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The Effect of Ultrasound Pre-treatment on the Yield, Chemical Composition and Antioxidant Activity of Essential Oil from Wild *Lavandula stoechas* L.

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Abstract: In this present work, the aim is to evaluate the effect of different times of ultrasound pre-treatment prior to hydrodistillation (US-HD) on the yield, chemical composition and antioxidant activity of essential oils of two wild *Lavandula stoechas* L. from the North of Algeria. The results indicate that ultrasound treatment engenders a rapid release of essential oils (1.59 %) recovery after only 10 min of sample of Adekar treated by ultrasound and followed by 90 min of hydrodistillation (HD) versus 180 min of hydrodistillation of untreated sample (1.17 %). However, the yields of Keddara sample treated by 45 min versus untreated samples were 0.87 % versus 0.62 %. 94.30 % and 88.26 % of total compounds were identified using chromatography-mass spectrometry (GC-MS) in samples of Adekar and Keddara treated by ultrasound versus untreated samples (92.64 % and 88.75 % respectively). A difference in chemical composition between the essential oils of the two harvesting sites and between the extracts obtained by HD and by US-HD was found. The percentage of the most of the major compounds (fenchone, camphor, 1,8-cineole, bornyl-acetate, myrtenyl-acetate and viridiflorol) and other compounds identified is higher in treated *L. stoechas* L. than untreated *L. stoechas* L. The study of antioxidant power was carried out by 2, 2 diphenyl-1-picrylhydrazyl (DPPH) method. The results showed that antioxidant power of treated samples is superior to antioxidant power of untreated samples. Antioxidant activity of both samples (treated and untreated) is less effective compared with antioxidant activity of ascorbic acid.

Key words: Ultrasound pre-treatment, *Lavandula stoechas* L. essential oil, yield, GC-MS, antioxidant activity.

Introduction

Belonging to the genus *Lavandula*, of the *Lamiaceae* family, *Lavandula stoechas* L. occurs naturally in Mediterranean countries. Its essential oils rich in bioactive compounds with intense and pleasant aroma are the cause of their wide use in medicine, pharmaceutical preparations, food industries, cosmetics and perfumery. Up to now, several conventional extraction techniques have been reported for the extraction of essential oils from *L. stoechas* like Hydrodistillation (HD). These conventional extraction techniques may

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either cause the degradation of the targeted compounds due to high temperature and long extraction times. In recent years, various novel extraction techniques have been developed and the use of ultrasound sonication has opened up some great expectations with a promising results. Different plant extracts and bioactive metabolites have been obtained with this technique [19,23]. Ultrasound assisted extraction utilizes acoustic cavitation to cause molecular movement of solvent and sample, offering advantages like improved efficiency, high level of automation, reduced extraction time and energy consumed and contribute to environmental preservation by reducing the use of water and solvents, elimination of wastewater, fossil energy and generation of hazardous substances, as compared to conventional extraction techniques [11,13,18]. In this direction, for the first time, various pretreatment times by ultrasound before hydro distillation (US-HD) have been used for the extraction of essential oil from *L. stoechas* in order to develop a more advantageous alternative in terms of quality and quantity of essential oils. This study evaluates the effect of ultrasound treatment of *L. stoechas* on extraction time, yield, chemical composition and antioxidant activity.

Materials and methods

Plant material

The flowering tips of *L. stoechas* were collected at maximum flowering stage in April 2014, from wild populations located in two regions in northern Algeria: Keddara (Location: Boumerdes, Latitude: 36°39'0 N, Longitude: 3°24'35 E, Altitude: 644 m, Slope: > 15 %) and Adekar (Location: Bejaia, Latitude: 36°42'44 N, Longitude: 4°34'20 E, Altitude: 86 6m, Slope: > 15 %). The botanical authentication was performed at the herbarium of the Botany Department of National School of Agriculture (Algiers), where voucher specimen was deposited. The plant material was washed and dried in the dark and well-ventilated area at room temperature and stored in paper bags.

Hydrodistillation procedure (HD)

50 g of the ground flowering tips of the plant were hydrodistilled for 180 min using a modified Clevenger-type apparatus according to the European Pharmacopoeia. The essential oils were collected, dried with anhydrous Na_2SO_4 and stored at 4°C until analysis. Extractions were performed at least three times, and the mean values are reported.

Ultrasound assisted extraction prior Hydro distillation (US-HD)

Ultrasounds were applied on the plant materials as a pre-treatment before hydrodistillation according to Ait-kaci Aourahoun, *et al*.[2] methodology. 50 g of the ground flowering tips was mixed with 0.8 L deionized water in Erlenmeyer flask. The mixture was submitted to ultrasound at the fixed-frequency of 26 kHz for different times: 10, 20, 30, 45 and 60 min. A glass rod was used for homogenization of the mixture. The resulted treatments following different times were then hydrodistillated for 90 min. For applying ultrasonic waves, an ultrasound cleaning bath (pulse system 270, Italy, 26 kHz, 150 W) was used. To ensure a rigorous comparison, the same glassware and same operating conditions were used for conventional Hydrodistillation. The procedure was performed at least three times, and the mean values are reported.

GC-MS analysis

To evaluate the effect of ultrasonic pre-treatment of *L. stoechas* flowering tips on the chemical composition of distillated oils, AEO and KEO samples obtained at 10 min and 45 min of ultrasonic pre-treatment extraction, respectively, were chosen for their high efficiency. The qualitative and semi-quantitative analysis of essential oil samples were carried out using a Hewlett Packard Agilent 6890N gas chromatograph (GC) coupled to a 5973 mass spectrometer (MS) detector and equipped with a fused-silica-capillary column with a non-polar stationary phase HP-5MS™ (30 m × 0.25 mm × 0.25 µm film thickness). GC-MS were obtained using the following conditions: carrier gas He; flow rate 1 ml/min; split 1:30; injection volume 1 µl; injection temperature 250°C; oven temperature progress from 50°C to 290°C at 3°C/min; the temperature of the mass spectrometer (280°C), ion source (230°C), quadrupole (150°C). The ionization mode used was

electronic impact at 70 eV.

Retention indices (RI) were calculated for all constituents related to the retention time (TR) of n-alkanes that were analyzed under the same chromatographic conditions [30]. The identification of the essential oil constituents was based on the comparison of the mass spectra with those of the NIST05 and NIST libraries (computer matching) and published mass spectra [1]. The identification was confirmed by comparing the RI with those of previously published RI [1].

DPPH Free radical-scavenging activity

The antioxidant capacity of the *L. stoechas* essential oils was performed using 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay as previously described by Thakral *et a*l.[29]. Briefly, 1 ml of different concentrations of the essential oils (200, 400, 600, 800, and 1000 µg/ml) were added to 1 ml of 0.1 mM ethanol solution of DPPH and incubated in obscurity at 27°C during 30 min. The disappearance of the DPPH radical was read spectrophotometrically at 517 nm against a blank (EtOH solution). DPPH radical-scavenging activity, expressed as inhibition percentage (I %), was calculated using the following formula:

$$I\ \% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the control reaction containing all reagents except the essential oil and A_{sample} is the absorbance of the test reaction containing also the oil. Ascorbic acid was used as a reference antioxidant. The $EC_{50(DPPH)}$ value (µg/ml) represented the concentration of essential oil that scavenged 50 % of the DPPH radicals and was used as an estimate of the radical-scavenging activity. It was calculated from the plot of I % against the essential oil concentrations. All tests were carried out in triplicate and the $EC_{50(DPPH)}$ values were reported as means ± SD.

Statistical analysis

Results of essential oil yield and antioxidant activity are reported as mean±SD. Significant differences between the means was made by one-way analysis of variance (ANOVA) followed by Tukey's pair wise test. Differences with $P<0.05$ were considered statistically significant

Results and discussion

Effect of ultrasonic pre-treatment time on the yield

Figure 1 shows the influence of pre-treatment time by ultrasound on the yield of essential oils of *L. stoechas* from harvesting regions Adekar (AEO) and Keddara (KEO). Samples showed different yield extraction profiles. Adekar *L.*

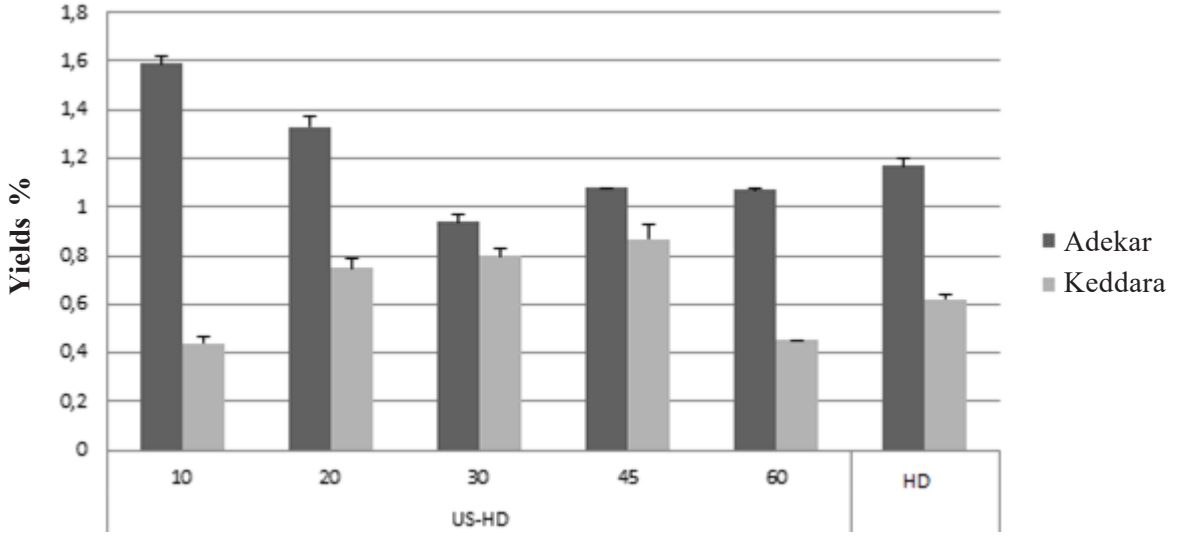

Fig. 1. Yields of wild Adekar and Keddara *Lavandula stoechas* essential oils obtained by different times of ultrasound pre-treatment prior Hydrodistillation (US-HD) and by Hydrodistillation (HD)

stoechas reached maximal oil yield of (1.59± 0.03 %) at only 10 min of ultrasound pre-treatment, followed by a significant decrease ($p<0.05$) of yield at 20 min (1.33± 0.04 %) and 30 min (0.94 ± 0.03 %) of pre-treatment. A significant ($p<0.05$) improvement in oil yield when prolonging the ultrasonic pre-treatment time up to 45 min (1.08± 0.00 %) and 60 min (1.07 ± 0.01 %). In keddara samples, the EO extraction yield was improved by rising the ultrasonic pre-treatment time from 10 min to 45 min with (0.44± 0.03 %) and (0.87± 0.06 %), respectively. The extension of sonication pre-treatment to 60 minutes resulted in signi-ficant decrease ($p<0.05$) in yield of KEO by 48.28 %. Similar variations in the pattern of extraction yield depending on sonication treatment time have previously reported for the phenolics of star fruits [3].

The decrease in EO yield after the extended sonication time duration (starting from 20 min for AEO and at 60 min for KEO) might be attributed to the temperature increase of the medium process [28], which influences the stability of EO compounds depending on their chemical structure. Moreover, a probable degradation that could have occurred due to the generation of free radicals, mainly the highly reactive hydroxyl radicals as reported by Annegowda *et al*.[3]. On another hand, our two samples could exhibit varied results probably due to their different oil gland structure and density as well as chemical characteristics of their EOs, which could be specifically impacted by the ultrasound treatment. In fact, the distribution and structure of oil secretory gland may vary depending on plant species even within the genus [4], organ [27], phenological stage [10]. Similarly, significant relationships have been reported between the essential oil composition and the eco-geographical origins of the populations [6]. The impact of ultrasonic pre-treatment on extraction oil efficiency might be related to different mechanisms involved during ultrasound extraction (fragmentation, erosion, capillarity, detexturation and sonoporation). For example, Comparison of extraction yields of boldo leaves showed enhancement of extraction yield from 20 % for conventional maceration to 25 % with ultrasound extraction [25]. Knowing that boldo leaves possess trichomes on the surface of leaves, the authors showed that theses structure have been damaged or removed from the leaf after ultrasound treatment, which is not the case of leaves submitted to maceration. Jacotet-Navarro *et al*.[16] also showed that ultrasound considerably improve the mass extraction yield of ginger, as a rise of 126 % was noticed between conventional maceration and ultrasound assisted extraction while reducing the extraction time from 540 to 110 min. Mechanical impacts of ultrasound and other influencing parameters factors have been very good reviewed by Chemat *et al*.[12].

Comparison of *L. stoechas* essential oils obtained by HD and US-HD

Conventional hydrodistillation method was compared to hydrodistillation preceded with different times of pre-treatment by ultrasound (Figure 1). Results shows that in sample of Adekar, only 10 min of ultrasound pre-treatment followed by 90 min of hydrodistillation provide (1.59± 0.03 %) of yield comparing to (1.17± 0.03 %) of yield ($p<0.05$) of untreated sample submitted to 180 min of hydrodistillation. However, overall yield of essential oils of Keddara sample treated by 45 min (0.87 ± 0.06 %) and untreated sample (0.62 ± 0.02 %) are not significantly different.

Few studies have evaluated the effect of pre-treatment of ultrasound prior the hydrodistillation on essential oils yield. Assami *et al*.,[5] showed that only 30 min are required to recover 80 % of total essential oil from caraway seeds pretreated with ultrasound, against 90 min for untreated samples. Ultrasound treatment of the air dried aerial parts of *Thymus daenensis* had significant effect on extraction efficiency of its essential oil by hydrodistillation [28]. Ultrasonic extraction followed by hydrodistillation of *Elettaria cardamomum* seeds facilitated short time extraction, improved extraction efficiency and produced good quality cardamom essential oil [22]. on the other hand, this technique, have not provide a positive effect on extraction kinetics of Lavandin essential oil and gives a maximum yield after 60 min of steam distillation preceded by 30 min of ultrasound pre-treatment [24]. Among the new oil extraction techniques, ultrasound assisted extraction, has attracted significant attention due to its positive impacts on

extraction time, yield and solvent consumption [8]. These positive effects by ultrasound waves are mostly attributed to the acoustic cavitation event. The cavitation effects cause directly disruption and thinning of biological membranes and cell walls, consequently heating increasing the mass transfer rate of organic substances from the solid matrix to the solvent [28]. Furthermore, ultrasonic pre-treatment could cause the glandular walls to crumble or rupture more rapidly and more efficiently than in conventional hydrodistillation. Among the novel extraction methods application of ultrasound attracted lots of attentions due to a short time and enough extraction efficiency. The comparison of the novel methods and their possible effects on the chemical structure of extracted essential oil could provide useful information in the term of possibility of approach about other similar essential oil.

Chemical composition of essential oils

Table 1 reports the chemical analysis of both AEO and KEO obtained by US-HD and HD, and giving the best yield (at 10 min and 45 min, respectively, of ultrasonic pre-treatment). Forty-seven compounds were identified. Thirty-seven compounds were identified similarly in both Adekar and Keddara samples untreated by ultrasound versus 36 and 35 compounds in treated samples, respectively. 94.30 % and 92.64 % of the total oil were identified in Adekar US-HD and HD samples, respectively, with most dominant compounds fenchone and camphor. In Keddara, 88.26 % and 88.75 % of the total oil was determined in treated and untreated samples with predominant constituents fenchone and 1,8-cineol. These present data are in agreement with those reported by Benabdelkader *et al*.[9] in relation to major EO components from several wild populations of Algerian *L. stoechas*.

Although AEO and KEO of treated and untreated samples contain almost the same principal compounds but their behavior was different. In AEO obtained with US-HD, the relative amount of fenchone (26.66 %) was higher than in HD (21.31 %) (Fig. 2). Whereas in KEO,

Table 1. Chemical composition of *Lavandula stoechas* L. essential oils obtained by US-HD and HD

No.	Compounds[a]	RI[e]	RI[L]	Composition (%)			
				AEO		KEO	
				US-HD	HD	US-HD	HD
1	Tricyclene	917	921	-	0.20	-	-
2	α-Pinene	927	932	0.18	0.17	0.16	0.23
3	Camphene	944	946	1.37	2.18	1.09	1.39
4	ρ-Cymene	1017	1020	0.40	0.49	-	-
5	Limonene	1021	1024	-	0.25	-	-
6	1.8-cineole	1026	1026	4.14	2.11	17.24	16.48
7	γ-Terpinene	1049	1054	-	-	0.15	0.30
8	*cis*-Linaloloxide	1073	1067	0.63	-	-	-
9	Fenchone	1093	1083	26.66	21.31	22.30	23.90
10	Linalool	1101	1095	0.50	0.23	-	-
11	α-Fenchol	1118	1114	1.41	0.95	1.39	1.36
12	Camphor	1146	1141	23.73	26.53	10.33	9.57
13	Borneol	1166	1165	2.03	1.42	-	-
14	Terpinen-4-ol	1172	1174	0.61	0.30	0.67	0.66
15	p-Methylacetophenon	1176	1179	0.18	0.19	0.98	1.72
16	p-cymene-8-ol	1181	1179	0.90	0.65	0.93	-
17	Myrtenal	1186	1195	0.62	0.77	0.58	1.39
18	Myrtenol	1190	1194	1.22	-	1.02	-

table 1. (continued).

No.	Compounds[a]	RI[e]	RI[L]	Composition (%) AEO US-HD	AEO HD	KEO US-HD	KEO HD
19	Verbenone	1198	1204	0.86	0.49	-	0.80
20	α-Fenchyl acetate	1208	1218	0.75	1.42	0.72	0.66
21	*trans*-Carveol	1213	1215	0.42	0.20	0.28	0.29
22	Carvone	1239	1239	0.61	0.40	0.41	0.58
23	Bornyl acetate	1287	1287	6.84	10.34	3.77	3.43
24	Myrtenyl acetate	1324	1324	4.46	4.09	2.22	2.70
25	Eugenol	1347	1356	-	-	0.22	0.15
26	Cyclosativene	1359	1369	0.51	0.61	0.71	0.67
27	α-Copaene	1365	1374	0.18	0.13	0.40	0.44
28	Geranyl acetate	1368	1379	-	-	-	0.17
29	Sativene	1379	1390	-	-	0.12	0.09
30	*cis*-Caryophyllene	1403	1408	-	-	0.14	0.14
31	Aromadendrene	1447	1439	-	-	0.29	0.16
32	*allo*-Aromadendrene	1460	1458	0.24	0.13	-	-
34	γ-Cadinene	1512	1513	1.27	1.41	2.18	2.50
35	Calamenene	1515	1521	0.59	0.55	0.73	0.79
36	Cadina-1.4-diene	1524	1533	2.20	2.61	4.22	2.89
37	α-Calacorene	1534	1544	0.50	0.52	0.71	0.77
38	*cis*-α-Copaene-8-ol	1556	-	0.52	0.45	0.68	0.64
39	Palustrol	1562	1567	0.32	0.30	0.45	0.46
40	Caryophyllene oxide	1575	1582	0.68	0.65	1.03	0.87
41	Viridiflorol	1592	1592	4.23	4.84	5.43	5.32
42	Ledol	1601	1602	1.01	1.12	1.41	1.33
43	Guaiol	1606	1600	1.9	2.31	2.57	2.55
44	1-*epi*-Cubenol	1623	1627	0.77	0.84	1.29	1.36
45	t-Cadinol	1635	1638	0.91	1.04	1.44	0.70
46	α-Cadinol	1649	1652	-	0.44	-	0.75
47	Cadalene	1666	1675	-	-	-	0.54
	Total identified (%)			94.30	92.64	88.26	88.75
	Monoterpene Hydrocarbons			1.95	3.29	1.40	1.92
	Oxygen-containing Monoterpenes			76.39	71.21	62.08	62.14
	Sesquiterpene Hydrocarbons			3.59	3.65	6.93	5.91
	Oxygen-containing Sesquiterpenes			10.34	11.99	14.30	14.52
	Others			0.18	0.19	0.98	1.72
	US treatment time (min)			10	00	45	00
	Extraction time (min)			90	180	90	180
	Yield (%)			1.59	1.17	0.87	0.62

[a] Components listed in order of elution on HP5-MS column

RI[e]- Experimentally determined retention indices on the mentioned column by co-injection of a homologous series of n-alkanes C8-C21; RI[L]- Literature retention indices (Adams, 2007);

AEO (Adekar Essential Oil); KEO (Keddara Essential Oil);

US-HD (Ultrasound assisted extraction prior Hydrodistillation); HD (Hydrodistillation).

fenchone concentration was higher in untreated samples (23.90 %) than in treated ones (22.30 %) (Fig. 2). On the other hand, relative abundances of camphor and bornyl-acetate were slightly highest in KEO/US-HD samples than those of HD (10.33 - 3.77 % and 9.57 - 3.43 %, respectively). Whereas, inversed concentrations patterns were obtained in the case of AEO samples. As can be seen in Figure 2, 1,8-cineol was the only main compound which the relative amount was higher in both AEO (4.14 %) and KEO (17.27 %) extracted with US-HD process compared to HD (2.11 % and 16.48 %, respectively). It should be also noted that some minor compounds, such as myrtenol, were detected only in EOs obtained with US-HD (Table 1). Inversely, other minor compounds such as α-cadinol were detected only in EOs obtained with HD. The effect of ultrasonic treatment on chemical composition of both AEO and KEO could be connected with temperature properties and solubility of their various components [21]. It has been found that relative abundances of fenchone and camphor were highest at 150°C, in Turkish *L. stoechas*, whereas solubility of myrtenol and bornyl acetate was highest at 125°C. On the contrary fenchyl alcohol reached maximum relative amount at only100°C [15].

Previous studies showed that generally US-HD process might enhance EO extraction yield or provide more valuable EO components than conventional HD. Additionally, the behavior of main volatile compounds could present different patterns in the same plant species according to the organ material [2]. It is also important to note the influence of extraction time on the extraction kinetics for major components obtained with developed extraction methods, such as ultrasonic extraction [5] and subcrtical water (SbCW) extraction, both combined with HD. Indeed, extraction by SbCW of flower EOs from Turkish *L. stoechas* for most abundant two components, fenchone and camphor, was mostly completed at 15 min. Other major components, 1,8-cineol, myrtenol and myrtenyl acetate were reached the relative maximum amount at 20 min of the extraction [15]. Jacotet-Navarro *et al.*[16] showed that quantity of ginger phenolics compounds extracted was improved by 29 %, by comparing conventional maceration (CM) to ultrasound assisted extraction (UAE).

Antioxidant activity

The effects of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [26]. Results of DPPH free radical scavenging activity by essential oils of pre-treated samples giving the best yield and untreated samples compared to ascorbic acid as a standard antioxidant are summarized in Table 2 and Figures 3A and 3B. The reducing power of essential oils of treated and

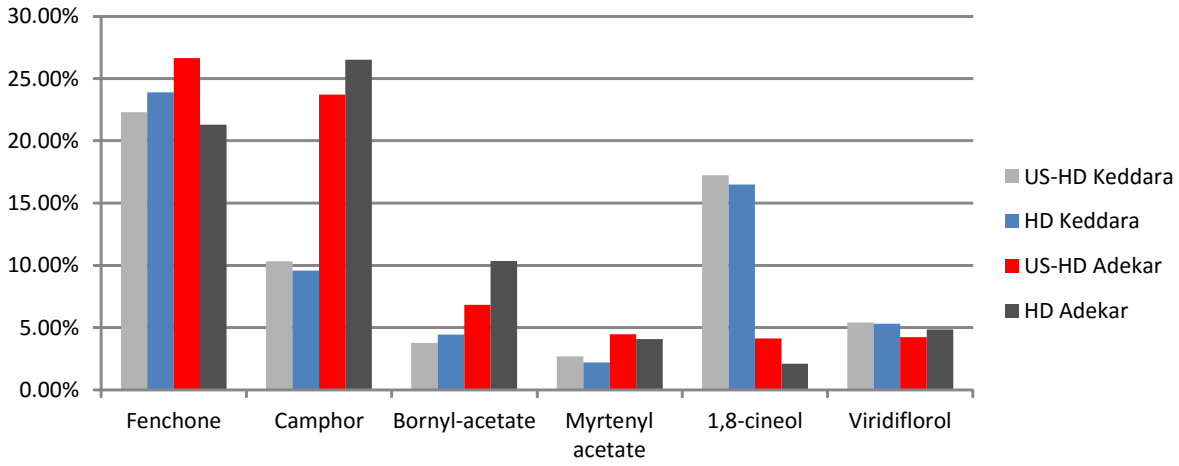

Fig. 2. Main compounds of Adekar and Keddara *Lavandula stoechas* essential oils obtained by hydrodistillation (HD) and ultrasound assisted extraction prior hydrodistillation (US-HD)

Table 2. Antioxidant activity of *Lavandula stoechas* essential oils obtained by US-HD and HD

Essential oil	EC50 ± SD (µg/ml)
Keddara HD	2786.94 ± 17.32
Keddara US-HD	2564.10 ± 16.45
Adekar HD	1663.96 ± 9.69
Adekar US-HD	1547.29 ± 42.23
Ascorbic acid	16.16 ± 1.16

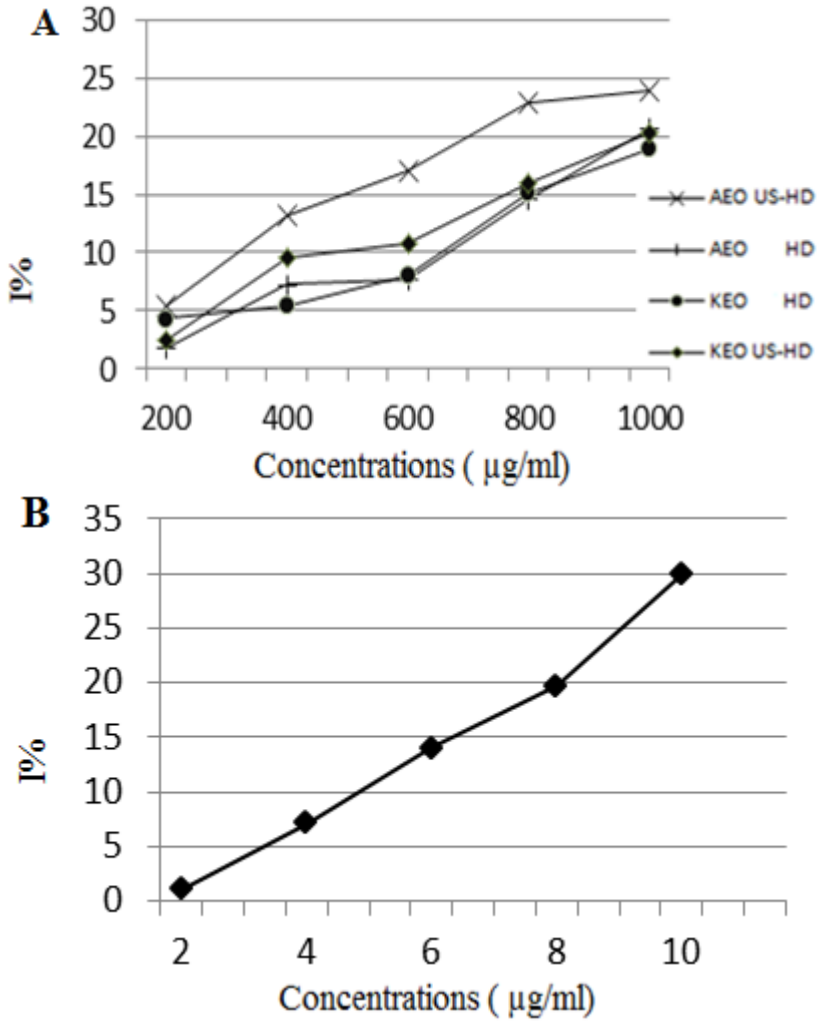

Fig. 3. DPPH radical-scavenging activity (I %) of (**A**) essential oils of *Lavandula stoechas*, (**B**) ascorbic acid

untreated *L. stoechas* was concentration dependent. As the concentrations tested for EOs samples were very powerful in the case of the reference ascorbic acid which is a strong antioxidant, hence the following concentrations were tested: 2; 4; 6; 8 and 10 µg/ml. The percentages of antioxidant activity of all samples were very lower than that ascorbic acid. At the concentration of 1000 mg/ml, KEO and AEO of treated and untreated *L. stoechas* samples have revealed a percent of inhibition of DPPH free radical of (20.22 ± 1.72 %) and (18.88 ± 2.08 %), (23.96 ±

3.08 %) and (20.70 ± 4.41 %) respectively. These results clearly show that antioxidant power of essential oils obtained by US-HD was higher than antioxidant power of essential oils obtained from untreated samples. The EC_{50} values studied graphically, express the effective concentration of the antioxidant extract necessary for trapping and lowering 50 % of moles of DPPH dissolved in ethanol. According to these values which have shown an efficacy very inferior to that of ascorbic acid (16.16 ± 1.16 µg/ml), EC_{50} of untreated samples is always higher than EC_{50} of treated samples of the two harvest sites (Table 2) with an significant difference ($p<0.05$) between KEO/US-HD and KEO/HD, and insignificant difference between AEO/US-HD and AEO/HD, which means that samples treated by ultrasound is more effective antioxidant compared to untreated samples. In spite of this insignificant difference of AEO, it is important to note that the treatment with ultrasound saved us time by reducing the time of hydrodistillation of the half. Our EOs have a low antioxidant activity comparing to EOs of Mohammedi *et al.*,[20] and Barkat *et al.*,[7], who found EC_{50} of (1852.76 ± 55.74 µg/ml) and (584 ± 0.58 µg/ml) of leaves and flowers of *L. stoechas* harvested from Tlemcen and Constantine (Algeria), respectively.

Antioxidant activities of the essential oils from aromatic plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of the main constituents, but also to the presence of other constituents in small quantities or to synergy among them [17]. Ultrasound assisted extraction not only improved yield but as the method is fast and run at low temperature, the final product usually showed less thermal degradation than traditional or microwave assisted method. This is especially important in case of food or cosmetic because in these two fields, essential oils have to be of high quality and good flavor [14]. Due to the different in constituents of different essences, different behaviors are observed in their antioxidant properties but in general, the ultrasound method has improved the antioxidant properties compared to the hydro distillation method and this finding is consistent with the researcher's statements.

Conclusion

The objective of this work is to show the advantage of ultrasound treatment of plant material before hydrodistillation process, in order to recover essential oils from two wild populations of Algerian *L. stoechas* L. US-HD extraction took shorter time compared to conventional hydrodistillation method. Total extraction time of 100 min and 135 min (consisting of 10 min and 45 min time, respectively, of ultrasonic pre-treatment plus 90 min of hydrodistillation) was used during US-HD whereas 3 h was required in HD extraction. The comparative study of the main compounds showed a qualitative and quantitative difference between AEO and KEO, as well as between EOs obtained by HD and US-HD. The antioxidant activity of EOs from samples pre-treated by ultrasound was higher compared to untreated samples.

The novel method US-HD provided useful informations in the term of possibility of approach with other wild *L. stoechas* populations and other similar essential oils.

In relation to green aspects, the use of ultrasound as a pre-treatment prior hydrodistillation improved the extraction of essential oil with the same amount of biomass and water, but in shortened extraction time without loss of quality of the final product. So, the energy consumption, although it is not calculated, should be lower, reflecting in less pollutants emission, making this method greener than conventional hydrodistillation. Other key extraction parameters (frequency and intensity of ultrasound, temperature of the water) should be studied in the future to scalling up the US-HD extraction process of lavender (*L. stoechas*) essential oils.

Green extraction processing especially for essential oils such as ultrasound pre-treatment prior hydrodistillation, could be a new concept to protect both the environment and consumers, and in the meantime enhance competition of industries to be more ecologic, economic and innovative.

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References

1. **Adams, R.P. (2007).** Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Co. Illiois, USA.
2. **Ait-kaci Aourahoun, K., Fazouane, F., Benayache, S. and Souici, F. (2016).** Glandular Hairs and Essential Oils of Stem and Leave from the Promosing Medicinal Legume *Cytisus triflorus* L'Hérit. Using Ultrasounds: Comparison with Conventional Hydrodistillation. Ind. J. of Natural Sciences. 6 (35): 10911-10922.
3. **Annegowda, H.V., Bhat, R., Min-Tze, L., Karim, A.A. and Mansor, S.M. (2012).** Influence of sonication treatments and extraction solvents on the phenolics and antioxidants in star fruits. J. of Food Science and Technologie.49: 510-514.
4. **Arnold, N. and Bellomaria, B. (1993).** Observations morpho-anatomiques et histochimiques dans les *Salvia willeana* (*Holmboe*) Hedge et *Salvia fruticosa* Mill. de Chypre. Flora Mediterranea. 3: 283-297.
5. **Assami, K., Pingret, D., Chemat, S., Meklati, B.Y. and Chemat, F. (2012).** Ultrasound induced intensification and selective extraction of essential oil from *Carum carvi* L. seeds. Chemical Engineering and Processing: Process Intensification. 62: 99-105.
6. **Bajalan, I., Rouzbahani, R., Ghasemi Pirbalouti, A. and Maggi, F. (2016).** Variation in chemical composition and antibacterial activity of the essential oil of wild populations of *Phlomis olivieri*. Chemistry and Biodiversity. 14(5): 1-8 (e1600444).
7. **Barkat, M. and Laib, I. (2012).** Antioxidant activity of the essential oil from the flowers of *Lavandula stoechas*. J. of Pharmacognosy and Phytotherapy. 4(7): 96-101.
8. **Barrera Vázquez, M.F., Comini, L.R., Martini, R.E., Núñez Montoya, S.C., Bottini, S. and Cabrera, J.L. (2014).** Comparisons between conventional, ultrasound-assisted and microwave-assisted methods for extraction of anthraquinones from *Heterophyllaea pustulata* Hook f. (*Rubiaceae*). Ultrasonics Sonochemistry. 21: 478-484.
9. **Benabdelkader, T., Zitouni, A., Guitton, Y., Jullien, F., Maitre, D., Casabianca, H., Legendre, L. and Kameli, A. (2011).** Essential oils from wild populations of Algerian *Lavandula stoechas* L.: composition, chemical variability and *in vitro* biological properties. Chemistry and Biodiversity. 8(5): 937-953.
10. **Bosabalidis, A. M. and Sawidis, T. (2014).** Glandular and non-glandular hairs in the seasonally dimorphic *Origanum dictamnus* L. (*Lamiaceae*) as a means of adaptation to cold stress. Acta Agrobotanica. 67(1): 15-20.
11. **Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Périno-Issartier, S., Fabiano-Tixier, A.S. and Vian, M. (2017a).** Review of Green Food Processing techniques. Preservation, transformation, and extraction. Innovative Food Science and Emerging Technologies. 41: 357-377.
12. **Chemat, F., Rombaut, N., Sicaire, A.G., Meullemiestre, A., Fabiano-Tixier, A.S. and Abert-Vian, M. (2017b).** Ultrasound assisted extraction of food and natural products: Mechanisms, techniques, combinations, protocols and applications. Ultrasonics Sonochemistry. 34: 540-560.
13. **Chen, F., Sun, Y., Zhao, G., Liao, X., Hu, X., Wu, J. and Wang, Z. (2007).** Optimization of ultrasound-assisted extraction of anthocyanins in red raspberries and identification of anthocyanins in extract using high performance liquid chromatography-mass spectrometry. Ultrasonics Sonochemistry. 14: 767-778.
14. **Da porto, C., Decorti, D. and Kikic, I. (2009).** Flavour compounds of *Lavandula augustifolia* L., to use in food manufacturing: Comparison of three different extraction methods. Food Chemistry. 112: 1072.
15. **Giray, E.S., Kirici, S., Kaya, D.A., Türk, M., Sönmez, O. and Inan, M. (2008).** Comparing the effect of sub-critical water extraction with conventional extraction methods on the chemical

composition of *Lavandula stoechas.* Talanta. 74: 930-935.

16. **Jacotet-Navarro, M., Rombaut, N., Deslis, S., Fabiano-Tixier, A.S., Pierre, F.X., Bily, A. and Chemat, F. (2016).** Towards a "dry" bio-refinery without solvents or added water using microwaves and ultrasound for total valorization of fruit and vegetable by-products. Green Chemistry.18(10): 3106-3115.
17. **Joshi, R.K. (2010).** *In vitro* antimicrobial and antioxidant activities of the essential oil of *Craniotome furcata*. J. of Applied and Natural Science. 2(1): 57-62.
18. **Luque-Garcia, J.L. and Luque de Castro, M.D. (2003).** Ultrasound: A powerful tool for leaching. Trends in Analytical Chemistry. 22: 41-47.
19. **Luque-Garcia, J.L. and Luque de Castro, M.D. (2004).** Ultrasound assisted Soxhlet extraction: an expeditive approach for solid sample treatment. Application to the extraction of total fat from oleaginous seeds. J. of Chromatography A. 1034: 237-242.
20. **Mohammedi, Z. and Atik, F. (2012).** Pouvoir antifongique et antioxydant de l'huile essentielle de *Lavandula stoechas* L.. Nature and Technologie. 06: 34-39.
21. **Mullakaev, M.S., Volkova, G.I. and Gradov, O.M. (2015).** Effect of ultrasound on the viscosity-temperature properties of crude oils of various compositions. Theoretical Foundations of of Chemical Engineering. 49(3): 287-296.
22. **Nashwa, F. S.M. (2015).** A short extraction time of high quality hydrodistilled cardamom (*Elettaria cardamomum* L. Maton) essential oil using ultrasound as a pretreatment. Industrial Crops and Products. 65: 287-292.
23. **Palma, M. and Barroso, C.G. (2001).** Ultrasound assisted extraction and determination of tartaric and malic acids from grapes and winemaking by-products. Analytical Chemistry. 458: 119-130.
24. **Périno-Issartier , S., Ginies, C., Cravotto, G., Chemat, F. (2013)**. A Comparison of Essential Oils Obtained From Lavandin via Different Extraction Processes: Ultrasound, Microwave, Turbohydrodistillation, Steam and Hydrodistillation. J. of Chromatography A. 1305 : 41-47.
25. **Petigny, L., Perino, S., Wajsman, J. and Chemat, F. (2013).** Batch and Continuous Ultrasound Assisted Extraction of Boldo Leaves (*Peumus boldus* Mol.). Int. J. of Molecular Sciences. 14(3): 5750-5764**.**
26. **Soares, J.R., Dins, T.C.P., Cunha, A.P. and Almeida, L.M. (1997).** Antioxidant activity of some extracts of *thymus zygis*. Free Radical Research. 26: 469- 478.
27. **Sulborska, A. (2013).** Structure and distribution of glandular and non-glandular trichomes on above-ground organs in *Inula helenium* L. (*Asteraceae*). Acta Agrobotanica. 66 (4): 25-34.
28. **Tavakolpour, Y., Moosavi-Nasab, M., Niakousari, M., Haghighi-Manesh, S., Hashemi, S.M.B., Khaneghah , A.M. (2016**). Comparison of Four Extraction Methods for Essential Oil from *Thymus daenensis Subsp. Lancifolius* and Chemical Analysis of Extracted Essential Oil. J. of Food Processing and Preservation. 41(4): e13046.
29. **Thakral, J., Borar, S., Kalia, R.A.N. (2010).** Antioxidant Potential Fractionation from Methanol Extract of Aerial Parts of *Convolvulus arvensis* Linn. (*Convolvulaceae*). Int. J. of Pharmaceutical Sciences and Drug Research. 2: 219-223.
30. **Van den Dool, H., and Kratz, P.D. (1963)**. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatography A. 11: 463- 471.