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DEVELOPMENT OF AN ANTIOXIDANT COSMETIC USING *Bactris guineensis* PULP AS AN ACTIVE

León Mendez, D.[1*]; De la Espriella Angarita, S.[2]; Leon Mendez, G.[1,3], Pacheco-Galván, S.[1], González-Cardona, M.[1], Tapia-Carmona, Y.[1], Misas-Castro, M.[1], Granados-Conde, C.[2]

[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@curnvirtual.edu.co

Abstract

Introduction: The current relationship between the consumption of fruits with high levels of antioxidant compounds and disease prevention has been demonstrated. Among the antioxidant properties of many fruits highlights the ability to regulate alterations related to oxidative stress induced by reactive oxygen species (ERO) and free Radicals (RL), so have gained the interest of Scientists for their potential internal and external use. **Objective:** Develop an antioxidant using bactris guineensis pulp as an active ingredient. **Methods:** To the pulp obtained from the fruit of corozo; they were determined the antioxidant activity by the technique of antiradicalaria activity by the method DPPH and ABTS. + and the content of total phenols was performed by the method colorimetric Folin-Ciocalteu. Later the development was carried out a phytocosmetic type emulsion fluid. At the time of elaborating each one of the formulations, it was evaluated organoleptic, rheological and chemical characteristics; and the in-vitro antioxidant activity of each finished product was determined; Likewise, sensory evaluation was carried out on each product with a view to evaluating its acceptability by an expert panel. **Results:** It was found that the corozo pulp showed high antioxidant power, according to the techniques used. Subsequently, the development of a phytocosmetic type emulsion o/w fluid, non-greasy feeling, with pulp of corozo, and the same tests of antioxidant activity practiced to the pulps were carried out, observing Whereas such activity is retained in the final product; The results of this work contribute to the development of stable and functional cosmetic formulations using fruit pulps as cosmetic assets. **Conclusions:** The pulp of the corozo is promising to develop phytocosmetics, due to its high antioxidant activity and its high content of total phenols. It should be pointed out that the products designed, using as an active corozo pulp, showed good stability and acceptability on the part of the evaluation panel.

Keywords: *Phytocosmetic, antioxidant activity, fruits*

Introduction

The flora in Colombia is so varied so it is indicated that it is the second country in the world with the greatest richness of plants [1], it is estimated that of the 300 thousand species of plants in the world, more than 40 thousand of them are in Colombia; a little more than 13%, currently having 1,500 endemic plant species [2,3]. Some of these plant species have active ingredients with potential biological activity, with broad prospects to carry out research and development of new pharmaceutical products and natural cosmetics.

Among the typical species of the Andean region, one of the most relevant from the food field is the corozo (*Bactris guineensis*), which is a species of plant of the palm family (*Arecaceae*) [3], distributed geographically from Nicaragua to Colombia and Venezuela.

The fruits almost spherical, 1.5-2 cm in diameter, purple almost black; mesocarp juicy, whitish, bittersweet; flattened endocarp3. The color of the corozo is due to the presence of anthocyanins, which were isolated and identified by countercurrent chromatography and HPLC (high-pressure liquid chromatography) preparation, where the compounds cyanidin-3-rutinoside and cyanidin-3-glucoside are the main components (87.9%) and other pigments in small amounts [4,5].

Polyphenols are secondary plant metabolites that are characterized by the presence of phenolic rings with one or more hydroxylated groups including functional derivatives that are capable of removing reactive oxygen species (ROS), among which are hydrogen peroxide (H_2O_2), superoxide ion (O_2^-) and hydroxyl radical (OH^-) thanks to their ability to donate electrons, generating stable phenoxyl radicals [6,7]. These metabolic properties have brought an increase in the development of research around neurodegenerative diseases, such as cancer, cardiovascular diseases, diabetes, neurodegenerative diseases and premature aging [7-10].

Skin aging is an irreversible biological process, which depends on a series of complex factors, both endogenous and exogenous (inclement weather, environmental pollution, cold, heat, ultraviolet light)[11]. 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 bactris guineensis as an active pulp.

Methods

Collection of plant material

The corozo fruits (*Bactris guineensis*), were collected in the city of Cartagena (10°25′25″N 75°31′31″W). The fruits were selected taking into account that they were free of external damage and presented commercial maturity; they were washed and scalded at 90°C for 5 minutes. The pulps were obtained by mesh refiner 1.5mm aperture; they were packed in airtight bags and then refrigerated to a temperature of 4°C[12].

Bromatological characterization of the corozo

The chemical characterization of the corozo was carried out, to which the content of nutrients and micronutrients was determined by means of the tests described below. For protein, the Kjeldahl method was used according to AOAC 955.04; ash was determined by the direct method according to AOAC 924.05; moisture; by means of the drying method at 100±2 °C according to AOAC 925.0910; fiber; by the gravimetric enzymatic method[13]; carbohydrates[14]; fat; by the Soxhlet method according to AOAC 936.15 and vitamin C.

Determination of total phenols

The total phenol content was determined by the Folin-Ciocalteu colorimetric method. A mixture of phosphowolfroic and phosphomolybdic acids in basic medium was used as a reagent, which is reduced by oxidizing phenolic compounds, resulting in blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}). Consequently, a standard curve was initially constructed using as standard gallic acid between 50-500 µg / mL. Subsequently, the pulp extract from each fruit was diluted to a concentration at which the phenol content would be within the range of the standard curve. The results were expressed as mg of gallic acid / 250 mL of sample. Absorbance readings were performed at 760 nm on a Thermo Scientific ™ GENESYS 10S visible UV spectrophotometer [13].

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DPPH• radical method

DPPH• free radical scavenging activity was determined using the method described by Silva et *al.* [16], with some modifications. 75 µl of sample were added to 150 µL of a methanolic solution of DPPH$^{\bullet}$ (100 µg / mL) and incubated at room temperature for 30 min, after which the disappearance of the DPPH$^{\bullet}$ radical was determined spectrophotometrically at 550 nm in reader of Microplates Multiskan Ex (Thermoscientific). The percentage of inhibition (% Inh) was calculated using equation (1).

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

Where A_0 and A_f are the target absorbance values (DPPH solution in alcohol) and the sample (DPPH solution plus antioxidant dissolved in ethanol), respectively.

ABTS$^{\bullet+}$ radical method

The ABTS free radical scavenger activity was determined using the method described by Re et al. [17], with some modifications. The ABTS radical was formed following the reaction of ABTS 3.5mM with 1.25 mM potassium persulfate (final concentration). Therefore, the samples were incubated at 2-8 ° C and in the dark for 16-24h. Once the $ABTS^{+}$ radical was formed, it was diluted with ethanol until an absorbance of 0.7 ± 0.05 at 734 nm was obtained. To a volume of 190 µL of the dilution of the $ABTS^{+}$ radical was added 10 µL of the sample under study and incubated at room temperature for 5 minutes, after which time the disappearance of the $ABTS^{+}$ radical was determined spectrophotometrically 734 nm in the Multiskan Ex microplate reader (Thermoscientific).

Preformulation study

In order to determine that there are no incompatibilities between the active ingredient and the auxiliaries formulation which affect the stability of the fine product, a preformulation study was carried out. This was done by reviewing the technical data sheets of each raw material, to verify possible interactions between the components and to take the necessary measures (Table 1) [18-20].

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[19].

Viscosity determination

The apparent viscosity of the gel was measured at 25°C in a Brookfield viscometer (United States) until the reading stabilized[19].

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 [19,20].

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 quality of a fruit includes in addition to its physical characteristics – size, weight, color and texture – its nutritional content (Table 2). In the results (Table 2) it can be seen that the pulp of Bactris guineensis evaluated has a high moisture content (80.22±0.20%), Vitamin C (240.2± 0.45) and ashes of (2.3±0.30%), the latter parameter evidences a high mineral content.

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The water content in food, the molecular shape and its location within the food product are factors that significantly affect specific characteristics such as appearance, texture, color, etc. [21]. All foods contain water to a greater or lesser extent. Water content figures vary between 60 and 95% in natural foods. In plant and animal tissues, there are two general forms: free water and bound water. Free or absorbed water, which is the predominant form, is released very easily and is estimated in most methods used for the calculation of water content (moisture).

It should be noted that the differences between the moisture values according to different authors may be due to the degree of maturity in which the products were evaluated, since as the degree of maturity increases, the amount of water in the fruit or vegetable decreases; to the variety used, since they report it as the species in general; to the method used, etc. [21]

The lipid content does not usually exceed 1g/100g in fruits. The lipid fraction of fruits comprises acylglycerides, glycolipids, phospholipids, carotenoids, triterpenoids and waxes [21].

Dietary fiber or dietary fiber is made up of soluble fiber (pectins) and insoluble fiber (cellulose) in a proportion that varies according to the vegetable [22]. The soluble fraction of fiber, that is, pectins, are mainly found in the skin of fruits, and the organism being unable to break its bond, have no caloric value, so it can be used in the control of obesity, in addition to decreasing the glycemic response. In addition, it decreases the levels of LDL and total cholesterol, with the consequent preventive effect of cardiovascular alterations. On the other hand, they are also attributed purifying properties, by eliminating toxins [21].

Fruits contain 0.1-1.5% nitrogen compounds, of which proteins constitute 35-75% in fruits; amino acids are also well represented. The fraction of soluble nitrogen compounds consists on average of 50% free amino acids. All other nitrogenous compounds are quite scarce. It should also be noted that most of the protein fraction, which is subject to major changes depending on the type of fruit and its degree of maturity, is made up of enzymes [21].

Figure 1 shows the results of the antioxidant capacity of the crown pulp by $DPPH^{\bullet}$ and $ABTS^{\bullet +}$.

The biggest challenge is to achieve an effective emulsion-like cosmetic design that achieves permeation through the skin without presenting harmful effects [23,24]. Therefore, in the design of an emulsion 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 [23].

Figure 2 and 3 show the results of physicochemical parameters such as pH and viscosity of the emulsion-type cosmetic using corozo fruit extract 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 [20].

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 emulsion-type cosmetic using as an active corozo fruit extract using $DPPH^{\bullet}$ and $ABTS^{\bullet +}$.

Discussion

Plants are considered photosynthetic organisms that are exposed to very oxidative environments (solar radiation, oxygen, among others), so they have a very effective antioxidant system. Likewise, various metabolites have been reported, especially phenolic substances such as flavonoids, tannins and other substances that act synergistically to enhance the antioxidant effect such as tocopherols, catechins, organic acids and carotenoids [15]. Antioxidants can act by multiple mechanisms, depending on the reaction system or the radical or oxidant source used.

The phenolic compounds quantified in fruit extracts are of great importance because they constitute a group of secondary metabolites that are considered natural antioxidants with multiple biological benefits for humans, such as the prevention of cardiovascular and degenerative diseases. In fruits it has been found that the main compounds present are, for the most part, phenolic acids, flavonoids and tannins, however, phytochemicals such as vitamin C (ascorbic acid), folic acid (vitamin B) and β-carotenes (provitamin A) have also been found, which allows to establish that the consumption of fruits increases the intake of bioactive compounds with multiple properties for human health.

It is important to note that there are several internal and external factors that affect the quality and/or quantity of phenolic compounds in plants, such as genetic diversity (variety and origin of the sample), stage of maturity, environmental variables (light intensity, climate, temperature, use of fertilizers, wounds), method of extraction, processing and storage [15]. The corozo presented an excellent antioxidant activity. The IC_{50} values for the $DPPH^{\bullet}$ and $ABTS^{\bullet}$ assay found for the corozo were 75.76 µg/mL and 40.06 µg/mL respectively. The antioxidant activity of these fruit and vegetable products can be related to the content of total phenols [25].

It is very important to know that an antioxidant cosmetic is the synergism of many actives, which have different mechanisms of reducing action in their interactions with reactive oxygen species (ROS) or other radicals [26,27]. The measure of the antioxidant capacity of cosmetics has had a lot of relevance in recent years, due to the quality of the information that can be obtained; from the resistance to oxidation, the quantitative contribution of antioxidant substances and the antioxidant activity produced by natural products in the body at the time of application [28,29].

The results evidenced in Figures 2 and 3 indicate that the emulsion using as an active 5% of proposed corozo fruit extract remained stable, that is, all the physico-chemical indicators evaluated remained within the acceptance limits established in each one.

It is important to keep in mind that, in the preparations of application on the skin, the pH should be between 4.5-8.5, so that irritation and damage to the skin does not occur. The pH of the emulsion remained in a range of 8.2-7.97, which is within the recommended values, the above guarantees that the emulsion does not cause irritation at the time of application and, in addition, constitutes an indicator of physical stability. In the rheological analysis, a characteristic behavior was observed in this semi-solid pharmaceutical form, obtaining values between 5882 and 5908 cP. In addition, it was determined that the formulations correspond to a non-Newtonian fluid with positive thixotropy. Rheological properties should provide the preparation with adequate extensibility and adaptability to the surface and skin cavities.

It is important to highlight that antioxidant actives in the cosmetic industry have become a global trend for the creation of anti-wrinkle treatments. They can be derived from natural or synthetically manufactured resources [18-20]. Its key performance criteria are to counteract free radicals that can cause skin cells to mutate. Antioxidants can be supplied as water- or oil-soluble ingredients, although oil-derived or lipid-soluble ingredients can be absorbed more effectively in the stratum corneum. Antioxidants can help in several functions, including: reducing fine lines and wrinkles, supporting cell regeneration and stimulating collagen production [18-20].

With the evaluation of the physicochemical and antioxidant properties, the mechanical-structural stability of the emulsion could be demonstrated using 5% of corozo fruit extract as an active ingredient, which is an indication of its physical stability for 150 days.

The pulp of *B. guineensis* (corozo) is considered a promising ingredient for designing phytocosmetic products due to its high antioxidant activity and its high content of total phenols. The antioxidant emulsion using corozo as an active ingredient presented promising results; likewise, the physical and chemical indicators evaluated remained stable, all of the above contributes to the development of functional cosmetic formulations using Colombian fruit pulps.

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Table 1. Proposed formulation for emulsion design (%).

Component	Formulation (%)
Glyceryl stearate, PEG-100 stearate	10
Glycerin	5
Stearyl alcohol	2
Isopropyl myristate	5
Preserver	0,1
Corozo extract	5
Water	c.s.p. 100%

Table 2. Results of bromatological analyses of corozo pulp.

Fruit	Fiber %	Ash %	Fat content %	Protein %	Moisture %	Carbohydrates %	Total phenols (mg AG/100mg pulp)	Vitamin C (mg)
Corozo	1.25±0.30	2.3±0.30	0.11±0.22	1.15±0.50	80.22±0.20	16.22±0.15	81.4±0.25	240.2± 0.45

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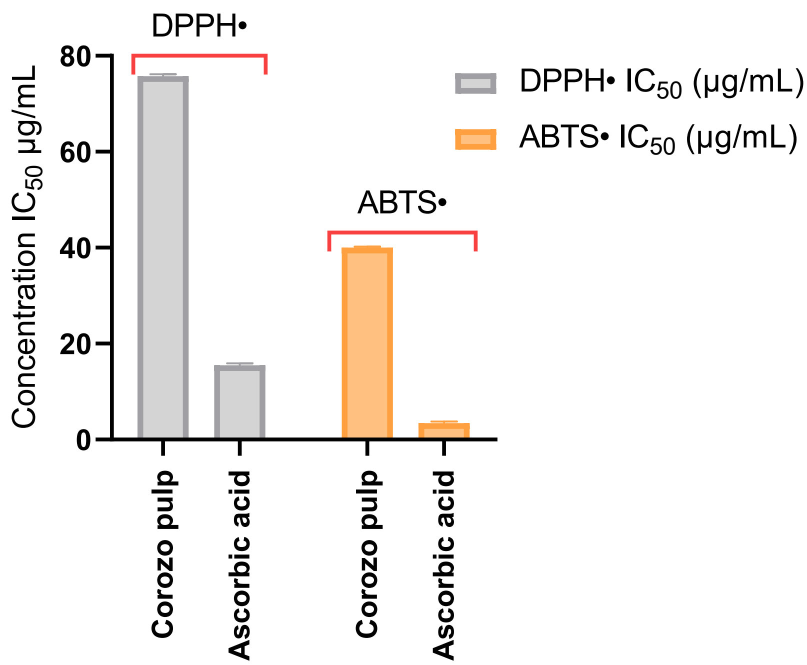

Figure 1. Antioxidant capacity of corozo pulp by DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ methods

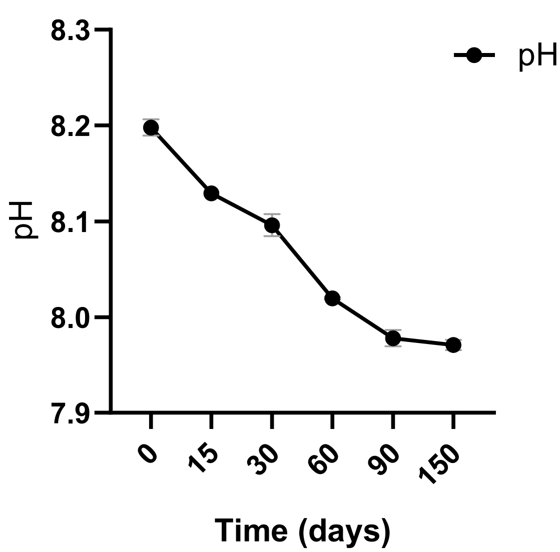

Figure 2. Variation of the pH of the emulsion type cosmetic of the corozo pulp over time.

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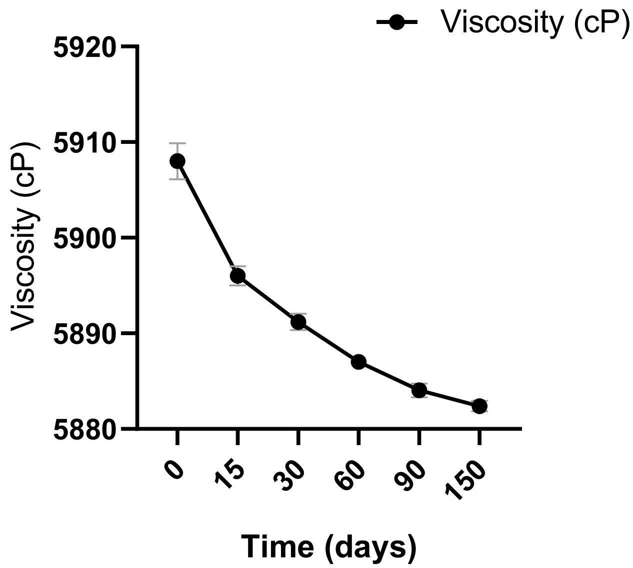

Figure 3. Variation of the viscosity of the emulsion type cosmetic of the corozo pulp over time.

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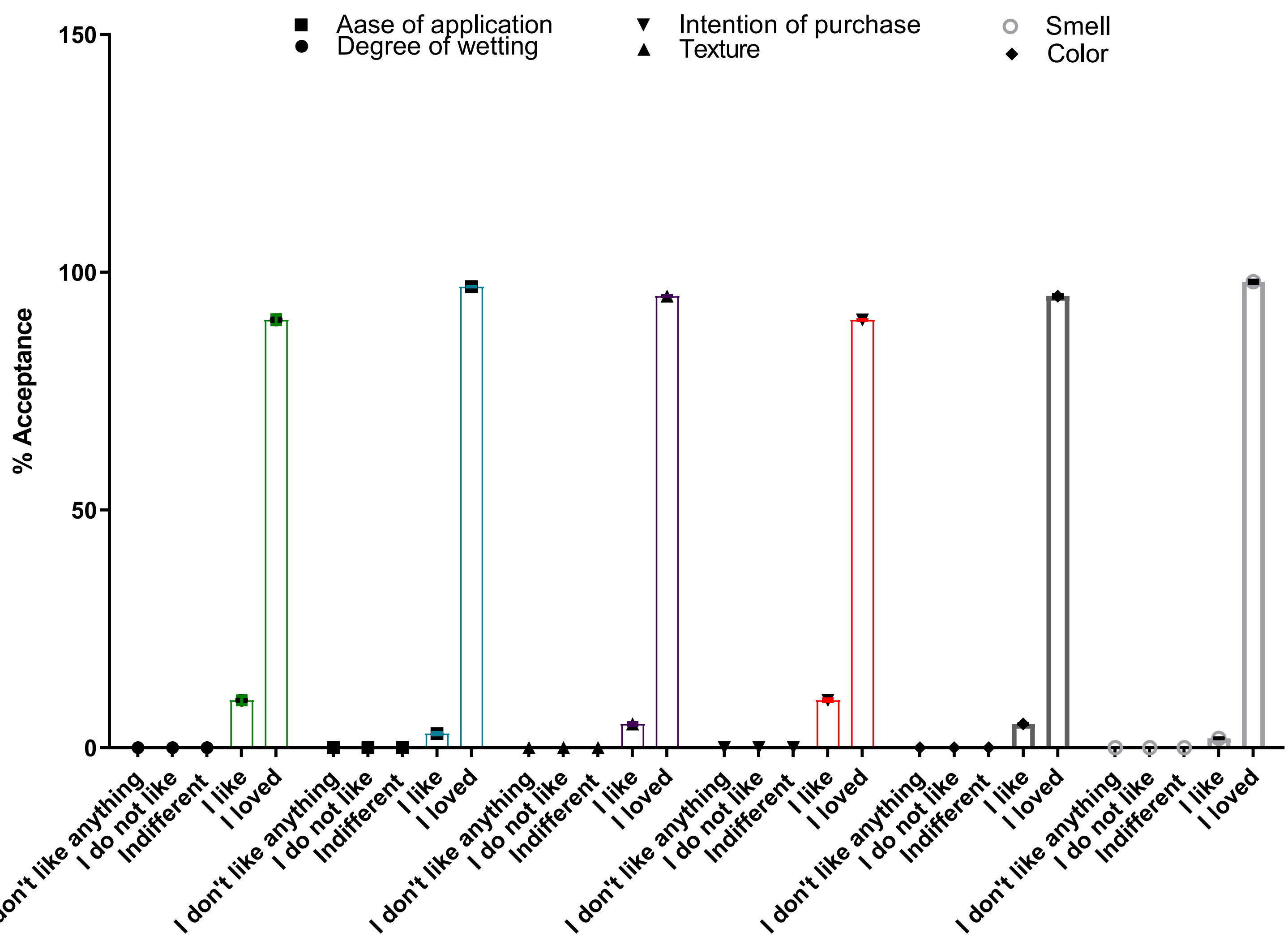

Figure 4. Percentage of acceptance by the panelists.

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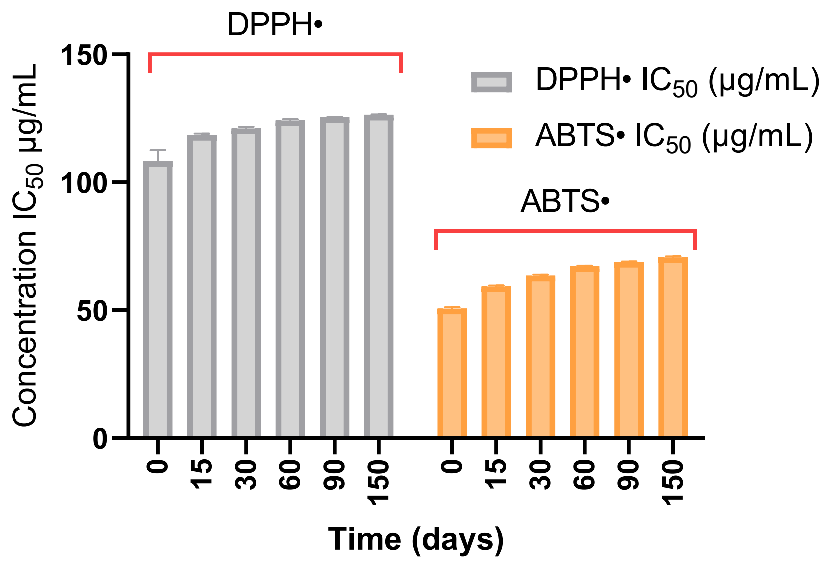

Figure 5. Antioxidant capacity of cosmetic type emulsion of corozo pulp.

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