Assessing properties of *Acantholippia deserticola* (phil.) moldenke essential oil: Comparison between hydrodistillation and microwave-assisted hydrodistillation extraction methods

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Abstract

Acantholippia deserticola (Rica-Rica) is a native shrub of the Chilean highlands used as a medicinal plant and food dressing. The objective of this study was to compare the physical, antioxidant and antimicrobial characteristics of its essential oil (EO), based on the process parameters and extraction methods using hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD), and assessing presence of fatty acids. The process performance as well as the colour parameters, refractive index, fatty acid profile, antioxidant activity and antimicrobial activity were evaluated. The best process conditions for HD were 90 min, 420 W and 150 g 1000 mL^{-1}; for MAHD, these were 90 min, 700 W and 200 g 1000 mL^{-1}, with yields of 0.45 and 0.49 mL 100 g^{-1} and antioxidant capacities of 2.38 and 3.92 μmol Trolox g^{-1} respectively. The collection season, extraction method and its parameters affected the yield and fatty acid profile, influencing EOs' activity. In terms of mass of herbs, process time as well as energy efficiency and environmental impact, the extraction of MAHD was 8% more efficient. EOs extracted by both methods show a slight inhibitory effect on *Streptococcus* sp. and *Bacillus cereus*, and MAHD EO showed a highly inhibitory effect on *Streptococcus* Group A. The type of extraction method and the process parameters could be set to obtain suitable EOs according to its potential industrial application.

Keywords: antimicrobial; antioxidant; fatty acids; Rica-Rica; yield

Introduction

Essential oils (EOs) are complex mixtures of organic substances, which are liquid at room temperature and contain volatile compounds. They are generally soluble in organic solvents, low soluble in water and can be obtained from the roots, stems, leaves and flowers of plants (El Asbahani *et al*., 2015). The properties of EOs, which depend on their composition, include antibacterial (Deng *et al*., 2016), antifungal (Falasca *et al*., 2016), anti-inflammatory (Rodrigues *et al*., 2016) and antioxidant (Sodeifian and Sajadian, 2017) effects. These properties allow EOs to be used in different fields (El Asbahani *et al*., 2015) such as the food industry (Atarés and Chiralt, 2016), in sanitising products (Falcó *et al*., 2018) or as pesticides (Benites *et al*., 2014; Zarria *et al*., 2010). EOs are products of agricultural industry and have great prospects for the future development. Traditional methods used for the extraction of EOs from plants include steam distillation (Périno-Issartier *et al*., 2013), hydrodistillation (HD) (Jiao *et al*., 2012; Li *et al*., 2012;

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Stanojević *et al*., 2015) and solvent extraction (Soran *et al*., 2009). New extraction methods favour green technologies, with a focus on reducing environmental impacts while improving energy efficiency (Chemat *et al*., 2012; Farhat *et al*., 2017). These methods include microwave-assisted hydrodistillation (MAHD) (Ajayi *et al*., 2016; Fardhyanti *et al*., 2019; Golmakani and Moayyedi, 2015; Jeyaratnam *et al*., 2016), ultrasound extraction (Hashemi *et al*., 2018), solvent-free extraction (Kusuma *et al*., 2018), supercritical fluid extraction (Shahsavarpour *et al*., 2017; Sodeifian and Sajadian, 2017) and ohmic-assisted HD (Gavahian *et al*., 2015), among others. The extraction methods of EO differ in their process parameters such as the size of particles (Shahsavarpour *et al*., 2017), the use of solvents (Okoh *et al*., 2010), the sample–solvent mass ratio and the time and power of the process (Kusuma *et al*., 2018). Several authors (Gavahian *et al*., 2015; Torres-León *et al*., 2017; Megawati *et al*., 2019) have reported that the MAHD method, which takes advantage of microwaves as a heat source in the extraction process, shows greater efficiency in comparison to HD. Taban *et al*. (2018) reported that the use of microwave energy to heat solvents produces a greater rupture of cells. Nazarni *et al*. (2018) agreed with this finding and noted that this breakage would facilitate the penetration of solvent into plant material, allowing the release of intracellular products, thereby accelerating mass transfer. However, fatty acids can be carried away during HD and present in EOs. This has been poorly studied (Rezaei *et al*., 2018; Zaïri *et al*., 2019), although fatty acids have interesting nutritional and bioactive properties (Koç *et al*., 2019; Ohlrogge *et al*., 2018).

Acantholippia deserticola (Rica-Rica [RR]) is an aromatic herb native to the Chilean highlands and used in the form of a decoction or infusion because of its medicinal properties and as a food dressing. Indeed, a number of *Acantholippia* species have been used in popular medicines for analgesic and anti-inflammatory effects (Díaz-Véliz and Mora, 2012; Larrazabal *et al*., 2018). Rica-Rica is registered as a medicinal herb by the Chilean Ministry of Health (Parada, 2012), and its hydroalcoholic extracts have shown antioxidant (Morales *et al*., 2008) and anti-inflammatory (Carro *et al*., 2015) effects. Its phytochemical compounds include flavonoids, tannins, saponins and triterpenes/steroids that could be potentially toxic to cellular systems at concentrations of 50–100 µg/mL (Carro *et al*., 2015). Rojo *et al*. (2009) and Sampietro *et al*. (2016) agree that the main compound of Rica-Rica EO (RR-EO), obtained by the HD method, is β-thujone, and the two studies found its contents to be 77.9% and 66.5%, respectively. However, the studies differed in the second highest compound, which was found to be α-thujone (10.5%) and trans-sabinyl acetate (12.1%), respectively. These compositional differences could be attributed to geographic, climatic, seasonal or other environmental factors, and these differences can affect the biological activity of RR-EO (Falasca *et al*., 2016; Gasparetto *et al*., 2017). Benites *et al*. (2013) extracted EOs by the HD method and reported sedative, anxiolytic and antidepressant effects. Rojo *et al*. (2006) did not find any antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* or *Candida albicans*. It was previously demonstrated that RR-EO extracted by MADH has antioxidant activity, showed strong inhibition against food-associated pathogenic bacteria and contained 13 compounds, being β-thujone (45%) the main compound (Larrazabal *et al*., 2019). However, it is necessary to characterise whether different methods of extraction of EOs change their physical and biological properties.

The objective of this study was to evaluate the effect of the extraction process parameters (time, power and herb:water ratio) on the yield and energy efficiency of the process, physical characteristics (colour parameters and refractive index [RI]), presence of fatty acids and biological activity (antioxidant and antimicrobial) of EOs when extracted by HD and MAHD methods.

Materials and Methods

Sample collection

Acantholippia deserticola (Rica-Rica) leaves and stems were collected from the Toconao locality within the San Pedro de Atacama community (22.9087°S, 68.1997°W) of the Antofagasta region, Chile. Samples were collected in two stages, September (stage I) and January (stage II); these months, which correspond to the austral spring and summer respectively, were selected to evaluate the impact of season on EO composition. Moisture (g water 100 g^{-1}), fat content (g 100 g^{-1}) and the leaf:stem ratio were determined for stage I and II samples. The herbs were authenticated and registered by Dr. Roberto Rodríguez, Department of Botany, School of Natural Sciences and Oceanography, University of Concepción (Universidad de Concepción), Chile, under specimen number CONC 182473.

EO extraction

Stage I

The stage I samples were used to evaluate the effect of the process parameters of each extraction method (HD and MAHD) on the yield and EO characteristics. For HD extraction, the samples were distilled using a Clevenger apparatus equipped with a heating blanket. For MAHD extraction, a microwave (Model MS-1948 JL, LG) was used following the methodology described by Golmakani and Moayyedi (2015) and Ferhat *et al*. (2006). The methodology consists of introducing a flask, with the

sample and water as appropriate for each treatment, into a microwave oven modified for this purpose, as seen in Figure 1. In both cases, a 2^3 factorial experimental design was used, where the effects of time, 90–120 min (HD) and 45–90 min (MAHD); heating potency, 240–420 W (HD) and 420–700 W (MAHD); and herb:water ratio, (120–200 g 1000 mL^{-1}), were evaluated. To project the future industrial applications of these extraction processes, energy density (ED) was determined for each of the treatments evaluated. Depending on the mass of herb and water (g) and the power used (W), the ED varied between 0.200 and 0.365 W g^{-1} for HD and between 0.350 and 0.609 W g^{-1} for MAHD.

The extracted EOs were separated from the aqueous layer using sodium sulphate, filtered, and recovered with ethyl ether before finally heated at 40°C for 1 h in an oven (BOV-V-125F, Biobase). For both extraction methods, the process yield (mL 100 g^{-1} wd) and some characteristics of EO, such as the colour parameters (L^*, a^* and b^*), RI, antioxidant capacity (AC) and fatty acid profile (GC), were evaluated using dried samples (30°C for 48 h). The results were expressed on a dry basis. For each extraction method (HD and MAHD), the process parameters that showed the greatest yield and AC were selected.

Stage II

The stage II samples were used to: (i) evaluate the effect of harvesting season on extraction performance and EO characteristics, and (ii) preliminarily evaluate the EO antibacterial activity and determine the energy efficiency of the extraction process of HD and MAHD methods, according to the parameters selected in stage I. The same analyses that were used in stage I were carried out; in addition, antimicrobial activities against 32 pathogenic bacteria were also determined.

Analytical methods

Physical parameters

The RI was measured according to the Food Chemical Codex (FCC, 1996) at 20°C. The colour parameters, L^*, a^* and b^*, were measured using a Colorflex Tristimulus Color Plot (Model 11491, Hunter Lab).

Presence of Fatty acid

Presence of fatty acid was determined using a Shimadzu GC-2010 gas chromatographer with the Supelco 37 Component FAME Mix as the standard and an RTX-225 column (30 m × 0.32 mm I.D. × 0.2 μm; 90% biscyanopropyl/10% phenylcyanopropyl polysiloxane). An initial oven temperature of 120°C was maintained for 2 min before increasing by 4°C every 2 min until reaching 230°C. The nitrogen carrier gas flow was 19.9 cm s^{-1}, while sample injection was 1 μL (splitless analysis). The methodology described by Folch *et al*. (1957) was used for fatty acid methylation.

Antioxidant capacity

The AC was determined via the ferric ion reducing antioxidant power assay (FRAP) using acetone as the solvent and Trolox as the standard. The calibration curve standards (between 10 and 80 μM) and the samples were measured at 593 nm at 4 min after the start of the reaction.

Antimicrobial activity

The antimicrobial activity was assessed using the paper-disc agar-plate method. The following 21 Gram-negative bacteria were assessed: *Enterobacter aerogenes*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Providencia alcaligenes*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Aeromonas hydrophila*, *Aeromonas veronii*, *Yersinia enterocolitica*, *Serratia rubidaea*, *Salmonella typhi*, *Salmonella paratyphi* B, *Shigella flexneri*, *Klebsiella pneumoniae*, *Escherichia coli*, *Vibrio cholerae*, *Proteus vulgaris*, *Proteus mirabilis*, *Alcaligenes* sp. and *Vibrio alginolyticus*. The following 10 Gram-positive bacteria were assessed: *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Corynebacterium* sp., *Bacillus cereus*, *Enterococcus* sp., *Staphylococcus aureus*, *Enterococcus* sp., *Streptococcus* Group A, *Micrococcus* sp., *Streptococcus* sp., and the yeast fungus *Candida* sp. Strains were obtained from the collection of Departamento de Tecnología Médica of Universidad de Antofagasta, Chile, and clinical isolates were kept in a cepary at –20°C in medium with 20% glycerol and in a cepary with Brain-Heart Agar medium at room temperature. Müller-Hinton agar medium (20 mL; Liofilchem) was poured onto petri dishes, and the cultured bacteria (100 μL) were plated at an approximate final concentration of 10^6 bacteria mL^{-1}. After 15 min, a Whatman No. 1 paper disc (6 mm; Whatman International) impregnated with the EO (10 μL) was placed on the agar surface. The plates were incubated at 37°C for 24 h and the inhibition diameter was measured. All analyses were performed in duplicate.

Statistical analysis

Analysis of variance was applied using Statgraphics Centurion software to compare the characteristics of the oils obtained according to the collection time, the process parameters (time, temperature, power and herb:water ratio) and the extraction method.

Results and Discussion

Raw material

While both sample groups presented a stem:leaf mass ratio close to 70:30 (Table 1), the stage II specimens

(January, austral summer) had significantly higher humidity and fat contents than those from stage I (September, austral spring). Notably, between January and March, the north Chilean highlands undergo a climatological phenomenon known as the 'highland winter', which is characterised by heavy rains over the semiarid soil. Several authors have evidenced a strong relationship between the climatological conditions that are present at the time of collection and the yield of the extracted EO. Sá *et al*. (2016) and Gasparetto *et al*. (2017) reported higher EO yields from plants harvested in the months that had the highest rainfall, that is, between December and March. Furthermore, Gasparetto *et al*. (2017), together with Falasca *et al*. (2016), reported important seasonal effects on the composition of oils and their biological properties. In this work, the highest fat content was obtained from the plants collected in stage II, coinciding with the summer raining period, having EO yields that were approximately three times higher than those collated in stage I.

Stage I: Evaluation of HD and MAHD extraction conditions

HD method

The physical and biological EO properties extracted with the HD method are shown in Figure 1. In terms of the colour parameters, L^* values (Figure 2A) were markedly low (close to zero), meaning that the EOs were very dark, and the a^* and b^* coordinates (Figure 2B) were near to the axes of the CIELAB colour sphere. This implies very low colour saturation, close to achromaticity. Treatments with a lower power (240 W) produced a more greenish tone ($a^* < 0$), while the EOs obtained with a greater power level had redder tones ($a^* > 0$). The a^* value was significantly affected in a directly proportional way by the time and power factors ($P < 0.05$). The other colour parameters, L^* and b^*, and the RI, were not significantly affected by any of the factors (time, power and herb:water ratio) or their interactions ($P > 0.05$). The RI (Figure 2C) was between 1.451 and 1.453, values within the characteristic intervals reported for other herbal EOs (Gavahian *et al*., 2015; Torrenegra *et al*., 2015; Ud-Daula *et al*., 2016). The highest RI values were obtained when a larger amount of sample mass was used.

Extraction with HD, using a factorial design of 2^3, presented a yield ranging between 0.32 and 0.46 mL 100 g^{-1} (Figure 2D). The yield was significantly higher ($P < 0.05$) in the treatments with a greater power (red figures) and a lower herb mass (empty figures). The latter finding was consistent with that reported by Stanojević *et al*. (2015), who demonstrated that by increasing the amount of water with respect to the mass, or in this case at a lower herb:water ratio, the resistance to the transfer of mass was reduced and the contact of sample with water was improved, promoting extraction. Regarding the extraction time, in most of the cases the yields were increased in the treatments with longer extraction period ($P > 0.05$, square figures), which was probably related to a higher degree of warming that favoured the transfer of mass (Li *et al*., 2012). The same phenomenon can explain the effect of power on yield. On the other hand, at lower power (blue figures), the process time increased the EO's AC, probably because of greater extraction of the compounds responsible for this activity. However, at high power levels (red figures), the opposite effect occurred. In such cases, the thermal degradation of those compounds could have occurred. In particular, an intense and/or prolonged thermal treatment may be responsible for a significant loss of natural antioxidants because most of these compounds are relatively unstable (Tomaino

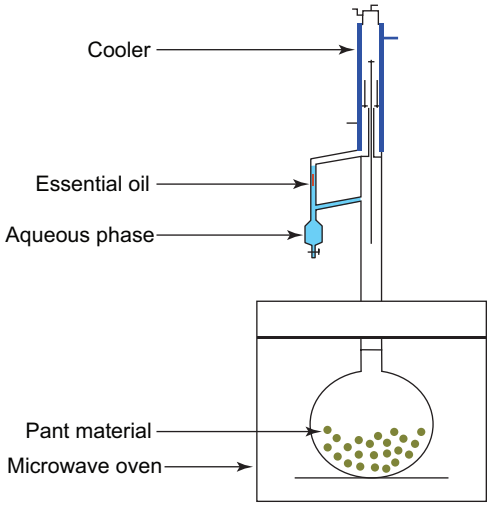

Figure 1. Microwave-assisted hydrodistillation (MAHD) method (Ferhat *et al*. 2006).

Table 1. Herb samples' characterisation.

Stage	Period	Stem* (%)	Leaf* (%)	Humidity (%)	Fat (%)
I	September (Spring)	70 ± 2	30 ± 2	11.7 ± 0.1	0.37 ± 0.28
II	January (Summer)	71 ± 2	29 ± 2	17.1 ± 0.1	1.14 ± 0.10

*g total weight (100 g^{-1}).

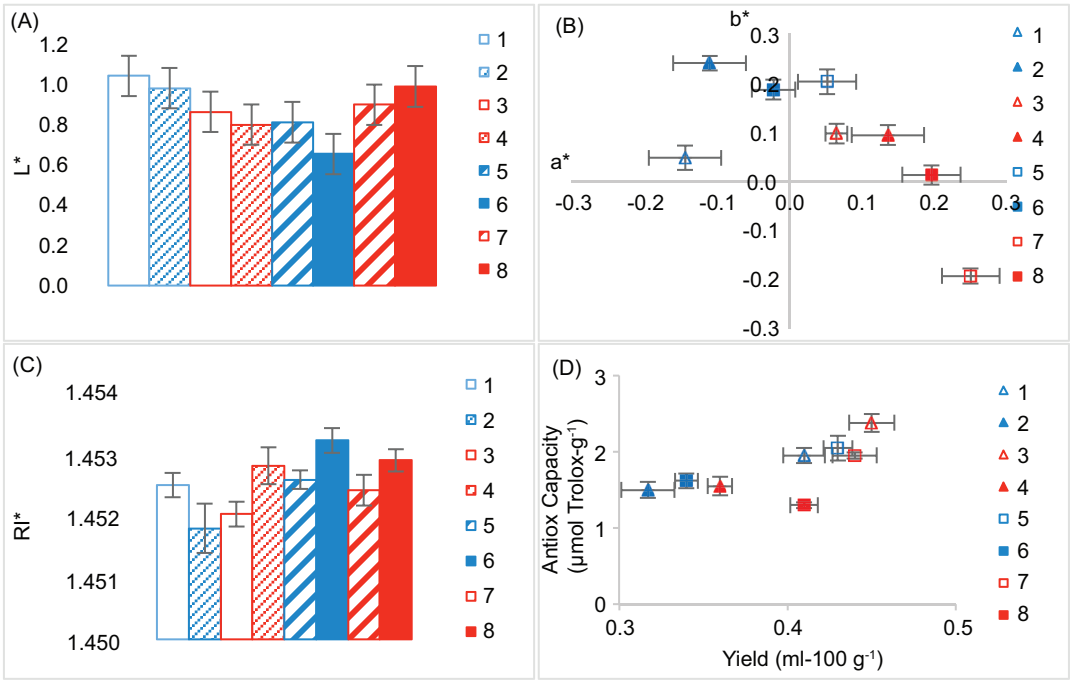

Figure 2. Colour parameters L* (A), a* and b* (B), refractive index (RI; C) and antioxidant capacity and yield (D) of Rica-Rica essential oil obtained through hydrodistillation (HD) for each treatment (1: 90 min 240 W 150 g; 2: 90 min 240 W 200 g; 3: 90 min 420 W 150 g; 4: 90 min 420 W 200 g; 5: 120 min 240 W 150 g; 6: 120 min 240 W 200 g; 7: 120 min 420 W 150 g; and 8: 120 min 420 W 200 g).

et al., 2005). Similar to the observations of process parameters on yield, the herb mass was found to exert an inverse effect on AC. Although treatments 3 and 7, both with the lowest herb mass and the highest potency, presented similar yields, treatment 3 (shorter period) had a greater AC. Apparently, with this treatment, most of the antioxidant compounds could be extracted, reducing the thermal degradation that could occur under longer periods. Tomaino *et al.* (2005), and Olmedo *et al.* (2015) reported that the thermal stability of the antioxidant activity of EOs depends on their composition. While for some EOs, the AC may decrease due to thermal heating, in other cases this property is not affected or may even increase due to greater extraction or transformation of responsible compounds.

More than 70% of the extracted fatty acids were saturated (Table 2), and the main fatty acid was caprylic acid (C8:0), which has been reported to have antifungal, antibacterial and antiviral effects (Gadotti *et al.*, 2014; Ruiz-Rico *et al.*, 2015). In turn, the main component of monounsaturated fatty acids was palmitoleic acid (C16:1), followed by elaidic acid (C18:1n9t). Beneficial biological effects have been reported for palmitoleic acid, related to improved hyperglycaemia and hypertriglyceridemia (Yang *et al.*, 2011). Elaidic acid, the trans isomer of oleic acid, could be formed due to the heating process during EO extraction (Valenzuela, 2008a, 2008b). Also among the polyunsaturated fatty acids (PUFAs) was linolelaidic acid (C18:2n6t), the trans isomer of linoleic acid, which appeared only when longer treatment period was used. This finding coincides with that of Bhardwaj *et al.* (2016) regarding the generation of trans isomers in intense technological processes applied to oils. In treatments with lower power levels, no PUFAs were detected. This could be explained by the lower heating intensity sufficient for their extraction. While the EO AC of some species can decrease due to thermal heating, in other species this property is not affected by heating. This is a crucial point to consider when assessing possible applications, particularly for the food industry.

MAHD method

The colour parameters of EOs extracted by the MAHD method were similar to those obtained by the HD method. The L* values (Figure 3A) were close to 0 (i.e. the EOs were dark), and the a* and b* coordinates (Figure 3B) were near to both axes; however, in the MAHD extraction, all the values of a* were positive, implying that these EOs were more brown than greenish. No significant effects were found for the herb:water ratio, time and power, or their interactions. The RI (Figure 3C) for MAHD varied between 1.452 and 1.453, which is within the ranges reported for other EOs obtained through

Table 2. Fatty acids profile for Rica-Rica essential oil obtained through hydrodistillation treatment (1: 90 min 240 W 150 g; 2: 90 min 240 W 200 g; 3: 90 min 420 W 150 g; 4: 90 min 420 W 200 g; 5: 120 min 240 W 150 g; 6: 120 min 240 W 200 g; 7: 120 min 420 W 150 g and 8: 120 min 420 W 200 g).

Fatty acids	Treatment (%)							
	1	2	3	4	5	6	7	8
C8:0	70.2 ± 0.1^{bcd}	75.3 ± 0.2^{d}	65.1 ± 2.7^{b}	71.3 ± 0.8^{cd}	70.2 ± 0.2^{cd}	68.2 ± 1.3^{bc}	58.2 ± 5.4^{a}	67.1 ± 0.9^{bc}
C12:0	–	–	–	–	–	–	–	1.1 ± 0.8^{a}
C16:0	6.1 ± 0.6^{bc}	5.1 ± 0.3^{b}	10.1 ± 0.4^{e}	8.1 ± 0.3^{d}	6.0 ± 0.1^{b}	4.2 ± 0.3^{a}	7.0 ± 0.6^{c}	6.3 ± 0.2^{b}
C16:1	8.2 ± 1.0^{ab}	6.0 ± 0.1^{a}	6.2 ± 1.6^{a}	5.2 ± 0.2^{a}	6.2 ± 0.7^{a}	11.3 ± 0.5^{b}	7.1 ± 3.3^{a}	7.1 ± 0.2^{a}
C18:1n9t	8.3 ± 0.6^{a}	6.2 ± 1.4^{a}	6.2 ± 3.4^{a}	4.1 ± 0.2^{a}	5.4 ± 0.5^{a}	4.1 ± 0.5^{a}	8.2 ± 4.2^{a}	5.4 ± 1.4^{a}
C18:2n6t	–	–	–	–	5.4 ± 0.4^{a}	5.1 ± 0.3^{a}	5.2 ± 0.0^{a}	5.3 ± 0.1^{a}
C21:0	4.2 ± 0.3^{bc}	3.1 ± 0.2^{a}	5.2 ± 0.5^{cd}	5.4 ± 0.7^{cd}	4.3 ± 0.3^{ab}	3.2 ± 0.4^{a}	6.1 ± 0.1	4.1 ± 0.3^{bc}
C24:1	4.1 ± 0.5	5.0 ± 0.5	9.1 ± 1.4	7.1 ± 0.7	5.2 ± 0.3	4.0 ± 0.2	9.2 ± 1.4^{d}	6.0 ± 0.8
Saturated	80	83	79	84	80	76	71	78
Monounsaturated	20	17	21	16	15	20	24	18
Polyunsaturated	–	–	–	–	5	5	5	5

Different superscript letters in columns indicate significant differences ($P < 0.05$).

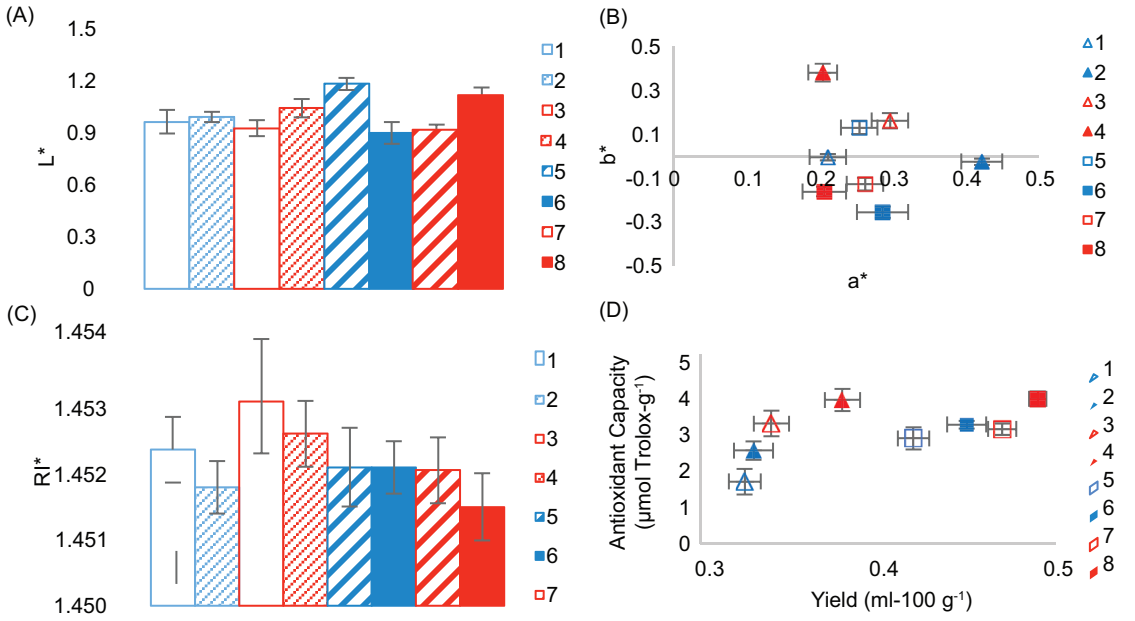

Figure 3. Colour parameters L* (A), a* and b* (B), refractive index (RI; C) and antioxidant capacity and yield (D) of Rica-Rica essential oil obtained through microwave-assisted hydrodistillation (MAHD) for each treatment (1: 45 min 420 W 150 g; 2: 45 min 420 W 200 g; 3: 45 min 700 W 150 g; 4: 45 min 700 W 200 g; 5: 90 min 420 W 150 g; 6: 90 min 420 W 200 g; 7: 90 min 700 W 150 g; 8: 90 min 700 W 200 g).

this extraction method, for example 1.46 (oregano herb; Torrenegra *et al.*, 2015) and 1.431 (*Mentha piperita*; Gavahian *et al.*, 2015). There are no previous reports of *A. deserticola* EOs extracted using the MAHD method. Similar results were reported by Golmakani and Moayyedi (2015) who did not find a significant effect of either extraction method (HD or MAHD) on the colour coordinates or RI of EOs.

In this work, when the MAHD method was used, the yields varied between 0.31 and 0.49 mL 100 g^{-1} (Figure 3D), which were higher than those obtained by

the HD method (shown previously). The higher yields resulted from the higher extraction rates enabled by microwaves and could be due to a synergy of two transfer phenomena, mass and heat (Golmakani and Moayyedi, 2015). The microwave energy increases the molecular movement, thus improving the penetration of solvent, which favours extraction of EO from biological matrix (Torres-León *et al*., 2017). All factors evaluated in the MAHD method (time, mass and power) were directly related to higher yields.

The AC varied between 1.68 and 3.89 μmol Trolox 100 g^{-1} and directly depended on the mass of the sample and the power used ($P < 0.05$). The time factor caused a greater increase in AC when lower power levels were used. The highest antioxidant capacities were achieved with treatment 4 (45 min) and treatment 8 (90 min); however, the highest yield was also obtained with the latter. The longer process time used in this case promoted increased EO extraction, but, apparently, it did not contribute to increase in AC. This coincides with the findings of Jiao *et al*. (2012), who suggested a maximum power of 700 W which allowed the complete extraction of EO while avoiding the loss of volatile compounds.

The fatty acid profiles of the RR-EOs obtained through MAHD (Table 3) revealed a significantly higher proportion of saturated fatty acids (84–93%) than of monounsaturated fatty acids (6–15%). Interestingly, PUFAs were not detected in any of the eight treatments, in contrast to the findings when using traditional HD (see Table 2). Of the saturated fatty acids, caprylic acid (C8:0) was the most abundant (69%), while among monounsaturated fatty acids, elaidic acid was the most abundant (4–12%). The conditions of the MAHD process affected both type of fatty acids and their concentrations in EOs. Therefore, a critical factor worth evaluating is the optimal conditions for extracting certain fatty acids. As mentioned above, PUFAs were only obtained by the HD method after 120 min. Shorter extraction periods but higher power levels are used in the MAHD method than used in the HD process. The absence of PUFAs in the EOs extracted by MAHD could be due to (i) a short extraction time that is insufficient to promote the transfer of compounds such as PUFAs from the plant tissue to water (Kusuma *et al*., 2018), (ii) harsher treatment conditions that degrade PUFAs during extraction (Bhardwaj *et al*., 2016; Li *et al*., 2012; Valenzuela, 2008a, 2008b) and (iii) compounds having a low interaction with microwaves, resulting in less extraction by MAHD than by HD (Orio *et al*., 2012). Because the yield and AC were higher in the MAHD method, it is unlikely that the process time was insufficient to extract PUFAs; therefore, it is considered more likely that their absence is caused by thermal degradation.

Stage II: Comparative analysis of HD and MAHD

Yield and AC

The process parameters for each technique were selected based on the best performance and AC obtained in stage 1. These were treatment 3 for HD (90 min, 420 W and 150 g 1000 mL^{-1}) and treatment 8 for MAHD (90 min, 700 W and 200 g 1000 mL^{-1}). For the samples collected in stage I (spring), the AC was 2.38 μmol

Table 3. Fatty acids profile for Rica-Rica essential oil obtained through microwave-assisted hydrodistillation treatment (1: 45 min 420 W 150 g; 2: 45 min 420 W 200 g; 3: 45 min 700 W 150 g; 4: 45 min 700 W 200 g; 5: 90 min 420 W 150 g; 6: 90 min 420 W 200 g; 7: 90 min 700 W 150 g; 8: 90 min 700 W 200 g).

Fatty acids	Treatment (%)							
	1	2	3	4	5	6	7	8
Caprylic acid (C8:0)	82.1 ± 0.0^{f}	79.2 ± 4.0^{de}	69.1 ± 0.0^{a}	71.2 ± 0.1^{ab}	73.2 ± 0.2^{bc}	76.1 ± 1.7^{cd}	78.3 ± 1.4^{de}	81.4 ± 0.1^{ef}
Undecanoic acid (C11:0)	11.3 ± 0.7^{c}	7.2 ± 0.7^{b}	11.1 ± 0.0^{c}	12.3 ± 1.1^{c}	7.1 ± 0.7^{b}	4.4 ± 2.1^{a}	–	–
Lauric acid (C12:0)	–	–	2.2 ± 0.0^{b}	–	2.2 ± 0.1^{b}	2.0 ± 0.0^{a}	–	–
Palmitoleic acid (C16:1)	–	–	–	–	–	6.2 ± 0.3^{a}	–	–
Elaidic acid (C18:1n9t)	7.1 ± 0.7^{ab}	13.1 ± 6.7^{b}	8.3 ± 0.0^{ab}	8.3 ± 0.3^{ab}	6.2 ± 1.6^{a}	4.2 ± 0.1^{a}	5.1 ± 0.0^{a}	5.0 ± 0.8^{a}
Heneicosanoic acid (C21:0)	–	3.0 ± 0.1^{a}	4.2 ± 0.0^{ab}	4.2 ± 0.5^{a}	6.1 ± 1.0^{c}	5.1 ± 0.4^{bc}	6.2 ± 0.9^{c}	5.2 ± 0.1^{c}
Nervonic acid (C24:1)	–	–	6.3 ± 0.0^{a}	5.2 ± 0.4^{a}	6.2 ± 0.4^{a}	5.3 ± 0.7^{a}	10.4 ± 0.5^{c}	8.3 ± 1.6^{b}
Saturated	93	88	86	87	88	85	84	87
Monounsaturated	7	12	14	13	12	15	16	13

Different superscript letters in rows indicate significant differences ($P < 0.05$).

Trolox g^{-1} and 3.98 µmol Trolox g^{-1} and the yield was 0.45 and 0.49 Ml 100 g^{-1} for HD and MAHD methods, respectively. For the samples collected in stage II (summer), a significant increase in yield was observed 0.66 mL 100 g^{-1} (HD) and 0.71 mL 100 g^{-1} (MAHD), while a slight reduction in AC was observed, 2.35 µmol Trolox g^{-1} (HD) and 3.89 µmol Trolox g^{-1} (MAHD). These results highlight two important points. The first is the effect of extraction method. In both stages, both yield and AC were higher with the MAHD technique. This could be explained as a combination of the following factors. (i) Power: the higher heating power used in MAHD can accelerate mass transfer to a certain extent, thus increasing the EO yield (Li *et al*., 2012); (ii) microwaves: these produce a greater rupture of plant tissues, which has been verified by scanning electron microscope (SEM; Farhat *et al*., 2017) and results in a more efficient heat flow affecting the release rate (Gavahian *et al*., 2015; Torres-León *et al*., 2017) and (iii) herb mass: 33% more herb mass was used with this MAHD extraction. There is no complete agreement among authors on the extraction method (HD or MAHD) that demonstrates a greater performance. Some authors have found results similar to those obtained in this work, with yields significantly higher in the MAHD method, such as 0.92% (MAHD) and 0.09% (HD) for oregano EO (Torrenegra *et al*., 2015), among others (Jiao *et al*., 2012; Li *et al*., 2012). However, other studies, such as Ajayi *et al*. (2016), have reported 0.73% for HD but only 0.64% for MAHD. Other authors report similar yields with both methods but with considerably shorter process period for the MAHD method (Bousbia *et al*., 2009; Gavahian *et al*., 2015). On the other hand, AC showed a significant increase in the EO obtained through MAHD compared to HD, which can be explained by the same factors—microwave energy, a greater power level (but at levels that do not reduce the content of antioxidant compounds) and higher herb:water ratios. The second important point highlighted by our results is the effect of the collection season. Differences were found in extraction process yield and the composition and properties of EOs. In one context, the higher yields obtained in stage II were directly related to the higher fat content of the samples collected in this stage (Table 1), and on the other side, the reduced AC observed for both techniques could be related to the compositional differences that occurred in the plants and therefore in their EO properties, depending on seasonality. Different authors have reported differences in yield, composition and properties, depending on seasonality. While de Alencar *et al*. (2017) and Delfine *et al*. (2017) reported a negative correlation between the rainy season or irrigation at the time of harvest and the EO yield, Moghaddam and Farhadi (2015) did not find any correlations. Jemâa *et al*. (2012) have found that the collection season in which the highest yield of eucalyptus is obtained also depends on the species. Regarding the relationship between EO properties and the collection season, de Alencar *et al*. (2017), and Jemâa *et al*. (2012), respectively, reported that antibacterial and fumigant activities were significantly higher in summer. Falasca *et al*. (2016) reported that in January they obtained the lowest EO performance and the best antifungal activity. Dhouioui *et al*. (2016) reported great variation in the amount of compounds present in EOs that depended on the month of collection; a significantly higher antimicrobial activity was found when the plants were collected in September. In the current work, the AC of samples collected in summer was slightly lower ($P > 0.05$) than those of stage I. The reported differences in the water and fat composition of plants between the seasons could be an indicator that other compounds also vary over time, of which some could be related to AC.

Colour parameters

Regarding the colour parameters (Table 4), it was observed that in both collection seasons, and for both extraction methods, the EOs were markedly dark. Most EOs had yellow–orange tones, coinciding with the results of Delfine *et al*. (2017), but were extremely close to the axis, that is they were nearly achromatic. The only significant colour differences were for the L^* values ($P < 0.05$); these where found in stage I, and the MAHD samples were lighter than the HD samples. In stage II, the opposite trend occurred. Notably, the colour properties are highly dependent on the composition of the analysed material.

Fatty acid profile

Important changes in the fatty acid profile were observed (Table 5) as a function of both the extraction method and

Table 4. Physical characteristics of Rica-Rica essential oils extracted through the selected conditions for traditional hydrodistillation and microwave-assisted hydrodistillation, with samples collected in stage II (summer).

Extraction	Colour			Refractive Index
	L*	a*	b*	nD^{20}
HD	0.60 ± 0.00^{b}	0.12 ± 0.10^{a}	0.49 ± 0.20^{a}	1.453 ± 0.100^{a}
MAHD	0.54 ± 0.00^{a}	0.09 ± 0.10^{a}	0.45 ± 0.20^{a}	1.452 ± 0.100^{a}

Different superscript letters in rows indicate significant differences ($P < 0.05$).
nD^{20} = refractive index at 20 °C

the collection season. Regarding the effect of extraction method, MAHD produced more saturated fats and a lower number of identified compounds than the HD method. When MAHD was applied, tridecanoic acid and palmitoleic acid were not detected, and the concentrations of caprylic acid, palmitic acid and elaidic acid were increased compared to HD. These differences are due to the use of different extraction techniques. Farhat *et al*. (2017), also reported fewer compounds obtained by MAHD than HD when extracting *Rosmarinus officinalis* L. In contrast, Ajayi *et al*. (2016) reported fewer compounds in EOs extracted from *Cymbopogon citrates* when HD was applied (n = 7) compared to MAHD (n = 16). Notably, other works have not reported considerable differences in the components of EOs, but differences have been found in the percentages of some compounds depending on the extraction method used (Li *et al*., 2012; Périno-Issartier *et al*., 2013). The present results coincide with those of previous studies that highlight the importance of considering component differences when selecting the optimal extraction method (Sodeifian and Sajadian, 2017). Regarding the effect of collection season, consistent with other authors (Dhouioui *et al*., 2016), EO compositional differences for both methods were observed between the collection periods. For the stage II samples (summer) and HD extraction, tridecanoic acid (C13:0) was found, but this compound was not detected in any of the stage I samples. In addition, although caprylic acid was still the principal fatty acid, its content was considerably reduced in both seasons and methods in stage II. In both stages and in all EOs there were a high proportion of saturated fatty acids. However, for stage II samples, an especial significant reduction was observed when the HD method was applied.

Table 5. Fatty acids profile for Rica-Rica essential oils extracted through traditional hydrodistillation and microwave-assisted hydrodistillation.

Fatty acids	%	
	HD (treatment 3)	MAHD (treatment 8)
Caprylic acid (C8:0)	40.1 ± 0.7[a]	57.3 ± 0.9[b]
Tridecanoic acid (C13:0)	3.8 ± 0.0	–
Palmitic acid (C16:0)	15.4 ± 1.2[a]	19.4 ± 0.5[b]
Palmitoleic acid (C16:1)	10.7 ± 0.2	–
Elaidic acid (C18:1n9t)	15.6 ± 1.0[b]	7.3 ± 0.0[a]
Heneicosanoic acid (C21:0)	4.0 ± 1.4[a]	4.0 ± 0.3[a]
Nervonic acid (C24:1)	12.2 ± 0.6[a]	12.0 ± 1.1[a]
Saturated	60	80
Monounsaturated	40	20

Different superscript letters in columns indicate significant differences ($P < 0.05$).

Antimicrobial activity

In the evaluation of the antimicrobial activity of RR-EOs, the preliminary analyses of 32 microorganisms revealed that 20 were sensitive and had varying degrees of inhibition (Figure 4). Rota *et al*. (2008) noted that the antimicrobial effectiveness of EOs depends on the type of microorganism and classified this effect into three categories of growth inhibition (disk diameter (in mm) included): ≥20-mm zone indicates strong inhibition; <20–12-mm zone indicates moderate/mild inhibition and <12-mm zone indicates no inhibition. Notable sensitivity results include the following microorganisms: sensitive, *Streptococcus* Group A; intermediate sensitivity, *Streptococcus* and *Bacillus cereus*; and the others were resistant. While the extracted EOs from both methods appeared to exert equivalent effects on most of the bacteria, differences were observed for Group A *Streptococcus* and *Salmonella paratyphi* B, where MAHD EOs showed a greater antibacterial effect. In contrast, for *Salmonella typhi*, *Staphylococcus epidermidis* and *Pseudomonas fluorescens*, the HD EO exerted greater inhibitory effects. The distinct compositions of the EOs caused by collection season, extraction method or other factors may have determined different antimicrobial or antifungal effects (Falasca *et al*., 2016; Zantar *et al*., 2015). Jiao *et al*. (2012) and Okoh *et al*. (2010) reported greater antimicrobial activity in EOs obtained by MAHD than by HD. Ajayi *et al*. (2016) reported that the extraction of certain compounds would be promoted by MAHD. Some research suggests that antimicrobial activity can be influenced by the mixture of compounds that form part of EO rather than by the effects of each individual compound (Deng *et al*., 2016). The present preliminary study supports the relevance of finding compounds or mixture of compounds with antimicrobial capacities to combat multi-resistant bacteria, such as *Pseudomonas*, one of the principal opportunistic pathogens in intra-hospital infections, and *Staphylococcus aureus*, which is known to be virulent and antibiotic-resistant. Moreover, the current results suggest that determining the minimum inhibitory concentration is crucial for establishing the future applications of RR-EOs in economic sectors such as food, cosmetic and pharmaceutical industries.

Energy efficiency of HD and MAHD processes

Finally, considering that the power level and the mass of grass used for the MAHD method were higher than those used for HD, the energy consumption, the mass of grass required and the process time required to obtain 1 mL of EO were determined for the selected treatments (treatment 3 for HD and treatment 8 for MAHD). Additionally, the mass of CO_2 released into the atmosphere was determined for each of the processes, considering that to obtain 1 kWh from coal or fuel, 800 g of CO_2 will be

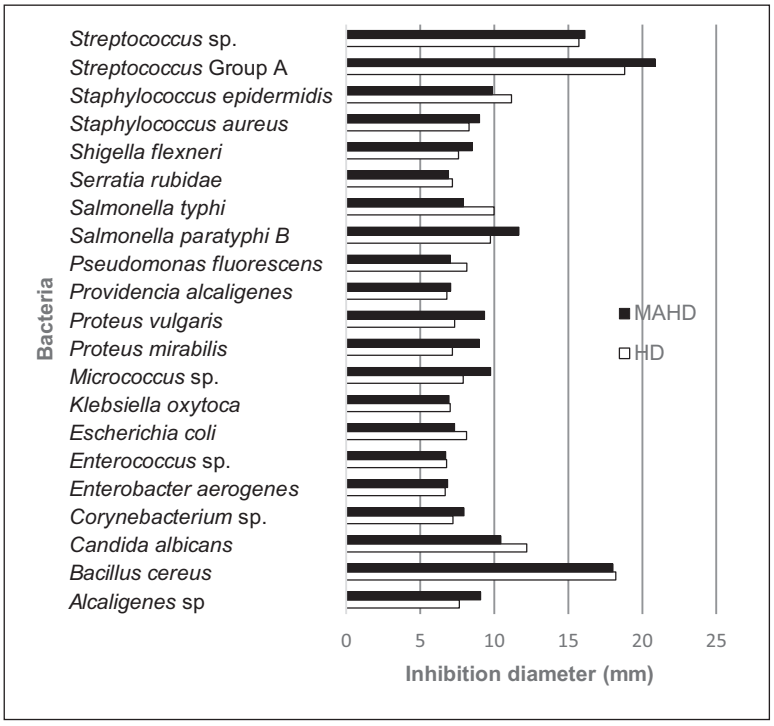

Figure 4. Sensitivity of microorganisms to Rica-Rica essential oil obtained through traditional hydrodistillation (HD) and microwave-assisted HD (MAHD).

rejected in the atmosphere during combustion of fossil fuel (Bernard, 2001, Elyemeni *et al*., 2019; Ferhat *et al*., 2006). Table 6 shows the respective data and results, and it was observed that the selected MAHD method (700 W and 90 min) was 8% more efficient than the HD method (420 W and 90 min) for different calculated parameters. Depending on the harvest season, MAHD requires between 141 and 204 g of herb compared to 152 to 222 g required by HD. Similarly, the process time is reduced, which, depending on the harvest season, ranges from 127 to 184 min for MAHD, in contrast to 136 and 200 min required by HD (420 W and 150 g). The above is directly related to energy consumption and, consequently, to the estimation of the mass of greenhouse gases, such as CO_2, produced during the extraction processes. These results coincide with those found by other authors, who have

Table 6. Comparison of the efficiency of the methods of extraction of essential oils through hydrodistillation and microwave-assisted hydrodistillation, depending on the required mass, process time, energy consumption and estimation of CO_2 production.

		HD (treatment 3)	MAHD (treatment 8)
Experimental results	Extraction time (min)	90	90
	Heating power (W)	420	700
	Sample weight (Herb + water) (g)	1150	1200
	Energy density (w g^{-1})	0.37	0.58
	Yield (mL EO 100 g^{-1})	0.45	0.49
	Electric consumption (W)*	1000	1000
Conditions for 1 mL EO	Herb mass required (g)	222	204
	Extraction time (min)	200	184
	Electric consumption (kwh)	3.33	3.06
	CO_2 rejected (kg)	2.67	2.45

*Declared by manufacturer.

reported greater energy efficiencies and better environmental impacts of MAHD compared to HD (Golmakani and Moayyedi, 2015; Jeyaratnam *et al*., 2016; Solanki *et al*., 2019). It is essential to consider energy efficiency and environmental impact together with other characteristics of EOs, such as their composition, presence of fatty acid and biological activity, when evaluating and selecting different extraction techniques and their respective process parameters.

Conclusions

The activity and fatty acid profile of EOs varied according to the collection season and the extraction method used. The MAHD method (90 min, 700 W and 200 g 1000 mL^{-1}) resulted in a higher percentage of oil extracted in relation to initial mass, as well as greater antioxidant and antimicrobial capacities, compared to the traditional HD method. However, the traditional HD method (90 min, 420 W and 150 g–1000 mL^{-1}) performed better in terms of energy costs and environmental impact. When selecting the extraction method for RR-EO, the yield in relation to the required initial mass, presence of certain fatty acids, antioxidant activity, energy costs and environmental impacts should all be considered carefully. This work shows that the parameters of the processes and extraction technique used significantly affect the performance, energy expenditure, physical and biological characteristics of OEs and their fatty acid profile. Depending on specific requirements related to OEs' potential industrial uses, these parameters are established and the extraction technique selected.

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