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Assessment of the Antioxidative Potential of *Rosmarinus officinalis* L. (*Lamiaceae*) Irrigated with Static Magnetic Field-Treated Water

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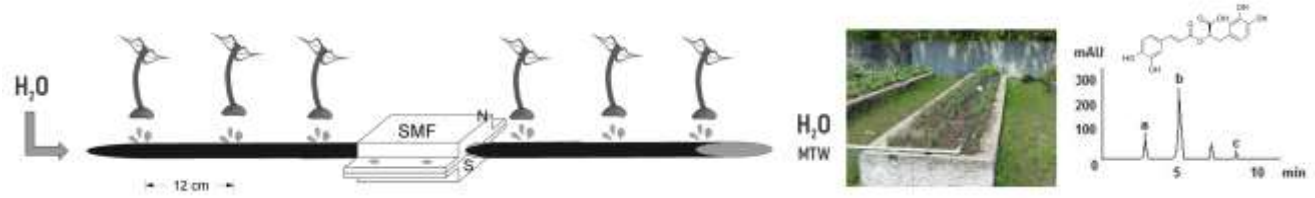
HIGHLIGHTS

- Irrigation with SMF-treated water stimulated an increase in polyphenol content.
- Rosmarinic acid concentration was higher in plants irrigated with SMF-treated water.
- Leaf extracts from rosemary subject to SMF-treated water showed higher antioxidant activity.

Abstract: Phenolic compounds are one of the main groups of secondary metabolites in plants and are known for their antioxidant activity. *Rosmarinus officinalis* L. (rosemary) contains different phenolic compounds including carnosol, carnosic acid and rosmarinic acid. In Cuba, rosemary cultivation is limited because it is difficult to propagate and has a low yield. As a result, it was removed from the Herbal Medicine National Formulary. However, the National Public Health System has a strong interest in rosemary because of its value as a natural antioxidant medicine. Irrigation with water treated with a static magnetic field (SMF) is a possible strategy to increase rosemary yield. This technology has been applied to accelerate plant growth and increase crop quality. The aim of this study was to evaluate the content of phenolic compounds and antioxidant activity in aqueous leaf extracts from plants irrigated with SMF-treated water in comparison to control plants. Significant differences in phenolic content and antioxidant activity were observed between aqueous extracts of control plants and plants irrigated with SMF-treated water. Therefore, irrigation with SMF-treated water is a promising technology to improve the cultivation of rosemary as a raw material to obtain pharmaceutical products with high antioxidant activities.

Keywords: static magnetic field; phenols; antioxidant activity; active plant extracts; rosmarinic acid; natural plant product.

GRAPHICAL ABSTRACT



INTRODUCTION

Polyphenols are widely distributed throughout the plant kingdom, constituting one of the most abundant classes of plant secondary metabolites. More than 8,000 chemical structures of phenols are known, belonging to different groups of secondary metabolites [1,2]. As a result, they are an important part of the human diet. Significant amounts of polyphenols are reported in vegetables, fruits, condiments and beverages [2,3].

Furthermore, polyphenols are known for their antioxidant properties, inhibiting lipid peroxidation and scavenging free radicals such as hydroxyl, superoxide and alkoxy radicals. They play an important role in the prevention of atherosclerosis and thrombosis by inhibiting platelet aggregation and reducing capillary permeability and fragility. In addition, phenolic compounds have anticarcinogenic potential, as they can promote the pumping of certain carcinogens out of cells and activate detoxification enzymes [4].

Rosmarinus officinalis L., rosemary, is a very important source of polyphenols [5] and is known as one of the spices with the highest antioxidant activity [6]. Rosemary has been used in traditional medicine, chemistry, cosmetic and food industry [7,8]. Nowadays, it is the most used aromatic and medicinal plant worldwide. Its importance is mainly related to the fact that it had many bioactive compounds. The most significant antioxidant polyphenolic compounds in rosemary leaves are rosmarinic acid, carnosic acid and related stable compounds such as carnosol, rosmanol, epirosmanol and 7-metylepirosmanol [6,9,10].

Rosemary is a perennial evergreen herb with fragrant, needle-like leaves and belongs to the mint family (*Lamiaceae*). Rosemary propagation presents some difficulties because flowering only takes place sporadically throughout the year and seeds have a low viability [11]. However, it has been cultivated successfully by adding plant growth regulators and using different substrates [10] and plant tissue culture techniques [12]. Irrigation using water treated with a magnetic field could possibly increase rosemary yield, as it changes certain physical and chemical plant properties, thereby affecting plant growth and reproduction. Indeed, some authors demonstrated that magnetic treatment of the irrigation water had the potential to improve early seedling growth and nutrient content in *Cicer arietinum* [13,14]. Similarly, Boix and coauthors [11] reported that the use of water treated with a static magnetic field (SMF) (100-150 mT) improved the growth and development of *R. officinalis*. Furthermore, in another study, Boix and coauthors [15]. showed the presence of monoterpene and sesquiterpenes compounds in leaf extracts of rosemary cultivated with SMF-treated water. However, the number of studies on the effects of irrigation with SMF-treated water on rosemary cultivation is still very limited. Therefore, the aim of this study was to evaluate the phenolic content and antioxidant activity in aqueous leaf extracts of rosemary plants irrigated with SMF-treated water (100-150 mT).

MATERIAL AND METHODS

Experiments were conducted on the experimental plots of the National Centre of Applied Electromagnetism (CNEA) in Santiago de Cuba (Cuba) and at the Centre for Environmental Sciences of Hasselt University (Belgium).

Plant material

Rosmarinus officinalis plants (voucher 21324 from the Centre for Biodiversity and Ecology of Santiago de Cuba) of approximately 0.5 m long and 6 months old were grown in an experimental plot system under natural conditions at 30ºC and a relative humidity ranging between 70 and 80%. The substrate was composed of soil, organic matter and clay soil (1:2:1), according to the recommended conditions for the growth and development of rosemary [16]. Some chemical and physical characteristics of the substrate are presented in Table 1.

Table 1. Physico-chemical characteristics of the substrate used in this study.

Mineral composition (mg/L)	
Ca	30178
Mg	10866
K	55.2
Na	15.0
Fe	39966
Cu	116.72
Physical properties	
Conductivity	0.28 mS/cm
pH	6.8
Total hardness	158.5 mg/L

The plants were irrigated with a dropping system through an air microjet system of 10 m long with irrigation points at every 12 cm. Irrigation was performed twice a day for 30 min, using an ITUR water pump at a flow rate ranging between 2.54 and 2.91 m^3/h.

For magnetic treatment of the water, an external permanent magnet was used, which was designed, built and calibrated at the CNEA using Nuclear Magnetic Resonance and a 0.41 T type Teslameter. The magnetic induction in the central area of the magnetizer ranged between 100 and 150 mT [17] (Figure 1).

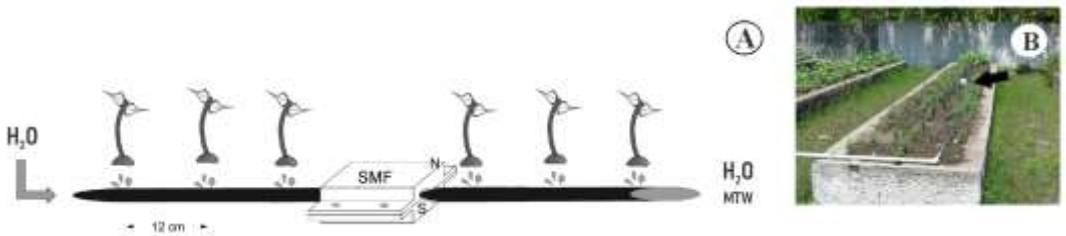


Figure 1. Cultivation system with a magnetizer dispositive in the irrigation system. **A.** A dropping system through an air microjet system of 10 m long with irrigation points at every 12 cm where water is treated (MTW) with a static magnetic field (SMF). **B.** Plants of *Rosmarinus officinalis* in the soil, under natural environmental conditions, showing the irrigation system containing static magnetic field (SMF) – arrow.

Plants were either irrigated using water treated with SMF (100-150 mT) or with non-treated water. After 180 days of treatment, the leaves of 60 plants per condition were collected. Leaf material was dried in an oven at 40 °C until a constant weight was reached. The dried leaves were homogenized and the dry vegetal powder was kept at 4 °C until further analysis.

Preparation of rosemary extracts

In order to prepare aqueous rosemary extracts, 3 g of dried leaf was added to 100 mL dH_2O. The solution was heated to 70 °C for 1 h. Subsequently, it was centrifuged for 3 min at 3000 x *g* and the supernatant was filtered through a Whatman paper (GF/A, 110 mm) and lyophilized. The final extract (≈0.3%), expressed as mg of dry extract per mg of dry sample, was kept at 4°C until further analysis.

Standard calibration curve

Determination of the content of the compounds in plant material was performed by the external standard method. The calibration curves were evaluated by analyzing three authentic curves, constructed with the standards solution at five concentration levels. The results were analyzed by linear regression using the least squares method, in order to define the coefficient of determination (R^2). The calibration curve of quercetin showed the linearity of the detector over the tested range between 1 and 100 µg/mL with an R^2= 0.9943. The curve of ferulic acid was in the linearity range of 2.5-20 mg/mL (R^2= 0.0994). The concentrations of reference substance rosmarinic acid used for the calibration curve was 0.05 to 0.5 mg/mL (R^2= 0.0999).

High Performance Liquid Chromatography (HPLC) analysis

The content of ferulic acid, quercetin and rosmarinic acid in aqueous leaf extracts was determined using HPLC as described by Okamura and coauthors (1994). The analysis was performed using an Agilent 1100 HPLC instrument equipped with a RP-C18 4.6 x 150 mm column with a 5 µm particle size and 300 Å pore

size. The system was further composed of a binary pump, an autosampler, temperature-controlled column compartments and an ultraviolet detector. The mobile phase consisted of 90% solvent A (840 mL purified water, 8.5 mL acetic acid and 150 mL acetonitrile) and 10% solvent B (methanol) over a period of 30 min. Compounds were detected at 284 nm.

Antioxidant activity: total phenol assay, DPPH radical-scavenging activity (RSA) assay, Ferric Reducing Antioxidant Power (FRAP) assay, Phosphomolybdenum method and Reducing power

The total phenolic concentration in the aqueous plant extracts was determined using the Folin-Ciocalteu method [18,19] with some modifications. Briefly, a 10 µL aliquot of rosemary extract was added to 990 µL Milli-Q water. Then, 500 µL of Folin-Ciocalteu agent was added and the solution was stirred vigorously and incubated for 5 min. Finally, 1.5 mL of a saturated sodium carbonate solution was added, after which the solution was again stirred vigorously and incubated at room temperature for 1 h. Absorbance was determined at 760 nm using a ShimadzuUV-1602 spectrophotometer. Calculations were based on a gallic acid calibration curve (2.50 to 20 mg/mL, y= 0.1828x – 0.0104, R^2= 0.9983).

The capacity of the aqueous extracts to scavenge the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was evaluated. Two millilitres of a dilution series from 0.5 to 500 µg/mL aqueous extract was added to 1 mL of DPPH (0.125 mM) in ethanol. The reaction mixture was incubated in a dark room for 30 min. Subsequently, the absorbance at 517 nm was kinetically monitored using a Shimadzu UV-1602 spectrophotometer. The percentage inhibition of activity (AA%) was calculated using the following equation: AA% = (A_0-A_1/A_0) x 100, where A_0 is the absorbance of the control (DPPH in ethanol) and A_1 is the absorbance of the extract. The half maximal inhibitory concentration (IC_{50}) values were calculated from the linear regression obtained by plotting the concentration (µg/mL) against the AA%. The antioxidant potential is inversely proportional to the IC_{50} value and is a parameter widely used to measure radical-scavenging efficiency.

The ferric reducing antioxidant power (FRAP) assay was used to provide an indication of the reducing ability of the aqueous plant extracts [20]. This assay is based on the measurement of the ability of a substance to reduce Fe(III) to Fe(II) and provides an indication of the total antioxidant capacity of extracts. Fe(II) is measured spectrophotometrically via detection of its complex with (TPTZ), which has a maximal absorbance at 595 nm.

The FRAP reagent was freshly prepared by mixing 100 mM TPTZ and 200 mM ferric chloride in 0.25 M Na acetic buffer (10:1:1 v/v/v) with pH 3.6. 10 µL of the aqueous extract was added to 17.6 µL of Na acetic buffer and 150 µl of FRAP reagent in 96-well plates. After 4 min incubation at room temperature, the absorbance at 593 nm was kinetically monitored for 15 min using a microplate reader (FLUOstar Omega, BMG LABTECH). A calibration curve of Trolox (100 to 1000 µM) was used and results were expressed in µM Trolox per gram dry weight (DW).

Total antioxidant capacity assay is a spectrophometric method for the quantitative determination of the antioxidant capacity, through the formation of a phosphomolybdenum complex. The phosphomolybdenum method is based on the reduction of molybdenum Mo(VI) to Mo(V) and the subsequent formation of a green phosphate-Mo (V) complex at acidic pH [21]. One mL of reagent was added to 0.2 mL of 100 µg/mL aqueous extract and incubated for 90 min at 100 °C. Subsequently, the absorbance at 695 nm was determined. Ascorbic acid was used as a standard. The antioxidant capacity was estimated according to the following equation:

$$\text{Antioxidant effect } (\%) = \left\lfloor \frac{(\text{Abs } standard - \text{Abs } sample)}{\text{Abs standard} \times 100} \right\rfloor \quad (1)$$

In the reducing power assay, antioxidant compounds form a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700 nm [22]. One mL extract was added to 2.5 mL phosphate buffer (0.2 M, pH 6.6). Subsequently, 2.5 mL 1% potassium ferricyanide was added and the mixture was incubated at 50 °C for 20 min. Then, 2.5 mL 10% trichloroacetic acid was added to the mixture, which was subsequently centrifuged at 1800 x *g* for 10 min. 2.5 mL of supernatant was mixed with 2.5 mL dH_2O and 0.5 mL 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. Ascorbic acid was used as a standard (50, 100, 200, 250 and 500 µg/mL). A higher absorbance of the reaction mixture indicates a higher reducing power. The calibration curve for ascorbic acid is linear in the concentration range of 50-200 µg/mL and had R^2= 0.9968.

Statistical analysis

The Kolmogorov-Smirnov test was used to verify normal distribution of the data. Results were statistically analyzed using a student's t-test or One AWAY with ANOVA followed by a *post hoc* Tukey-Kramer test to correct for multiple comparisons.

RESULTS

Results of the HPLC analysis of aqueous rosemary extracts (Table 2, Figure 2) show that rosmarinic acid (4.97 min), ferulic acid (3.38 min) and quercetin (9.03 min) concentrations were significantly higher in plants irrigated with SMF-treated water (hereafter referred to as SMF plants) as compared to plants irrigated with non-treated water (hereafter referred to as control plants). Furthermore, it is interesting to note that the relative abundance of these compounds differed between control plants and SMF plants.

Table 2. Rosmarinic acid, ferulic acid and quercetin concentrations (mg/L) in aqueous extracts of leaves from *Rosmarinus officinalis* control plants and SMF plants.

Treatments	**Polyphenols (mg/mL)**		
	Ferulic acid	Rosmarinic acid	Quercetin
Control	0.19 ± 0.005	0.09 ± 0.02	0.01 ± 0.0001
SMF	2.79 ± 0.02*	8.38 ± 0.01*	0.51 ± 0.005*

Data represent the mean ± SD of three replicates. *Significant from t-Student ($p < 0.05$).

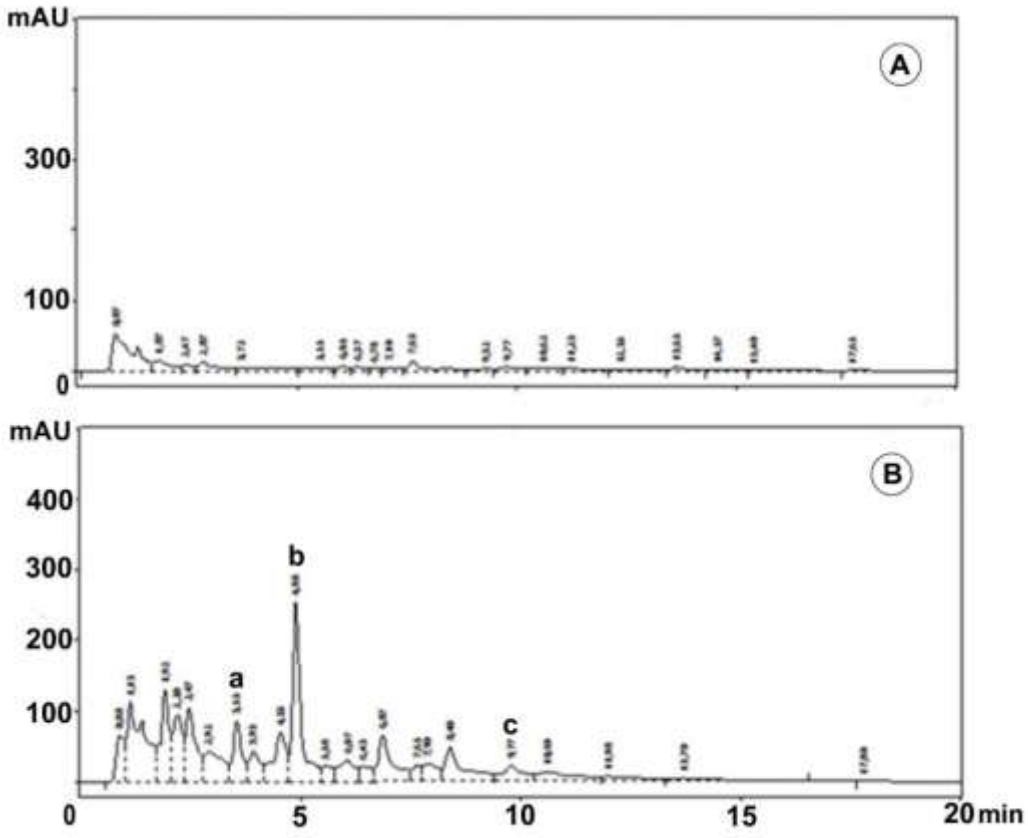


Figure 2. Chromatographic profile (HPLC) of *Rosmarinus officinalis* aqueous extracts. The first chromatogram (A) is control plant extract. Below, the chromatogram of MTW-plant extract (B): (a) ferulic acid (RT 3.4 min), (b) rosmarinic acid (RT 4.8 min), (c) quercetin (RT 9.7 min) measured at 284 nm. RT: retention time (min).

Whereas ferulic acid was the most abundant compound in control plants, rosmarinic acid concentrations exceeded those of ferulic acid in SMF plants. The presence of these compounds in *R. officinalis* was confirmed in literature [5,23].

Antioxidant activity

Total phenolic concentrations in aqueous *R. officinalis* extracts are presented in Table 3. The data indicate that irrigation with SMF-treated water significantly increased the antioxidant activity and total phenolic content in leaves.

Table 3. Antioxidant tests using leaf aqueous extracts of *Rosmarinus officinalis* after irrigation with SMF-treated water.

Treatment	mg GAE/g DW	IC_{50} (µg/mL)	TAC (µmol Trolox/g DW)	µmol ascorbic acid/ g DW	Max Effect (Abs ± SD)
Control	98.64 ± 16.99	42.24 ± 9.67a	7861.05 ± 197.20	96.77 ± 0.24	1.80 ± 0.1
SMF	121.21± 20.40*	17.69 ± 6.45b	8015.62 ± 226.70	173.00 ± 0.03*	2.37 ± 0.06*
Quercetin	--	5.65 ± 1.20c	--	--	--

Values represent the mean±SD of three replicates. *Significant by t-Student and letter significant for ANOVA followed Tukey-Kramer test ($p<0.05$)

The DPPH radical-scavenging activities are presented in terms of the IC_{50} values (Table 2). Data demonstrate that aqueous extracts of SMF plants exhibited a significantly lower IC_{50} – and therefore higher antioxidative activity – as compared to those of control plants. The DPPH radical-scavenging activity of *R. officinalis* was previously demonstrated Kasparavičienė and coauthors [24] Interestingly, results of Luis and coauthors [25] also indicated that the free radical-scavenging activity of *R. officinalis* extracts was proportional to the rosmarinic and carnosic acid concentrations, confirming their role in antioxidative defense. Results obtained by the FRAP assay are shown in Table 2. Data show a slight but non-significant increase in total antioxidative capacity of SMF plants as compared to control plants. Nevertheless, these results are in agreement with the significantly increased DPPH radical-scavenging activity and higher phenol content measured in SMF plants. Results obtained using the phosphomolybdenum method (Table 2) also indicate that the antioxidant activity – expressed as ascorbic acid equivalents per gram dry weight – was significantly higher in SMF plants as compared to control plants, thereby confirming the results obtained using the methods described above.

Furthermore, results indicate that irrigation with SMF-treated water significantly increased the reducing power of *R. officinalis* leaves (Table 2). The value of SMF plants was significantly higher with a value of 2.37 ± 0.06 than the value in the control plants 1.80 ± 0.1.

DISCUSSION

In general, results obtained in this study indicate that irrigation with SMF-treated water increased the phenolic content and antioxidative activity of *R. officinalis*. The enhanced total phenolic content (Table 2) is in agreement with the increases in rosmarinic acid, ferulic acid and quercetin detected by HPLC analysis (Table 2).

Hozayn and coauthors [26] reported a positive effect of irrigation with magnetically treated water on the concentrations of photosynthetic pigments, carotenes and polyphenols in *Triticum aestivum* (wheat). Similarly, they demonstrated that magnetically treated water increased phenol and indole acetic acid concentrations in *Cicer arietinum* (chickpea). In addition, total phenol concentrations of *Vicia faba* were also significantly increased by irrigation with magnetically treated water [14].

Many authors reported that treatment of water with SMF can affect its chemical-physical properties. Pang and coauthors [27], for example, demonstrated that magnetic treatment of water can produce an increase in the solubility of minerals and change its surface tension, electrical conductivity and pH. Furthermore, magnetization of water can cause polarization of its atoms and affect the dipole moment, the transition state of the electrons and the vibrational state of the water molecule. This can in turn result in a better water assimilation of the plant and an increase of enzyme activity and endogenous hormone levels. Indeed, Phirke and coauthors [28] reported that the effects of treatment with a magnetic field could be due to biochemical changes or alterations of enzyme activities [26]. Magnetic treatment of the irrigation water was shown to increase the concentrations of photosynthetic pigments and secondary metabolites in leaves of *R. officinalis* [11,15]. Ferrer and coauthors [29] showed that aqueous extracts of *Solanum lycopersicum* plants irrigated with SMF-treated water (150-300 mT) had a higher phenolic content and antioxidant activity as compared to those of control plants. Similar observations were made when lentil plants were irrigated with SMF-treated water, which significantly improved their growth and increased their phenolic content [30].

Several studies reported a higher total phenolic content in wheat plants irrigated with SMF-treated water as compared to control plants [31,32]. They explained that this increase may be attributed to the fact that the magnetic field changes cell membrane characteristics, cell reproduction and cell metabolism. Indeed, Formicheva and coauthors [33] reported that irrigation using magnetically treated water significantly induced cell metabolism and mitosis of meristematic cells of pea, lentil and flax. Moreover, the synthesis of new proteins could also underly the growth stimulation observed in SMF plants. Furthermore, the involvement of plant hormones on growth of plants irrigated with magnetically treated water needs further attention, as it has been demonstrated that irrigation with SMF-treated water increased the gibberellin (GA_3) and kinetin contents in broad bean [34]. An increased GA_3 content was also observed in sunflower plants irrigated with SMF treated water [35].

Regarding the antioxidant activity of leaf aqueous extracts, a higher DPPH radical-scavenging activity was observed in SMF plants as compared to the control plants (Table 2), which corresponds to the higher concentrations of rosmarinic acid, ferulic acid and quercetin (Table 2). The DPPH-scavenging activity in control plants measured in this study was in the same range as that reported by Avila Sosa and coauthors [36]. In addition, Btissam and coauthors [37] obtained an IC_{50} concentration in 42.08 mg/mL of essential oil of *R. officinalis* that is highly similar to the value of control plants 42.24 mg/mL obtained in the present study. They indicated that the anti-radical activity related to the presence of phenolic compounds. However, the total activity is not only attributed to the major compounds, e.g. rosmarinic and ferulic acid, because interactions between different compounds can exist in a synergistic way to reduce free radicals. Besides, other phenolics not identified as carnosic acid, a diterpene of *R. officinalis*, has potent antioxidant activity *in vitro* [38].

Albayrak and coauthors [39] showed that infusion and maceration of *R. officinalis* could be used to extract phenolic compounds and the major antioxidant activity with DPPH method was observed when using maceration. Gómez-Estaca and coauthors [40] also demonstrated that the antioxidant activity – as measured by the FRAP method in aqueous extracts of both *Origanum vulgare* and *R. officinalis* was related to their phenolic content. In another study, the antioxidant activity, evaluated by the FRAP method in *R. officinalis* was related to the phenolic compounds because they have the ability to scavenge free radicals, donate hydrogen atoms and chelate metal cations [41].

This investigation corroborated that phenolic compounds participate in the growth and development of *R. officinalis* in normal conditions. Moroever, it is demonstrated with experimental evidence that irrigation with magnetically treated water can stimulate the production of metabolites with antioxidant activity.

CONCLUSION

The results presented in this study demonstrate that aqueous leaf extracts of *R. officinalis* plants irrigated with SMF-treated water have a higher polyphenol content and *in vitro* antioxidant activity than those of control plants. The polyphenolic content of rosemary is very important regarding to its use in nutritional supplements and natural antioxidant medication (to prevent diseases like liver cancer, atherosclerosis or heart diseases) and as an antioxidant substitute of synthetic compounds in food and pharmaceutical industries. Therefore, irrigation with SMF-treated water is an interesting strategy, as it not only improves rosemary yield but also increases its antioxidant properties.

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Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Middleton EJr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells, implications for inflammation, heart disease and cancer. Pharmacol Rev. 2000 Dec; 52(4):673–751.
2. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents, Mechanism and actions. Mutation Res. 2005 Nov; 579:200–13.
3. Williamson G. The role of polyphenols in modern nutrition. Nutr Bull. 2017 Sep; 42(3):226–35.
4. Mileo AM, Miccadei S. Polyphenols as modulator of oxidative stress in cancer disease, new therapeutic strategies. Oxid Med Cell Longev. 2016 Nov; 1-17.
5. Rashid KI, Ibrahim KM, Hamza SJ. Effect of some biotic and abiotic elicitors on phenolic acids and diterpenes production from rosemary (*Rosmarinus officinalis* L.) leaf and callus analyzed by High Performance Liquid Chromatography (HPLC). J Al-Nahrain University. 2011 Sep; 14:104-9.

6. Nieto G, Ros G, Castillo J. Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinalis* L.), a review. Medicines. 2018 Sep; 5(3):98.
7. Ijaz Hussain A, Anwar F, Shahid Chatha SA, Jabbar A, Mahboo, S, Singh Nigam P. *Rosmarinus officinalis* essential oil, antiproliferative, antioxidant and antibacterial activities. Braz J Microbiol. 2010; 41:1070-78.
8. Yesil-Celiktas O, Sevimli C, Bedir E, Vardar-Sukan F. Inhibitory effects of rosemary extracts, carnosic acid and rosmarinic acid on the growth of various human cancer cell lines. Plant Foods Hum Nutr. 2010 Jun; 65(2):158-63.
9. Almela L, Sánchez-Munoz B, Fernández-López J, Roca MJ, Rabe V. Liquid chromatographic-mass spectrometric analysis of phenolics and free radical scavenging activity of rosemary extract from different raw material. J Chromatogr A. 2006 Mar; 1120:221-9.
10. Silva AMO, Andrade-Wartha ERS, Carvalho EBT, Lima A, Novoa AV, Mancini J. Effect of aqueous rosemary extract (*Rosmarinus officinalis* L.) on the oxidative stress of diabetic rats. Rev Nutr. 2011 Jan/Fev; 24:121-30.
11. Boix YF, Aleman EI, Dubois AF, Botta AM. Riego con agua tratada magnéticamente en *Rosmarinus officinalis* L. (romero) como alternativa en la propagación convencional. Centro Agrícola. 2008 Mar; 35:23-7.
12. Boix YF, Arruda RCO, Defaveri ACA, Sato A, Lage CLS, Victório CP. Callus in *Rosmarinus officinalis* L. (Lamiaceae), a morphoanatomical, histochemical and volatile analysis. Plant Biosyst. 2013 Dec; 147:751-7.
13. Grewal HS, Maheshwari BL. Magnetic treatment of irrigation water and snow pea and chickpea seeds enhances early growth and nutrient contents of seedlings. Bioelectromag. 2011 Jan; 32:58-65.
14. El Sayed H, El Sayed A. Impact of magnetic water Irrigation for Improve the growth, chemical composition and yield production of broad bean (*Vicia faba* L.) plant. Amer J Exp Agric. 2014 Jan; 4:476-96.
15. Boix YF, Alemán EI, Torres JM, Chávez ER, Arruda RCO, et al. Magnetically treated water on phytochemical compounds of *Rosmarinus officinalis* L. Int J Agric Environ Biotechnol. 2018 Jan-Feb; 3:297-303.
16. Sturdivant L, Blakley T. The Bootstrap Guide to Medicinal Herbs in the Gardn, Field & Marketplace. Friday Harbor, WA:San Juan Naturals; 1999.
17. Gilart F, Deas D, Ferrer D, Lopez L, Ribeaux G, Castillo J. Hight flow capacity devices for anti-scale magnetic tretament water. Chem Eng Process, Process Intensif. 2013 Aug; 70:211-16.
18. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Vitic. 1965 Jan;16:144-58.
19. Dorman HJD, Peltoketo A, Hiltunen R, Tikkanen MJ. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chem. 2003 Nov; 83:255-62.
20. Penarrieta JM, Alvarado JA, Äkesson B, Bergenståhl B. Total antioxidant capacity and content of flavonoids and other phenolic compounds in canihua (*Chenopodium pallidicaule*), an andean pseudocereal. Mol Nutr Food Res. 2008 Jun; 52:708–17.
21. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex, specific application to the determination of vitamin E. Anal Biochem. 1999 May; 269:337-41.
22. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Jpn J. Nutr.* 1986, 44, 307-15.
23. Kiarostami, K., Mohseni, R., Saboora, A. Biochemical changes of *Rosmarinus officinalis* under salt stress. J Stress Physiol Biochem 2010; 6(3):114-22.
24. Kasparavičienė G, Ramanauskienė K, Savickas A, Velžienė S, Kalvėnienė Z, et al. Evaluation of total phenolic content and antioxidant activity of different *Rosmarinus officinalis* L. ethanolic extracts. Biologija. 2013; 59(1):39–44.
25. Luis JC, Johnson CB. Seasonal variations of rosmarinic and carnosic acids in rosemary extracts. Analysis of their *in vitro* antiradical activity. Span J Agric Res. 2005; 3(1):106-12.
26. Hozayn M, Qados AMSA. Magnetic water application for improving wheat (*Triticum aestivum* L.) crop production. Agric Biol J North Amer. 2010; 1:677-82.
27. Pang XF, Deng B. Investigation of changes in properties of water under the action of a magnetic field. Sci China Ser G Phys Mech Astro. 2008 Oct; 51:1621–32.
28. Phirke PS, Kubde AB, Umbarkar SP. The influence of magnetic field on plant growth. Seed Sci Technol. 1996; 24(2):b375-92.
29. Ferrer AE, Leite OG, Rocha JBT. Irrigation of *Solanum lycopersicum* L. with magnetically treated water increases antioxidant properties of its tomato fruits. Electromag Biol Med. 2012 Jan; 1-8.
30. Amira MS, Hozayn M. Magnetic water technology, a novel tool to increase growth, yield and chemical constituents of lentil (*Lens esculenta*) under greenhouse condition. J Agric Environ Sci. 2010; 7:457-62.
31. Goodman EM, Greenabaum B, Morron TM. Effects of electromagnetic fields on molecules and cells. Intern Rev Cytol. 1995; 158:279-325.
32. Atak C, Emiroglu O, Aklimanoglu S, Rzakoulieva A. Stimulation of regeneration by magnetic field in soybean (*Glycine max* L. Merrill) tissue cultures. J Cell Mol Biol 2003; 2:113-19.

33. Formicheva VM., Zaslavskii VA, Govorun RD, Danilov VT. Dynamics of RNA and protein synthesis in the cells of the root meristems of the pea, lentil and flax. Biophysics. 1992; 37:649-56.
34. Mohamed AI, Ebead BM. Effect of magnetic treated irrigation water on salt removal from a sandy soil and on the availability of certain nutrients. Int J Eng Appl Sci. 2013; 2(2):36-44.
35. Turker M, Temirci C, Battal P, Erez ME. The effects of an artificial and static magnetic field on plant growth, chlorophyll and phytohormone levels in maize and sunflower plants. Phyton-Ann Rei Bot. 2007; 46:271-84.
36. Avila Sosa R, Navarro Cruz AR, Vera López O, Dávila Márquez RM, Melgoza Palma N, et al. Romero (*Rosmarinus officinalis* L.), una revisión de sus usos no culinarios. Cienc Mar. 2011 Mar; XV(43):23-36.
37. Btissam R, Rajae R, Amina A, Brigitte V, Mohamed NHIRI. In vitro study of anti-glycation and radical scavenging activities of the essential oils of three plants from Morocco, *Origanum compactum*, *Rosmarinus officinalis* and *Pelargonium asperum*. Pharmacogn J. 2015 Mar/Apr; 7:124-35.
38. Rasoul A, Maryam HGK, Taghi GM, Taghi L, dehghan Rasle. Antioxidant activity of oral administration of *Rosmarinus officinalis* leaves extract on rat's hippocampus which exposed to 6-hydroxydopamine. Braz Arch Biol Technol. 2016 Jan; 59:e16150354.
39. Albayrak S, Aksoy A, Albayrak S, Sagdic O. *In vitro* antioxidant and antimicrobial activity of some Lamiaceae species. Iran J Sci Technol. 2013; 37:1-9.
40. Gómez-Estac, J, Bravo L, Gòmez-Guillén MC, Alémán A, Montero P. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. Food Chem. 2009 Jan; 112(1):18-25.
41. Teruel RM, Garrido DM, Espinosa CM, Linares BM. Effect of different format-solvent rosemary extracts (*Rosmarinus officinalis*) on frozen chicken nuggets quality. Food Chem. 2015 Sep; 172:40–6.