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QUALITATIVE EVALUATION OF MONOTERPENES IN *Rosmarinus officinalis* CULTIVATED WITH MAGNETICALLY TREATMENT WATER

Evaluación cualitativa de monoterpenos en *Rosmarinus officinalis* cultivados con agua tratada magnéticamente

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ABSTRACT. Lamiaceae (labiatae *labiadas)* family have many species for example *Rosmarinus officinalis* L., it is one of the species with the highest antioxidant power by the presence of essential oils, monoterpenes and phenolic compounds. Its vegetative propagation in Cuba is one of its difficulties and in recent years, this species has been removed from the National Formulary of Phytopharmaceuticals because of its availability. The magnetically treated water has been used for irrigation because it stimulates the growth and development of plants, showing that its application can activate metabolism of cells; however, studies have been very limited in these respects. The aim of this work was to evaluate monoterpen bioactive compounds present in the species *Rosmarinus officinalis* L., cultivated with magnetically treated water in Santiago de Cuba. N-hexane extracts were analysed by the method of thin layer chromatography of high-resolution (HPTLC) silica gel (fluka plates, foils analytical Alu thickness 0, 2 mm) with a solvent system of toluene and ethyl acetate. Monoterpen standard solutions as cineol, borneol, geraniol, linalool, citral, eucalyptol, citronellal were used and two types of developers vanillin in 10 % ethanol and a solution of sulphuric acid in ethanol, the observation was with white light. The results showed that hexane extracts from treated plants with 120 mT and control plants had the presence of monoterpens, and it was identified with retention factor (Rf) between 0,08 to 0,93.

Key words: chromatography, magnetic field, lamiaceae, medicinal plants

RESUMEN. La familia Lamiaceae (labiatae *labiadas),* está formada por numerosas especies entre ellas el *Rosmarinus officinalis* L. La misma constituye una de las especies con mayor poder antioxidante por la presencia de sus aceites esenciales, monoterpenos y compuestos fenólicos. Su propagación vegetativa en Cuba es una de sus dificultades y en estos momentos se encuentra retirada del Formulario Nacional de Fitofármacos, debido a la disponibilidad en el país. El agua tratada magnéticamente ha sido muy utilizada en el riego ya que estimula el crecimiento y desarrollo de las plantas, mostrando que su aplicación puede activar el metabolismo de las células; sin embargo, los estudios han sido muy limitados en estos aspectos. El objetivo del trabajo fue evaluar los compuestos bioactivos monoterpenos presentes en la especie *Rosmarinus officinalis* L., cultivadas con agua tratada magnéticamente en Santiago de Cuba. Los extractos n-hexano fueron analizados a través del método de cromatografía de capa fina de alta resolución (HPTLC), en sílica gel (placas Fluka, alufoils analítica espesor 0,2 mm), con sistema de solvente de tolueno y acetato de etilo, soluciones estándares de monoterpenos: cineol, borneol, geraniol, linalol, citral, eucaliptol, citronelal y dos tipos de reveladores la vainillina en etanol al 10 % y una solución de ácido sulfúrico en etanol, la observación fueron con luz blanca. Se obtuvo como resultado que los extractos de las plantas tratadas con 120 mT y las plantas control, se identificaron la presencia de monoterpenos con un factor de retención (Rf) entre 0,08-0,93.

Palabras clave: cromatografía, campo magnético, lamiaceae, plantas medicinales

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INTRODUCTION

Rosmarinus officinalis is among the plants used in traditional medicine, due to the presence of different compounds, such as phenolic compounds, rosmarinic acid, flavonoids, monoterpenes, carnosic acid, carnosol and rosmanol (1). Due to its high content of these metabolites, it has a great antioxidant activity and its extracts are excellent free radical (oxidative stress) kidnappers (2), or molecules that reacting with proteins, fats and nucleic acids can provoke changes causing heart diseases, cancer, Alzheimer's disease and premature aging.

Ecological conditions in different countries may influence plant phytochemical compounds, since they can be particularly accumulated as a result of their responses to environmental conditions (3). Thus, depending on the geographic location where plants grow under climatic conditions, soil, humidity and height above sea level, they lead to different changes in quantity and types of bioactive molecules; for example, rosemary varieties coming from Portugal are characterized by high amounts of myrcene, while the greatest concentrations of camphor and cineol are recorded in France and Morocco, respectively (4, 5).

In Cuba, this plant grows spontaneously and it exists in private gardens. Such species blooms sporadically, but when it does, its seeds have little viability. Likewise, its vegetative propagation is mostly difficult, when cuttings are rooting or when it manages to generate roots, its growth is very slow (6). Now, it is withdrawn from the national registration of phyto and apimedicaments, due to crop availability[A]; in recent years, this species belongs to the list of prioritized plants, so as to develop natural and traditional medicine in our country.

Now, the use of magnetic fields to stimulate plant growth has increased, on account of its lower detrimental influence on the environment. Water treated with static magnetic field in irrigation systems is highlighted among the methods employed, for its beneficial effects on plant production and yield. Although these studies are still very limited, it is stated that magnetic field biological effects depend on magnetic induction levels, exposure time, ion content, quality, volume and water flow, as well as temperature (7).

Magnetically treated water has been applied in agriculture as irrigation to improve plant growth, development and productivity (8). So far, studies have demonstrated the significant role of irrigating plants with magnetically treated water and it has also been recommended to save water (9, 10).

Other studies on this subject have shown that by applying magnetic fields with irrigation, cell metabolism may be activated. Researches performed on bean plants irrigated with magnetically treated water increased growth, gibberellin (GA3) concentration, kinetin and photosynthetic pigments (chlorophyll a, b and carotenoids), compared to plants watered without magnetic treatment (8). Some studies performed on chickpea plants irrigated with magnetically treated water induced a positive effect on its germination percentage and yields (11, 12). Other authors used 125 mT in *Glyxine max* germination and obtained rubisco increase (13). Regarding *Raphanus sativus* species, lipid synthesis was stimulated in chloroplasts, mitochondria and cell membrane when the magnetic field was applied at a flux density of 500 μT (14). Positive increases were also recorded in seed yield and lipid synthesis was stimulated in radish (14, 15).

The objective of this work was to evaluate the presence of monoterpene bioactive compounds in *Rosmarinus officinalis* L. species, irrigated with magnetically treated water in Santiago de Cuba, by means of high resolution thin layer chromatography (TLC/HPTLC) techniques.

MATERIALS AND METHODS

Plants were grown at the experimental plot from the National Center of Applied Electromagnetism (CNEA) in Santiago de Cuba following the proposed methodology (16). The species was placed in the herbarium from the Biodiversity and Ecosystem (BIOECO) Center and registered by No. 21324.

Vegetal material

Leaves from adult *Rosmarinus officinalis* L. species were used as starting material. Six-month-old plants 50 cm long were cultivated in beds at CNEA experimental plot.

Meteorological data were recorded during the research period: temperature of about 30 $^{o}C+2$; average relative humidity between 60 and 70 %; rainfall from 55 to 60 mm^3. The substrate was composed of organic matter and soil (1: 2), as well as different types of minerals.

[A] Buró nacional de farmacia MINSAP. *Formulario nacional fitofármacos y apifármacos*. Edit. Ciencias médicas, la Habana, 2010.

Soil physical properties were:

Organic matter: >4,3 %
Electrical conductivity: 271 µs cm^{-1}
pH: 6,8

Plants were irrigated twice a day through a microjet drip system for 30 minutes, using an itur pump and a delivery system controlled by valves that enable to apply water per section.

Water characteristics for irrigation were:

Water rate: 1,4-1,6 m/s
Pump flow: 2,54-2,91 m3 h-1
Electrical conductivity: 208 µs cm-1
Ph: 7.50

Soil and water were chemically analyzed and the following average values were obtained (Table I).

Magnetic treatment

An external magnetizer with permanent magnets was designed, built and calibrated at CNEA for each magnetic treatment. An external magnetizer with permanent magnets 10 cm long was used at a magnetic induction of 120 mT, which was measured by a Microwebermeter 192041, of a lower relative measurement error than 5 %. Sample size was of 40 plants per treatment.

The following treatments were studied:

- T1: control plants that were not irrigated with magnetically treated water.
- T2: plants irrigated with magnetically treated water at a magnetic induction of 120 mT.

Extracts were obtained with hexane solvent from *R. officinalis* L. leaves, which were collected and subjected to a drying process in an oven at 40 °C up to constant weight, using 3 g of them as the starting weight of each treatment in 100 ml of the solvent.

Successive extractions were carried out with hexane as solvent, aimed to achieve a greater exhaustion of the crushed dry vegetable material. The extraction was performed by Soxhlet equipment during four hours of continuous reflow. The extracted material was filtered and concentrated in a Buchi Rotoevaporator model 461 at a temperature of 40 ^{o}C, until reducing it to a volume of 10 ml to be later stored for further analysis.

Biochemical analysis

Photosynthetic pigments of *R. officinalis* L. were determined according to a methodology (17). Thus, 100 mg of fresh leaves were weighed and placed in a mortar with acetone at 80 % to be macerated and transferred through a Whatman filter paper (GF/A, 110 mm), obtaining a final solution volume of 20 ml.

Measurements were recorded through an UV-1602 spectrophotometer at wavelengths of 663, 646 and 470 nm, and acetone at 80 % as target.

Calculations were performed using the following formulae:

Chl a (µg ml^{-1} extract) = $12{,}21*A_{663}-2{,}81*A_{646}$

Chl b (µg ml^{-1} extract) = $20{,}13*A_{646}-5{,}03*A_{663}$

Carotenoids (µg ml^{-1} extract) = $1000*A_{470}-3.27*Chla-104*Chl bL$

Qualitative evaluation was done through high resolution thin layer chromatography (HPTLC) techniques in silica gel (Fluke plates, analytical alufoil thickness 0.2 mm). Two microliters of standard monoterpene solutions were applied to chromate plate: cineol, borneol, geraniol, linalol, citral, eucalyptol and citronellal, in addition to both previously prepared extracts. Toluene and ethyl acetate was solvent system (93:7); once the plate was developed, it was revealed by two types of solutions: vanillin in ethanol and the second ethanol solution with sulfuric acid; later on, the plate was heated at 110 °C for five minutes observed under white light (18).

Table I. Soil and water chemical characteristics at CNEA experimental plot in Santiago de Cuba

Mineral composition of soil	mg L^{-1}	Mineral composition of water	mg L^{-1}
Fe	28966,20	HCO_3^-	130,58
Ca	29178,00	PO_4	1,33
Na	15	Na	12,42
K	67	Ca	39,88
Mg	10866,00	Mg	8,88
Co	17,35	K	2,54
Ni	11,30	Na	11,31
Cu	114,72	CI	33,18
Zn	100,20	$S0_4$	31,20
		Total hardness	158,5

Experimental data obtained from photosynthetic pigment concentration were statistically processed by means of a single classification variance analysis for 95 % confidence interval and a t-Student test. Results were shown as mean values ± standard error.

RESULTS AND DISCUSSION

Although there were no statistically significant differences in photosynthetic pigment concentration, from the biological point of view, the highest values of chlorophyll$_a$ (1.70±0.04 mg L^{-1}), chlorophyll$_b$ (0,60±0,04 mg L^{-1}) and carotenes (0,52±0,04 mg L^{-1}) were observed in treated plants, whereas the lowest values of chlorophyll$_a$ (1,40±0,06 mg L^{-1}), chlorophyll$_b$ (0,33±0,03 mg L^{-1}) and carotenes (0.40±0.03 mg L^{-1}) were recorded in the control plant (Table II).

The qualitative evaluation of monoterpenes present in hexane extracts of *R. officinalis* L. irrigated with magnetically treated water and water without magnetic treatment is shown in the figure, besides monoterpene standards of cineol, borneol, geraniol, linalol, citral, eucalyptol and citronellal, in addition to extracts.

The study of bioactive compounds in extracts derived from plants irrigated with magnetically treated water and water without magnetic treatment showed the presence of some standard compounds in its composition and there was coincidence in linalol (0.38), citral (0.25) and citronellal (0.50) with the extracts from plants irrigated with magnetically treated water and water without magnetic treatment (Table III).

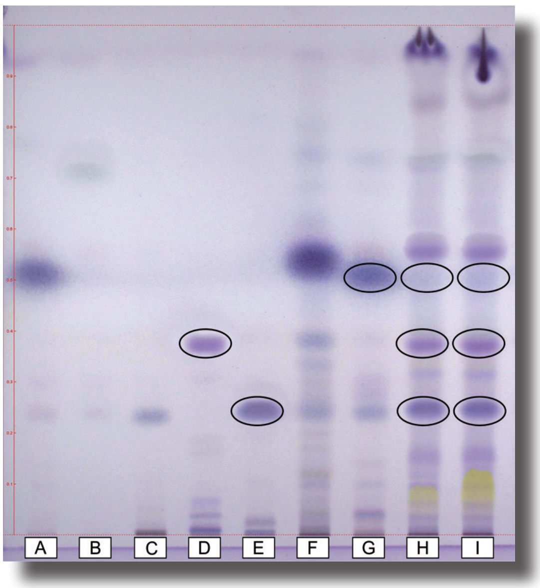
Results prove that some compounds present in our extracts are related to the chemical composition of essential oils in *Rosmarinus officinalis* species cultivated in Pakistan, which were determined by gas chromatography and mass spectrometry methods, where oxygenated monoterpene groups appear at 67 % and hydrogenated ones at 27 % (3).

As natural antioxidants are quite significant, there are some studies about different types of production and properties; however, their lack of diffusion and different experimental conditions lead to adjust certain extraction parameters as solvents as well as dosage, so that they can be fully used.

Table II. Effect of magnetically treated water on photosynthetic pigment concentration in *Rosmarinus officinalis* L. leaves (p≤0.05)

Treatments	Pigmentos fotosintéticos(mg L^{-1})		
	Chlorophyll $_a$	Chlorophyll$_b$	Carotenes
Control	1,40 ± 0,06	0,33± 0,03	0,40± 0,03
120 mT	1,70 ± 0,04	0,60 ± 0,04	0,52 ± 0,04

Mean±es (n=6)



A: cineole B: borneol C: geraniol D: linalol E: citral F: eucalyptol G: citronellal H: hexane extract of plants irrigated without magnetic field Control I: hexane extract of plants irrigated with magnetically treated water at 120 mT

Monoterpene profile identified by HPTLC in leaf extracts from *R. officinalis* irrigated with magnetically treated water

Table III. Retention factor (r_f) values of hexane extracts from *R. officinalis* L.

Compound	Rf
Cineol (A)	0,51
Borneol (B)	0,71
Geraniol (C)	0,23
Linalool (D)	0,38
Citral (E)	0,25
Eucalyptol(F)	0,53
Citronellal (G)	0,50
Hexane control extract (H),	(1) 0,03; (2) 0,15; (3) 0,25; (4) 0,38; (5)0,50; (6) 0,55; (7) 0,73; (8) 0,84
Treated hexane extract (I)	0,03; (2) 0,15; (3) 0,25; (4) 0,38; (5)0,50; (6) 0,55;(7) 0,73; (8) 0,84

In *Lamiaceae* family, the essential oil is produced in leaves by secretory structures, such as glandular, pelted and capitated trichomes. On this concern, several chemical compounds have been reported in *R. officinalis*, which have been generally grouped by several authors, who have identified the presence of α-pinene, β-pinene, camphene, terpene esters, such as 1,8-cineol, camphor, linalol, verbinol, carnosol, rosmanol, isorosmanol, 3-octanone, isobanyl-acetate and β-caryophyllene, as well as vanillyl, caffeic, chlorogenic, oleanolic, rosmarinic, carnosic, ursolic, butylenic, betulinic, betulin, α-amyrin, β-amirine, borneol, terpineol, linalol, citronellal and bornyl acetate acids (19, 20).

Studies conducted in *Melissa officinalis*, belonging to the same family as *R. officinalis*, reported similar compounds in its essential oils, such as linalol, citronellal and methylcitronelal, monoterpenes being the greatest amount of phytochemical composition (21).

Results showed that plants irrigated with magnetically treated water at an induction of 120 mT obtained better values of photosynthetic pigment concentration, compared with control plants. Likewise, the presence of linalol, citronellal and citral monoterpenes was proved in extracts from plants irrigated with magnetically treated water and control plants, which demonstrate that magnetic field did not vary any compound in both extracts and the absence of other standards in the plate could be due to the extracting method employed.

This study provided some knowledge about applying static magnetic field treated water and its influence on rosemary irrigation. Moreover, HPTLC method was used as a quick and economical tool for dry drug and natural extract analyses, which allow obtaining a better raw material in the field that can be used to manufacture natural antioxidant extracts.

CONCLUSIONS

This research work reached higher values of $chlorophyll_a$, $chlorophyll_b$ and carotenes compared to control plants. It was shown that *Rosmarinus officinalis* irrigated with magnetically treated water had the same compounds as plants without magnetic treatment. Through thin layer chromatography or high resolution thin layer chromatography (TLC/HPTLC) techniques, those substances found in natural extracts used as natural medicines or phyto-medicaments can be preliminarily and quickly authenticated.

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