), α-pinene (5.8%), α-copaene (1.9%) and δ-elemene (1.8%). Tchoumbougnang et al. [16] reported that the oil from the fruits of *P. nigrum* grown in Cameroon contained sabinene (11.2%), 3- α -carene (18.5%), limonene (14.7%) and β-caryophyllene (12.8%). In the study developed by Martins et al. [17], the essential oil of *P. nigrum* fruit from the west of Africa was constituted by limonene (18.8%), trans-β-caryophyllene (15.4%), sabinene (16.5%), and β-pinene (15.4%).

Differences in the composition of their essential oils could be due to natural chemical variations called chemotype, which occur in the secondary metabolism of plants and could possibly be induced by environmental factors such as soil type, altitude, sun exposure, rain, gather or seasonal variation, and in accordance with McGimpsey et al. [27], can also be influenced by genetic factors. β-caryophyllene was present in small amount in the essential oil of the literature. However, sabinene was present in higher amount. It is also important to mention the absence of limonene in our results.

In contrary, the quantity of hydrodistillation solvent induced several variations qualitative and quantitative on the chemical composition of MHD oils. Results are presented in Table 1 and Figure 4. At lower amounts of

solvent (50 mL and 100 mL), the oxygenated compound caryophyllene oxide was the major component. High amounts were found 32.52% and 40.94% respectively. When the volume of solvent increases to 200 mL the quantity of this constituent decreases to 7.46%. A low percentage 2.46% of this compound was also obtained by the CHD hydrodistillation [28]**.** Whereas, non polar constituent β-caryophyllene revealed the major essential oil composition 52.68%. In MHD extraction, the solvent volume should be sufficient enough to immerse the plant matrix completely in the solvent throughout the entire irradiation process. Oxygenated compounds amount 50% was higher at low quantity of an excellent microwave absorbing solvent. In case of MHD a higher ratio of solvent to matrix may not give better yield due to non-uniform distribution and exposure to microwaves. During absorption, the microwaves energy is converted into kinetic energy, thus enabling the selective heating of the microwave-absorbent parts of the plant material. The effect of microwave energy is strongly dependent on the nature of both the solvent and the solid matrix. Most of the time, the chosen solvent possesses a high dielectric constant and strongly absorbs microwave energy. Indeed, microwaves interact selectively with the polar molecules present in the plant [29]. Localized heating leads to the expansion and rupture of cell walls and is followed by the liberation of essential oils into the solvent [30].

Table 1: Influence of the solvent volume on the percentage of MHD essential oils compounds extraction.

Compounds	**Relative amount (%)**			
		Solvent volume (mL)[(b)]		
	RT[(a)]	**50**	**100**	**200**
Sabinene	9.11	11.73	4.76	2.04
α-copaene	22.92	7.17	7.60	9.11
Germacrene-D	23.39	1.30	1.45	1.84
Caryophylene	24.32	15.29	14.49	52.68
α-Humulene	25.30	1.21	1.27	3.88
Cubenol	27.20	3.18	4.51	5.01
Caryophylene oxide	29.08	32.52	40.94	7.46

(a) Retention time in GC analysis.
(b) Volume of water in microwave hydrodistillation.

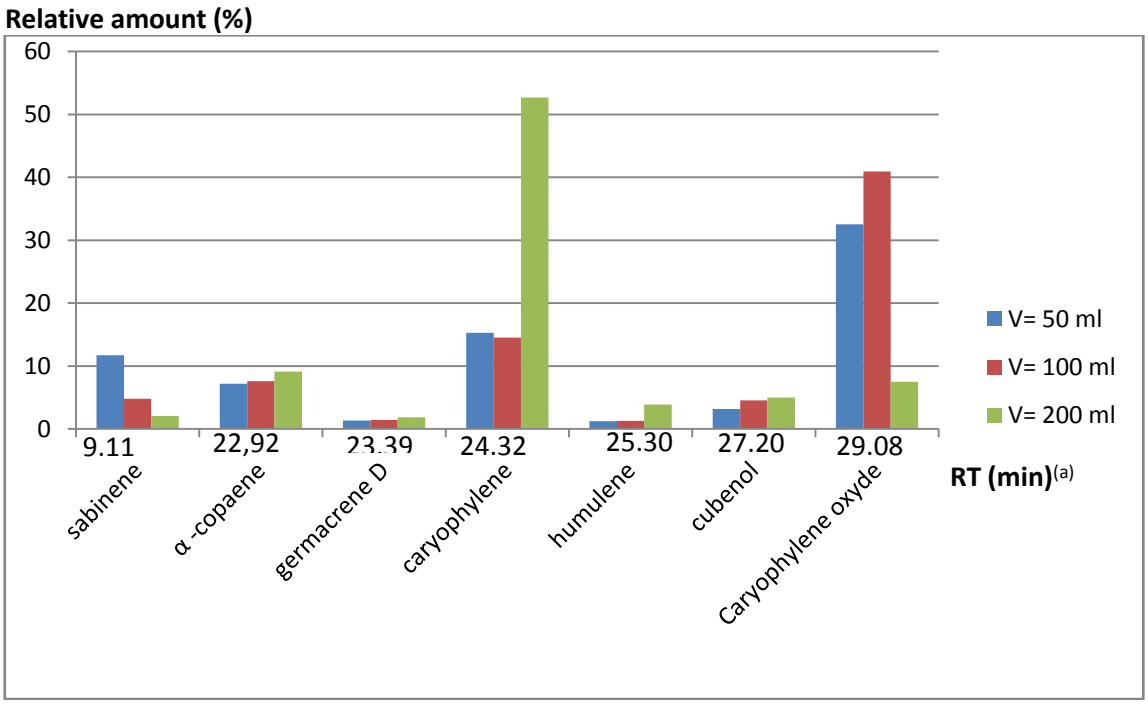

Figure 4: Influence of the solvent volume on the percentage of MHD essential oils compounds.
(a)Retention time in GC analysis.

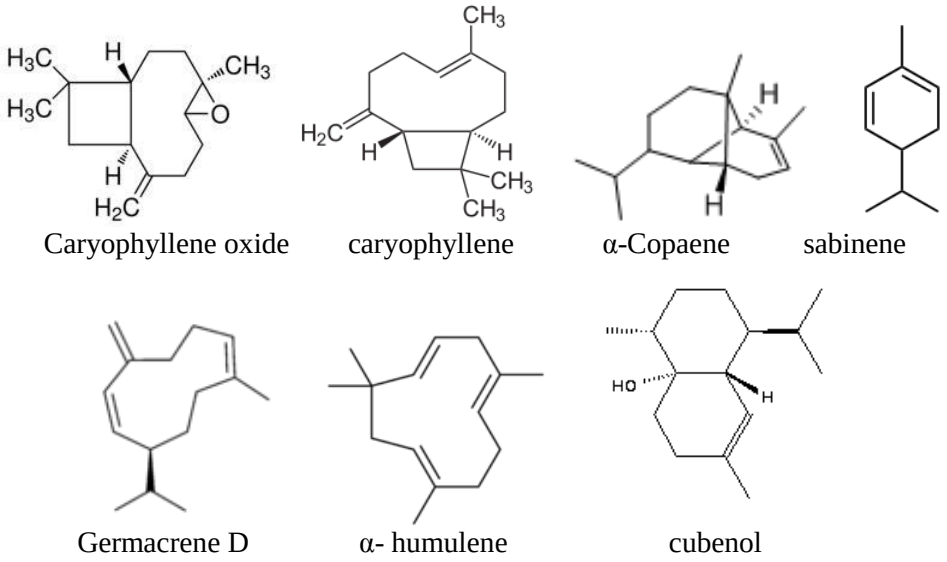

Figure 5: Major compounds of MHD essential oils extraction.

On the other hand, MHD extraction oil revealed an important influence of microwave power on the chemical composition of essential oils. Results are presented in Figure 6 and table 2. At low power 280W, the oxygenated compound caryophyllene oxide was selectively extracted with a high amount 63.13% because of his strongly microwave absorption [29]. However, non oxygenated compounds presented a lower percentage varying from 0.72% to 8.25%. When the microwave power increases to 700W, the oil was highly rich in caryophyllene (52.67%) and pour in caryophyllene oxide (4.79%). The high microwave power promotes selective extraction of non oxygenated compounds; however, low microwave power was favorable to obtain a high amount of oxygenated compounds. An irregularity was observed concerning the selectivity of extracted compounds for the intermediate microwave power. This investigation showed the importance of the choice of microwave power for carrying selective essential oil extraction.

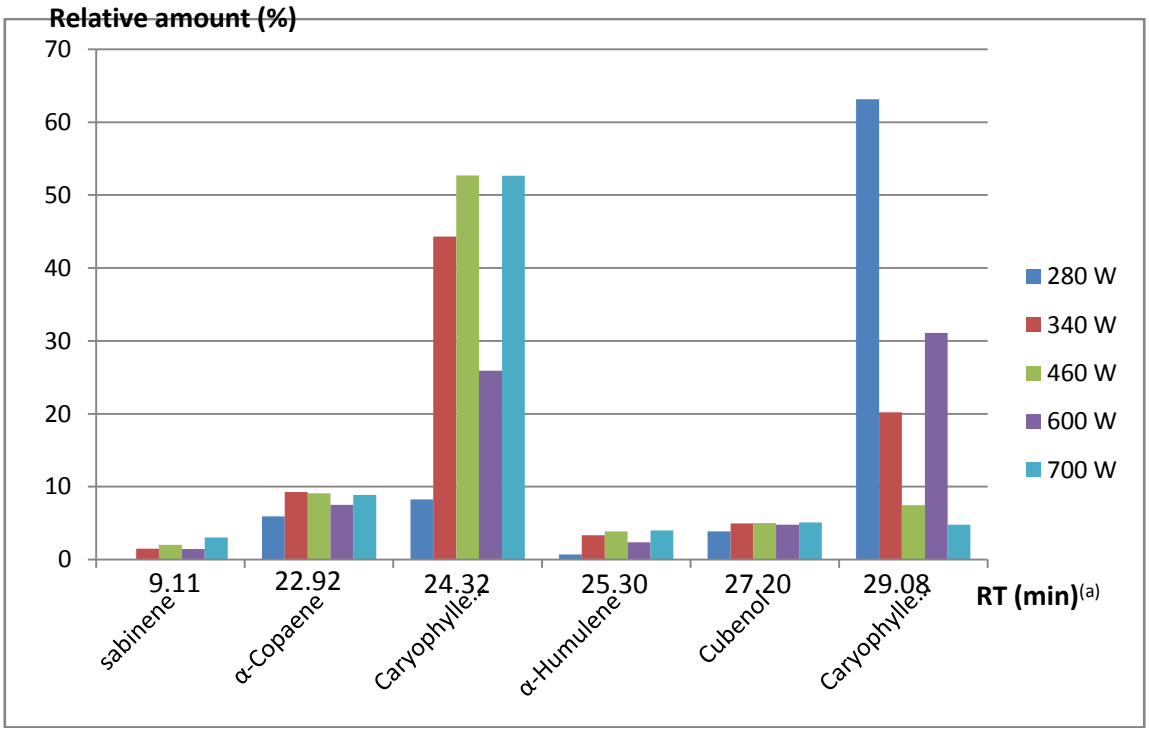

Figure 6: Influence of Microwave power on the percentage of MHD essential oils compounds.
(a) Retention time in GC analysis.

Table 2: Composition of major MHD essential oils compounds extraction under different microwave power values.

		Relative amount (%)				
		Microwave power (b)				
Compounds	**RT(a)**	**280w**	**340w**	**460w**	**600w**	**700w**
Sabinene	9.11	-	1.48	2.04	1.45	3.01
α-copaene	22.92	5.95	9.28	9.11	7.53	8.86
Germacrene-D	23.39	1.13	1.83	1.84	1.79	1.83
Caryophyllene	24.32	8.25	44.30	52.68	25.93	52.67
α-Humulene	25.30	0.72	3.32	3.88	2.39	4.00
Cubenol	27.20	3.85	4.97	5.01	4.80	5.10
Caryophyllene oxide	29.08	63.13	20.22	7.46	31.11	4.79

(a) Retention time in GC analysis.
(b) Microwave power used in microwave hydrodistillation of essential oils.

Conclusion

The main advantages of microwave assisted extraction over the conventional extraction technique is that it reduces solvent consumption, it has a shorter operational time, it gives higher yields of essential oils, has a good reproducibility and minimal sample manipulation for extraction process. The results show that no significant effect of the volume of conventional extraction solvent was found concerning the chemical composition oil. In contrary, the quantity of solvent and the microwave power used presented several variations in the chemical composition of microwave assisted extraction. Thus, this investigation showed the importance of the choice of microwave power and the quantity of solvent for carrying selective essential oil extraction.

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