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Co-detection of eugenol and butylated hydroxyltoluene by green and selective hydrodistillation of *Heliotropium europaeum* L. using ionic liquids as additives

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Abstract: This study is the beginning of the research that focuses on unconventional ionic liquids (ILs) hydrodistillation (HD) extraction of the essential oil of *Heliotropium europaeum L.* using ILs as additives. Two ILs based on 1-butyl-3-methylimidazolium were used by switching the anions (Cl^- and PF_6^-). The effect of mass percent of the added ILs on its yield and composition was evaluated. Compared to the conventional HD, ILs-HD gives a higher yield of essential oils (0.10–0.36 %). Particularly, with [C_4mim][PF_6], the observation of morphological changes using scanning electron microscopy (SEM) confirmed the effectiveness of the ionic liquid in this distillation process. The GC–MS analysis of essential oils (EOs) revealed the presence of sixty-six compounds in HD, ILs-HD methods. Gas chromatography–mass spectrometry analysis of the EOs revealed the predominance of eugenol (1.70–72.35 %), butylated hydroxytoluene (8.95–65.39 %) and phytol (18.20 %). The new distillation methods of *H. europaeum* with ILs identifies more compounds (50 compounds in ILs-HD [C_4mim][PF_6]; 22 compounds in ILs-HD ([C_4mim][Cl]) than conventional hydrodistillation (25 compounds in HD). Therefore, the ILs-based hydrodistillation approach is superior in improving the production of EOs. It is important to emphasize that the data presented in this study are not yet available for any of this Algerian *Heliotropium* species of genus and pre-

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sent the great potential of this medicinal plant as a source of novel bioactive extracts with possible therapeutic uses.

Keywords: Boraginaceae; medicinal plant; essential oils; GC–MS; Algeria.

INTRODUCTION

In recent years, the world has been increasingly interested in using medicinal plants to prevent or treat many diseases, especially during these recent years when plants and essential oils are increased. In fact, essential oils (EOs) are characterized to have several medicinal and biological activities, particularly anticancer, antidiabetic, antimicrobial, anti-inflammatory and antioxidant, which are also used in food preservation.[1] The EOs are a mixture of volatile molecules that include classes of compounds with concentration variability and characterized by the preponderance of terpenes especially, mono and sesquiterpenes.[2,3]

Among the most used methods in the extraction of essential oils, hydrodistillation (HD) is the most widely used method. For the purposes of enhancing recovery and decreasing extraction time, the traditional hydrodistillation can be facilitated with chemicals,[4] physical[5] or biological techniques.[6] Mainly, ionic liquids (ILs) have recently received great attention and they were considered as green solvents due to their excellent properties such as low vapour tension, non-flammable, thermal stability, selective extraction ability, comprehensive dissolving capacity and excellent structural designability. ILs can be used to pretreat plant material before hydrodistillation[7] or as an additive during distillation.[8] As an ideal substitute for volatile organic solvents, the widespread use of ILs can bring even more significant price cuts for EOs extraction. In fact, ILs have been successfully recovered and reused in the hydrodistillation of Eos,[9] thereby reducing costs.

The genus *Heliotropium* belong to Boraginaceae family includes 250–300 species; the native range of *Heliotropium europaeum* is Macaronesia, Europe to Mediterranean region, Arabian Peninsula and India.[10] The heliotrope name derives from the fact that these plants turn their leaves to the sun.[11] Additionally, in Algeria, seven species belong this genus are *H. luteum* Poiret, *H. supinum* L., *H. europaeum* L., *H. strigosum* Willd., *H. curassavicum* L., *H. anchusifolium* Poiret and *H. bacciferum* Forsk.[12] Also, this plant is an herbaceous annual, grows primarily in the subtropical biome and in the crop fields; it is known in Algeria under the name “Daharet ech chems and Aquerbana”.[12] *Heliotropium* genus have been used in traditional medicine for the treatment of acne and cattle wounds,[13] rheumatism, putrefaction, pyoderma and ringworm infection,[14] snake bites and scorpion stings,[15] ulcers, venereal diseases, sore throat,[16] cough and fever.[17] Also, it has been used externally for healing wounds, and treatment of warts.[18] The genus *Heliotropium* revealed the presence of many biological activities such as antimicrobial, antiviral and antitumor,[19] anti-plasmodial and antitrypano-

somal,[20] anti-inflammatory,[21–23] anti-tuberculosis[24] and wound healing.[25] Some species of *Heliotropium* have been investigated for their phenolic compounds: kaempferol, silybin, caffeic acid, genistein and apigenin,[26] quinones, sterols, flavonoids and triterpenoids.[27,28] Phytochemical reports on *H. europaeum* are very limited and majority of these studies are focused on the presence of toxins in pyrrolizidine alkaloids.[29]

In this study, two ILs, 1-butyl-3-methylimidazolium chloride [C_4mim][Cl] and 1-butyl-3-methylimidazolium hexafluorophosphate [C_4mim][PF_6], were used as additives in hydrodistillation which was applied for the extraction of EOs from *H. europaeum*. In addition, the proposed ILs- hydrodistillation (HD) method was compared to the HD method on the yields and chemical composition of essential oils of *H. europaeum*. Besides, ILs have been efficiently recycled and reused in the experiments. As far as we know, the distillation methods using ILs as additives of *H. europaeum* essential oil has not been reported previously in Algeria.

EXPERIMENTAL

Plant material

The aerial parts of *H. europaeum* were collected from Blida district, Algeria in the August 2020. The plant was identified by Dr. W. Khitri and confirmed through the Flora of Algeria[12]. The voucher specimen [He/2248QS] was deposited at the herbarium of Pharmacy Department, Faculty of Medicine, Ahmed Ben Bella Oran1 University. The aerial parts were washed to remove soil particles and then dried at room temperature in the shade. In addition, the aerial parts were cut into small pieces and then stored in glassware until use.

Synthesis of ILs

Synthesis of 1-butyl-3-methylimidazolium chloride [C_4mim][Cl]. [C_4mim][Cl] was obtained by reacting 1-methylimidazole with 1-chlorobutane.[30] Typically, under an inert atmosphere, a mixture of 1-methylimidazole (9.9997 g, 0.1218 mol, 1 eq.) and 1-chlorobutane (16.9110 g, 0.1872 mol, 1.5 eq.) was introduced in a 100 mL round bottom flask and heated under reflux at 85 °C for 72 h. A viscous pale-yellow liquid was obtained and after cooling to ambient temperature the residue was washed once with ethyl acetate and three times with diethyl ether. Afterwards, the solvent was removed by evaporation under vacuum at 70 °C for 2 h to get [C_4mim][Cl] as a white solid (19.1098 g) obtained in 89.82 % yield, and stored in closed desiccators before use. The purity of the IL was checked by proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectrometry and the NMR spectrum characteristic showed no organic impurities in the IL.

Synthesis of 1-butyl-3-methylimidazolium hexafluorophosphate [C_4mim][PF_6]. [C_4mim][PF_6] was obtained by metathesis of the chloride ion of [C_4mim][Cl] using potassium hexafluorophosphate.[31] 20.0000 g (0.1 mol) of [C_4mim][Cl] salt and 18.4060 g (0.1 mol) of potassium hexafluorophosphate are solubilized in acetonitrile using a 100 mL round bottom flask at room temperature. The mixture was maintained under vigorous agitation for 24 h. Two phases formed and were separated, and then the solvent was removed by evaporation under vacuum, the organic IL phase was washed with water (3×25 mL) until the negative silver nitrate test. The [C_4mim][PF_6] IL was then dried for 6 h under vacuum at 75 °C to afford 12.7717 g of

C_4mim][PF_6] as a light-yellow viscous liquid, and then stored in closed desiccators before use. The purity of the IL was checked by proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectrometry and the NMR spectrum characteristic showed no organic impurities in the IL.

Spectral data of the synthesized compounds are given in Supplementary material to this paper.

Sample preparation

Hydrodistillation was carried out for three hours using water recycling Dean-stark type apparatus, according to the European Pharmacopoeia. The plant material (20 g) was chopped into small pieces and mixed with different ionic liquids solutions (200 mL) of [C_4mim][Cl] with respective concentrations of 0, 2.5, 5 and 10 %. In the case of [C_4mim][PF_6], only a concentration of 5 % was used. The mixture was put in a 500 mL round bottom flask, and then a Dean-stark type apparatus was connected. After that, the round bottom flask was placed onto a heating mantle for 3 h. Then, the essential oil was collected, dehydrated with anhydrous Na_2SO_4, and filtered. It was then weighed and stored in amber glass vials at 4 °C in the dark until chromatographic analysis. The hydrodistillation of the aerial part of *H. europaeum* gave yellowish oil.

The weight of the volatile oils of the *H. europaeum* was calculated by weight according to the following equation:

$$\text{Essential oil}\ (\%) = \text{Weight volatile essential oil extracted} \times 100 / \text{Wight of sample} \quad (1)$$

Essential oil analysis

The chemical composition of *H. europaeum* essential oil was analysed by gas chromategraphy coupled to mass spectrometry (GC–MS, Shimadzu QP2020 Instruments) equipped with a capillary column (phase: Crossbond® 5 % diphenyl/95 % dimethyl polysiloxane) dimensions of which are 30 m×0.25 mm and 0.25 μm film thickness, and with the following conditions: a volume of 0.5 μL solution, prepared by 10 vol. % of the sample dilution in *n*-hexane, was injected in split mode (80:1). Injector and detector temperatures were maintained at 250 and 310 °C, respectively, the column temperature was programmed at 60 °C fixed for 3 min then increased to 310 °C with the increment of 2 °C/min, and then maintained at 310 °C for 10 min. Helium (99.995 % purity) was used as a carrier gas, with a flow rate of 1 ml/min. The conditions of the mass spectrometer are as follows: ionization voltage 70 eV, ion source temperature 200 °C, and electron ionization mass spectra were acquired over the mass range of 45–600 *m*/*z*.

Identification of essential oil components

Identification established was based on the molecular structure, molecular mass and calculated fragments. Linear retention indices (*LRI*) were calculated for separate compounds relative to a homologous *n*-alkanes serial (*n*-C_8–C_{40}). The name, retention time, area and base *m*/*z* of the components of the test materials were ascertained. Components were identified by comparison of their calculated with those of literature,[32] as well as their mass spectra with those recorded by the NIST17[33] (National Institute of Standards and Technology) and ADAMS[34] libraries. In this study, all chemicals were obtained from Sigma–Aldrich Chemical Co.

Scanning electronic microscopy (SEM)

To study the impact of the ILs used as an additive by the hydrodistillation of *H. europaeum* essential oil a scanning electron microscope (SEM) was used to examine the morphological changes of plant cells. The dry samples of *H. europaeum* were scanned before and after treatment by IL using the Quattro SEM system (Thermosientific Company, USA). The tested samples were fixed on a specimen holder with aluminium tape. Then the tested samples were puttered with gold and examined under high vacuum conditions at an accelerating voltage of 12.5 kV (20 μm, 1000 magnification).

Recovery and recycling of ILs

To exhibit the ability to recover the ILs used in this work and reused it subsequently in another extraction of *H. europaeum* essential oil, the biomass obtained from the hydrodistillation was removed by filtration, and the resulting aqueous solution was extracted three times by 10 ml of dichloromethane. Furthermore, the anhydrous $CaCl_2$ was added and filtered. However, a dark ionic liquid was obtained after evaporation under reduced pressure. Though, the analysis of the HNMR of the two ionic liquids after and before use presents no substantial difference. So, the reused ionic liquid was stored in desiccators for further extraction.

RESULTS AND DISCUSSION

During the distillation process, the efficiency depends on the EOs yields and chemical composition. In addition, this work was carried out to inspect the extraction of EO from the *H. europaeum* plant using ionic liquids as additives during the hydrodistillation methods. In this study, two ILs, hydrophilic and hydrophobic, were used, respectively, [C_4mim][Cl] and [C_4mim][PF_6], and two effects were examined.

Effect of [C_4mim][Cl] concentration on extraction efficiency of essential oil

The IL concentration is crucial in extracting analytics from drugs.[35] To study the effect of ILs concentration, the various concentrations of aqueous ILs (2.5, 5, 10 %) were used for the essential oil extraction. The results are summarized in Fig. 1, which presents distillation methods.

The yield of essential oil using the different concentrations of ILs (0, 2.5, 5 and 10 %) are 0,052, 0.1, 0.22 and 0.36 %, respectively. The result demonstrates that the yield of EO obtained without using [C_4mim] [Cl] was the lowest and that EO yields increased gradually from 2.5 to 10 %. A high EOs yield (0.36 %) was obtained when the concentration was 10 % with IL-HD. This result is explained by the fact that the use of IL as an additive during hydrodistillation improved the cell disruption.[36] When the concentration is further increased the viscosity is also augmented, which diminishes the penetration of ILs solution into the interior of the sample matrix, preventing more constituent extractions.[37] Furthermore, the butylated hydroxytoluene (BHT) extraction is higher with the use of 5 % of IL (Table S-I of the Supplementary material). Also, the reduction of the amounts of ILs in order to save costs seems more sensible to us. So, 5 % of the IL is chosen as the relevant concentration.

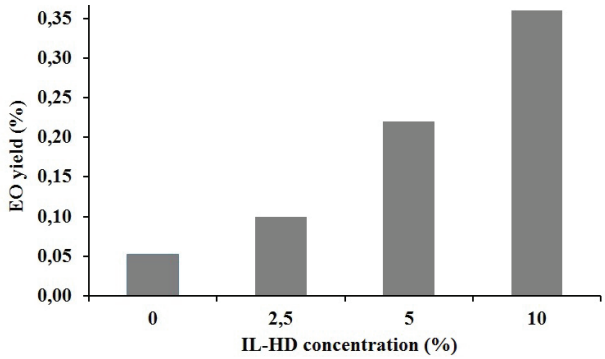

Fig. 1. Effect of ionic liquids concentration on yields of essential oils.

Effect of anion on extraction efficiency of essential oil

The choice of the IL is a crucial step for effective extraction. In fact, 1-butyl-3-methylimidazolium with PF_6^- was studied and compared to Cl^-. As an outcome, yield enhancement was observed with PF_6^-. It grows up to *ca*. 0.67 % with the same concentration. It seems that the ionic liquid [C_4mim][PF_6] can strongly interact with cellulose, which is the main component of the cell walls of the plant, causing the dissolution of cellulose, which leads to the release of more compounds.[38] The essential oil composition analysis obtained using PF_6^- leads to eugenol as the main product. It is acquired at *ca*. 72.35 %. In contrast, the use of Cl^- shows the butylated hydroxytoluene as the main component when all concentrations are used (Table S-I).

SEM observation

SEM was used to examine the morphological changes before and after extraction with different distillation methods.

As a result, when the ILs were used, the material morphology was significantly changed. In fact, the changes in morphological structure observed for the ILs-HD samples (c and d in Fig. 2) were markedly different from those examined by HD samples.

In the case of the ILs-HD sample, the external cells were severely damaged, there was a considerable destruction of cells, showing that most cell walls were crimped and broken, which explains the improvement in EOs yield and detection of more volatile compounds. However, it can be seen that the morphological structure of the HD (b in Fig. 2) sample became wrinkled, but there was no rupture of cell walls as observed in the ILs-HD methods.

Comparative analysis of different distillation procedures

The chemical composition of *H. europaeum* EOs extracted with or without using ILs showed significant differences and these results are contrary to those

observed by Chiappe.[8] In fact, not only the EO yield was affected by these different distillation methods and different concentrations, but also the relative peak area of each volatile compound, as shown in the typical gas chromatographic profile given in Figs. 3–6.

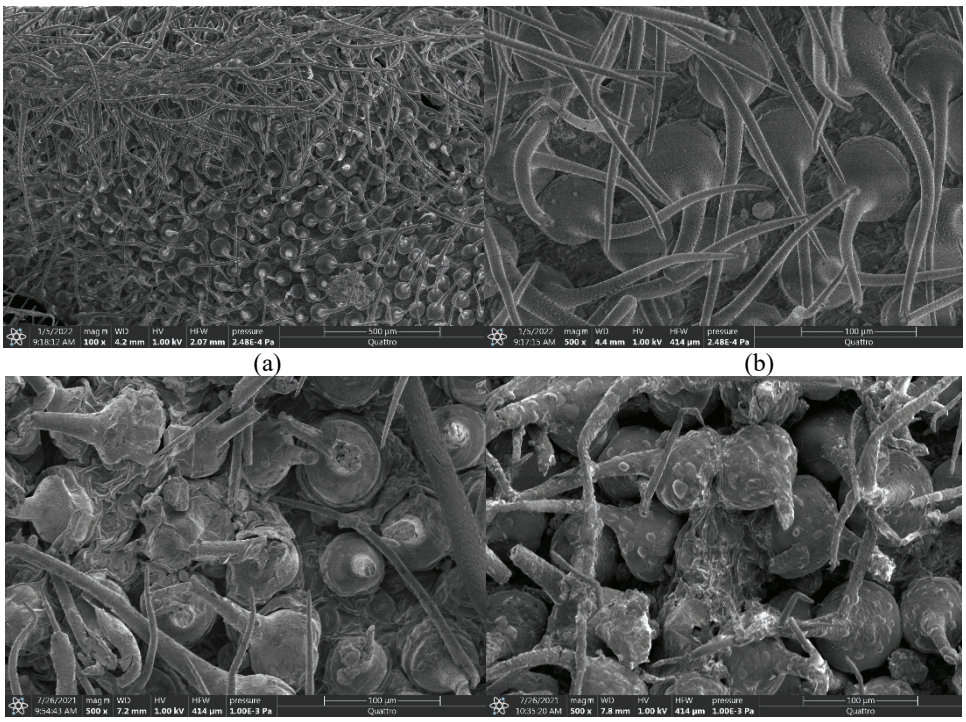

Fig. 2. Images of SEM of *H. europaeum* L: a) before extraction, b) treated by HD, c) treated by IL HD [C_4mim][Cl] and d) treated by IL HD [C_4mim][PF_6].

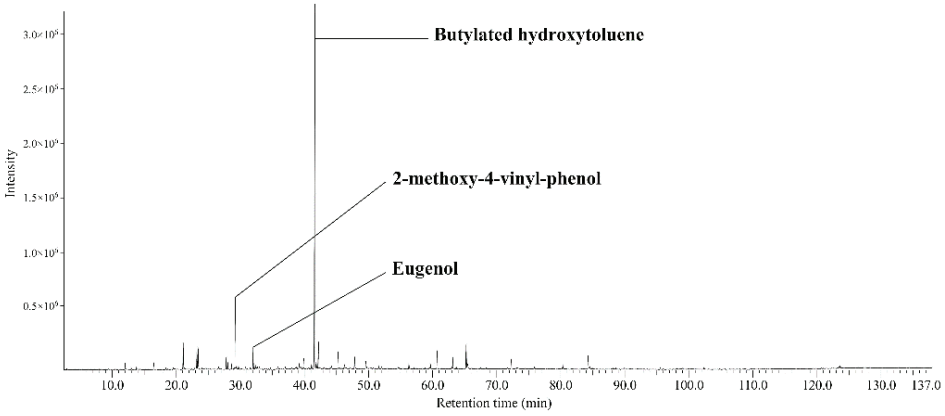

Fig. 3. TIC chromatogram of essential oils, HD method.

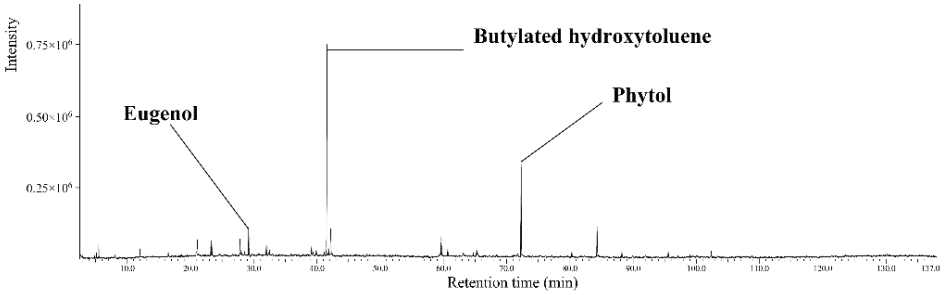

Fig. 4. TIC chromatogram of essential oils IL-HD [C_4mim][Cl] 2.5 % method.

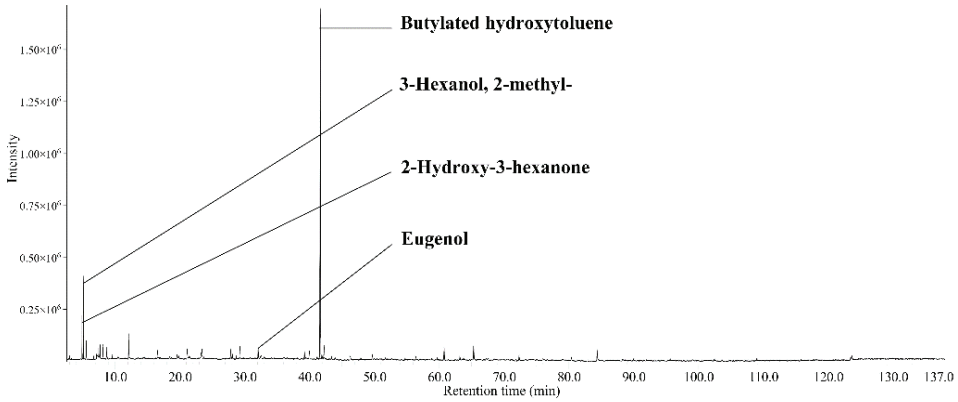

Fig. 5. TIC chromatogram of essential oils IL-HD [C_4mim][Cl] 5 % method.

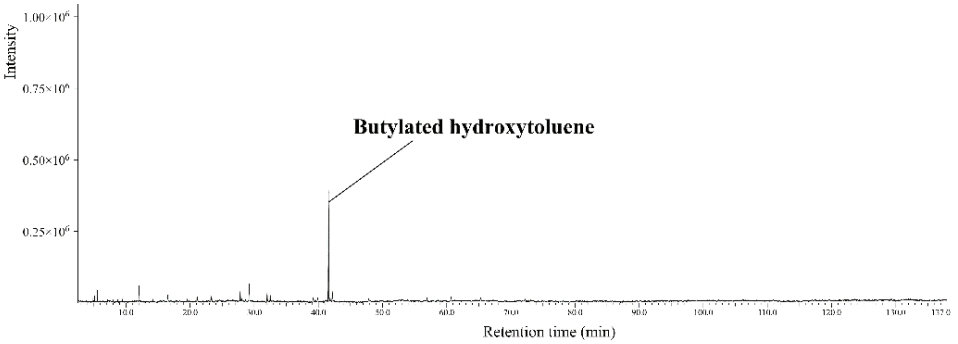

Fig. 6. TIC chromatogram of essential oils IL-HD [C_4mim][Cl] 10 % method.

The total number of volatile components detected was sixty-six compounds and they were identified in the present study of the EO of *H. europaeum* which includes both major and minor constituents. The main constituents in EOs obtained by different methods ILs-HD with [C_4mim][Cl] with different concen-

trations 2.5, 5 and 10 %, and HD method were: butylated hydroxytoluene with area percentages of 39.96, 65.39, 59.07 and 57.08 %, respectively. However, this compound was observed at only 8.95 % using [C_4mim][PF_6].

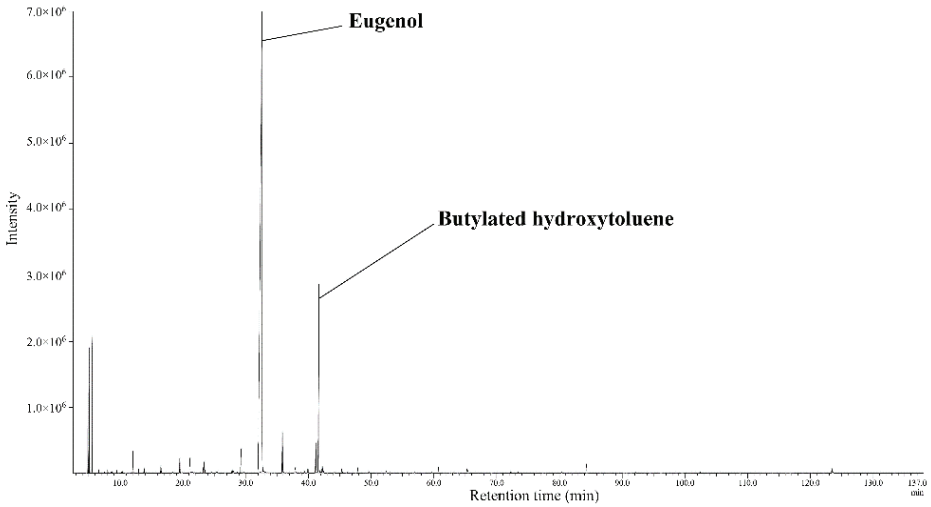

Fig. 7. TIC chromatogram of essential oils IL-HD [C_4mim][PF_6] method.

On the other hand, the use of [C_4mim][PF_6] gave eugenol as the main constituent with the area percentage of 72.35 %. On the contrary, this product was obtained with area percentages of 4.94, 1.65, 9.46 and 9.08 %, respectively, using [C_4mim][Cl] with different concentrations of 2.5, 5 and 10 %, and the HD methods. This selective extraction can be explained by the probability of dissolution of the eugenol in [C_4mim][PF_6] because eugenol is polar. It was observed in another study that the [C_4mim][PF_6] tended to interact more strongly with polar solutes.[39] Thus, [C_4mim][PF_6] can be able to aid fibre expansion and liberate the content in plant tissues so that more eugenol can be released.

There is no report in the literature on the composition of the EOs of *H. europaeum* with ILs. Only one publication was found, which showed the obtained EO through simple hydrodistillation without ILs.[18] Except for eugenol, phytol, *β*-ionone and hexacosane, the EO obtained by IL differed profoundly from the literature report.[18] Notably, compounds such as silphiperfol-6-en-5-one, geranyl acetone and *cis*-linoleic acid methyl ester were conspicuously absent in our oil samples.

Phytol was seen as the second most abundant constituent component in the essential oil obtained by IL-HD with [C_4mim][Cl] in 2.5 % with the area percentage of 18.20 %. Also, this component is present in the essential oil obtained by IL-HD with [C_4mim][PF_6], but with a small area ratio of 0.06 %, this compound is considered a good antimicrobial and anti-inflammatory agent.[39]

Additionally, higher concentrations of compounds have been observed in the EOs derived from IL-HD using [C_4mim][PF_6]. This outcome could be attributed to the physical and chemical characteristics of [C_4mim][PF_6], which are significantly influenced by water saturation and the presence of dissolved substances or ions, which naturally occur during liquid–liquid extraction involving ILs.[39] Moreover, these findings also highlight the significant role of the organic anion present in ILs, which might be attributed to the hydrogen bond interaction between ILs ions and the constituents of the cell wall (mainly composed of cellulose). This interaction serves as a key factor in inducing changes in plant tissue and facilitating the release of more volatile compounds.[40] Based on these results, it can be concluded that the composition of the major components in the EO of *H. europaeum* is dependant on the specific distillation process employed.

CONCLUSION

In this work, two ionic liquids were successfully used as additives to efficiently extract essential oils from *H. europaeum* L. by hydrodistillation. The unique properties of non-volatility enable these ionic liquids to be used as extractants in hydrodistillation. The anion's nature can significantly affect both the solubilization capacity and the selectivity of the ionic liquid. Among the studied ionic liquids, [C_4mim][PF_6] has a higher selectivity and extraction efficiency, indicating that ionic liquids can be used to recover volatile analytes. The observation of morphological changes using scanning electron microscopy confirmed the effectiveness of the ionic liquid in this distillation process. The new method brings about more identified compounds than the conventional hydrodistillation with the satisfactory essential oils yield and composition and the application of gas chromatography–mass spectrometry was effective in identifying sixty-six compounds from *H. europaeum.* The essential oil composition of *H. europaeum* growing naturally in Algeria revealed that these species are richer in volatile composition than genotypes from Iran.[11] This variation may be related to different environmental and climatic conditions. In addition, it is necessary to investigate in detail the reasons for the loss of some volatile components with ionic liquids in the future. These results shed light into the phytochemistry of this unexplored species of the Flora of Algeria. The second step will be to evaluate and valorise the biological activities of the essential oil of this plant.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12488, or from the corresponding author on request.

ИЗВОД

КОДЕСТИЛАЦИЈА ЕУГЕНОЛА И БУТИЛОВАНОГ ХИДРОКСИТОЛУЕНА ПРИМЕНОМ ЗЕЛЕНЕ СЕЛЕКТИВНЕ ДЕСТИЛАЦИЈЕ ЕТАРСКОГ УЉА ИЗ *Heliotropium europaeum* L. ВОДЕНОМ ПАРОМ УЗ ЈОНСКЕ ТЕЧНОСТИ КАО АДИТИВЕ

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У раду је описана дестилација воденом паром уз примену јонских течности као адитива (IL-HD) у циљу изоловања етарског уља *Heliotropium europaeum* L. Две јонске течности на бази 1-бутил-3-метилимидазола су коришћење уз измену анјона (Cl^- и PF_6^-). Испитан је утицај количине јонске течности на принос и састав уља. У поређењу са стандарном дестилацијом воденом паром (HD), IL-HD повећава принос етарског уља (0,10–0,36 %). Применом [C_4mim][PF_6], примећена је морфолошка промена, што је утврђено скенирајућом електронском микроскопијом (SEM). Тиме је потврђена ефективност јонских течности током дестилације. Анализа етарских уља гасном хроматографијом–масеном спектрометријом потврдила је присуство 66 једињења након HD и IL-HD. Доминантни су еугенол (1,70–72,35 %), бутиловани хидрокситолуен (8,95–65,39 %) и фитол (18,20 %). Новом методом дестилације са јонским течностима, у етарском уљу *H. europaeum* идентификовано је више једињења (50 након IL-HD [C_4mim][PF_6], 22 након IL-HD [C_4mim][Cl]) него стандардном дестилацијом воденом паром (25 једињења). Закључено је да се дестилацијом уз промену јонских течности повећавају приноси етарског уља. Резултати приказани у овом раду нису публиковани ни за једну врсту *Heliotropium* пореклом из Алжира, а литература указује на велики потенцијал ове медицинске биљке као извора нових биоактивних супстанци за терапеутске сврхе.

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