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Antioxidant and Antimicrobial Effect of Extracts of *Rosmarinus Officinalis L.*, Cultivated in Puebla, Mexico

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Abstract: There is a growing interest in technological and therapeutic applications of bioactive compounds found in medicinal plants. *Rosmarinus officinalis* L. stands out in this area because of its antimicrobial, antioxidant, and antifungal properties. The metabolites of this plant are affected by environmental conditions and the extraction method. In the present study, the antioxidant (DPPH assay) and antimicrobial (inhibition halo) properties of extracts from non-polar to polar solvents (from hexane to water) were evaluated. Plants were grown at the Botanical Garden-BUAP in open areas. The extracts with the highest antioxidant potential concentration of 10 µg/mL were ethyl ether (72.71%), methanol (84.51%), ethanol (83.14%), and at 1.0 µg/mL water (45, 44%). The antimicrobial activity stood out against Gram-negative bacteria, being active in most of the extracts at a lower level than the control. Differently, against Gram-positive, only the polar extracts were active. Even with E. fecalis, a greater inhibition was shown than the control. The extracts of Rosmarinus officinalis L. cultivated in Mexico, populated, and extracted in polar and non-polar conditions present a relevant activity for traditional medicine.

Keywords: solvent extract; antimicrobial; antioxidant; DPPH; traditional medicine.

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1. Introduction

The trend to use plant bioactive compounds for therapeutic and technological purposes has boosted the exploration of various plant species [1,2]. Among focused plants that have generated great interest, it is necessary to mention rosemary (Rosmarinus officinalis L.), a green perennial shrub, a member of the Lamiaceae family, possessor of a characteristic smell and taste [3]. Rosemary is native to the Mediterranean and sub-Himalayan areas. However, it is widely distributed worldwide, except in humid or cold geographical areas [4,5]. Likewise, many uses and applications of this plant are recognized: i) as a condiment in food preparation, ii) in the manufacture of cosmetics and fragrances, and as a natural preservative, iii) for ornament purposes, and iv) in traditional medicine (used to treat inflammation, stomach aches, dysmenorrhea and pain) [6-8]. Currently, it is used as an alternative to drug therapies in diseases such as diabetes, cancer, and its anti-inflammatory, antifungal, hypoglycemic,

hypolipidemic, hepatoprotective, hypotensive, antimicrobial, and antioxidant properties [9-12].

On the other hand, it is known that the content and type of secondary metabolites present in this plant depend on its geographical origin; variation in climatic conditions favors the content of some metabolites, such as rosmarinic and carnosic acid [13,14]. Different fertilizers in soil induce the formation of different phenolics derivatives, flavonoids, and terpenes in its essential oil [15]. Other geographical factors, such as duration and intensity of daylight and water availability, are essential to maintain the production of photosynthetic metabolites [16,17].

Figure 1. *Rosmarinus officinalis L.* at University Botanical Garden-BUAP, Puebla, Mexico.

An important biological activity of rosemary is related to its di- and triterpenes, phenolic components. Carnosic acid, carnosol and rosmarinic acid are related to an antioxidant, antimicrobial and hepatoprotective activity [18,19] while carnosic acid, carnosol, rosmanol, rosmarinic acid and ursolic acid have anticancer activity [18]. The metabolite concentration from extracts depends on factors such as the source of the tissue, age of the plant, weather and stress conditions, geographic area, and type of soil [20, 21]. This plant is widely used in cooking and traditional medicine in Mexico, mainly for its antimicrobial properties. This manuscript explores its antioxidant activity derived from the polarity of the used solvent in the extraction process of *Rosmarinus officinalis* L. cultivated in Puebla, Mexico (Figure 1).

2. Materials and Methods

2.1. Identification and recollection.

A specimen of *Rosmarinus officinalis* L. cultivated in the University Botanical Garden of the Benemerita Universidad Autonoma de Puebla, identified as "Romero" (herbarium identifier 20060002 A), was used in this study (Figure 1). The plant grew 2140 m above sea level, at 22 °C on average, in open areas. The collection was carried out from 8:00 to 10:00 am local time, in batches of 100 grams of leaves for each extraction, cutting in the mature region of the plant and preserving new shoots to allow the development of the plant.

2.2. Extraction.

For batches of 100 g of fresh Rosemary leaves, differential extraction was carried out in Soxhlet equipment. Extracts started using hexane, then increasing the polarity of the solvent each 3 h. Solvents (dielectric constant, D) were hexane (1.9), ethyl ether (4.3), ethyl acetate (6.0), dichloromethane (9.1), acetone (20.6), ethanol (22.4), methanol (32.6), and water (79.7)

[22]. A stock solution of each extract of 1.0 g in 10 ml was prepared, and dilutions of 1:10, 1:100, and 1:1000 were prepared for subsequent studies.

2.3. Antioxidant activity.

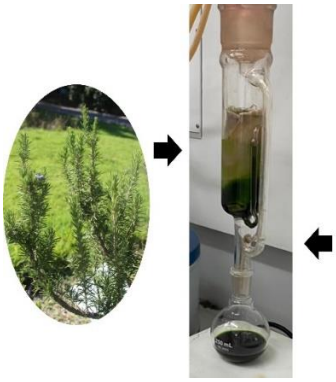
The antioxidant activity was tested through the inhibitory potential of DPPH radical oxidation evaluated according to the reported methodology [23,24], using 2.7 mL of the extract and 0.3 mL of the standard DPPH solution. Experiments were carried out at 515 nm at 60 min, and the inhibition value (% inhibition) was calculated through (%inh=(ABlank-AExtract)/ABlank).

2.4. Antimicrobial activity.

The antimicrobial susceptibility test was used to determine the minimal concentration (in µg/mL) of antimicrobial agent to inhibit or kill bacteria [25,26] by means of 10.0 µL of each extract, besides 3 bacteria Gram-positive: *Staphylococcus aureus* (ATCC25923), *Staphylococus saprophyticus* (ATCC19701), *Enterococus faecalis* (NCTC775), and 3 Gram-negative: *Escherichia coli* (ISSTE hospital), *salmonella typhimurium* (ISSTE hospital), *Pseudomona aeruginosa* (NCIB8295). The impregnated disks are placed on an inoculated Luria-Bertani Agar (LB) plate. Inoculated with each bacterium, a control disc of penicillin (10 mg) was incubated at 37°C for 48 h, and the disk diffusion zone diameter was subsequently determined.

3. Results and Discussion

The use of rosemary in traditional medicine and cooking depends on the process of manipulating the vegetable material; this ranges from tinctures in ethyl alcohol, extractions, or aqueous decoctions to oily extractions in vegetable fats. Given its reported potential, it is important to study different solvent properties, the most important being the antimicrobial activity, as well as the antioxidant, these two related to their medicinal uses for washing wounds and their antioxidant properties related to their food applications, so it is necessary to study these two properties based on the polarity of the extraction solvent used (Figure 2).



Solvent	*Gram positive bacteria*	*Gram negative bacteria*	*Antioxidant activity*
Hexene		✓	✓
Ethyl ether			
Ethyl acetate			
Dicloromethane		✓	
Acetone	✓	✓	✓
Ethanol		✓	✓
Methanol	✓		✓
Water		✓	

Figure 2. Extraction process; antioxidant and antimicrobial activity.

3.1. Extraction.

The extraction process was carried out in soxhlet equipment in a different way, using an increase in the polarity of the solvent from hexane (μ_D=1.9) to water (μ_D=79.7) with a total of 8 solvents to emulate the conditions extremes of polarity to which rosemary is traditionally

subjected. In Table 1, you can see the extraction percentages obtained in each solvent, highlighting that the extreme values are where the greatest extraction takes place, by hexane and ethyl ether in the solvents: non-polar and water in the polar, but values less than 6% indicating that most of the content is non-extractable fiber, that is, primary metabolites, indicating that the biological potential is at low concentrations.

Table 1. Solvent extraction percentage used by differential extraction in Soxhlet.

Solvent	Dielectric constant (D)	Extract %
Hexene	1.9	3.87 ± 0.51
Ethyl ether	4.3	5.21 ± 0.57
Ethyl acetate	6.0	1.44 ± 0.18
Dichloromethane	9.1	0.81 ± 0.09
Acetone	20.6	2.80 ± 0.38
Ethanol	22.4	0.80 ± 0.12
Methanol	32.6	1.30 ± 0.09
Water	79.7	4.63 ± 1.17

3.2. Antioxidant potential.

An important biological activity is the antioxidant one since excess oxidizing species in the body have a great damaging effect on the organism; for this reason, new alternatives for the consumption of antioxidants are constantly sought in nutraceuticals, although these depend largely on the extraction process and the polarity of the solvent used. In Figure 3, we can see the values obtained for each of the extracts and the control, which used resorcinol at 5.0 µg/mL, finding a value of around 50%. The extracts were standardized at 10.0 µg/mL, finding high values for almost all the extracts. It was even necessary to dilute the water extract 10 times to obtain a quantifiable value since at a dose of 10.0 µg/mL, and the value would correspond higher to 100 %.

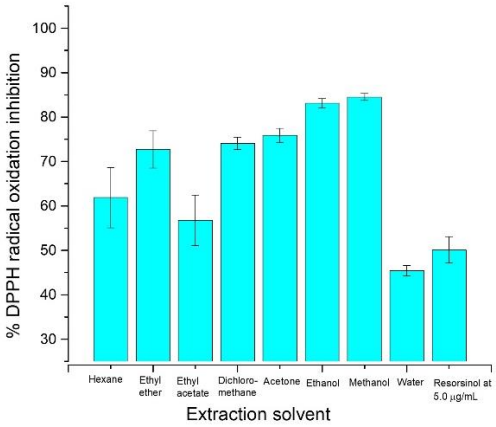


Figure 3. Antioxidant capacities by DPPH technique at 10.0 µg/mL and water at 1.0 µg/mL.

On the other hand, in the other solvents, we find values higher than 50% at a concentration of 10.0 µg/mL, mainly the ethereal extract can be highlighted, which had an extraction as well as a value higher than 70%, corresponding to the processes of oily extraction that rosemary is subjected to. In the same way, high values are obtained in polar solvents. An ANOVA statistical study was carried out with a Tukey test ($p < 0.05$) comparing extract by extract to determine if any extract is better. In Figure 4, we can observe this test, denoting that ethyl ether, methanol, ethanol, and water are the extracts with greater potential and statistical differences. However, it is worth considering that although no significant difference was observed between these solvents, the water is diluted by a factor of 10.

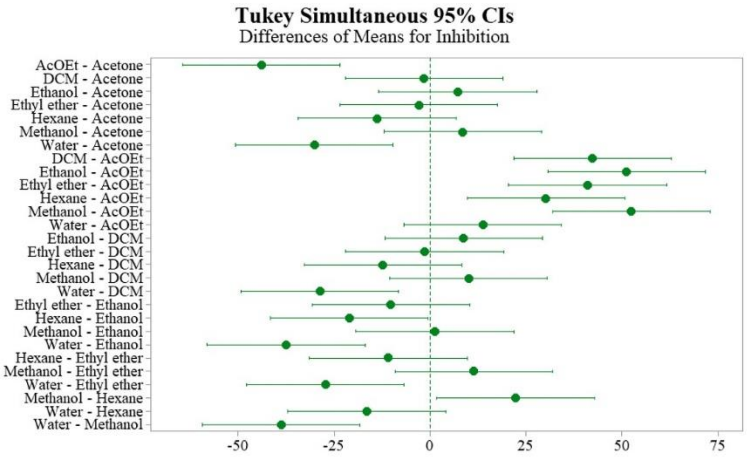


Figure 4. Antioxidant statistical analysis by Anova $p < 0.05$ post-Tukey.

3.3. Antimicrobial activity.

In contrast, antimicrobial activity has been attributed to the use of rosemary, particularly in bacteria associated with environmentally acquired infections. The results obtained were classified according to their response to the Gram test. In Gram-positive bacteria, two staphylococci and one enterococcus (Table 2), highly polar solvent extracts exhibited more biological activity. For *S. aureus* and *S. saprophyticus*, the hexane extract presented activity up to the concentration of 1.0 µg/mL, with less activity in *S. aureus* water extract, which had less activity than the hexane extract. While in *S. saprophyticus* the aqueous extract had twice the inhibitory effect than hexane, although in both cases lower than penicillin. Due to an extract being a complex mixture and not an isolated structure, so lower values are expected; however, against *S.* aureus, the ethanolic extract presents (10.0 µg/mL) an inhibition halo of the same order as penicillin, that is, alcoholic tinctures have potential against infections of this species. With respect to *E. fecalis*, only the polar solvents show biological activity since penicillin is not very effective against this bacterium; these extracts have great potential as they present an inhibitory effect superior to control.

Table 2. Halo inhibition (mm) at Gram-positive bacteria.

Bacteria	***S. aureus***			***S. saprophyticus***			***E. fecalis***		
Solvent	**10.0 µg/mL**	**1.0 µg/mL**	**0.1 µg/mL**	**10.0 µg/mL**	**1.0 µg/mL**	**0.1 µg/mL**	**10.0 µg/mL**	**1.0 µg/mL**	**0.1 µg/mL**
Hexene	1.2	0.7	-	1.5	0.5	-	-	-	-
Ethyl ether	0.7	0.4	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-	-	-
Dichloromethane	-	-	-	-	-	-	-	-	-
Acetone	1.1	-	-	-	-	-	-	-	-
Ethanol	2.5	1.3	0.6	-	-	-	0.6	-	-
Methanol	0.7	-	-	-	-	-	0.4	-	-
Water	0.5	0.3	-	2.9	1.1	0.7	0.7	-	-
Penicilin (10 mg)	2.6			6.1			0.1		

(-) means activity absence

4. Conclusions

Rosmarinus officinalis L has great pharmacological potential; its contents of metabolites depend on the cultivation conditions and the extraction method, including temperature and solvent. For this work, rosemary was grown in Puebla, Mexico, under special care provided by the University Botanical Garden-BUAP, but in open areas. Two important activities were studied and demonstrated as antioxidants and antimicrobial. For the first

activity, extracts stood out at a concentration of 10 µg/mL: ethyl ether (72.71%), methanol (84.51%), ethanol (83.14%), and particularly for water (44%) using a diluted solution of 1.0 µg/mL. For antimicrobial activity in Gram-negative tests, most of the extracts showed activity contrary to tests in Gram-positive essays. Extracts from polar solvents showed greater activity in *E. fecalis*, with values higher than the control. The above results reaffirm the pharmacological potential of *Rosmarinus officinalis* L, on bacteria that commonly affect humans.

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Conflicts of Interest

The authors declare no conflict of interest.

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