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Rosmarinus officinalis extract, complementary testing for quality control as active ingredient for formulations

Extracto de *Rosmarinus officinalis* y sus pruebas complementarias para el control de calidad como principio activo para formulaciones

ORTA-MARTINEZ, Felipe†´, RAMOS-GONZALEZ, Elsy Janeth´´, GARAY-HERNANDEZ, María del Carmen´* and MARTÍNEZ-ORTIZ, Rosa María´´´

´ *Universidad Autónoma de Zacatecas, Área de Ciencias de la Salud, Unidad Académica de Ciencias Químicas. México.*
´´ *Universidad Autónoma de Zacatecas, Unidad Académica de Preparatoria. México.*
´´´ *Universidad Autónoma de Zacatecas, Área de Ciencias de la Salud, Unidad Académica de Odontología, México.*

ID 1st Author: *Felipe, Orta-Martinez* / **ORC ID:** 0009-0007-4118-7106

ID 1st Co-Author: *Elsy Janeth, Ramos-Gonzalez* / **ORC ID:** 0000-0002-0572-3211

ID 2nd Co-Author: *Maria del Carmen, Garay-Hernández* / **ORC ID:** 0009-0003-0798-9958

ID 3rd Co-Author: *Rosa Maria, Martinez-Ortiz* / **ORC ID:** 0000-0001-7811-169X

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Abstract

Today contemporary alternative medicine continues to increase significantly around the world. Taking the above into account and given the need for new pharmaceutical formulations, techniques for the extraction of bioactive compounds from plants have been developed, such is the case of the extract of rosemary (*Rosmarinus officinalis*). The purpose of this work is to evaluate, through official and unofficial tests from the Pharmacopeia of the United Mexican States, a rosemary extract tablet. The extract was obtained by maceration in methanol and a simple distillation. Subsequently, using the direct compression method, prepare tablets and then evaluate them and determine the identity, disintegration, dissolution, titration and dose uniformity tests established by the Pharmacopeia of the United Mexican States (from spanish Farmacopea de los Estados Unidos Mexicanos, FEUM) for this type of pharmaceutical presentations. The results show that the tablets comply with the determinations of the pharmacopeial tests, which indicates that the tablets prepared in this work are of good quality, from a manufacturing point of view and can pass the validation process, so they could be used in the clinic.

Rosemary extract, *Rosmarinus officinalis*, Unofficial tests from FEUM

Resumen

La medicina alternativa contemporánea hoy en día sigue aumentando significativamente en todo el mundo. Tomando en cuenta lo anterior y ante la necesidad de nuevas formulaciones farmacéuticas, se han desarrollado técnicas de extracción de compuestos bioactivos de plantas, tal es el caso del extracto de romero (*Rosmarinus officinalis*). El propósito de este trabajo es evaluar, mediante pruebas oficiales y no oficiales de la Farmacopea de los Estados Unidos Mexicanos, un comprimido del extracto de romero. El extracto fue obtenido mediante maceración en metanol y una destilación simple. Posteriormente, utilizando el método de compresión directa, elaborar tabletas para después evaluarles y determinarles los ensayos de identidad, desintegración, disolución, valoración y uniformidad de dosis que establece la Farmacopea de los Estados Unidos Mexicanos (FEUM) para este tipo de presentaciones farmacéuticas. Los resultados muestran que los comprimidos cumplen con las determinaciones de los ensayos farmacopeicos, lo que indica que las tabletas elaboradas en este trabajo son de buena calidad, desde un punto de vista de manufactura y pueden pasar el proceso de validación, por lo que pudieran ser utilizadas en la clínica.

Extracto de Romero, *Rosmarinus officinalis*, Pruebas no oficiales de FEUM

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* Author Correspondence (Email: mcgarayh@uaz.edu.mx)
† Researcher contributing as first author.

Introduction

Plants have been used for thousands of years in many parts of the world for their nutritional and medicinal properties (Simental J et al., 1999). In recent years, the effect on health of potential bioactive compounds present in plants has been studied and it is possible to assure that there is more information on their functional, medicinal and/or toxicological properties (Shahidi F et al., 1992).

Rosemary (*Rosmarinus officinalis*) is a plant rich in active principles and with action on almost all organs of the human body. Having a high content of essential oils, whose active ingredients are flavonoids, phenolic acids and bitter principles, it generates a tonic and stimulating action on the nervous system, circulatory system and heart. In addition to being choleretic, cholagogue, antispasmodic, diuretic, emmenagogue and antigodanotropic (Musa OM et al., 2008). In studies carried out at the Autonomous University of Zacatecas, where the total extract of rosemary leaves was obtained and used in murine models with liver damage. It was observed that this extract regenerates hepatic cells, suggesting that rosemary acts as a hepatic antioxidant (Gutiérrez-Hernández R, 2010). In recent years, a large number of studies have been developed that provide information on the applications of rosemary beyond its culinary uses. The objective of this work was to provide rosemary extract with a pharmaceutical tablet presentation to subsequently perform evaluations to determine the official and unofficial (or complementary) tests established by the United Mexican States Pharmacopoeia for tablets, thus guaranteeing a good quality tablet.

Methodology

The study method used was cross-sectional and longitudinal. The rosemary was donated by the Academic Unit of Agronomy of the Autonomous University of Zacatecas, where an expert botanist certified the authenticity of Rosmarinus officinalis, two collections of the plant were made (Bruneton J. 2001 and González et al., 2006).

Preparation of the total rosemary extract

The preparation of the extract was performed using the technique described by Vajira (Vajira PB, 2013). From the dried rosemary leaves, the extract was obtained. Briefly, the leaves were pulverized to a fine powder, using an Oster brand blade mill. Subsequently, the corresponding extraction was carried out by maceration with methanol, where the powder was suspended in methanol and heated between 60 and 70 ºC for 2 hours. This mixture was filtered under vacuum repeatedly until all possible residue was removed from the powdered leaves. To remove the green pigments, activated charcoal was added and filtered again under vacuum until a light brown liquid was obtained. This methanol solution was concentrated to a final minimum volume by a simple distillation process for 20 to 30 minutes. A creamy yellow precipitate was produced, which was filtered and allowed to dry for 20 to 30 minutes in an oven at 40 ºC and a creamy yellow powder (rosemary extract) was obtained, which was reserved for further studies (Bruneton J. 2002).

Quality control tests of rosemary extract (as active ingredient)

Organoleptic properties

Appearance. This was carried out by direct observation under the microscope, in which the structure and morphology of the crystals that make up the extract were described in detail.

Color. The color was observed visually, which was determined based on the color of the rosemary extract plant. Odor. The odor was determined directly, being characteristic to the Rosmarinus officinalis plant (Lema, 2013).

Physical properties. Determination of refractive index and relative density. An aqueous solution of rosemary extract containing 50 mg in 50 mL of water was prepared, directly previously calibrated, calibrated refractometer and relative density with a pycnometer (FEUM, 2011).

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Determination of the refractive index. From an aqueous solution containing 1 mg of rosemary extract per mL, a drop was placed in the previously calibrated Abbé refractometer and the refractive index was measured, where the value was obtained directly from the reading of the equipment (Alonso, 2004; Lema, 2013). Determination of relative density. An empty, clean and dry 25 mL pycnometer was weighed, and the weight was recorded taking all the decimals marked by the analytical balance, then it was filled with distilled water and the weight was recorded in the same way, finally the same pycnometer was filled again but now with an aqueous solution of rosemary extract at a concentration of 1 mg/ mL and the weight was recorded and then the density was calculated (FEUM, 2011; Lema, 2013).

Results

Once the rosemary plant donated by the Agronomy Academic Unit of the Autonomous University of Zacatecas was collected, the leaves were separated from the stem and then dried in the shade for 2 and a half months. After this time, the rosemary (Rosmarinus officinalis) extract was obtained as described above.

Quality control tests of rosemary extract as PA. Rosemary extract

The extractions were performed with methanol, making some modifications to adapt it to the conditions of the laboratory (Industrial Pharmacy Laboratory) where the extractions were performed. Ten extractions were performed with different amounts of ground leaf in each extraction, where the amount of extract obtained is proportional to the amount of leaf (Table 1).

Extractions	Sheet (g)	Excerpt (g)	% Performance
1	50	2	4
2	60	2.7	4.5
3	60	2.4	4
4	70	2.9	4.14
5	60	2.2	3.66
6	70	3	4.2
7	78	2.9	3.71
8	110	3.7	3.36
9	60	2.5	4.33
10	60	2.6	4.33
x̄	67.8	2.69	4.023
S	16.7185	0.4771	0.3545
CV	24.6585	17.7360	8.8118
x̄ = average, S=standard deviation, CV= coefficient of variation.			

Table 1 Rosemary extracts and percent yield of each extract

It was found that there is no correlation between percent yield and leaves or extract. However, between ground leaves and amount of extract obtained there is a very significant correlation with a p-value of 0.000000200 and a correlation coefficient of 0.908 as shown by Pearson's correlation (figure 1).

Organoleptic properties

Appearance, color and odor. Table 2 shows the results of the visual comparison between the extracts, showing similarities between them.

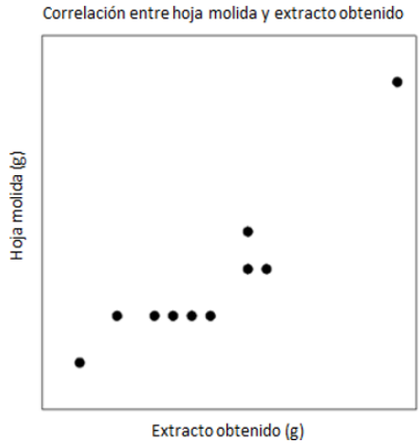

Figure 1 Correlation between ground leaf and extract. The increase is observed as the amount of leaf used increases

Organoleptic properties of rosemary extract (appearance, color and odor).

	Appearance	color	Odor
1	Amorphous crystals	Creamy yellow	Characteristic of the plant from which the extract was obtained
2	Amorphous crystals	Creamy yellow	Characteristic of the plant from which the extract was obtained
3	Amorphous crystals	Creamy yellow	Characteristic of the plant from which the extract was obtained
4	Amorphous crystals	Creamy yellow	Characteristic of the plant from which the extract was obtained
5	Amorphous crystals	Creamy yellow	Characteristic of the plant from which the extract was obtained
6	Amorphous crystals	Creamy yellow	Characteristic of the plant from which the extract was obtained
7	Amorphous crystals	Creamy yellow	Characteristic of the plant from which the extract was obtained
8	Amorphous crystals	Creamy yellow	Characteristic of the plant from which the extract was obtained

Table 2

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Appearance. They are creamy yellow amorphous crystals, they also show a slight agglomeration between them, which resembles small lumps of rosemary extract (Figure 2).

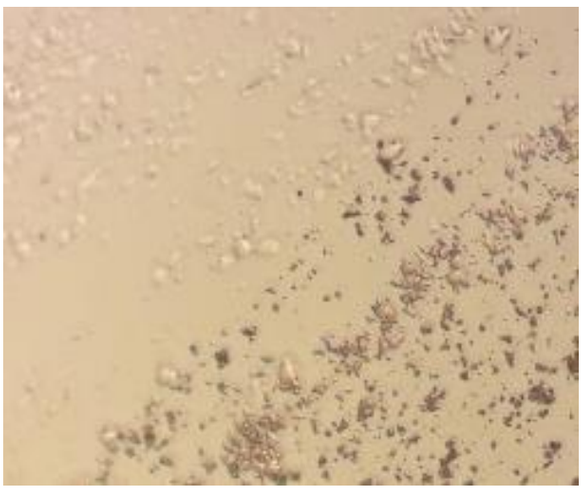

Figure 2 Microscopic view of rosemary extract

Color. A creamy yellow color is observed, characteristic of the dye that the plant presents in the extract (figure 3).

Figure 3 Rosemary extract showing a creamy yellow coloration

Odor. The rosemary extract presents a characteristic odor of the plant from which it was obtained (Rosmarinus Officinalis).

Physical tests (refractive index and relative density). For these determinations, a solution containing 1 mg/mL of rosemary extract in water was prepared. The refractive index was 1.325 ± 0.006, while the relative density was 1.25 ± 0.02 (Table 3).

Excerpt	Refractive index	Relative density
1	1.327	1.26
2	1.308	1.26
3	1.327	1.27
4	1.327	1.24
5	1.326	1.26
6	1.327	1.26
7	1.327	1.25
8	1.326	1.24
9	1.328	1.26
10	1.327	1.20
ẋ	1.325	1.25
S	0.006	0.02
CV	0.4528	1.6
ẋ = average, S=standard deviation, CV= coefficient of variation.		

Table 3 Physical properties of rosemary extract, refractive index and relative density

Conclusions

The method used to obtain an extract of Rosemary (Rosmarinus officinalis) of good quality is adequate. This extract can be used as an active ingredient for the preparation of tablets. The Rosmarinus officinalis extract has the desired characteristics and/or physical and organoleptic properties ensuring that this extract meets the requirements and acceptance criteria established by the FEUM.

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