Enhancement of total phenolic and flavonoids extraction from *Rosmarinus officinalis* L using electromagnetic induction heating (EMIH) process

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Abstract Electromagnetic induction heating (EMIH) assisted extraction of phenolic compounds from *Rosmarinus officinalis* L, and the antioxidant and antimicrobial activities of the plant extract were examined in this study. The extraction yield acquired with this process was found to be $25.1 \pm 2\%$, with maximum amounts of phenolic compounds: 127.87 ± 2.1 mg Gallic acid equivalents per g dry weight and total flavonoids contents 14.48 ± 1.5 mg quercetin equivalents per g dry weight, under optimum extraction conditions (extraction time 2 h, ratio of raw material to liquid 1:2 and 0% of NaCl). The antioxidant activity was assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH); 2, 2'-azinobis (3-ethylbenzothiazoline-6- sulfonic acid) radical cation (ABTS⁺) and ferric reducing power (FRAP) methods. The results indicate the extract derived through EMIH showed a strong antioxidant ability (89.25%; EC₅₀ of 0.0148 µg/mL). Besides, the antimicrobial bioassay demonstrated that the extract possessed a good antimicrobial activity against all tested fungi and bacteria.

Keywords ABTS⁺ · Antioxidant activity · Antimicrobial activity · DPPH Electromagnetic induction · Flavonoids · FRAP · Phenolic compounds · *Rosmarinus officinalis* L

Introduction

Natural antioxidants present in plants have received immense attention in recent years, and there has been a worldwide surge towards the identification and the use of antioxidants from medicinal herbs because of their beneficial effects on human health. (Akhavan et al. 2015).

Rosmarinus officinalis L, a member of the Lamiaceae family, is a perennial herb that is mainly found in the Mediterranean region. It has been customarily utilized as a culinary spice, essentially to enhance flavor, and also as a drug, due to its enormously restorative properties (Borras Linares et al. 2011).

Nowadays, this plant is one of the most appreciated sources of natural bioactive components, especially in the functional foods industry. In fact, this plant exerts a great number of biochemical and pharmacological properties, namely hepatoprotective (Ponce et al. 2008), antibacterial (Djeridane et al. 2006), antitumor, antioxidant (Sacchetti et al. 2005; Cavero et al. 2005 and Peshev et al. 2011), antiulcerogenic (Dias et al. 2000), antidiabetic, antinociceptive (Haloui et al. 2000), and anti-inflammatory (Beninca et al. 2011) properties. Most of these detected effects are associated to the phenolic contents of this herb. Particularly, its potent antioxidant activity is mainly due to phenolic diterpenes, such as carnosic acid, carnosol, rosmadial or rosmanol, among others. Furthermore, the presence of other antioxidant phenolic compounds in rosemary, namely flavonoids (genkwanin, cirsimaritin), and phenolic acids (rosmarinic acid) (Del bano et al. 2003), has also been reported.

Conventional extraction methods are applied to extract phenolic compounds and flavonoids from plant materials, such as maceration, heating or refluxing (Bimakr et al.

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2011). However, the disadvantages are the loss of desired compounds owing to hydrolysis, oxidation and degradation during extraction due to long extraction times. In recent years, various new and promising extraction techniques including ultrasound-assisted extraction (Guangyan et al. 2012), supercritical fluid extraction proposed by Babovic (Babovic et al. 2010), extraction assisted by microwave, pulsed electric field assisted extraction, extraction by a pressurized liquid (Zhang et al. 2013) and electromagnetic induction heating have been introduced and have drawn significant research attention (Languez Rivera and Vilarem 2007).

Many efforts have been made in reducing time of extraction of total phenolic compounds and enhancing antioxidant activity from eggplant peel by ultrasound-assisted extraction technique (Dranca and Oroian 2016). This technique resulted in an optimized extraction time of less than one hour, which was found to be very economical (Dranca and Oroian 2017). But this emerging extraction method still suffers from the use of organic solvents (alcohols) and the inconvenience of a supplementary step to eliminate the toxic solvent (methanol etc.) from the extracts so obtained. Hence, there is still a need for a method to extract bioactive substances from biomass that does not involve the use of organic solvents, and provides a greener, and more economical alternative.

The present work introduces the electromagnetic induction heating as a new approach for the extraction of flavonoids and total phenolic compounds and optimizes the operational parameters such as extraction time, the ratio of raw material to liquid and concentration of the electrolyte solution. This study focusses on the evaluation of extraction yield, total phenolic/flavonoids, and chemical compositions. Additionally, the antioxidant activities of *Rosmarinus officinalis* L extracts by DPPH, ABTS⁺ and FRAP methods have also been assessed. The results have also been compared to those obtained by conventional methods.

Materials and methods

Chemicals and materials

Chemical reagents were procured from Sigma-Aldrich, Germany. A rotary evaporator (Yamato, Rotary Evaporator, Model-RE 801) was used for sample preparations; electromagnetic induction heating process was realized with a commercial induction plate (157737DDQ100108, Samsung; France). UV Spectroscopy measurements were done with UV-1800 Shimadzu, spectrophotometer, Japan).

Plant material

Fresh mature leaves of *Rosmarinus officinalis* L were collected in April 2015 from the region of Medea in the center of Algeria (latitude 36°16'03"N, longitude 2°45'00"E, altitude 881 km) and identified by Dr KAR-AHACANE, belonging to the 'Life Sciences and Nature' faculty, University of Khemis Miliana, Algeria.

Samples were air-dried in a shady room at room temperature for two days, and ground into a small species.

Investigation of the suitable method for extracting of *Rosmarinus officinalis* L

Maceration

Powder of leaves of *Rosmarinus officinalis* L was macerated with 60% ethanol (1:2 w/v) for 72 h at room temperature with occasional shaking. Obtained extracts were filtered and re-macerated by the same process and solvent until exhaustion. A rotary evaporator functioning under reduced pressure at 4 °C was used to remove ethanol and the obtained precipitate was dried, weighed and dissolved in 5 mL of absolute methanol and saved at 4 °C.

Heat reflux extraction

The dried powdered leaves (1:2 w/v) were boiled with distilled water at 100 °C for 6 h, the extract was filtered through a Whatman No 1 filter paper, and evaporated by a rotary evaporator. Obtained precipitate was dried, and dissolved again in 5 mL of absolute methanol and stored at 4 °C.

Electromagnetic induction heating-assisted extraction

Plant material was introduced in a stainless cylindrical container, placed on an induction plate (Fig. 1) and immersed in distilled water. This apparatus contains 9 power heating levels.

In order to determine the conditions of maximum extraction yield, maximum phenolic/flavonoid contents, optimal extraction conditions were achieved by sequentially changing one experimental parameters while fixing the others.

The following extraction conditions were altered: Extraction time (30, 60, 90, 120 and 180 min); Ratio of raw material to water (1:10, 1:4, 1:2 and 3:4 w/v); concentration of electrolyte solution NaCl (0, 1.25, 5 and 10%).

The combined extract obtained from each extraction was weighed and kept in a tight container.

Fig. 1 Experimental device used for Electromagnetic induction heating process

Extraction yield was determined as follows:

$$Y_{\text{Extract}}(\%) = \frac{M_{\text{extract}}}{M_{\text{feed}}} \times 100$$

Y_{Extract} : extraction yield (%), m_{extract} : crude extract mass (g), m_{feed} : feed mass (g).

Total phenolic content (TPC)

The TPC was measured using Folin-Ciocalteu procedure (Pothitirat et al. 2009). A volume of 1 ml of the sample under study was mixed with 5 mL of the Folin-Ciocalteu reagent and 4 mL of sodium bicarbonate solution [7% (w/v)]. The obtained solution was maintained for 2 h in dark at room temperature. A UV-visible spectrophotometer was used to record absorbance at 765 nm. TPC was defined as a milligram of gallic acid equivalents (GAE) per g of dry weight.

Total flavonoid content (TFC)

TFC were evaluated using aluminium chloride colorimetric method as mentioned by Miliuskas et al. (2004) with few modifications. A sample of 2 mL was mixed with 2 mL of 2% aluminium chloride solution (AlCl_3). The mixture was held for 30 min at room temperature. Absorbance measurement was realized at 415 nm against a blank sample, not containing aluminium chloride, using UV-visible spectrophotometer. TFC was presented in milligrams of quercetin equivalents (QE) per g of dry weight.

Determination of antioxidant activity

Three methods were used to determine this activity for obtained extracts: DPPH-scavenging assay, ABTS^+ and

FRAP method. The results were compared with known antioxidant, ascorbic acid.

DPPH scavenging assay

A volume of 2.5 mL of extract was mixed with 1 mL of a methanol solution 0.1 mM of DPPH and incubated at 37 °C for 30 min. Sample absorbance was determined at 517 nm using UV-VIS spectrophotometer and compared to corresponding blank. Percent inhibition was calculated as follows:

$$\%(\text{inhibition}) = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100$$

A_{blank} represent the measured absorbance of the sample containing control reaction (all reagents without the test compound).

A_{sample} represents the measured absorbance of the test compound.

The EC_{50} value; defined as the necessary concentration of the sample for scavenging 50% of the DPPH free radical. It was calculated from the curve of percent scavenging plotted versus the concentration.

Scavenging activity of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS^+)

The antioxidant activity was also assessed by the ABTS^+ decolourisation assay (Siddhuraju 2006) which was produced by the reaction between 7 mM ABTS^+ in H_2O and 2.45 mM potassium persulfate. Test solutions with different concentrations in methanol were mixed with 0.8 ml of ABTS^+ solution. The mixtures were then incubated in a dark place at 37 °C for 30 min and the absorbance was measured at 734 nm by a spectrophotometer.

The radical scavenging activity of samples was defined in terms of the EC_{50} (the effective concentration at which 50% of the ABTS^+ radicals were scavenged). EC_{50} was calculated from the log-dose inhibition curve using ascorbic acid as the reference compound.

Ferric reducing antioxidant power (FRAP)

A mixture of 1 mL of extract solution, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate was incubated at 50 °C for 20 min. Then 2.5 mL of 10% trichloroacetic acid was then added to the previous solution and centrifuged at 3000 rpm for 20 min. A 1 mL aliquot of supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl_3 (0.1%), and absorbance was measured at 700 nm. All increase in absorbance

leads to a rise of ferric reducing activity (Hinneburg et al. 2006).

Determination of antimicrobial activity

The in vitro antibacterial and antifungal activities of extracts were evaluated by the disc diffusion method (DDM) using Mueller–Hinton agar for bacteria and Sabouraud Dextrose agar for fungi (Baron and Finegold 1990).

Discs containing 30 μ l of the extract were used and growth inhibition zones were measured after 24 and 48 h of incubation at 37 and 24 °C for bacteria and fungi respectively.

Gentamycin for bacteria and Nystatin for fungi were used as positive controls. The microorganisms used were: *Enterococcus faecalis* ATCC 15753, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC10536, *Aspergillus Niger* ATCC 16404 and *Candida albicans* ATCC 10231. These microorganisms were supplied by the microbiology laboratory of the Hospital of Medea, Algeria and evaluated using laboratory reference strains (American Type Culture Collection “ATCC” for bacteria).

Results and discussion

Optimization of EMIH extraction conditions

In this part of study, the effect of different experimental conditions on the extraction efficiency of *Rosmarinus officinalis* L extracts was evaluated.

Fig. 2 Effect of EMIH time on extraction yield, total phenolics and total flavonoids

Effect of EMIH time on extraction yield; total phenolics and total flavonoids

The results of Fig. 2 showed extraction yield, TPC and TFC. It shows highest values reached for an extraction time of 120 min (yield of $25.1 \pm 2\%$, 127.87 ± 2.1 mg GAE/g dw and 14.48 ± 1.5 mg QE/g dw, respectively) and then substantially decreased with prolonging EMIH time. Since flavonoids were located in the cytoplasm of the cells, it was clear that EMIH led to diffusion by the disruption of important cellular structures such as cell walls and cell membranes, which are responsible for controlling the mass transfer process.

This point was reported and demonstrated by Zouambia et al. (2017), they reported that structure of citrange albedos tissues treated by EMIH was greatly disintegrated and the surface morphology was visibly changed and became much looser than that by conventional heating. These results guide us to choose the value of 120 min as an optimum time of EMIH-assisted extraction.

Effect of ratio of raw material/water on extraction yield and total flavonoids

The result of Fig. 3 showed that extraction yield and total flavonoids increase with the change in ratio of raw material to water from 1:10 (w/v) to 1:2 (w/v) and then decreased.

The highest yield ($25.1 \pm 2\%$) value, TPC (127.87 ± 2.1 mg GAE/g dw) and TFC (14.48 ± 1.5 mg QE/g dw) were achieved for a ratio of plant material to the liquid of 1:2 (w/v). Attempts to operate at a lower ratio were not successful. In fact, small and non-reproducible amounts of flavonoids were obtained. Highest yield values were obtained when water covers plant material totally in the reactor and swelled it. Hence, when the ratio plant material/liquid becomes too high, material outside water

Fig. 3 Effect of ratio raw material to water on extraction yield, total phenolics and total flavonoids

was not concerned by auxiliary energetic phenomena that make the glands receptacles burst and consequently reduce extraction yield. Consequently, the optimum ratio of plant material to water was fixed at 1:2 (w/v).

Effect of electrolyte solution concentration on extraction yield and total flavonoids

The results pertaining to the extraction yield and total flavonoids for different NaCl concentrations at different ratios of plant material to water are presented in Table 1.

As can be seen, the extraction yield, total flavonoids and total phenolics increased significantly with the concentration of NaCl in the range of 0 to 1.25%, for a ratio of raw material to liquid of 1:10 (w/v) and 1:4 (w/v), and reached a peak value of extraction yield of $21 \pm 0.8\%$, TPC: 6.67 ± 1.23 mg GAE/g dw and TFC: 12.76 ± 1.45 mg QE/g dw at 1.25% of NaCl. However, after this optimum concentration value, the yield was inversely decreased. It seems that lower concentration of NaCl can accelerate the heating of the medium by increasing electric conductivity.

On the other hand, for a ratio of raw material to liquid of 1:2 (w/v) and 3:4 (w/v); the content of flavonoids and extraction yield decreased with increasing the concentration of NaCl. These results are in agreement with values previously reported by Languez Rivera and Vilarem (2007) in the case of extraction of essential oils by direct induction assisted-heating process.

Effect of extraction methods on extraction yield, total phenolics and total flavonoids of rosemary extracts

The result of extraction yield, TPC (total phenolic content) and TFC (total flavonoid content) of *Rosmarinus officinalis* L extracts obtained by different extraction methods are shown in Fig. 4.

Comparing extracts obtained by the different extraction methods of this study shows that EMIH process extract contained the highest amount of phenol and flavonoid followed by heat reflux and maceration extract.

EMIH process served the highest yield of extract ($25.1 \pm 2\%$) in a short time of 2 h with the maximum contents of total phenolics (127.87 ± 2.1 mg GAE/g dw) and total flavonoids (14.48 ± 1.5 mg QE/g dw). Conversely, maceration gave the lowest yield ($6.37 \pm 1.95\%$) and minimum content of phenolics and flavonoids (30.7 ± 1.9 mg GAE/g dw, 2.97 ± 1.8 mg QE/g dw respectively). This is in agreement with the results of Zaouali et al. (2013).

Although it is difficult to make a comparison with published works with different plant material sources, present results are better than those reported by Proestos and Komaitis (2008) using microwave-assisted extraction from *Rosmarinus officinalis*, where a value of total phenolic content of 23.8 mg Gallic acid/g dry sample was obtained.

Compared to the conventional heating method, the electromagnetic induction heating hand over a rapid heating through the plant by producing heat even within the material to be heated. Hence, energy is directly reproduced in the core of the plant material, which provides

Table 1 Effect of electrolyte solution concentration on extraction yield, total phenolics and total flavonoids

Concentration of NaCl (%)	Ratio of raw material to water (w/v)			
	1/10	1/4	1/2	3/4
0				
Extraction yield (%)	6.21 ± 1.10	11.74 ± 1.40	25.1 ± 2.3	24 ± 2.2
Total flavonoids (mg QE/g dw)	2.39 ± 0.92	4.466 ± 1.021	14.48 ± 1.50	9.85 ± 1.60
Total phenolics (mg GAE/g dw)	30.0 ± 1.9	104.05 ± 2.51	127.87 ± 2.11	126.01 ± 1.86
1.25				
Extraction yield	19.89 ± 1.5	21.0 ± 0.8	23.3 ± 2.6	22.20 ± 1.86
Total flavonoids	2.80 ± 0.65	12.76 ± 1.45	13.63 ± 2.60	9.75 ± 1.45
Total phenolics	35.56 ± 1.63	6.67 ± 1.23	125.03 ± 2.69	124.56 ± 1.56
2.5				
Extraction yield	14.90 ± 0.56	17.09 ± 1.86	21.1 ± 1.48	19.89 ± 2.01
Total flavonoids	2.20 ± 0.79	8.86 ± 1.45	13.03 ± 1.79	9.66 ± 0.78
Total phenolics	27.89 ± 1.79	3.123 ± 2.230	98.57 ± 1.96	111.14 ± 0.46
5				
Extraction yield	12.06 ± 1.27	15.00 ± 0.95	14.30 ± 1.56	13.75 ± 1.67
Total flavonoids	2.06 ± 0.46	6.74 ± 0.76	12.79 ± 1.49	9.49 ± 0.83
Total phenolics	25.36 ± 1.48	2.987 ± 0.460	88.74 ± 1.46	90.64 ± 1.67

Fig. 4 Effect of extraction methods on extraction yield, total phenolics and total flavonoids

consistency and homogeneity in heating. These results imply a substantial increase in extraction amount of polar compounds, like phenolics and flavonoids, which enhance yield and time-saving.

Antioxidant activities

The total antioxidant activity of *Rosmarinus officinalis* L. of obtained extracts by different studied separation method was determined by using three methods: DPPH-scavenging assay, ABTS⁺ and FRAP method. The results presented in Fig. 5 and Table 2 summarizes the EC₅₀ values obtained from the DPPH and ABTS⁺ based radical-scavenging assay.

DPPH scavenging assay

The Fig. 5a shows the DPPH radical scavenging capacity of flavonoids of *Rosmarinus officinalis* L. It was observed that the flavonoids from *Rosmarinus officinalis* L. exhibited notable DPPH scavenging activity and the DPPH radical scavenging effects were increased with increasing concentration. The antioxidant activity increased from 25.03 to 97.7% when the concentration increased from 0.03 µg/ml to 0.28 µg/ml. The results obtained in our work indicate that phenolics/flavonoids extracted by EMIH process had a strong DPPH radical-scavenging activity with an EC₅₀ value of 0.0148 µg/ml (Table 2).

However, DPPH free radical scavenging of maceration derived extract (EC₅₀ = 0.0779 µg/ml) was less than that of the ascorbic acid (EC₅₀ = 5.7 µg/ml) and the extracts derived by other extraction methods. Likewise, this extract had the lowest contents of total phenolic compounds (30.7 ± 1.9 mg GAE/g dw) and flavonoids (2.97 ± 1.8 mg QE/g dw). On the other hand, the high antioxidant activity in the extract prepared by the EMIH method could be attributed to the high phenolic content, most likely consisting of enriched concentrations of rosmarinic acid, carnosic acid and carnosol (Brieskorn and Domling 1969; Cuvelier et al. 1996).

ABTS⁺ based radical scavenging assay

Antioxidant activity was also performed for *Rosmarinus officinalis* L. extracts by ABTS⁺ method. It clear from the results (Fig. 5b) that by increasing concentration of samples, the inhibition also increased. The highest inhibition was attained by the EMIH sample; which was 97.107% (EC₅₀ = 0.00469 µg/ml) compared to the ascorbic acid. The maceration extract showed a lower antioxidant activity (88.37%; EC₅₀ = 0.0306 µg/ml) which may be related to its lower phenolic contents (30.7 ± 1.9 mg GAE/g dw).

Several investigations (Cottica et al. 2011; Sen and Chakrabort 2011) show a relationship between the antioxidant activity and the content of phenolic compounds of such as flavonoids. Hence, the present study approves the correlation between the polyphenolic content and the antioxidant activity, as shown by the EMIH extracts that contained the highest phenolic/flavonoid content and exhibited the higher antioxidant activity.

Ferric reducing antioxidant assay (FRAP)

The ferric reducing antioxidant power of the studied extracts is shown in Fig. 5c.

Absorbance values were taken against the sample concentrations. All tested samples showed a dose-dependent activity; there was an increase in the values with concentration, which demonstrate the rise in the ferric reducing power. The highest activity was attained by EMIH sample with an absorbance value of 1.05 at a concentration of 0.263 µg/ml.

The reducing power is generally related to the presence of reductones. The antioxidant action of reductones depends on the scissure of the free radical chain which give a hydrogen atom or reacting with certain precursors of peroxide to prevent peroxide formation (Gulcin et al. 2010; Liu and Yao 2007).

By comparing the antioxidant activity measured by three methods, i.e., DPPH, ABTS⁺ and FRAP, all tested samples showed similar trends in all used antioxidant determination methods. So, it is clear that the three methods are consistent with each other in evaluating the antioxidant activity.

In addition, our results showed much stronger antioxidant activity for all the samples compared to those obtained by some modern extraction methods such as supercritical carbon dioxide extraction (Babovic et al. 2010) and microwave-assisted extraction (Xie et al. 2015).

Although the difference of antioxidant ability could be interpreted in terms of different qualities of herbs, geographical origin, harvest date, climatic conditions, but extraction method has the major impact.

Fig. 5 Antioxidant activities of *Rosmarinus officinalis* L extracts obtained by different extraction methods: **a** DPPH free radical scavenging; **b** ABTS⁺ based radical scavenging; **c** FRAP

Table 2 EC₅₀ values of different *Rosmarinus officinalis* L extracts obtained by DPPH and ABTS⁺ methods

Extracts	EC ₅₀ values (μg/mL)	
	DPPH	ABTS ⁺
Maceration extract	0.00779	0.0306
Heat reflux extract	0.00485	0.0241
EMIH extract	0.00148	0.00469
Standard	Ascorbic acid	5.7

Antimicrobial activity

The different extracts from *Rosmarinus officinalis* L were tested against two Gram-positive and one Gram-negative bacteria, as well as two fungi. The result, presented in Table 3, shows that the extract obtained by EMIH process

exhibited a good biological activity against all tested fungi and bacteria except for a resistant gram-negative bacteria, *Echerichia coli*, as well as a fungi, *A.niger*.

The most sensitive microorganism was *Staphylococcus aureus* with inhibition zone of 24 mm for extract obtained by EMIH process. Other microorganisms, subjected to this study, were found to be less sensitive to the obtained extracts, showing inhibition zones ranging from 8 to 14 mm.

However, more activity on gram-positive bacteria than gram-negative bacteria was observed. This result shows the same trend reported for many other studies on other plant species (Kokoska et al. 2002). The higher resistance shown by gram-negative bacteria than gram-positive bacteria can be assigned to the differences in the hydrophobic/hydrophilic character of the surface of the cell membranes of these bacterial groups. Gram negative bacteria make their external membrane surfaces highly hydrophobic (Okoh

Table 3 Antimicrobial activity of *Rosmarinus officinalis* L extracts

Microorganisms	Zone of inhibition (mm) ^a				
	Extraction methods			Antibiotics	
	Maceration	Heat reflux	EMIH	Gentamycin	Nystatin
<i>E.coli</i>	NA	NA	NA	20	NT
<i>E.feacalis</i>	NA	NA	8.0 ±	9	NT
<i>S.aureus</i>	14 ± 0.5	15 ± 1	24 ± 1	17	NT
<i>C.albicans</i>	NA	13 ± 0.5	14 ± 0.5	NT	18
<i>A.niger</i>	NA	NA	NA	NT	16

NA not active; NT not tested

^ainhibition zone diameter (mm), including diameter of sterile disk (6 mm)

et al. 2010), while in gram-positive bacteria, the lipophilic ends of the lipoteichoic acids, may help in the penetration of hydrophobic compounds (Cox et al. 2000; Ultee et al. 1999).

Conclusion

In this study, EMIH process assisted extraction of phenolic compounds from *Rosmarinus officinalis* L was optimized and compared to conventional extraction methods: Maceration and heat reflux. It promoted high yield ($25.1 \pm 2\%$) of crude extract, the highest contents of total phenolic (127.87 ± 2.1 mg GAE/g dw) and total flavonoids (14.48 ± 1.5 mg QE/g dw) with an interesting antibacterial and antifungal activities against some strains tested in this study. Additionally, the phenolic compounds obtained by EMIH process under optimal conditions (extraction time 2 h, ratio of raw material to liquid 1:2 and 0% of NaCl) exhibited a remarkable free radical scavenging (89.25%, $EC_{50} = 0.0148$ µg/mL) and antioxidant activity, which may be assigned to the presence of a high polyphenolic content.

In light of these experiments, it can be concluded that the EMIH process represents a new way to extract phenolics/flavonoid compounds from plant sources, and thereby derive extracts that have enhanced antioxidant and antimicrobial properties.

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