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*Research Article*

# EFFECT OF ULTRASOUND PRETRAITMENT ON THE QUALITY AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM FOUR WILD POPULATIONS OF ALGERIAN *LAVANDULA STOECHAS* L.: COMPARISON WITH CONVENTIONAL HYDRODISTILLATION

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**ABSTRACT.** This work presents the influence of ultrasound pretreatment of *Lavandula stoechas* on the extraction process of essential oils (EOs), compared with conventional hydrodistillation (HD). This study aims to improve the green techniques for obtaining EOs from aromatic and medicinal plants.
The tests were carried out on *L. stoechas* harvested from 04 sites in the north of Algeria. These samples were pretreated by sonication times of 10, 20, 30, 45 and 60min followed by 90min of HD (US-HD) and compared with untreated samples (180min of HD) in terms of extraction duration, yield, quality, chemical composition and antimicrobial activity of EOs.
10min of pretreatment time gives yields of 1.60% and 1.59% of Kodiat and Adekar EO's, respectively. This pretreatment time proved to be the best compared with the other pretreatment times. The US-HD yields are higher compared to those obtained by HD for the four samples. The US-HD EO's has a darker color and a more intense odor compared with HD EO's. GC/MS analysis showed a qualitative and quantitative difference in the chemical composition of treated and untreated samples. Our extracts showed good antimicrobial activity against most of the 08 pathogenic strains tested. This antimicrobial potential remains more interesting in the US-HD case.

**Key words:** *Ultrasound pretreatment; essential oils; yield; GC/MS; MIC.*

## INTRODUCTION

To cope with a growing need for natural products, especially essential oils (EOs), the industrial processes for obtaining them are subject to a constant improvement in their performance. The optimization of these processes takes into account the following criteria: the increase of the yield, the quality of the oils as well as the reduction of the time and the production costs. In addition, in a context of sustainable development, the

reduction of pollution has become a necessity. Furthermore, an adaptation of the processes is necessary for the production of thermosensitive EOs or EOs containing compounds of thermosensitive interests.

In recent years, the use of ultrasound sonication has opened high expectations with promising results. Various plant extracts and bioactive metabolites have been obtained with this technique [1], [2]. Ultrasonic assisted extraction uses acoustic cavitation to cause the molecular displacement of the solvent and sample, offering advantages such as improved efficiency, high level of automation, reduced extraction time, energy consumption and contributes to the preservation of the environment by reducing the consumption of water and solvents, the elimination of fossil energy, wastewater and the generation of hazardous substances, compared to conventional extraction [3, 4, 5].

*Lavandula stoechas* is a species belonging to the family of *Lamiaceae,* it has the largest spread since it covers almost the entire Mediterranean except a small arc east from Lebanon to Libya. Its biodiversity center, and probable origin, is the south of the Iberian Peninsula [6]. This species of lavender is very common in the northern part of Algeria where it meets spontaneously at quite variable altitudes.

Many studies using ultrasound for the extraction of EO's have been conducted [7, 8, 9]. But, very few studies have applied ultrasound as pretreatment before hydrodistillation (US-HD) for EO's extraction [10, 11, 12], and the study of the effect of US-HD on the antimicrobial activity has never been investigated. It is in this context that the objective of our work was oriented towards a comparative study of the conventional technique of hydrodistillation (HD) with that of US-HD in terms of yield, quality, chemical composition and antimicrobial activity of *L.stoechas* EO's harvested from 04 different sites situated in the North of Algeria.

## 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 four regions in northern Algeria: Adekar (Location: Bejaia, Latitude: 36°42′44″N, Longitude: 4°34′20″E, Altitude: 866m, Slope: > 15%), Keddara (Location: Boudouaou, Latitude: 36°39′00″N, Longitude: 3°24′35″E, Altitude: 644m, Slope: > 15%), Kodiat (Location: Taouarga, Latitude: 36°47′39″N, Longitude: 3°58′10″E, Altitude: 536m, Slope: > 12%) and El-kahla (Location: Larbaatache, Latitude: 36°33′27″N, Longitude: 3°21′23″E, Altitude: 861m, Slope: > 12%). 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 a 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 [13]. 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 yield values were reported as means±SD.

### *Ultrasound assisted extraction prior hydrodistillation (US-HD)*

Ultrasounds were applied on the plant materials as a pre-treatment before hydrodistillation according to Ait-kaci Aourahoun et *al.* (2016) methodology. 50 g of the ground flowering tips was mixed with 0,8L deionized water in Erlenmeyer flask. The mixture was submitted to ultrasound for different times: 10, 20, 30, 45 and 60 min. A glass rod was used for homogenization of the mixture. These samples treated by the US were then hydrodistilled 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 yield values were reported as means±SD.

### *GC/MS analysis*

To evaluate the effects of ultrasonic pre-treatment of *L. stoechas* flowering tips on the chemical composition of HD samples and US-HD samples of the best pretreatment time giving the best yield, 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 × 0.25µm film thickness). GC-MS was obtained using the following conditions: carrier gas He; flow rate 1ml/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.

### *Component identification*

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 [14]. 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. The identification was confirmed by comparing the RI with those of previously published RI [15].

### *Antimicrobial activity*

Minimum inhibitory concentration (MIC) values for microorganisms tested. This choice of microorganisms affects both phyto-pathogenic microorganisms and those which are pathogenic to humans (two bacteria Gram+: *Bacillus subtilis* and Methicillin-resistant *Staphylococcus aureus*, two bacteria Gram-: *Escherichia coli* and *Pseudomonas aerugenosa*, three molds: *Umbelopsis ramanniana*, *Aspergillus carbonarius* and *Fusarium culmorum* and the yeast *Candida albicans*) were determined in vitro using the conventional agar dilution method [16]. Serial dilutions of *L.stoechas* EO's obtained by HD and US-HD of the best pretreatment time giving the best yield, were prepared with sterile nutrient agar, melted and enriched with agar (10 g / l) and with the Tween-80 (0.5%, v / v) to cover a concentration range from 0.14 to 18mg / ml (This concentration range was chosen after realizing antimicrobial power tests of our samples at different

concentrations by the disk method). The nutrient agar solutions obtained were immediately poured into Petri dishes after stirring. These dishes were allowed to dry at room temperature and then inoculated with a spot of 1 µl of each suspension of target microorganism in two repetitions. The microorganism suspensions were prepared from cultures of 24 h for bacteria and 72 h for fungi and were adjusted to about $10^6$ CFU / ml. The same test was performed without EO's as a negative control. The inoculated dishes were incubated at 30°C for 24 h for the bacteria and 48 h for the fungi. After incubation, observation of the range makes it possible to determine the minimum inhibitory concentration, which corresponds to the lowest concentration of EO's able to inhibit the growth of microorganisms.

### *Statistical analysis*

Results of EO's yield and antimicrobial 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. The smaller the p value, the lower the probability of making a mistake. A limit value of 0.05 is often used. In other words, if the p value is less than 0.05, the differences are considered statistically significant.

## RESULTS AND DISCUSSION

### *Effect of ultrasonic pre-treatment time on the yield and quality of EO's (comparison with the HD method)*

Extractions by conventional HD and by US-HD at different times: 10; 20; 30; 45 and 60 min of the flowering aerial parts of *L. stoechas* were optimized and then carried out under strictly identical operating conditions on 04 wild Algerian populations. The EO yields of *L. stoechas* populations are shown in Fig. 1. All populations studied showed variable yields, ranging from 0.50% to 1.17% for HD extraction (180 min) and from 0.44% to 1.60% for pretreatment extraction at different times (10; 20; 30; 45 and 60 min) by the US followed by 90 min of HD. It should be noted that the best yields (1.60 and 1.59%) were obtained after 10 min of ultrasound pretreatment followed by 90 min of HD from the Kodiat and Adekar populations, respectively. For Keddara and El-kahla, the best yields (0.87% and 1.03%) were obtained after 45 and 60 min of ultrasound pretreatment, respectively. The best yields obtained by US-HD showed significant differences ($p < 0.05$) compared to those obtained by HD. Fig. 1 also shows the influence of ultrasonic pretreatment time on the yield of *L. stoecha*s EO's for each harvest region. The samples showed different extraction yield profiles. *L. stoechas* from Adekar achieved a maximum yield of 1.59 ± 0.03% at only 10 min of ultrasound pretreatment, followed by a significant ($p < 0.05$) decrease in yield at 20 min (1.33 ± 0.04%) and 30 min. (0.94 ± 0.03%) pretreatment. Significant ($p < 0.05$) improvement in oil yield by extending sonication pretreatment time to 45 min (1.08 ± 0.00%) and 60 min (1.07 ± 0.01%) was noticed.

For keddara samples, the extraction yield of EOs was improved by increasing the ultrasound pretreatment time from 10 min to 45 min with (0.44 ± 0.03%) and (0.87 ± 0.06%), respectively. Extending ultrasound pretreatment time to 60 minutes resulted in a significant ($p < 0.05$) decrease in KEO yield of 48.28%. For Kodiat *L. stoechas*, it reached

a maximum yield of (1.60 ± 0.1%) at only 10 min of ultrasound pretreatment, followed by a non-significant decrease ($p>0.05$) of the yield at 20 min (1.19 ± 0.03%), followed by a significant increase ($p = 0.05$) of the yield at 30min (1.37 ± 0.09%). A significant decrease ($p = 0.05$) in the yield during the extension of ultrasound pretreatment time up to 45 min (0.61 ± 0.03%) and 60 min (0.62 ± 0.04%) was seen. In the El-kahla sample, the extraction yield of the EOs was more or less stable by increasing the ultrasound pretreatment time from 10 min to 45 min with (0.48 ± 0.01%) and (0.53 ± 0.01%). ), respectively. Extending ultrasound pretreatment time to 60 minutes resulted in a significant ($p <0.05$) increase in EEO yield (1.03 ± 0.07%).

Extractions by conventional hydrodistillation (HD) and ultrasonic pretreatment before hydrodistillation (US-HD) of the four samples of *L. stoechas* provided EOs with variable colorations and odors. In general, the EOs obtained by US-HD has a darker color and a more intense odor compared to the EOs obtained by HD.

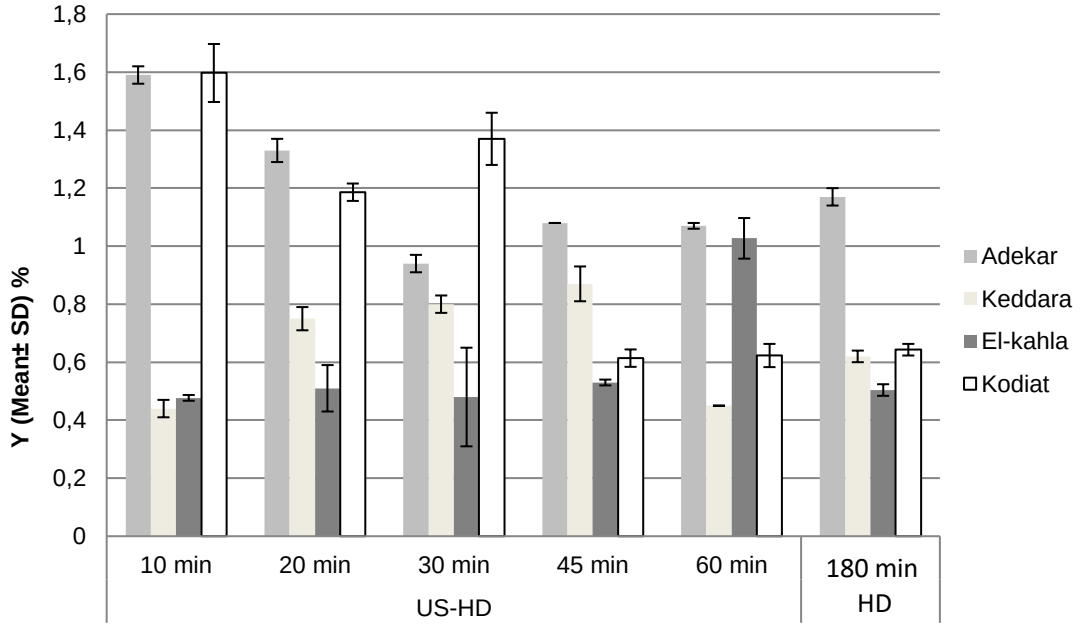


***Fig. 1.*** *Variation of extraction yields of 04 populations of L. stoechas essential oils obtained by hydrodistillation (HD) and by ultrasound pretreatment (US-HD) at different times.*

Few studies have evaluated the effect of pretreatment by US prior to HD on the yield and quality of EOs. Similar variations in the model of extraction yield as a function of ultrasonic treatment time have already been reported for *L.stoechas* EO's [11, 12], phenolic star fruit compounds [17]. Kimparis and *al*. [18] showed that the use of 30 min of ultrasound treatment for the extraction of EOs of *Allium sativum* has made it possible to improve the extraction by significantly reducing the time of extraction, which is in agreement with our results for the majority of the pretreatment times studied. Also, Assami and *al*. [10] showed that only 30 min of US pretreatment is required to recover 80% of caraway seed EOs, compared to 90 min of untreated samples. This increase in yield by ultrasound pretreatment was explained by the phenomenon of sonoporation in the studies of Meullemiestre and *al*. [19] who compared a wet yeast (*Yarrowia Lipolityca*)

treated with ultrasound with another untreated. The treated yeast has a highly impacted cell surface and perforations of the visible membrane have been observed, giving a higher yield compared to conventional extraction.

The best yields of AEO and TEO were obtained after only 10 min of ultrasound treatment, which can be explained by the work of Pingret and *al*. [20] for the recovery of total polyphenols from apple mark by ultrasound and by maceration. The authors were able to show that the kinetics of extraction is improved under ultrasound; this improvement seems to occur in the first 10 min of extraction. These results have already been confirmed by the works of Chekoual et *al*. [11, 12]. In addition, ultrasound pretreatment could cause the glandular walls to crumble or rupture faster and more efficiently than in conventional hydrodistillation.

Jacotet-Navarro and *al*. [21] have also shown that ultrasound greatly improves the extraction yield of ginger, an increase of 126% is observed between conventional maceration and ultrasound-assisted extraction while reducing the extraction time from 540 to 110 min. Ultrasonic treatment of air-dried aerial parts of *Thymus daenensis* had a significant effect on the extraction efficiency of its essential oil by hydrodistillation [9]. On the other hand, this technique had no positive effect on the extraction kinetics of Lavandin essential oil and gave a maximum yield after 60 minutes of steam distillation preceded by 30 min of ultrasound pretreatment [22].

Our four samples had varied results, probably because of their different structure and densities, as well as the chemical characteristics of their EOs, which could be specifically affected by the ultrasound treatment. In fact, the distribution and structure of the oil-secreting gland can vary depending on the plant species, even within the same genus [23], same organ [24] and even phenological stage [25]. For example, the comparison of boldo leaf extraction yields showed an increase in extraction yield of 20% for conventional maceration at 25% with ultrasound extraction [26]. Knowing that the boldo leaves have trichomes on the surface of the leaves, the authors have shown that these structures were damaged or removed from the leaf after sonication, which is not the case with leaves subjected to maceration. The mechanical effects of ultrasound as well as the influence of their parameters have been very well studied and published by Chemat and *al*. [27]. Concerning the difference of the quality of the extracts obtained by HD and US-HD, Kimparis and *al*. [18] showed in their comparative study of the extraction of EOs from fresh garlic (*Allium sativum*) by conventional distillation, by microwave assisted hydrodistillation and by ultrasound-assisted extraction that ultrasound causes less damage to the molecules. This makes this technique a better approach for extracting compounds that are primarily responsible for the characteristic odor and taste of freshly chopped garlic. The authors also indicated that ultrasound decreases the thermal degradation of sensory aromas.

***Chemical composition of essential oils***

Table 1 reports the GC/MS analysis of AEO, KEO, TEO and EEO obtained by HD and US-HD at the best pretreatment time giving the best yield (10, 45, 10 and 60min, respectively). 55 compounds were identified, with cumulative areas that correspond to 76% - 92.64% for extracts obtained by HD and 88.26% - 94.35% for extracts obtained by US-HD. Concerning the extracts obtained by HD, 37 compounds were identified in AEO, KEO and EEO and 42 compounds in TEO. As for the extracts obtained by US-HD, 35compounds were identified in the KEO, 36 compounds were identified in AEO and

EEO and 39 compounds were identified in TEO. It should be noted that in all the extracts obtained by US-HD, the total content of identified compounds is greater than the total content of compounds identified in the extracts obtained by HD. This result is reversed in the KEO (Table 1). So, this analysis showed the predominance of oxygenated monoterpenic compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, phenol ethers) with a combined content ranging from 62.08% to 76.39% for the EOs obtained by US-HD and from 46.61% to 71.21% for the EOs obtained by HD. This fraction is dominated either by fenchone (from 22.3 to 26.66% and from 13.66 to 23.9%, respectively) or by camphor (26.53%) in the only case of AEO obtained by HD. The profiles of oxygenated monoterpenes demonstrate the importance of 1,8-cineol in the samples of most populations (from 4.14 to 17.24% and 2.11 to 16.48%, respectively) where it was detected as the second or third majority component. Next, bornyl acetate (1.89 to 7.52% and 1.76 to 10.34%, respectively) and myrtenyl acetate (2.22 to 4.46% and 2.25 to 7.36%, respectively) are the other most represented oxygenates monoterpenes.

Oxygenated sesquiterpenes represent the second most abundant class of molecules (from 10.34 to 16.47% and from 11.99 to 22.23%, respectively). Among these, viridiflorol is the most abundant, representing from 4.23% to 5.75% and from 4.84 to 7.02, of EOs constituents, respectively, followed by guaiol (from 1.9 to 2.57% and from 2.31 to 3.23%, respectively). Fig. 2 clearly shows the content of the major compounds of the four populations of *L.stoechas* EO's obtained by HD and US-HD. The hydrocarbon sesquiterpenes come in third class with the abundance of cadina-1,4-diene (from 0.24 to 4.22% and from 0.38 to 2.89%, respectively). Hydrocarbon monoterpenes are very minor in all EOs analyzed (Table 1). It is important to note that GC / MS analysis of *L.stoechas* EO's showed that some compounds such as cis-linaloloxide and isoborneol were identified only in US-HD extracts, whereas other compounds such as tricyclene and limonene were identified only in the extracts obtained by HD (Table 1).

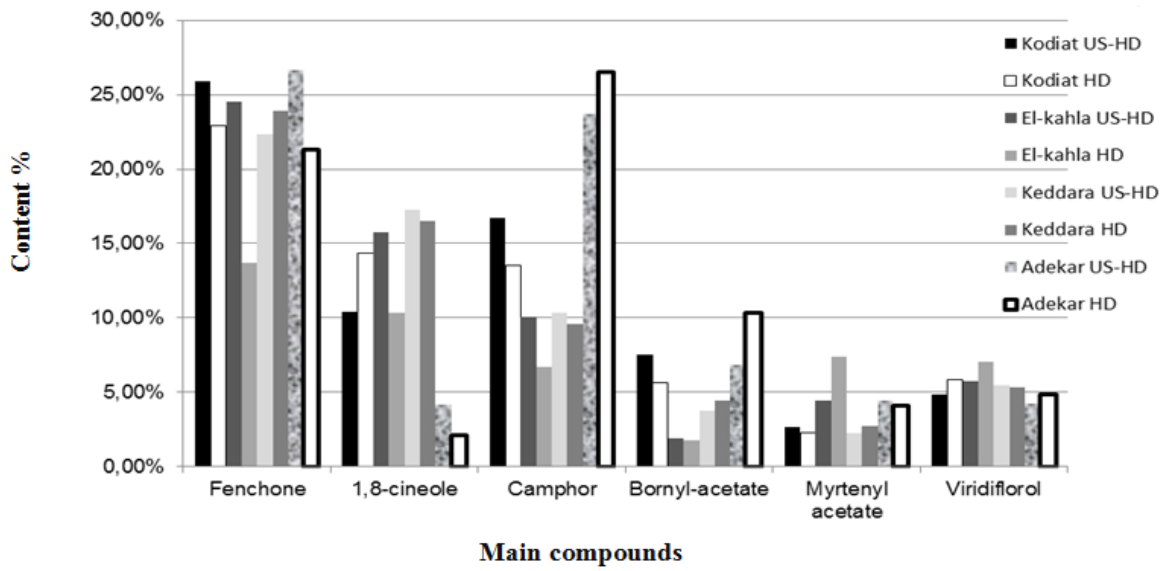


***Fig.2.*** *Main compounds of the 04 populations of L. stoechas essential oils obtained by hydrodistillation (HD) and by ultrasound pretreatment (US-HD).*

***Table 1.*** *GC / MS analysis of the 04 populations of L.stoechas essential oils obtained by hydrodistillation (HD) and by ultrasound pretreatment (US-HD).*

| N° | Compounds* | $IR_e$ | $IR_L$ | Composition (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | AEO | | KEO | | TEO | | EEO | |
| | | | | US-HD | HD | US-HD | HD | US-HD | HD | US-HD | HD |
| **1** | Tricyclene | 917 | 921 | - | 0,2 | - | - | - | 0,18 | - | - |
| **2** | *α*-Pinene | 927 | 932 | 0,18 | 0,17 | 0,16 | 0,23 | 0,47 | 0,36 | 0,15 | |
| **3** | Camphene | 944 | 946 | 1,37 | 2,18 | 1,09 | 1,39 | 2,07 | 1,95 | 0,59 | 0,32 |
| **4** | *ρ*-Cymene | 1017 | 1020 | 0,4 | 0,49 | - | - | 0,65 | - | - | - |
| **5** | Limonene | 1021 | 1024 | - | 0,25 | - | - | - | - | - | - |
| **6** | **1,8-cineole** | 1026 | 1026 | **4,14** | **2,11** | **17,24** | **16,48** | **10,39** | **14,33** | **15,77** | **10,36** |
| **7** | *γ*-Terpinene | 1049 | 1054 | - | - | 0,15 | 0,3 | - | - | - | - |
| **8** | *cis*-Linaloloxide | 1073 | 1067 | 0,63 | - | - | - | - | - | - | - |
| **9** | **Fenchone** | 1093 | 1083 | **26,66** | **21,31** | **22,3** | **23,9** | **25,88** | **22,89** | **24,54** | **13,66** |
| **10** | Linalool | 1101 | 1095 | 0,5 | 0,23 | - | - | - | 0,01 | | 0,32 |
| **11** | *α*-Fenchol | 1118 | 1114 | 1,41 | 0,95 | 1,39 | 1,36 | - | 1,29 | 1,85 | 1 |
| **12** | **Camphor** | 1146 | 1141 | **23,73** | **26,53** | **10,33** | **9,57** | **16,73** | **13,52** | **10,08** | **6,72** |
| **13** | Pinocarvone | 1156 | 1160 | - | - | - | - | - | 0,25 | 0,28 | - |
| **14** | Borneol | 1166 | 1165 | 2,03 | 1,42 | - | - | 0,22 | 1,78 | - | - |
| **15** | Isoborneol | 1167 | 1155 | - | - | - | - | 1,88 | - | - | - |
| **16** | Terpinen-4-ol | 1172 | 1174 | 0,61 | 0,3 | 0,67 | 0,66 | 0,42 | 0,38 | 0,64 | 0,54 |
| **18** | *p*- Methylacetophenol | 1176 | 1179 | 0,18 | 0,19 | 0,98 | 1,72 | 0,42 | 0,88 | 0,39 | 0,27 |
| **19** | *p*-cymene-8-ol | 1181 | 1179 | 0,9 | 0,65 | 0,93 | - | 0,98 | 0,97 | 0,87 | 0,61 |
| **20** | Myrtenal | 1186 | 1195 | 0,62 | 0,77 | 0,58 | 1,39 | 0,39 | 0,33 | - | 0,66 |
| **22** | Myrtenol | 1190 | 1194 | 1,22 | - | 1,02 | - | 0,7 | 0,57 | 3,67 | 1,66 |
| **23** | Verbenone | 1198 | 1204 | 0,86 | 0,49 | - | 0,8 | 0,69 | 1,14 | 0,96 | 0,53 |
| **24** | *α*-Fenchyl acetate | 1208 | 1218 | 0,75 | 1,42 | 0,72 | 0,66 | 1,45 | 1,14 | 0,69 | 0,58 |
| **25** | *trans*-Carveol | 1213 | 1215 | 0,42 | 0,2 | 0,28 | 0,29 | 0,32 | 0,32 | 0,37 | 0,36 |
| **26** | Carvone | 1239 | 1239 | 0,61 | 0,4 | 0,41 | 0,58 | 0,5 | 0,44 | 0,56 | 0,29 |
| **27** | **Bornyl acetate** | 1287 | 1287 | **6,84** | **10,34** | **3,77** | **3,43** | **7,52** | **5,62** | **1,89** | **1,76** |
| **28** | *p*-Cymen-7-ol | 1296 | 1289 | - | - | - | - | - | 0,16 | - | - |
| **29** | Thymol | 1306 | 1289 | - | - | - | - | - | 0,11 | - | - |
| **30** | **Myrtenyl acetate** | 1324 | 1324 | **4,46** | **4,09** | **2,22** | **2,7** | **2,65** | **2,25** | **4,4** | **7,36** |
| **31** | Eugenol | 1347 | 1356 | - | - | 0,22 | 0,15 | 0,11 | - | 0,31 | 0,2 |
| **32** | Cyclosativene | 1359 | 1369 | 0,51 | 0,61 | 0,71 | 0,67 | 0,63 | 0,61 | 0,62 | 0,51 |
| **33** | *α*-Copaene | 1365 | 1374 | 0,18 | 0,13 | 0,4 | 0,44 | 0,14 | 0,14 | 0,26 | 0,25 |
| **34** | Geranyl acetate | 1368 | 1379 | - | - | - | 0,17 | - | - | - | - |
| **35** | Sativene | 1379 | 1369 | - | - | 0,12 | 0,09 | - | 0,07 | - | - |
| **36** | *β*-Caryophyllene | 1403 | 1408 | - | - | 0,14 | 0,14 | 0,2 | 0,12 | 0,25 | 0,31 |
| **37** | Aromadendrene | 1447 | 1439 | - | - | 0,29 | 0,16 | - | - | - | - |
| **38** | *epi*-bicyclosesquiphellandrene | 1460 | | 0,24 | 0,13 | - | - | 0,11 | - | - | 0,43 |
| **39** | Butylated Hydroxytoluene | 1495 | 1514 | - | - | - | - | 0,23 | - | 0,43 | 0,47 |
| **40** | *γ*-Cadinene | 1512 | 1513 | 1,27 | 1,41 | 2,18 | 2,5 | 1,45 | 1,36 | 1,78 | 2,92 |
| **41** | Calamenene | 1515 | 1521 | 0,59 | 0,55 | 0,73 | 0,79 | 0,62 | 0,57 | 0,84 | 0,73 |
| **42** | **Cadina-1,4-diene** | 1524 | 1533 | **2,2** | **2,61** | **4,22** | **2,89** | **0,24** | **0,78** | **0,43** | **0,38** |
| **43** | *α*-Calacorene | 1534 | *1544* | 0,5 | 0,52 | 0,71 | 0,77 | 0,47 | - | 0,7 | 1,04 |
| **44** | *cis*-*α*-Copaene-8-ol | 1556 | | 0,52 | 0,45 | 0,68 | 0,64 | 0,35 | 0,46 | 0,99 | 2,27 |
| **45** | Palustrol | 1562 | 1567 | 0,32 | 0,3 | 0,45 | 0,46 | 0,34 | 0,42 | 0,47 | 0,53 |
| **46** | Caryophyllene oxide | 1575 | 1582 | 0,68 | 0,65 | 1,03 | 0,87 | 0,64 | 0,74 | 0,97 | 1,65 |
| **47** | Cis-β- Elemenone | 1582 | 1589 | - | - | - | - | - | 0,63 | 0,75 | 0,82 |
| **48** | **Viridiflorol** | 1592 | 1592 | **4,23** | **4,84** | **5,43** | **5,32** | **4,82** | **5,85** | **5,75** | **7,02** |
| **49** | Ledol | 1601 | 1602 | 1,01 | 1,12 | 1,41 | 1,33 | 1,11 | 1,42 | 1,39 | 1,79 |
| **50** | **Guaiol** | 1606 | 1600 | **1,9** | **2,31** | **2,57** | **2,55** | **2,26** | **2,78** | **2,52** | **3,23** |
| **51** | *1-epi-Cubenol* | 1623 | 1627 | 0,77 | 0,84 | 1,29 | 1,36 | 0,81 | 0,11 | 1,11 | 1,59 |
| **52** | *t*-Cadinol | 1635 | 1638 | 0,91 | 1,04 | 1,44 | 0,7 | 0,69 | 0,65 | 1,37 | 2,26 |
| **53** | epi-α-Muurolol | 1640 | 1640 | - | - | - | - | - | 0,34 | - | - |
| **54** | *α*-Cadinol | 1649 | 1652 | - | 0,44 | - | 0,75 | 0,7 | 0,73 | 0,72 | |
| **55** | Cadalene | 1666 | 1675 | - | - | - | 0,54 | 0,44 | 0,61 | - | 0,6 |
| **Total identified(%)** | | | | 94,35 | 92,64 | 88,26 | 88,75 | 90,69 | 89,26 | 89,36 | 76,00 |
| **Monoterpene Hydrocarbon** | | | | 1,95 | 3,29 | 1,4 | 1,92 | 3,19 | 2,49 | 0,74 | 0,32 |
| **Oxygen -containing Monoterpenes** | | | | 76,39 | 71,21 | 62,08 | 62,14 | 70,83 | 67,5 | 66,88 | 46,61 |
| **Sesquiterpenes Hydrocarbon** | | | | 5,49 | 5,96 | 9,5 | 8,45 | 3,86 | 3,65 | 4,88 | 6,57 |
| **Oxygen -containing Sesquiterpenes** | | | | 10,34 | 11,99 | 14,3 | 14,52 | 12,39 | 14,74 | 16,47 | 22,23 |
| **Others** | | | | 0,18 | 0,19 | 0,98 | 1,72 | 0,42 | 0,88 | 0,39 | 0,27 |
| **US treatment (min)** | | | | 10 | 0 | 45 | 0 | 60 | 0 | 10 | 0 |
| **Extraction time (min)** | | | | 90 | 180 | 90 | 180 | 90 | 180 | 90 | 180 |
| **Yield (%)** | | | | 1,59 | 1,17 | 0,87 | 0,62 | 1,03 | 0,5 | 1,6 | 0,64 |

*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), TEO (Kodiat Essential Oil), EEO (El-kahla Essential Oil), US-HD (Ultrasound assisted extraction prior Hydrodistillation), HD (Hydrodistillation).

We already know that soil composition, climate, age, vegetative cycle and extraction method can affect essential oil composition [28] and [29], but recent studies showed that generally US-HD process might enhance EO extraction yield or provide more valuable EO components than conventional HD. Indeed, by working on Turkish *L.stoechas*, Giray and *al*. [7] found that the yield of the EOs with a content of the major compounds fenchone and camphor was greater in the samples obtained by UAE by comparing with those obtained by HD. Jacotet-Navarro and *al*. [20] showed that quantity of ginger phenolics compounds extracted was improved by 29%, by comparing conventional maceration (CM) to ultrasound assisted extraction (UAE).

Additionally, the fraction of oxygenated monoterpenes is the most abundant fraction with the predominance of fenchone; these present data are in agreement with those reported by Benabdelkader and *al*. [30] in relation to major EO components from several wild populations of Algerian *Lavandula stoechas*. except that, the content of this common compound varies between the four samples studied, but above all, it varies between the two extraction methods. US-HD is shown to be more interesting for the extraction of this majority compound and the other main volatile compounds. The behavior of main volatile compounds could present different patterns in the same plant species according to the organ material [31]. The effect of ultrasonic treatment on chemical composition of EOs of the four samples studied could be connected with temperature properties and solubility of their various components [32]. 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 only 100°C [7]. In general, Ultrasonic pre-treatment increases the content of the major compounds and even that of other compounds [12].

### *Antimicrobial Activity*

The results of the in vitro study of the antimicrobial activity of AEO, KEO, TEO and EEO obtained by HD and US-HD at the best pretreatment time giving the best yield (10, 45, 10 and 60min, respectively) are summarized in the **Table 2**. In agreement with the great variability of their chemical compositions, the EOs obtained by the two methods of extraction of the 04 populations that we tested, were differentially effective towards the target microorganisms.

For bacteria, MIC values ranging from 1.12 mg / ml to values greater than 18 mg / ml were noted for the HD EO's, for the US-HD EO's, the MIC values vary between values below 0.14 mg / ml to values above 18 mg / ml. This same MIC result was obtained on the molds for the EOs of the two extraction methods. Concerning the yeast *Candida albicans*, the MIC of the HD EO's takes values lower than 0.14 mg / ml to a value greater than 18 mg / ml, whereas that of the US-HD EO's, it takes lower values Than 0.14 mg / ml to a value of 1.12 mg / ml. Based on these results, *L.stoechas* EO's of the 04 populations obtained by the two extraction methods have low to moderate antimicrobial potency, but generally, US-HD EO's have more effective antimicrobial activity on the bacteria and the yeast *Candida albicans* than HD EO's.

***Table 2.*** *Minimum inhibitory concentration (MIC) of the 04 populations of L. stoechas essential oils obtained by hydrodistillation (HD) and by ultrasonic pretreatment (US-HD).*

| microorganisms | | MIC (mg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | EEO HD | EEO US-HD | KEO HD | KEO US-HD | AEO HD | AEO US-HD | TEO HD | TEO US-HD |
| Gram + | ***Staphylococcus aureus*** | > 18 | < 0,14 | 18 | > 18 | > 18 | > 18 | > 18 | > 18 |
| | ***Bacilus subtilis*** | > 18 | 2,25 | 18 | 0,14 | > 18 | 18 | 1,12 | 9 |
| Gram- | ***Pseudomonas aerogenosa*** | > 18 | < 0,14 | 9 | 0,14 | 1,12 | 4,5 | > 18 | > 18 |
| | ***E. coli 25922*** | > 18 | > 18 | 18 | > 18 | > 18 | > 18 | > 18 | > 18 |
| Mold | ***Umbilopsis ramaniana*** | > 18 | 4,5 | 4,5 | > 18 | 1,12 | > 18 | > 18 | 4,5 |
| | ***Fusarium culmorum*** | > 18 | 18 | 18 | > 18 | > 18 | 4,5 | > 18 | > 18 |
| | ***Aspergilus carbonarius*** | > 18 | 0,14 | < 0,14 | < 0,14 | < 0,14 | < 0,14 | 1,12 | 1,12 |
| Yeast | ***Candida albicans*** | > 18 | 0,14 | 9 | 4,5 | 1,12 | 1,12 | 0,28 | 1,12 |

AEO (Adekar Essential Oil), KEO (Keddara Essential Oil), TEO (Kodiat Essential Oil), EEO (El-kahla Essential Oil), US-HD (Ultrasound assisted extraction prior Hydrodistillation), HD (Hydrodistillation).

Fig. 3. shows the cumulative MICs of each *L. stoechas* EO's against the 08 strains tested, the EEO obtained by US-HD has, in vitro, good inhibitory activity against microorganisms tested with a cumulative MIC of 43.31 mg / ml, whereas this same EO obtained by HD has, in vitro, the lowest antimicrobial activity with a cumulative MIC of 144 mg / ml. KEO, AEO and TEO obtained by HD and US-HD show practically the same antimicrobial potency with cumulative MICs of 94.64; 76.92 mg / ml, 75.5; 82.26 mg / ml and 92.52; 87.74 mg / ml, respectively. It is important to note that unlike AEO, KEO and TEO obtained by US-HD remain more efficient compared to those obtained by HD.

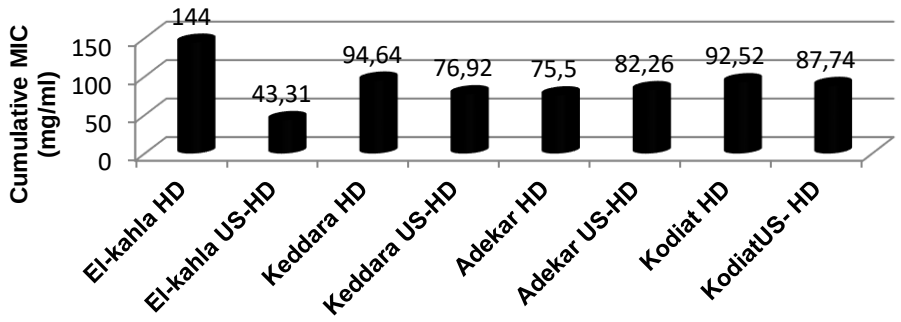


***Fig. 3.*** *Cumulative MIC of each Essential oil of the 04 populations of L. stoechas obtained by hydrodistillation (HD) and by ultrasound pretreatment (US-HD) against the 08 target strains.*

Fig. 4. shows the cumulative MIC of the wild populations of *L. stoechas* EO's obtained by HD and US-HD on each of the microorganism strains tested. The microorganisms studied did not show the same sensitivity against the EOs tested. All strains were found to be more sensitive to *L. stoechas* EO's obtained by US-HD than to EOs obtained by HD except for *U. ramanniana* which, unlike other strains, was found to be more sensitive to HD/EOs, as well for *E. coli* 25922 which exhibited the same sensitivity towards EOs of the two extraction methods. This bacteria is the most resistant strain to our EOs with a cumulative MIC of 72 mg / ml. In contrast, *A. carbonarius* was found to be the most sensitive strain to our HD and US-HD EO's with cumulative MICs of 19.4 and 1.54 mg / ml, respectively. *C. albicans* was also very sensitive to our EOs with cumulative MICs of 28.4 and 6.88 mg / ml, respectively. It should be noted that, on the one hand, *S.aureus* and *F. culmorum* showed almost the same tolerance against our EO's with cumulative MICs of 72 and 54.14 mg / ml and 72 and 58.5 mg / ml, respectively. On the other hand, *P. aerugenosa*, *B. subtilis* and *U. ramanniana* also showed the same tolerance against our EOs with cumulative MICs of 46.12 and 22.78 mg / ml, 55.12 and 29.39 mg / ml and 41.62 and 45 mg / ml, respectively.

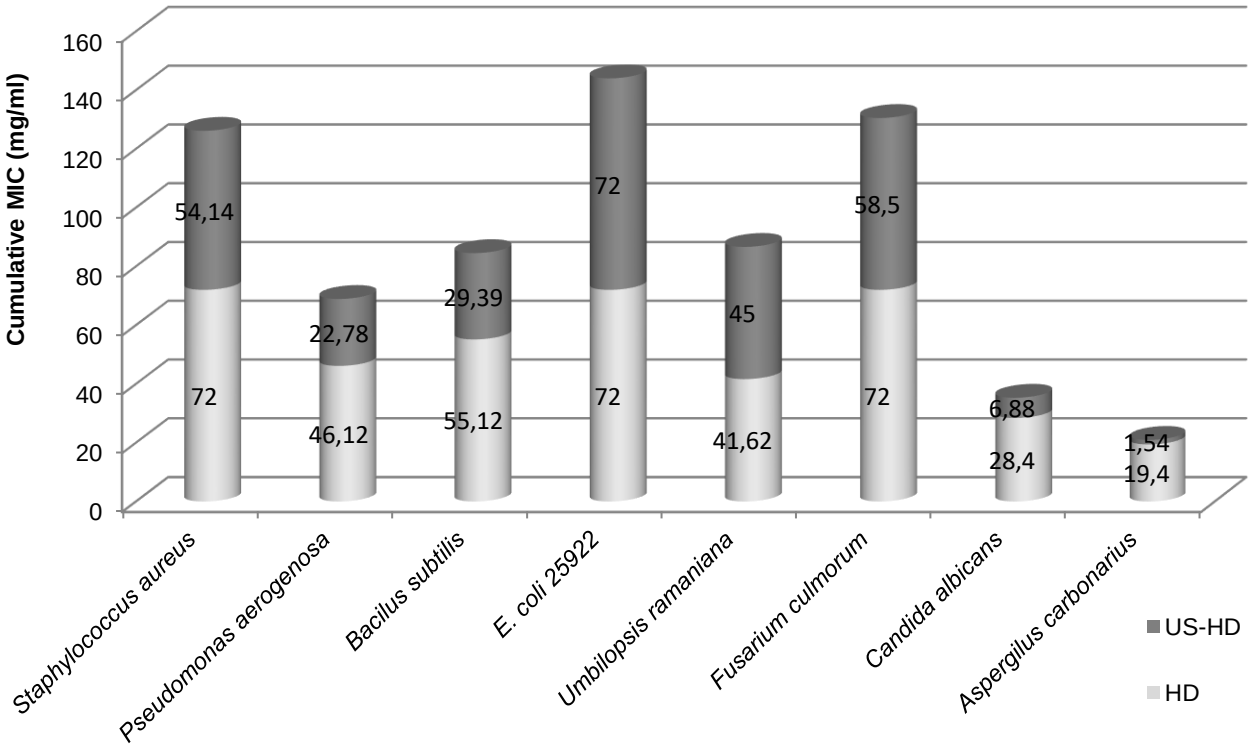


***Fig. 4.*** *Cumulative MIC of the 04 populations of L. stoechas essential oils obtained by hydrodistillation (HD) and by ultrasound pretreatment (US-HD) on each strain of target microorganism.*

Numerous studies have shown that the biological activities of EOs of aromatic plants are related to their chemical composition and in particular to the majority compounds. However, minority compounds can interact directly, or in a synergistic or antagonistic

manner, to create a mixture with biological activities. Recently, attempts have been made to identify the compounds responsible for the antimicrobial activity of EOs.

Oxygen molecules that are part of the composition of EOs are generally more active than hydrocarbon molecules that are, by contrast, known for their weak antimicrobial powers [33]. Indeed, all our extracts obtained by US-HD revealed a greater antimicrobial activity compared to that of extracts obtained by HD, except for AEO where the antimicrobial power of EOs obtained by HD was slightly higher than that of EOs obtained by US -HD (Fig. 3). These extracts (KEO, TEO, EEO and AEO) show higher levels of oxygen compounds (76.38, 83.22, 83.35 and 86.73%) compared to the extracts obtained by HD (76.66, 82.24, 68.84 and 83.2%) (Table1). Among these, camphor, linalool, 1,8-cineol [34], carvacrol, eugenol, perillaldehyde [35], terpinene 4-ol [36], caryophyllene oxide, spathulenol [37], borneol [38], viridiflorol, trans-pinocarveol, lédol [39] and myrtenal [40], which show powerful antiseptic activity.

The EEO obtained by US-HD, which had the highest antimicrobial potency, was characterized, like the other EOs, by the dominant presence of fenchone. Fenchone is, however, known to be little active [41]. It cannot, therefore, be responsible for the effects generated by EOs on microorganisms. The work of Joshi [42] indicated that the majority compounds may not necessarily be responsible for the antibacterial activity of EOs. However, several compounds known for their antimicrobial activities are present in our EOs as majority or minority constituents. For this purpose, we can suggest that the good antimicrobial activity of EOs obtained by US-HD may be due to the richness of camphor and 1,8-cineol compared to the EOs obtained by HD, except for AEO / HD where the good antimicrobial activity may be due to the richness of camphor and bornyl-acetate. Although the use of ultrasound has been widely analyzed until now, the study of the effect of ultrasound extraction on the antimicrobial power of EOs remains rare. It has been reported that EOs obtained by ultrasound-assisted extraction of *Zingiber officinale* Rose, of *Bosenbergia pandurata* Holtt and of *Curouma longa* Linn have greater antimicrobial activity toward *Salmonella Typhimurium* and low antimicrobial activity towards *Listeria monocytogenes* compared to EOs of the conventional methods [43]. The application of ultrasound has been successfully used for the microbial inactivation of high lipid food samples compared to conventional methods, with the advantage of applying mild temperatures that prevent the degradation of heat-sensitive compounds [44, 45]. On the other hand, it has been reported that the application of high intensity ultrasound for the extraction of varieties of biologically active compounds reduces the antimicrobial activity in a time and power dependent manner [46], but in general, ultrasound has proved to be a highly effective innovative technique applicable to many processes, such as emulsification, crystallization, homogenization, hydrolysis, extraction and microbial inactivation. This promising technique has many advantages over conventional techniques [47]. 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 [11].

## CONCLUSION

The objective of this work is to show the advantage of ultrasound treatment of plant material before hydrodistillation process (US-HD), in order to recover essential oils from four wild populations of Algerian *L. stoechas* L. Extraction of the EOs with (US-HD)

provides more valuable essential oils than the traditional method of hydrodistillation (HD). Ultrasonic pretreatment of 10 min followed by 90 min of HD of *L.stoechas* of the Kodiat population provided a quantity of EOs greater (1.6%) than that of EOs obtained by 180 min of HD (0.64%). This same pretreatment time could also provide a yield of 1.59% in the case of US-HD against a yield of 1.17% in the case of HD for the Adekar sample. 45 min and 60 min of ultrasound pretreatment could provide very high EOs (0.78 and 1.03%) yields compared to those obtained by HD (0.62 and 0.5%) for the Keddara and El-kahla samples, respectively. In general, these extracts obtained by US-HD are endowed with a more remarkable organoleptic quality.

The chemical analysis by GC / MS of EOs of the four populations studied showed a significant chemical variability. All EOs obtained by HD and US-HD were dominated by the fenchone except for the AEO obtained by HD where the fenchone took a second place after the camphor. The content of this major compound was higher in the extracts obtained by US-HD with the exception of EEO. These EOs are also characterized by the important presence of bornyl acetate, 1,8-cineol and viridiflorol with, in most cases, a higher content in the extracts obtained by US-HD. A large qualitative and quantitative variation of all the constituents in the different samples was observed, giving rise to a more interesting total content in the extracts obtained by US-HD with the exception of the KEO.

The antimicrobial activity of the extracts obtained by the two extraction methods was very variable in relation to the variation in chemical composition. All the EOs obtained by US-HD were more efficient against all microorganisms except for *Fusarium culmorum* which was shown to be more sensitive to EOs obtained by HD and for *E. coli* which showed an identical sensitivity towards the EOs of the two extraction techniques. Extraction with ultrasonic pretreatment of *L.stoechas* EO's provides richer extracts with bioactive molecules that has an antimicrobial effect and can therefore have greater application potential in the pharmaceutical, cosmetic, food and crop protection industries.

## REFERENCES

[1] Palma, M., 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.

[2] Luque-Garcia, J.L., 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. Journal of Chromatography A 1034: 237-242.

[3] Chen, F., Sun, Y., Zhao, G., Liao, X., Hu, X., Wu, J. 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. Ultrasonic Sonochemistry 14: 767-778.

[4] Chemat, F., Rombaut, N., Sicaire, A.G., Meullemiestre, A., Fabiano-Tixier, A.S., Abert-Vian, M. (2017b): Review of Green Food Processing techniques. Preservation, transformation, and extraction. Innovative Food Science and Emerging Technology 41: 357- 377.

[5] Luque-Garcia, J.L., Luque de Castro, M.D. (2003): Ultrasound: A powerful tool for leaching. Trends in Analytical Chemistry 22: 41-47.

[6] Upson, T., Andrews, S. (2004): The genus *Lavandula*. Portland and Oregon. USA. Timber Press 442p.

[7] Giray, E.S., Kirici, S., Kaya, D.A., Türk, M., Sönmez, O., 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.

[8] Khosravi, M., Mortazavi, S. A., Karimi, M., Sharayie, P., Armin, M. (2013): Comparison of ultrasound assisted and Kelavenger extraction methods on efficiency and antioxidant properties of Fennel's oil essence and its optimization by response surface methodology. International Journal of Agricultural and Crop Sciences 5 (21): 2521-2528.

[9] 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. Journal of Food Processing and Preservation 41(4): e13046.

[10] Assami, K., Pingret, D., Chemat, S., Meklati, B.Y., Chemat, F. (2012): Ultrasound induced intensification and selective extraction of essential oil from *Carum carvi* L. seeds. Chemical Engineering and Processing: Process Intensification Journal. 62: 99-105.

[11] Chekoual, L., Aissat, A., Ait-Kaci Aourahoun, K., Benabdelkader, T. (2018a): The Effect of Ultrasound Pre-treatment on the Yield, Chemical Composition and Antioxidant Activity of Essential Oil from Wild *Lavandula stoechas* L. Journal of essential oils and medicinal plants, 21(1): 253-263.

[12] Chekoual, L., Aissat, A., Ait-Kaci Aourahoun, K., Benabdelkader, T. (2018b): Comparative Study of Yield, Chemical Composition and Antioxidant Activity of Wild Algerian *Lavandula stoechas* L. Obtained by Ultrasound Pre-treatment and by Conventional Hydrodistillation. Phytothérapie 16(1): 109-118.

[13] Pharmacopée européenne 1. (1996): conseil de l'Europe. Maison neuve S. A. Editions, Sainte Ruffine.

[14] Van den Dool, H., Kratz, P.D. (1963): A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. Journal of Chromatography A 11:463-71.

[15] Adams, R.P. (2007): Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectroscopy, USA, Allured Publishing Co. Illiois, 804 p.

[16] Sökmen, A., Tepe, B., Daferera, D., Sökmen, M., Polissiou, M. (2004): The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control 15:627-634.

[17] Annegowd, H.V., Bhat, R., Min-Tze, L., Karim, A.A., Mansor, S.M. (2012): Influence of sonication treatments and extraction solvents on the phenolics and antioxidants in star fruits. International Journal of Food Science and Technology 49: 510-514.

[18] Kimparis, A.C., Siatis, N.G., Daferera, D., Tarantilis, P.A. (2006): Comparison of distillation and ultrasound-assisted extraction methods for the isolation of sensitive aroma compounds from garlic (*Allium sativum*). Ultrasonic Sonochemistry 13:54-60.

[19] Meullemiestre, C., Breil, M., Albert Vian, M., Chemat, F. (2016): Microwave, ultrasound, thermal treatments, and bead milling as intensification techniques for extraction of lipids from oleaginous *Yarrowia lipolytica* yeast for a biojetfuel application. Bioresource Technology 211: 190-199.

[20] Pingret, D., Fabiano-Tixier, A.S., Chemat, F. (2012): Lab and pilot-scale ultrasound-assisted water extraction of polyphenols from apple pomace. Journal of Food Engineering 111: 73-81.

[21] Jacotet-Navarro, M., Rombaut, N., Deslis, S., Fabiano-Tixier, A.S., Pierre, F.X., Bily, A., 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: 3106–15.

[22] Périno-Issartier, S., Ginies, C., Cravotto, G., Chemat, F. (2013): A Comparison of Essential Oils Obtained From Lavandin via Different Extraction Processes: Ultrasound, Microwave,

Turbo-hydrodistillation, Steam and Hydrodistillation. Journal of Chromatography A 1305:41-47.

[23] Arnold, N., Bellomaria, B. (1993): Observations morpho-anatomiques et histochimiques dans les *Salvia willeana* (Holmboe) Hedge et *Salvia fruticosa* Mill. de Chypre. Flora Mediterranea 3: 283-97.

[24] 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.

[25] Bosabalidis, A.M., 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 67:15-20.

[26] Petigny, L., Perino-Issartier, S., Wajsman, J., Chemat, F. (2013): Batch and continuous ultrasound assisted extraction of boldo leaves (*Peumus boldus* Mol.). International Journal of Molecular Sciences 14: 5750-5764.

[27] Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Périno-Issartier, S., Fabiano-Tixier, A.S., Vian, M. (2017a): Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. Ultrasonic Sonochemistry 34: 540-560.

[28] Hashemi, M.B., Niakousari, M., Saharkhiz, M.J., Eskandari, M.H. (2011a): Influence of *Zataria multiflora* Boiss. essential oil on oxidative stability of sunflower oil. European Journal of Lipid Science and Technology 113: 1520-1526.

[29] Hashemi, M.B., Niakousari, M., Saharkhiz, M.J., Eskandari, M.H. (2011b): Antioxidant activity of *Satureja bachtiarica* Bunge essential oil in rapeseed oil irradiated with UV rays. European Journal of Lipid Science and Technology 113: 1132-1137.

[30] Benabdelkader, T., Zitouni, A., Guitton, Y., Jullien, F., Maitre, D., Casabianca, H., Legendre, L., Kameli, A. (2011): Essential oils from wild populations of Algerian *Lavandula stoechas* L.: Composition, chemical variability, and in vitro biological properties. Chemical Biodiversity 8(5), 937-953.

[31] Ait-kaci Aourahoun, K., Fazouane, F., Benayache, S., Suici, F. (2016): Glandular hairs and essential oils of stem and leave from the promising medicinal legume *Cytisus triflorus* L'Hérit. using ultrasounds: comparison with conventional hydrodistillation. Indian Journal of Natural Sciences 35: 10911-10922.

[32] Mullakaev, M.S., Volkova, G.I., Gradov, O.M. (2015): Effect of ultrasound on the viscosity-temperature properties of crude oils of various compositions. Theoretical Foundations of Chemical Engineering 49(3): 287-296.

[33] Kalemba, D., Kunicka, A. (2003): Antibacterial and antifungal properties of essential oils. Current Medicinal Chemisry 10: 813-829.

[34] Pattnaik, S., Subramanyam, V. R., Bapaji, M., Kole, C.R. (1997): Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios 89: 39-46.

[35] Burt, S. (2004): Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology 94: 223-253.

[36] Carson, C. F., Mee, B. J., Riley, T.V. (2002): Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrobial Agents and Chemotherapy 46(6): 1914-1920.

[37] Ulubelen, A., Topcu, G., Eris, C., Sonmez, U., Kartal, M., Kurucu, S., Bozok- Johansson, C. (1994): Terpenoids from Salvia sclarea. Phytochemistry 36: 971-974.

[38] Tabanca, N., Kirimer, N., Demirci, B., Demirci, F., Husnu Can Baser, K. (2001): Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. phrygia and the enantiomeric distribution of borneol. Journal of Agricultural and Food Chemistry 49: 4300-4303.

[39] Guinoiseau, E. (2010): Molécules antibactériennes issues d'huiles essentielles: séparation, identification et mode d'action. Thèse de Doctorat de l'université de of Corse.

[40] Angion, A., Barra, A., Coroneo, V., Dessi, S., Cabras, B. (2006): Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and flowers. Journal of Agricultural and Food Chemistry 54(12): 4364-4370.

[41] Lis-Balshin, M., Roth, G. (2000): Composition of the essential oils of *Pelargonium odoratissimum*, *P. exstipulatum* and *P. x fragras* (*Geraniaceae*) and their bioactivity. Flavour Fragrance J. 15: 391-394.

[42] Joshi, R.K. (2010): In vitro antimicrobial and antioxidant activities of the essential oil of *Craniotome furcata*. Journal of Applied Natural Sciences 2(1): 57-62.

[43] Thongson, C., Davidson, P.M., Mahakarnchanakul, W., Weiss, J. (2004): Antimicrobial activity of ultrasound assisted solvent-extracted spices. Letters in Applied Microbiology 39: 401-406.

[44] Chemat, F., Huma, Z., Khan, M.K. (2011): Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrasonic Sonochemistry 18(4): 813-835.

[45] Piyasena, P., Mohareb, E., McKellar, R.C. (2003): Inactivation of microbes using ultrasound: a review. International Journal of Food Microbiology 87(3): 207-216.

[46] Soria, A. C., Villamiel, M. (2010): Effect of ultrasound on the technological properties and bioactivity of food: a review. Trends in Food Sciences and Technology 21: 323-331.

[47] Pingret, D., Fabiano-Tixier, A.S., Chemat, F. (2013): An improved ultrasound clevenger for extraction of essential oils. Food Analytical Methods 7: 9-12.