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Impact Of Electromagnetic Induction Heating On Antimicrobial Capacity Of Hydro-Distilled Leaves Of Pelargonium Graveolens

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Abstract

In this work, the usefulness of electromagnetic induction heating assisted hydrodistillation was investigated for Pelargonium graveolens leaves as a natural source of high value agents. In vitro antimicrobial activity of the extracts was evaluated against six bacterial strains and two fungal strains respectively using disc diffusion and Liquid Macrodilution methods. A comparative study on quantitative aspects over extraction time proves that conventional hydrodistillation (C-HD) gives an identical extraction kinetic behavior to electromagnetic induction heating assisted hydrodistillation (HD-EMI) with high extraction rates recorded when using HD-EMI. This latter increases the EOs yield for both fresh and dried leaves to (0.41 ± 0.04) % and (0.64 ± 0.03) % respectively versus (0.117± 0.04) % and (0.35± 0.02) % using C-HD. Compared to conventional heating, electromagnetic induction treatments result in 50 % lower extraction time and HD-EMI essential oils show an improved antibacterial activity. Against Escherichia coli and Staphylococcus aureus, HD-EMI and C-HD EOs show minimal inhibition concentrations (MICs) of 0.17 µg.ml^{-1} and 0.34 µg.ml^{-1}, respectively. Their MICs reach 0.34 µg.ml^{-1} and 0.68 µg.ml^{-1} against Klebsiella pneumoniae and Pseudomonas aerogenosa. The inhibition of Candidas albicans and Rhizopus arrhizus cannot occur at MIC values below 0.68 µg.ml^{-1}. Electromagnetic induction heating is thus a green and effective method for extracting high-value antibacterial compounds from Pelargonium graveolens leaves. Using the DPPH test, Pelargonium leaf EOs show antioxidant activity close to that of gallic acid, further demonstrating its potential as a promising source of preservatives in the food industry.

Keywords: antibacterial activity; antioxidant activity; essential oil; geranium; kinetic behavior.

Introduction

The use of essential oils, despite the age of its birth, has always been of keen interest that never ceases to occupy the headlines of the exponential development of plant biotechnology. Essential oils (EOs) are currently used on a scientific and rational basis to develop new products for various sectors: food, medical, veterinary and cosmetic. In fact, their extraction from plants would lead to explore new antimicrobial agents as safe alternatives of the conventional and synthetic ones that have become ineffective or produce side effects in the form of residual and mammalian toxicity when used for preservation of stored foods (Huttner et al. 2010; Lo et al. 2020; Samara et al. 2021).

Therefore, the need to continuously develop new and efficient methods for extracting essential oils has become paramount. Several methods are promising for essential oil extraction, offering advantages such as effective heating, fast energy transfer, time-saving, low operating costs and no adverse alterations in chemical compositions. For instance, electromagnetic induction heating can be recommended as a potential approach for plant extraction in a short period of time with a high yield while preserving the composition and physicochemical features of the Citrange pectin (Zouambia et al. 2017) and rhizome powder of R. palmatum (Epifano et al. 2018). Regarding the use of this last heating as simple and easy to handle method in hydrodistillation procedure, there is no study dealing with its effect on the yield and the antimicrobial activity of essential oils obtained from Pelargonium graveolens leaves, nor on the kinetic behavior of hydrodistillation.

Pelargonium. graveolens (family of Geraiaceae) belongs to the category of perennial plants with fragrant foliage. The genus Pelargonium has more than 280 species. A remarkably diversity in aromas of odorant species exist such as mint, rose, ginger, nutmeg, lemon and many other scents, underlying a richness of scented compounds produced in Pelargonium (Blerot et al. 2018; Lo et al. 2020). The main compounds of the essential oil of Pelargonium graveolens cultivated in Algeria are citronellol, geraniol and citronellyl formate (Attailia and Djahoudi 2015; Boukhatem et al. 2013; Chraibi et al. 2021). The Pelargonium's essential oil has anti-infective, anti-inflammatory, spasmolytic, hemostatic, curative, antioxidant properties and an antitumor potential on metastatic cancer cell lines (Boukhatem et al. 2022; El-Garawani et al., 2019; Fayoumi et al. 2022). Several studies validate that Pelargonium extracts can potentially be used as valuable anti-oxidant and antibacterial agents in the food. Due to its fungicidal activity against phytopathogenic fungi, it can be an alternative to the use of chemicals as postharvest fungicides of roses for the control of postharvest diseases (Stegmayer et al. 2022). Furthermore, in agricultural fields Pelargonium graveolens is suggested to be a valuable source of promising botanical pesticide.
In this study, the usefulness of electromagnetic induction heating as a simple and more cost-effective technology for the hydrodistillation of plants has been assessed for this potential source of food preservatives. As a standard approach, the essential oil of this plant was also extracted using conventional hydrodistillation. Furthermore, this study looked at hydrodistillation kinetics to highlight the variability in essential oil output, antimicrobial activity throughout two different growth stages of Pelargonium graveolens across extraction time.

Experimental

Plant material

Fresh leaves of Pelargonium graveolens were harvested during two growth stages: from the pre-flowering period to the early flowering period, in BENI ATTALI region located in Medea, a mountainous region in the north of Algeria. The leaves were washed and split into fresh and air-dried parts.

Essential oil extraction

Both of fresh and air-dried leaves were subjected to hydrodistillation during 3 hours. The hydrodistillation was undertaken under two different heating modes: the first one for classical hydrodistillation (C-HD) using a heating mantle (Faithful, China), while the second one was assisted by electromagnetic induction. Electromagnetic induction hydrodistillation (HD-EMI) was carried out on a magnetic induction glass-ceramic hotplate (Samsung, south Korea) having 9 different power levels where a stainless pot with lid was used as a container for plant material. In our study, a power level of 7 was chosen to achieve the same temperature used for conventional heating. In order to recover significant and measurable quantities, the EOs were separated

with diethyl ether. from distilled water obtained. The extracted oil was collected and kept in dark in capped glass vials at 4 – 5 °C prior to further use. The essential oil yield was calculated by the following formula:

$$\text{Yield in essential oil} = \frac{\text{mass of extracted essential oil}}{\text{mass of dried plant used}} \times 100 \quad (1)$$

In one of our concerns, the hydrodistillation kinetics were explored by taking fractions of essential oil at different hydrodistillation times.

Essential oil characterization

All essential oils obtained had been undergone an analytical study as different organoleptic characteristics (appearance, colour, smell), measurement of some basic physical and chemical parameters following standard procedures: relative density at 20°C (ISO:279, 1999), refractive index (ISO:280, 1999), ester index (ISO:709, 2001), acid index (ISO:1242, 1999), miscibility in ethanol (ISO:875, 1999) and pH measurement using electronic pH-meter (BOECO BT-675, Germany).

Antibacterial activity tests

The essential oil was tested against six clinical isolates of bacteria: three Gram-negative bacteria Escherichia coli (E.C), Klebsiella pneumonia (K.P), Pseudomonas aeruginosa (P.A), and three Gram-positive Bacteria Enterococcus faecalis (E.F), Staphylococcus aureus (S.A), Bacillus subtilis (B.S). All the strains were collected by swabbing from the environment of different departments of Mohamed Boudiaf hospital of Medea (Algeria), then transferred to the laboratory in nutrient broth (Fluka) for incubation at 37 °C during 24 h and 48 h for bacterial and fungi strains respectively.

The method of disc diffusion in an Agar medium was used. bacterial suspension was diluted and adjusted to turbidity equal to 0.5 McFarland standard turbidity corresponding to a size of inoculum of 1 x 10^{14} CFU.m^{-3}). The Mueller Hinton Agar was poured into Petri dishes. The Agar surface was seeded with the prepared bacterial suspension. Paper discs of 6 x 10^{-3} m in diameter; impregnated separately with three decreasing doses of Pelargonium graveolens leaves EOs; were deposited on the Agar surface to investigate the (dose-dependent) action of the EOs on the growth of germs. After incubation at 37 °C for 24 h, an area or clear halo is present around a disc if the essential oil inhibits bacterial growth. The larger the diameter of this zone, the more sensitive to the essential oil the strain is. The smaller the diameter, the more resistant the strain is. All tests were repeated three times.

Three Pelargonium graveolens leaves EOs concentrations diluted in ethanol: c_0, c_1 = 0.34 μg.ml^{-1}, c_2 = 0.17 μg.ml^{-1} were tested. c_0 corresponds to the solution containing crude essential oil.

Determination of Minimum inhibitory concentration against bacterial strains

The minimal inhibition concentration (MIC) was determined by the liquid macrodilution method. 0.2 x 10^{-6} m^3 of Pelargonium graveolens leaves EOs were placed in a sterile tube containing Mueller Hinton broth (Himedia) supplemented with 0.01 % Tween 80 (Merck). A cascade dilution was performed in Mueller Hinton Broth-Tween 80; so as to obtain a concentration range between 0.106 μg.ml^{-1} and 0.68 μg.ml^{-1}. A bacterial inoculum volume of 0.5 x 10^{-6} m^3 was deposited in each of the tubes of the range. A control of the bacterial growth was also carried out without EOs. The tubes were incubated at 37 °C for 24 hours after vortex agitation. The MIC corresponds to the lowest concentration of essential oil where microorganism growth was not observed. The control and all the tubes showing no visible growth with the naked eye after incubation were then centrifuged at 6000 RPM for 5 minutes to confirm the absence of bacterial precipitate.

Antifungal activity tests

To investigate the measurement of the antifungal activity of the studied essential oil, the disc method described in the previous section was used, but in this case the bacterial strains were replaced by the fungal strains. Sterile 9 mm in diameter discs impregnated with different dilutions of essential oils are placed in petri dishes containing PDA inoculated with the relevant fungal strains and incubated at 25 °C for 48 hours. The tested fungi included two clinical isolates: Candida albicans (C.A) and Rhizopus arrhizus (R.A).

Determination of Minimum inhibitory concentration against fungal strains

It consists of inoculating a range of decreasing EOs concentrations between 0.106 μg.ml^{-1} and 0.68 μg.ml^{-1}.with a fungal suspension. After incubation under a temperature of 25 °C for 48 hours, an observation of the

lowest concentration of extract able to inhibit the yeast suspension growth allows the determination of the minimum inhibitory concentration (MIC) against the studied fungal strains.

Determination of antioxidant activity

The antioxidant activity of the obtained Pelargonium graveolens leaves essential oils was determined using the [2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl] (DPPH) test (Rajurkar and Hande 2011). This test serves on the determination of the end-Product concentration of Antioxidant reaction (neutralization of DPPH). The reaction is accompanied by a change in the DPPH colour measured at 517 nm being as an antioxidant activity indicator. The antioxidant activity has been reported as the effective concentration required to reduce initial DPPH concentration by 50 %, EC50.

Results and discussion

Physico-chemical characterization of Eos

The obtained EOs was limpid liquid of greenish yellow color, with a pronounced scent of rose and lemon. The physico-chemical parameters of Pelargonium graveolens (P.G) leaves EOs are mentioned in Table 1. The measured relative density is lower than 1 and shows that essential oils are lighter than water. The high acid index indicates the high amount of free acids in this extracted oil. The high measured ester index, 65.33 ± 0.14 mg of KOH in g of essential oil, indicates the abundance of ester and glycerin in P.G leaves oil.

Table 1: Physico-chemical properties of Pelargonium.graveolens Eos

Acid Index, mg of KOH in g of essential oil.	10.02
Ester Index, mg of KOH in g of essential oil	65.33
Ethanol miscibility, $cm^3\ cm^{-3}$	3
Relative density at 20 °C	0.85
Refractive Index	1.468
PH	6

Our results indicate that P.G EOs yield (measured in $g.g^{-1}$ %) varies from (0.117 ± 0.04) to (0.64 ± 0.03)%. From previous works, dried leaves of Pelargonium graveolens produce 0.06 x 10^{-3} kg pale yellow color of Geranium oil in 0.15 % recovery (Lo et al. 2020). In other surveys, P.G oil's yield varies from 0.1 to 0.90 % and several parameters influence the EOs yield and quality like distillation time that can affect the EOs due to the isomerization and hydrolysis of esters to alcohols and acids (Rabesiaka et al. 2013; Rana et al. 2002).

Impact of heating method on EOs extraction

The impact of the heating method on the extracted essential oil amount was clearly visible as shown in Fig. 1 which represents the monitoring of the EOs yield obtained from fresh and dried Pelargonium graveolens leaves by using both of classical C-HD and the HD assisted by electromagnetic induction HD-EMI. Indeed, HD-EMI is significantly more efficient than conventional HD as it allows to increase the EOs yield for fresh and dried P.G up to (0.41± 0.04) % and (0.64 ± 0.03)% respectively, compared to (0.117 ± 0.04)% and (0.35 ± 0.02)% when using conventional HD. Electromagnetic induction heating allows homogeneous and fast heating, thanks to its principle of producing heat within the material to be heated. It is believed; through the earlier studies on this method; to have an influence on the progress of hydrodistillation and consequently on the yield of essential oil and the duration of the extraction (Youcef-Ettoumi et al. 2020). Some studies tried to increase the yield and/or enhance the EOs quality by using different extraction methods like supercritical CO_2 (Peterson et al. 2006), microwave extraction or by introducing cellulolytic enzyme mixtures containing glucosidases (Blerot et al. 2018; Kahriman et al. 2010).

Our results show that for both methods, air drying leads to a significant increase in the amounts of EOs compared with fresh leaves due to the fact that drying removes the water present in the leaves and thus allows the release of the EOs. This increase can reach 3 times in the case of C-HD while it can't exceed 2 times when HD-EMI was used. In agreement with our findings, a previous study indicated that the air drying of P.G leaves caused an improvement in the recovery of the oil components and the best drying period for the distillation of Geranium plants is 48 hours of drying after harvesting (Abouelatta et al. 2021). They found that the air drying of Geranium plants had also a significant effect on the chemical composition of the different components of the Geranium oil obtained by using steam distillation and hydrodistillation.

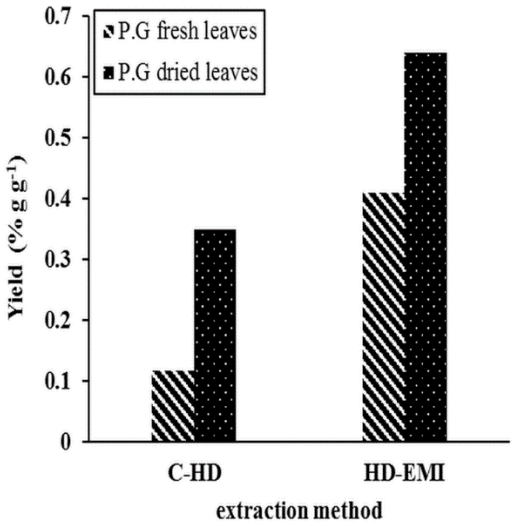

Fig. 1. Effect of heating method on the yield of Pelargonium graveolens leaves EOs.

Kinetic study of extraction

Fig. 2 depicts the kinetic study regarding the effect of hydro-distillation time on the accumulative amount of C-HD P.G essential oils for fresh and air dried leaves and HD-EMI essential oils for P.G air dried leaves. It is clear that both of heating methods recorded similar behavior with the same extraction steps. These behaviors had been also reported in the case of orange peels (Rana.et al. 2002).

As presented in the same figure, all the curves experience a very closer slopes in the first 20 minutes representing the period of the surface oil release which is an easily accessible fraction. In that stage, conventional HD allows to achieve a yield of (0.063 ± 0.004) % and (0.160 ± 0.012) % for fresh and air dried leaves respectively. While HD-EMI leads to reach (0.149 ± 0.043) % for air dried P.G leaves which is close to that obtained in the case of C-HD. It appears that the hydro-distillation release of the first EOs which constitute a highly volatile fraction; renting the leaf surface and readily available; cannot be affected by either the heating method or the presence of internal water in the leaves. In the second period, hydrodistillation is very affected by both of the plant material freshness and the kind of heating method: from 20 to 90 minutes of conventionnal hydrodistillation the yield increases sharply with 2.6 times when switching from fresh to air dried leaves while it increases with 64 % when moving from C-HD to HD-EMI. For this period, the essential oil bearing parenchymatous cells of the leaves surface had already been opened and essential oil diffused easily from the inner part to the leaves surface leading to a rapid oil removal by the boiling water and water vapor. The difference in extraction rates between fresh and dried leaves starts after 50 minutes reflecting the time needed to access the release of the EOs fraction that was held back by the presence of internal water in the fresh leaves. As expected in the case of HD-EMI kinetic study and compared to C-HD, the HD-EMI rate superiority starts earlier up to 20 minutes. This phenomenon can be explained by the fact that electromagnetic induction heating leads to a high, fast and homogenous heating, making extraction more efficient from the deeper localizations of EOs.

Up to 90 minutes of hydro-distillation process, as extraction time went by, the accumulated oil yields do not increase significantly. The depletion of essential oils and their release from deeper localizations in the P.G leaves may be likely the cause of this phenomenon. Beyond 120 minutes, the oil yields start to be nearly constant especially for conventional HD.

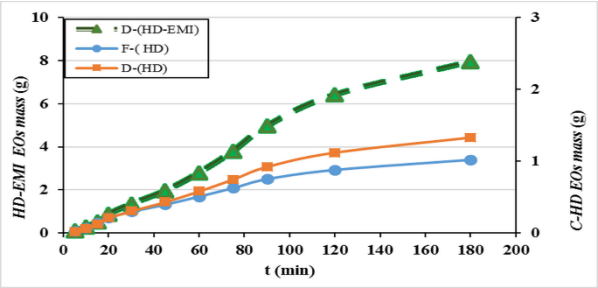

Fig. 2. Cumulative amounts profiles of Pelargonium graveolens leaves essential oils as a function of time.

Antimicrobial activity

From the examination of the Fig. 3 and Fig. 4, inhibition zone diameters vary between 10 mm to 14 mm for the plant material harvested before flowering stage reflecting slight inhibitory effect. While they range between (11± 1) mm to (23± 1) mm for the beginning flowering stage reflecting therefore the improvement of the antibacterial powerfull from slight to strong. It can be concluded that the antimicrobial potent vary with the variation of the plant freshness, the plant phenological stage and the concentration of EOs. The crude EOs of dried P.G leaves of the beginning flowering stage could be richer in agents responsible of the inhibition of these strains than those obtained from fresh and dried P.G leaves harvested before flowering stage. It has been widely demonstrated through a multitude of previous works that oils' activity would be expected to relate the plant's chemical quality of essential oils especially the major compounds. This composition is affected by the drying and the harvesting period (Abouelatta et al. 2021; Verma et al. 2013).

Our results show antibacterial activities of the EOs isolated by C-HD against at least one of the bacterial strains tested, with the exception of two Gram-positive bacterial strains: Entercoccus fecalis (E.F) and Bacillus subtilis (B.S). In opposite of our results, Entercoccus fecalis cannot resist Pelargonium graveolens essential oil and Bacillus subtilis shows sensitivity to this EO (Hsouna et al. 2012; Said et al. 2011). This discrepancy may be attributed to the concentration of the extracts used, the plant maturity and the abiotic parameters surrounding the plant. In our work, the most important antibacterial potent are recorded against Gram-positive and Gram-negative bacterial strains in the following decreasing order: Staphylococcus aureus (S.A), Escherichia coli (E.C), Pseudomonas aerogenosa (P.A), Klebsiella pneumoniae (K.P). This result reveals the gram-independence of the sensitivity to EOs of the strains and in the other hand the antibacterial effect unveiled is strain- dependent. Likewise, our findings are consistent with the statement made by previous works (Al-Mijalli et al. 2022; Ben ElHadj et al. 2020). In another way of statement, other studies looked into the oil's preference of Gram-positive bacteria over Gram-negative bacteria. They indicated that the Gram-positive bacteria is preferred because they lacked the hydrophilic polysaccharide chain that acts as a barrier for Gram-negative bacteria, this difference is what allows Gram-negative bacteria to be less susceptible to the Pelargonium graveolens oil (Hsouna et al. 2012).

The results reported by other authors on the Algerian P.G leaves of identical harvesting period examined in this work showed that this oil can exhibit antibacterial activity against Gram-positive and Gram-negative bacteria (Boukhatem et al. 2013). Bactericidal power is more important against the strains Staphylococcus aureus, Streptococcus agalactiae and Enterococcus faecalis (Rabesiaka et al. 2013). Our findings are also in line with the results previously found that highlighted the highest inhibitory effect of this essential oil against Staphylococcus. Aureus (Al-Mijalli et al. 2022). However, this bacterial strain had been found to be resistant to Pelargonium graveolens essential oil (Said et al. 2011).

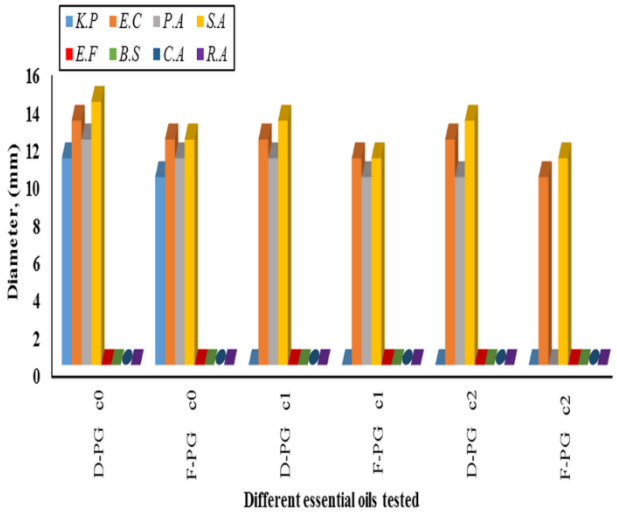

Fig. 3. Inhibition zone Diameters of essential oils of Pelargonium graveolens leaves harvested before flowering stage against different microbial strains, D-PG: dried Pelargonium graveolens leaves EO; F-PG: fresh Pelargonium graveolens leaves EO leaves; c0: crude essential oils; c1: 0.34 µg.ml^{-1}; c2:0.17 µg.ml^{-1}.

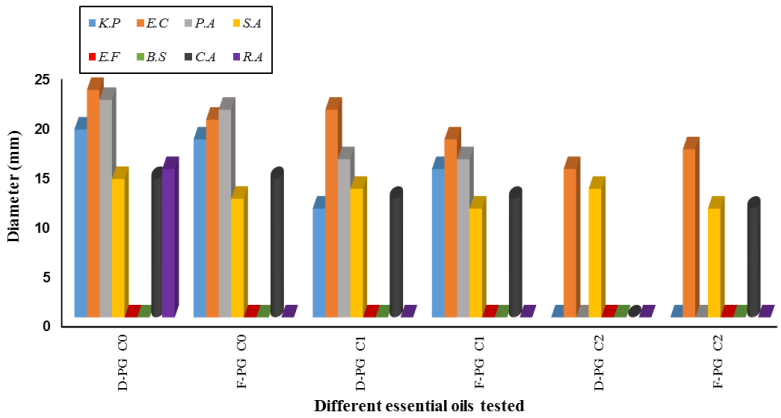

Fig. 4. Inhibition zone Diameters of essential oils of Pelargonium graveolens leaves harvested at early flowering stage against different microbial strains: D-PG: dried Pelargonium graveolens leaves EO; F-PG: fresh Pelargonium graveolens leaves EO leaves; c0: crude essential oils; c1: 0.34 $\mu g.ml^{-1}$; c2:0.17 $\mu g.ml^{-1}$.

From the above experimental data, it appears that the bacterial inhibition of P.G essential oil is more significant at increased EOs concentrations in the case of Staphylococcus aureus, Escherichia coli, Pseudomonas aerogenosa, Klebsiella pneumoniae. The highest inhibition zone diameters are exhibited by pure EOs, while the diameters decrease with decreasing EOs' concentration. In a previous work, crude essential oil of Pelargonium.graveolens showed a significant antibacterial activity against Escherichia coli and Staphylococcus Aureus but at lower concentrations, this oil became inactive (. Androutsopoulou et al. 2021).

Minimum inhibitory concentration (MIC)

In addition to the antibacterial activity, dried Pelargonium graveolens leaves essential oils of the beginning flowering stage has proven to be slightly antifungal against two tested pathogenic fungi: Candidas albicans, Rhizopus oryzae. The resistance of Candidas albicans to P.G EOs was also revealed in other previous work (Kabera et al. 2013). Furthermore, regarding the MIC values of dried P.G leaves extracted by the conventional HD and the HD-EMI methods against four bacterial and two fungal strains (Table 2), it is clear that the susceptibility of Klebsiella pneumoniae, Escherichia coli, Pseudomonas aerogenosa, Staphylococcus aureus, can be improved when the essential oils are obtained using HD-EMI. The observed MICs vary with respect to extraction method and the type of bacteria. In particular, Escherichia coli and Staphylococcus aureus show high sensitivity with identical MIC values of around 0.34 and 0.17 $\mu g.ml^{-1}$ respectively to C-HD and HD-EMI essential oils. While for Klebsiella pneumoniae and Pseudomonas aerogenosa, higher MIC values (indicating moderate sensitivity) are found 0.68 $\mu g.ml^{-1}$ and 0.34 $\mu g.ml^{-1}$ for C-HD and HD-EMI essential oils respectively. In contrast, the inhibitory effect of these EOs against the fungal strains: Candidas albicans and Rhizopus oryzae cannot occur at MIC below 0.68 $\mu g.ml^{-1}$ because they remain inactive in the range of the used concentration (0.106 – 0.68 $\mu g.ml^{-1}$).

Table 2: MIC values of dried Pelargonium.graveolens leaves essential oils against microbial strains.

	Minimum inhibitory concentrations MIC, $\mu g.ml^{-1}$	
	HD	**HD-IM**
Bacterial strain		
Gram negative :		
Klebsiella pneumoniae	0.68	0.34
Escherichia coli	0.34	0.17
Pseudomonas aerogenosa	0.68	0.34
Gram positive :		
Staphylococcus aureus	0.34	0.17
Fungal strain		
Candidas albicans	> 0.68	> 0.68
Rhizopus aryzae	> 0.68	> 0.68

Previous results showed that the essential oil of Pelargonium.graveolens has great antimicrobial activity against a panel of microorganisms. The maxima inhibition zone diameters and MIC values for bacterial strains,

which are sensitive to the P.G EOs, had been found in the range of 13 – 26 mm and 0.312–10 $g.L^{-1}$, respectively (Hsouna et al. 2012). At early flowering stage, a MIC (in $m^3.m^{-3}$ %) of 1 % and 0.25 % was observed for Escherichia coli and Staphylococcus aureus respectively (Al-Mijalli et al. 2022). The improvement of HD-EMI proven in this study is consistent with the work conducted on Citrus.sinensis essential oil in which the enhancement of the antibacterial effectiveness exhibited by HD-EMI EOs compared to conventional HD EOs is also proven (Youcef-Ettoumi et al. 2020). In recent work which investigated the P.G essential oils using the EP-SFME method (enzymatic pretreatment combined with a solvent-free microwave extraction method), higher antimicrobial activity is shown against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, and Candida albicans, than that from the conventional hydrodistillation (Wei et al. 2022).

Effect of distillation time on the antimicrobial potent

In the aim of investigating the effect of distillation time on the antimicrobial potent, P.G dried leaves EOs obtained at various time frames were tested for their antimicrobial activity against four bacterial strains and two fungal strains. The most active EOs fraction is that obtained in the time interval 45 to 60 minutes giving a moderate inhibition level of bacterial and fungal growth with inhibition zone diameters of 18 mm, 18 mm, 13 mm for Escherichia coli, Staphylococcus aureus and Candidas albicans respectively (Fig. 5). Rhizopus oryzae is slightly susceptible to P.G EOs obtained at C-HD time range of 60 to 75 minutes. However, no antibacterial activity is observed against Klebsiella pneumoniae and Pseudomonas aerogenosa for the whole of time frames studied along 90 minutes of C-HD.

Our results indicate that P.G dried leaves EOs using C-HD are found to be inactive for C-HD time interval [75 - 90] minutes against Escherichia coli and Candidas albicans respectively, and for C-HD time interval [60- 90] minutes against Staphylococcus aureus.

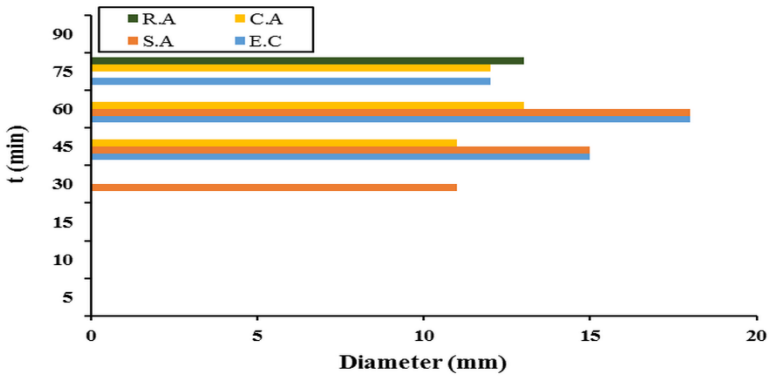

Fig. 5. Effect of Hydrodistillation time on inhibition zone diameters of essential oils extracted from P.G leaves of early flowering stage against microbial strains

Antioxidant activity

The antioxidant activity screening of C-HD essential oils of P.G dried leaves at early flowering stage was performed using 2,2-diphenyl-1-picrylhydrazy (DPPH). It reveals EC_{50} value of 6.4± 0.15 x 10^{-9} $g.L^{-1}$ close to that of gallic acid as positive control for which the EC_{50} is of 6.5± 0.12 x 10^{-9} $g.L^{-1}$. Our results; which testify the important antioxidant activity of P.G oil; are in agreement with those obtained in earlier works for Pelargonium Asperum oil originating from Comoros (Said et al. 2011). They gave an EC_{50} value of 6.675 x 10^{-9} $g.L^{-1}$. Other previous surveys found that DPPH values of ethanol extracts can reach EC_{50} = 91.84 ± 0.1 x 10^{-9} $g.L^{-1}$. Their results confirm the richness of extracts in phenolic compounds, which may strongly be correlated with DPPH values (Harzellah et al. 2022).

Conclusion

Among the objectives outlined, the main aim of the present study was to evaluate whether the use of electromagnetic induction assisted heating could exert an improvement in the production of Pelargonium graveolens essential oils with an additive antimicrobial activity. More importantly, the combination HD-EMI showed improvements in quantity and antibacterial potency of EOs extracted with reducing time of hydrodistillation. Moreover, this is the first study investigating biological activity and kinetic study of Pelargonium graveolens essential oils extraction using HD-EMI. Another important issue that deserves to be

highlighted is that quantitative differences also appear in EO's yield and in their biological potency as differences in plant freshness, phonological stage and distillation time occur. The exact timing of this influence was revealed by the kinetic studies, the essential oil fractions most likely to be affected by varying heating methods and plant freshness are those extracted after 20 and 50 minutes respectively. Pelargonium graveolens essential oil inhibitory effect on microbial growth is limited for defined time frames of hydrodistillation depending on strain. Our quantitative DPPH assay results confirm that P.G EOs exerted a potentiated antioxidant activity. These results could serve to promote the diversification of essential oils to provide a multitude of alternative antimicrobial and antioxidant agents for further use in agrochemicals food processing and other medicinal fields. Further research is required to isolate, characterize and identify bioactive constituents responsible for the observed activity depending time of hydrodistillation.

Conflit of interest

The authors declare that they have no conflit of interest.

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