PharmacologyOnLine

Newsletter • 2021 • vol.1 • 49-59

DEVELOPMENT OF AN ANTIOXIDANT COSMETIC USING AS AN ACTIVE ESSENTIAL OIL OF *Rosa spp.*

León Mendez, D.[1*]; Perez Pomares, T.[1]; De la Espriella Angarita, S.[2]; Granados Conde, C.[2]; Leon Mendez, G.[3]

[1]Corporación Universitaria Rafael Núñez, Programa de Tecnología en Estética y Cosmetología, GITEC, Cartagena, Colombia.

[2]University of Cartagena, Faculty of Engineering, Research in Engineering, Innovation, Quality Food and Health Group (INCAS).

[3] Fundacion Universitaria Tecnologico Comfenalco, Faculty of Engineering, Research Group CIPTEC, Cartagena, Bolívar, Colombia.

*deisy.leon@cumvirtual.edu.co

Abstract

Introduction: In particular, the rose is an economically important crop as a source of essential oils for the perfumery and fragrance industry. The production of its essential oil has generated interest for its multiple compounds which are able to eliminate free radicals. Objectives: To develop an antioxidant cosmetic using *Rosa spp* essential oil as an active. Methods: The essential oil was obtained by hydrodistillation assisted by microwave radiation, from the roses, later the antioxidant activity by the technique of anti-radical activity by the DPPH and ABTS. + method. Next, the development of a gel-type cosmetic was carried out. At the time of preparing the formulation, organoleptic, rheological and chemical characteristics were evaluated; and the in-vitro antioxidant activity of each finished product was determined; similarly, sensory evaluation was performed on each product with a view to evaluating its acceptability by a panel of experts. Results: It was found that rose essential oil demonstrated high antioxidant power, according to the techniques used. Subsequently, a gel-type cosmetic was developed using the essential oi as an active ingredient, and the same antioxidant activity tests were performed on the essential oil, observing that this activity is preserved in the final product; the results of this work contribute to the development of stable and functional cosmetic formulations using fruit pulps as cosmetic actives. Conclusions: The antioxidant gel from *Rosa spp* essential oil presented promising results; likewise, the physical and chemical indicators evaluated remained stable during the 150 days of study.

Keywords: *Antioxidant activity; chemical composition; design; natural products;.*

Introduction

In Colombia the cultivation of the Rose is of great importance due to the amount of area dedicated. The high demand for this flower in international markets and the excellent quality of rose produced in the country, largely define the competitiveness of Colombian floriculture [1,2].

The genus Rosa belongs to the Rosaceae family and consists of more than 100 species, divided into four subgenera: Hulthemia Dumort (Focke), Rosa Focke, Hesperhodos Cockerell and Platyrhodon (Hurst) Rehder. In total, 95% of the species are found in the subgenus Rosa, which is divided into 10 sections [1,3].

In particular, the rose is an economically transcendent crop as a source of essential oils for the perfumery and fragrance industry. The production of its essential oil has generated interest for its multiple compounds such as acyclic monoterpene alcohols (citronellol, geraniol and nerol), as well as aromatic alcohol, phenyl ethyl alcohol and long-chain hydrocarbons [1].

Some components of rose essential oil are able to eliminate free radicals, it is important to note that free radicals can cause oxidative stress on the skin, which, in turn, can generate premature aging of the skin. It should be noted that free radicals, also known as reactive oxygen species (ROS), are very reactive oxygen molecules: the byproduct of biochemical reactions existing in the body. These begin to damage cell membranes, proteins and DNA in a process known as oxidative stress4-7. Among the damaged cells are those responsible for the production of substances such as collagen, elastin and hyaluronic acid, which give young skin its firm structure. The production of this substance is naturally reduced as we age and oxidative stress accelerates this process [8,9].

While aging is an all-natural process, oxidative stress can cause the skin to age prematurely: it can begin to develop visible signs of aging, such as fine lines and wrinkles, and to denote a dull, tired appearance ahead of time. Therefore, the proper use of cosmetics with natural active ingredients has become a global trend, preferring every day more effective and respectful products with the care of the Environment. As described above, the objective of this research was to develop an antioxidant using *Rosa ssp* as an active ingredient.

Methods

Collection of plant material

The roses (*Rosas spp*), were collected in the city of Cartagena (10°25′25"N 75°31′31"W) and those that were fresh, whole, without signs of deterioration were selected. They were immediately chopped, weighed and processed.

Extraction of Essential Oil.

The EO was obtained by the method: microwave assisted hydrodistillation. For that, it was used a hydrodistillation equipment with 4 liters of capacity. 500 g of plant material were taken, then they were introduced into the extraction flask, which contained 500 mL of distilled water, the extraction time was 3-4 hours. As a source of microwave radiation, a conventional modified oven brand Samsung was used, with a 1-cycle irradiation of 60 minutes and a power of 70% [10-12]. The EO was collected in a DeanSteark type vessel.

Determination of the major components of EO by gas chromatography-mass spectrometry (GC / MS).

An Agilent 7890A / 5975C chromatograph was used. Each EO sample (50 µL) was dissolved in 450 µL dichloromethane, the injector temperature was 250°C, a HP-5MS 5% Phenyl Methyl Silox capillary column was used; Helium was used as carrier gas at constant flow rate of 1 mL / min, pressure of 7.6354 psi and linear velocity of 36 cm / sec. Initial temperature 45°C and transfer line temperature 280°C. Mass spectra were obtained by electron ionization (70 eV), with automatic scanning at a range of m / z 30-400 u.m., at 3.85 scan / s. The components identities were assigned by comparison of each spectrum with the database standards reported in the literature [13].

DPPH• radical method

Free radical scavenging activity DPPH was determined using the method described by Silva et al. [14] (with some modifications 75 µL of sample were added to 150 µL of a methanol solution of DPPH (100 ppm) and they were incubated at room temperature for 30 min, after the disappearance of the DPPH radical was determined spectrophotometrically at 405 mm in microplate reader Multiskan Ex (Thermoscientific®, USA). The percentage of inhibition (% Inh) was calculated using equation (1).

$$\%\ \text{inhibition} = \frac{(A_0 - A_f)}{A_0} * 100 \text{ (Equation 1)}$$

Where Ao and Af are the target absorbance values (DPPH solution in alcohol) and the sample (DPPH solution plus antioxidant dissolved in ethanol), respectively.

ABTS•+ radical method

The free radical scavenging activity ABTS was determined using the method described by Re *et al.* [15] with some modifications. The ABTS radical was formed following the reaction of 3.5 mM ABTS with 1.25 mM of potassium persulfate (final concentration). The samples were incubated at 5°C and in darkness for 16 h. Once the ABTS radical was formed, it was diluted with ethanol until having an absorbance of 0.7 ± 0.05 at 734 nm. To a volume of 190 µL of the ABTS radical dilution was added 10 µL of the sample under study and incubated at room temperature for 5 minutes. After this time, the disappearance of the ABTS radical at 734 nm was determined spectrophotometrically in the microplate reader Multiskan Ex (Thermoscientific®, USA).

Design of a cosmetics with antioxidant activity.

A preformulation study was carried out to determine that there were no incompatibilities between the active ingredient and the formulation auxiliaries, which affected the stability of the final product. This was done by reviewing the technical data sheets of each raw material to verify possible interactions between the components and take the necessary measures Table 1 [16].

To ensure that the formulation maintains its organoleptic characteristics, as well as its physical and chemical characteristics such as pH, viscosity and antioxidant activity in-vitro over time, the control of the same was carried out, at the time of preparing the formulation and at different times from its elaboration (0, 15, 30, 60, 90 and 150 days).

Determination of pH

10 g of the gel was taken with constant stirring to a moderate rat for 5 minutes, to which the pH was determined using a previously calibrated potentiometer[16].

Viscosity determination

The apparent viscosity of the gel was measured at 25°C in a Brookfield viscometer (United States) until the reading stabilized[16].

Sensory Analysis.

The samples kept were studied for its sensory characteristics. Hedonic scale ratings were used to evaluate the sensory attributes of the formulation cosmetics by expert panel members. The measure of the degree of acceptance of the product was obtained by the use of the
hedonic scale. Panelists were asked to their degree of likes or dislikes in terms of which best describes their perception about the product. The term may

be given numerical values to enable the results to be scored [17-19].

Statistical Analysis.

All trials were performed by sextupled. The results were expressed as the mean ± SD (standard deviation). Significant differences were determined by ANOVA analysis followed by Dunnett's or Tukey's test or as deemed appropriate.

Results

The efficiency of extracting *rosas spp* essential oil is presented in Figure 1.

Table 2 presents the chemical composition of *Rosas spp* essential oil which was carried out by gas chromatography/mass spectrometry (CG/EM).

The biggest challenge is to achieve an effective gel-like cosmetic design that achieves permeation through the skin without presenting harmful effects [22,23]. Therefore, in the design of a gel it is essential to choose the formulation that presents sensory, physicochemical and rheological characteristics ideal for topical administration, that is, with appropriate pH, viscosity, permeability, extensibility and texture. It is important to ensure that the cosmetic product is easy to use and aesthetically acceptable to the user [22].

Figure 2 and 3 show the results of physicochemical parameters such as pH and viscosity of the gel-type cosmetic using rose essential oil as an active ingredient.

Sensory evaluation is the standardized analysis of the organoleptic properties of a product, which is performed with the sense organs. It is usually called "standardized" in order to reduce the subjectivity that the evaluation can give through the senses, they are evaluations carried out in conditions similar to those of use of the product and are necessary in some areas where there are criteria or characteristics that cannot be measured with the instruments that exist. In cosmetics, properties such as softness, emollience, texture, ease of application, etc. are analyzed. For the present study, this type of test was carried out to determine the acceptability of the products developed by potential customers or users [19].

The results of the sensory evaluation of the developed product are shown below (Figure 4).

Figure 5 shows the results of the antioxidant capacity of the gel-type cosmetic using rose essential oil as an active ingredient through $DPPH^{\bullet}$ and $ABTS^{\bullet+}$.

Discussion

The result obtained in the extraction kinetics shows a final yield by the MWHD method of (0.666%). Similarly, the microwave-assisted hydrodistillation technique uses less extraction time, being attributed mainly to the breaking of the "vacuole" plant structures by means of applied electromagnetic radiation, allowing the extraction of the main components of greater abundance in the essential oil.

According to the scientific literature "microwave-assisted hydrodistillation uses three forms of heat transfer within the sample: irradiation, conduction, and convection. As a result, it produces heat more quickly inside and outside the glands [19,21].

Baydar [22], identified that particularly rose petals generally contain very little essential oil compared to other oilseeds, so the yield of rose oil is approximately 0.03 – 0.04%.

Noriega et al,[1] evaluated the yield of 21 essential oil samples from rose varieties by hydrodistillation; the varieties of roses belonged to the groups of Shrub, Bourbon, Damascena, Damask perpetual, Floribunda, Gallica, Grandiflora, Perpetual Hybrid, Mossy and Climbing, obtaining a yield between 0.005 and 0.072%. It is important to note that the variation in the yield of the essential oil can be due to various factors such as geobotanical, genetic and environmental [1].

Rose essential oil has a high content of monoterpenes; highlighting the presence of limonene and α-pinene. Noriega et al,[1] found as major compounds of the essential oils of the rose varieties identified by GC/MS geraniol, citronellol, nerol and phenyl ethyl alcohol.

Baser and Arslan[23] identified as compounds of the essential oil of roses citronellol (31-44%), geraniol (9 – 15 %), nonadecan (8–15 %), nerol (5–11 %), 1-nonadecene (2–5%), methyl eugenol (2–4 %), heneicosan (3–4 %), geranium acetate, phenylethyl alcohol, β-caryophyllene, citronellyl acetate, germacreno D and linalool in the range of 1 – 2 % each.

The antioxidant activity of EOs and fruit pulps was evaluated by three different methods: DPPH• and ABTS•+. Antioxidants can act by multiple mechanisms, depending on the reaction system or the radical or oxidant source used [25]. The results are expressed as anti-radical activity or IC_{50} (Effective Concentration 50), which is defined as "the concentration of the antioxidant that decreases the absorption of the radical to 50% of the initial amount". There is an inversely proportional relationship that indicates that the higher the value of the IC_{50}, the lower the anti-radical activity [24].

These major compounds define the biological activity of the essential oil, where not a single one can be framed as responsible for it, which explains the antioxidant activity found in the gel.

The stability of pharmaceutical products depends on environmental factors and factors related to the product, among which the physico-chemical properties, the pharmaceutical form and its composition stand out.[26-28]

These make it possible to ensure that the physical and chemical properties remain constant over a period of time. According to the results obtained, the antioxidant gel elaborated maintains its physicochemical characteristics (pH) unchanged for a time of 150 days, except for the viscosity that showed significant differences from day three compared to day 0, observing that the viscosity decreases over time, this behavior can be attributed to a change in the molecular conformation of the product, in fact, in other studies this characteristic has also been evidenced. [17,29,30]

It is important to keep in mind that, in preparations for application to the skin, the pH should be between 4.5-8.5 so that no irritation and damage to the skin occurs.[26,27] The pH of the antioxidant gel remained in a range of 7.94-8.0 which is within the recommended values. By performing a detailed analysis of the sensory evaluation, we can indicate that there is a direct relationship between the variables evaluated, evidencing an adequate extensibility and adaptability to the surface and skin cavities.

The cosmetic developed using as an active 5% rose essential oil has an excellent antioxidant activity and this is mainly due to the fact that rose oil contains many antioxidants among which are some monoterpenes.

Acknowledgments

The authors thank the Corporación Universitaria Rafael Nuñez, University of Cartagena and Fundación Universitaria Tecnologico Comfenalco for providing space, resources, and time for researchers

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Table 1. Proposed formulation for the design of the gel (%).

Component	Formulation (%)
Acrylate copolymer	1
Glycerin	5
Triethanolamine	1
Preserver	0,1
Active (Rose essential oil)	5
Water	c.s.p. 100%

Table 2. Majority components detected in the OE of Roses spp obtained through the microwave assisted hydrodistillation method (MWHD)

Component	% Relative abundance (rt, min)*
	Rosas spp
α-pinene	**0,48 (11,40)**
Limonene	**41,22 (11,68)**
Phenethyl alcohol	**58,3 (16,33)**

*Retention time (rt) and relative abundance (%) of essential oils, identified by comparison with reference mass spectrum of the NIST database - 2008.

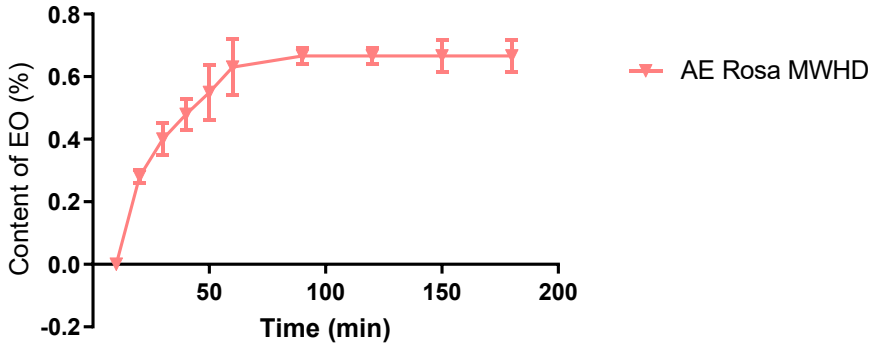

Figure 1. Extraction kinetics of the EO obtained through the microwave-assisted hydrodistillation method (MWHD)

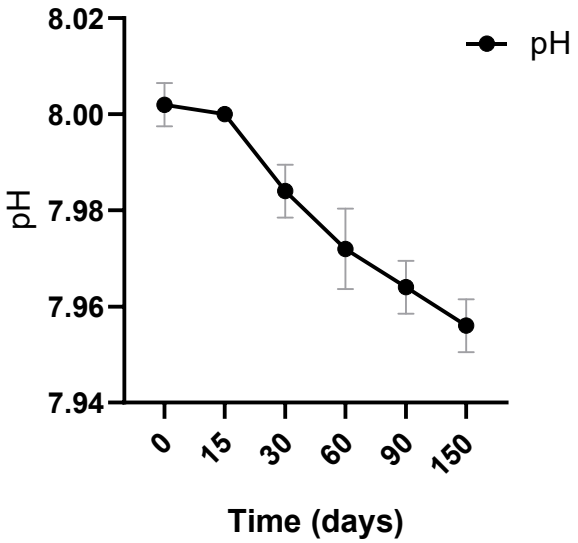

Figure 2. Variation of the pH of the gel-type cosmetic using rose essential oil as an active ingredient over time.

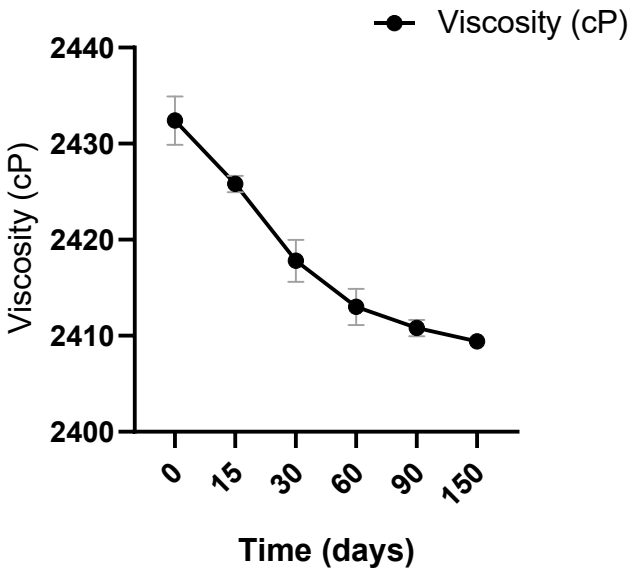

Figure 3. Variation of the viscosity of the gel-type cosmetic using rose essential oil as an active ingredient over time.

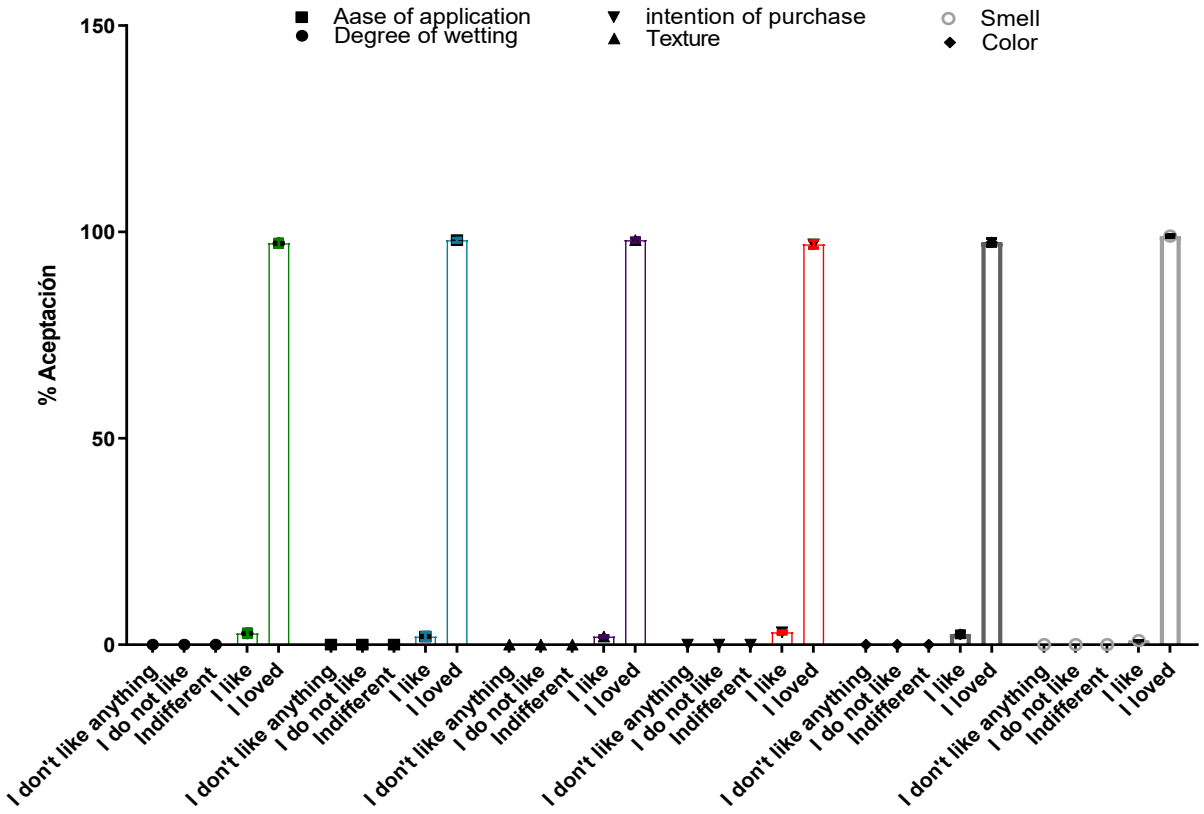

Figure 4. Percentage of acceptance by the panelists.

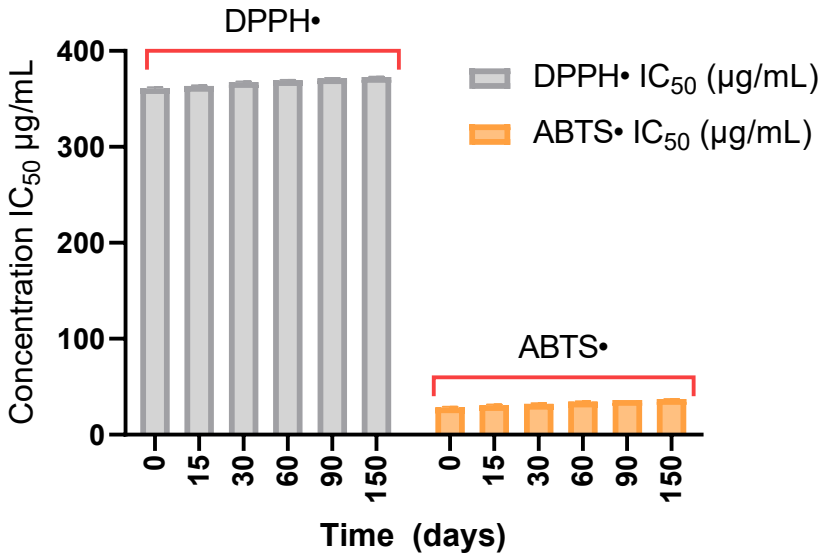

Figure 5. Antioxidant capacity of the gel type cosmetic using rose essential oil as an active ingredient over time.