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# Formulation of an Antioxidant Cosmetic Cream Containing *Coffea arabica* Fractions

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***Abstract*****—***Coffee is a very popular drink, which is traditionally used to supplement meals and for hedonistic purposes beyond of its functional and health properties. The coffee provides a multitude of bioactive by-products that can be used for the formulation of dermocosmetics. In this context, this work aimed to develop an emulsion using extracts of Coffea arabica as well as evaluate its physicochemical stability and antioxidant activity. Two formulations were developed with incorporation of different fractions of green seeds from Coffea arabica. Then, both formulations were submitted to extremes temperatures (5 and 45°C) for 15 days, and pH, electrical conductivity, organoleptic characteristics and presence of viable microorganisms were determined. Total phenolic content was estimated by Folin-Ciocalteu method. Afterwards, fractions and cream were tested by 2,2-diphenyl-1-picrylhydrazyl (•DPPH) radical scavenging assay. The formulation 2 showed no instability for all parameters evaluated. The hexane, glycolic, methanol, chloroform and ethyl acetate fractions, and cream at 150 µg/mL deactivated •DPPH radical at 24.7, 51.8, 86.2, 89.5, 90.9 and 79.8%, respectively. Furthermore, the emulsion kept its antioxidant activity even under accelerate tests. The results confirmed the stability of cream containing coffee fractions, suggesting its use as natural antioxidant cosmetic cream and further experiments to evaluate its photo protective potential.*

***Keywords*****—** ***coffee; cosmetics; antioxidant; cosmetic cream; stability test.***

## I. INTRODUCTION

According to International Coffee Organization data (ICO, 2016), in the period from 1996 to 2015, Brazil was the world's largest coffee producer (varying from 20.8% to 39.4% of the world production) and the third world consumer (after the European Union and the USA), but second if considering the individual countries of the European Union. In Brazil, between 2016/2017, there was a total production of 49,640 thousand bags of 60 kg and a total domestic consumption of 20,500 thousand bags of 60 kg of coffee. The State of Bahia has played an important role in the production of Arabica coffee and is the fourth largest producer in Brazil, contributing to Brazilian position as largest producer and exporter in the last three decades. Compared to other producing states, Bahia has been standing out by employing high technology and obtaining excellent productivity rates (Bliska *et al*., 2009; Frederico, 2012).

Besides to be a drink, the search for alternatives to coffee has established several by-products such as coffee oils (Tango, 1971), which attracts attention from cosmetic industry as a source of bioactive products (Esquivel, Jiménez, 2012). Since they are rich in phytosterols and essential fatty acids, coffee oils are rapidly absorbed and have good adhesion in cutaneous applications, whose properties go beyond dermal emollients (Wagemaker *et al*., 2012).

The major unsaturated fatty acids present in coffee oils are linoleic acid and palmitic acid (Wagemaker *et al*., 2011). They are often employed in cosmetic products to improve skin appearance, and especially treat aging signs (Webber, Ribeiro, Velásquez, 2005; Nosari et al., 2015). In fact, vegetable oils have been widely used as a basic

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component or an active compound of cosmetic formulations, due to their low toxicity and biocompatibility with human skin (Ambroisine *et al*., 2007; Durán et al., 2017).

The addition of active substances to topical products with a hydration function has become a very common practice in the cosmetics industry (Baby *et al*., 2008). Cosmetic cream are one of the most important classes of cosmetic products and body hygiene since they are used preventively or also as therapeutic adjuvants in various skin disorders, especially inflammation, infection and aging (Ferreira, Costa, 2011).

The incorporation of secondary metabolites from plants in the topical formulations may assist in skin protection by reducing oxidative damage to biomolecules caused by sun exposure. Also, the aging process itself has as physiopathogenic mechanism an imbalance between formation more than oxidants and its removal by endogenous antioxidants. Oxidative DNA lesions are believed to be the major mechanism in promoting aging (Palmer, Kitchin, 2010; Birben *et al*., 2012).

In the skin, the oxidative lesions stimulate a greater production of melanosomes by melanocytes, which induces the splitting of elastin and collagen fibers (Fernandes, Castro, Oliveira, 2011). Thus, the development of antiaging products has the challenge of reducing or inhibiting oxidative damage caused by species, generically called reactive oxygen species (ROS) and nitrogen (RNS), which include hydrogen peroxide, hydroxyl radical, peroxynitrite, among others (Bispo, 2008; de Souza et al., 2013).

According to Wagemaker *et al*. (2012), the use of exogenous compounds to counterbalance the excess of ROS and RNS inside the cells may constitute a promising strategy for prevention and treatment of a variety of redox-based diseases and disorders. Thus, ideal antioxidants employed by cosmeceutical industry should be thermoresistent, nontoxic and present organoleptic neutrality. These criteria justify the research of new natural sources of antioxidants because commonly natural bioactive compounds are well-tolerated by organisms (Louli, Ragoussis, Magoulas, 2004; Fries and Frasson, 2010).

In this context, this study aimed to develop a moisturizing cream containing fractions extracted from *Coffea arabica* grains with good stability and antioxidant property.

## II. MATERIAL AND METHODS

### *A) Preparation of coffee extracts*

The green coffee beans of *Coffea arabica* were kindly provided by Moinho Paquetá, Jequié, Bahia. The solvents were purchased from Merck (PA grade or better). Three hundred grams of coffee were subjected to cold maceration with 80% ethanol for 72 hours and filtered under vacuum. The grain residue (milestone) was pressed until exhaustion, resulting in the ethanolic extract of the coffee (EEC), which was concentrated in rotavaporator. The milestone was initially dried in a hood with exhaustion and then in an oven (Microprocessor, 40L, SX12 DTME) at 45ºC to determine its mass. The crude extract was diluted in methanol/$H_2O$ (3:1), obtaining the hydrometanolic fraction (HMFC), and then fractionated by a liquid-liquid partition with solvents hexane, chloroform, and ethyl acetate, providing the fractions hexane (HFC), chloroform (CFC) and ethyl acetate (EAFC).

Additionally, three hundred grams of coffee beans were crushed and subjected to steeping with glycerin/propylene glycol (1:1) for 72 hours. The macerate was then vacuum filtered, and the milestone was pressed until exhaustion. Thus, the glycolic extract of coffee (GEC) was collected.

### *B) Development of cream formulation*

The development of the formulation involved the elaboration of an emulsion containing different proportions of GEC, HFC, HMFC, EAFC and CFC. All fractions were employed for development of the emulsion because each one has a pharmacotechnical property. HFC is an emollient agent, and it confers hydration power (Wagemaker, 2009). CFC provides consistency for oil phase because it is rich in long chain fatty acid (Turatti, Lucas, 2001). GEC contains glycols and is used as wetting agent. HMFC and EAFC usually contain bioactive compounds with potential antioxidant and antiaging activities (Speer, Kolling, 2006).

Two formulations containing different concentrations of the cosmetic ingredients were prepared, according to following procedure (Table I). The water and hydroxyethylcellulose (Natrosol®) were heated under agitation (step 1) at 72°C. After gel formation, methylparaben (Nipagin®), previously solubilized in propylene glycol, was added. Separately, phase 2 was heated at 72 °C. Then, phase 2 was mixed into phase 1 with continuous stirring until cooling. Finally, the fractions were incorporated at 40°C. The product was packed and kept under ambient conditions (25 °C). This sample was considered as control product (day 0).

### *C) Preliminary assessment of formulation stability*

Stability was evaluated according to Stability Guide for Cosmetic Products established by Agência Nacional de

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Vigilância Sanitária (ANVISA) (2010). For pre-screening purposes, regarding physical stability and to obtain some stability prognoses, 2 g of the formulation were submitted to three cycles of 3000 rpm for the duration of 30 minutes. At the end of each cycle, the samples were checked to see whether there was any change in both formulations. Also, formulations were stored in a fridge (5 ± 1ºC) and oven (45 ± 2ºC) for 15 days. The samples were analyzed for appearance, color, odor, pH, conductivity and presence of viable microorganisms at 1st, 3rd, 5th, 10th and 15th days. As a control, the product was maintained at room temperature (25°C).

The emulsion was diluted to 10% in distilled water for pH and conductivity measurements using a digital pHmeter (SONAMBRA SNMPA-210; Brasil) and conductivity meter (Alpax.Mod. ACA-150; Brasil), respectively. Total viable microorganisms were determined according to IV Edition of the Brazilian Pharmacopoeia (2010) in the formula at 25°C within 0 and 15 days.

*D) Determination of total phenolic concentration*

The total phenolic content was determined by Folin-Ciocalteau spectrophotometric assay (Kujala *et al*., 2000). Samples were diluted into respective solvents to a final concentration of 1 mg/mL. An aliquot of 125 µL of extract or cream was mixed with 125 µL of the Folin-Ciocalteau reagent and 1 mL of distilled water. After 3 min, 125 µL of the $Na_2CO_3$ saturated solution was added to the mixture and incubated for 30 min at 37 °C. The absorbance was measured spectrophotometrically at 750 nm. A standard curve with quercetin at concentrations of 5, 20, 50, 100 and 200 µg/mL was used to quantify total phenolics. The line equation was obtained by linear fitting of the calibration curve ($Abs_{750nm}$ = 0.0061 x [µg of quercetin] + 0.0122, $R^2$ = 0.9999). The results were expressed as µg quercetin equivalent (QE) per mg of sample.

*E) Determination of antioxidant activity*

The antioxidant capacity was assessed by the sequestration method of the 2,2-diphenyl-1-picryl-hydrazyl radical (•DPPH) according to Brand-Willians *et al*. (1995) with modifications. Samples were diluted into respective solvents to a final concentration of 1 mg/mL, and 300 µL were incubated with 1700 µL of •DPPH (70 µM). After 20 min at 25 °C, in the dark, the absorbance was measured 517 nm. The negative control corresponds to the reaction mixture with the only solvent.

The percent inhibition was calculated using the formula:

$$\bullet DPPH\ scavenging\ activity\ (\%) = 100\ x\ \frac{(Ac-As)}{Ac},$$

where:

Ac = absorbance of the control.
As = absorbance of the sample.

*F) Statistical analysis*

All data were expressed as the mean ± standard deviation or $IC_{50}$ values based on three independent experiments. Significant differences ($p < 0.05$) were detected by one-way ANOVA with Tukey's post-test using IBM SPSS Statistics for Windows (IBM SPSS, 21.0, 2012, Armonk, NY: IBM Corp.). Pearson test was used for analysis of correlation.

## III. RESULTS AND DISCUSSION

Glycolic extract (EGC) and hexane (HFC), hydromethanolic (HMFC), ethyl acetate (EAFC) and chloroform (CFC) extracts were obtained from green coffee beans of *Coffea arabica*. These extracts were used as ingredients for preparation of an emulsion due to their potential emollient, humectant, consistency and antioxidant properties associated with essential fatty acids and phenolic derivatives, respectively (Athar, Nasir, 2005; Speer, Kolling, 2006; Esquivel, Jiménez, 2012; Wagemaker, 2009; Ribeiro *et al*., 2015). Esterols and fatty acids are important for protection and recovery of skin lipid barrier and, therefore, they have renewers and stimulants properties (Alvarez, Rodriguez, 2005). Polyphenolic compounds are excellent antioxidants and may deaccelerate the aging process because it reduces the oxidative damage to biomolecules (D' Leon, 2000; Ribeiro *et al*., 2015).

For emulsion development, two formulations with different concentrations of ingredients (formulas 1 and 2) were prepared (Table I). First, both formulas were centrifugated to accelerate sedimentation, cremation and/or coalescence processes of the biphasic system (Rieger, 1996). The emulsion obtained from formula 1 was not homogeneous and after centrifugation presented phase separation. On the other hand, formula 2 was homogenous and stable under centrifugation and, therefore, used for later experiments.

The formula 2 contained a higher concentration of a self-emulsifying wax, Polawax®, improving the incorporation of the oil phase into an aqueous phase and increasing the emulsion viscosity. As a consequence, the sedimentation rate of the emulsion slows down, avoiding phase separation. CFC concentration was also increased because it contained coffee-extracted waxes which improve cream stability (Godefroot, Verzele, 1981).

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*Table 1. Composition of Coffea arabica-based formulations*

| Components | Formula 1 (%) | Formula 2 (%) |
|---|---|---|
| AQUEOUS PHASE | | |
| Nipagin® | 0.2 | 0.2 |
| Propylene glycol | 5.0 | 5.0 |
| Natrosol® | 1.5 | 1.5 |
| Water qsp | 100 | 100 |
| OIL PHASE | | |
| Polawax® wax | 5.0 | 8.0 |
| Nipasol® | 0.1 | 0.1 |
| CFC | 4.0 | 5.0 |
| PHASE AT 40°C | | |
| MFC | 1.0 | 1.0 |
| EAFC | 1.0 | 1.0 |
| HFC | 5.0 | 5.0 |
| GFC | 5.0 | 5.0 |

CFC= chloroform fraction from coffee MFC= methanolic fraction from coffee; HFC= hexane fraction from coffee; EAFC= ethyl acetate fraction from coffee; GFC= glycolic fraction from coffee. Two formulations containing different concentrations of the cosmetic ingredients were prepared. The water and Natrosol® were heated under agitation at 72°C. After gel formation, Nipagin®, previously solubilized in propylene glycol, was added. Separately, phase 2 was heated at 72 °C. Then, phase 2 was mixed into phase 1. Finally, the fractions were incorporated at 40°C.

The formula 2 was then subjected to extreme temperature conditions (5 and 45°C) for 15 days, and its organoleptic (e.g. odor, appearance, and color), physicochemical and microbiological characteristics were assessed (Gray, White, 1978; Aluyor, Ori-Jesu, 2008; Anvisa, 2010).

The macroscopic characteristics of the emulsion under both temperature conditions were similar to product stocked at room temperature (25ºC). The emulsion remained viscous, homogeneous and shiny without any change in precipitation or turbidity (Table II). Since viscosity is directly associated with cream stability, our formulation was stable even after accelerating test (Aluyor, Ori-Jesu, 2008). The brownish color and odor of the cosmetic cream were characteristics of the coffee oil for at least 15 days of the experiment (Table II) (Lambers *et al*., 2006).

*Table 3. Physicochemical characteristics of the cream subjected to preliminary stability assay.*

| Time (day) | Treatment (°C) | pH | *p*-value* | Conductivity (µS/cm) | *p*-value* |
|---|---|---|---|---|---|
| 0 | 25 | 4.34±0.00 | | 290.04±0.02 | |
| 1st | 5 | 4.26±0.05 | 0.115 | 289.80±5.30 | 0.954 |
| | 45 | | | | |
| | | 4.29±0.04 | 0.166 | 290.80±6.90 | 0.847 |
| 3th | 5 | 4.27±0.04 | 0.094 | 294.73 ±11.49 | 0.550 |
| | 45 | | | | |
| | | 4.27±0.04 | 0.094 | 284.70±12.30 | 0.537 |
| 5th | 5 | 4.27±0.03 | 0.078 | 274.30±8.31 | 0.065 |
| | 45 | 4.27±0.05 | 0.136 | 276.73±4.91 | 0.069 |
| 10th | 5 | 4.25±0.05 | 0.089 | 269.43± 14.67 | 0.136 |
| | 45 | | | | |
| | | 4.27±0.05 | 0.118 | 275.30±7.45 | 0.077 |
| 15th | 5 | 4.27±0.03 | 0.056 | 276.60± 11.43 | 0.071 |
| | 45 | | | | |
| | | 4.29±0.03 | 0.102 | 276.40± 6.03 | 0.060 |

*The results were compared to cream at initial conditions (0 day), using one-way ANOVA with Tukey's post-test.

The pH of the emulsion subjected to extreme temperatures ranged from 4.0 to 4.5 over 15 days of the assay (Table III), but no significant difference was found when compared to emulsion stocked at 25°C ($p > 0.05$).

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Therefore, our coffee emulsion is likely biocompatible with the dermal application because the human skin has acidic character and pH values between 4.0 and 6.5, depending on local (Kaufman, Hamsagar, 1962).

The electrical conductivity allows determining the process of emulsion inversion by measuring free water into formulation (RDC 67, 2007). The values of electrical conductivity of the emulsion 2 are described in Table III. The results demonstrated that the electrical conductivity of the emulsions stocked at 5 and 45°C for 15 days were relatively higher (269-280 µS/cm), as expected for oil-in-water emulsions, and no significant difference to cream at initial conditions (25°C, first day) ($p > 0.05$).

The ANVISA (Resolution 481/1999) also recommend microbiological assay for emulsions to ensure the product quality and to end user safety (Rodrigues *et al*., 1997, Morais *et al*., 2008). In our experimental conditions, the total aerobic microbial counting was less than $10^3$ CFU/g in the emulsions stocked at 25°C up to 15 days, which is acceptable for topical formulations according to ANVISA protocols.

Emulsions are considered stable when its quality and phase characteristics did not alter under thermal, agitation and gravity stresses. Also, the safety is primarily guaranteed by the absence of microorganisms in the formulation determined by standard protocols. Therefore, our data confirm the stability and safety of our coffee extract-based cream (formula 2).

Green coffee oils have been used in the cosmetics industry for its ability to help maintain natural skin humidity. Furthermore, they contain a series of lipophilic substances with important antioxidant characteristics, such as tocopherols, and can protect the skin against oxidative damage caused by UV exposure or aging (D' Leon, 2000; Martinez, Lecha, 2002). Among the preventive aspects, the correlation between polar antioxidant activity and the ability to delay the aging of cells, as well as inhibit the appearance of cancer cells is highlighted (Barreiros, David, David, 2006). Phenolic compounds have received much attention because of its ability to retard oxidative reactions *in vitro* and *in vivo* (Esquivel, Jiménez, 2012; Moser *et al*., 2001).

In this context, we investigated the ability of the fractions and emulsion of deactivating •DPPH radicals. All samples (150 µg/mL) exhibited some *radical scavenging* activity against •DPPH (Table IV). The hexane, glycolic, methanolic, chloroform and ethyl acetate fractions reduced •DPPH concentration at 24.7, 51.8, 86.2, 89.5 and 90.9%, respectively. Surprisingly, the final emulsion stocked at 5, 25 and 45°C for up to 15 days decreased •DPPH at around 80%. Therefore, the antioxidant properties of coffee fractions are maintained even when incorporated into the cosmetic cream and submitted to extreme conditions.

The phenolic compounds were measured in all samples by Folin-Ciocalteau method (Table IV). Nevertheless, there was no correlation between the concentration of phenolic compounds and antioxidant activity in the fractions or cream ($r = 0.00816$ and $p < 0.98776$) (Table IV footer), which suggests that other compounds as carotenoids and tocopherols may be responsible for their antioxidant activity than phenolics.

*Table 4. Antioxidant activity and phenolic content in Coffea arabica fractions and cream containing coffee.*

| Samples | •DPPH inhibition (%) | Total phenolic (µg QE/mg) |
|---|---|---|
| MFC | 86.2±2.3 | 100.3±12.8 |
| HFC | 24.7.±2.3 | 50.9±13.2 |
| CFC | 89.6±2.7 | 33.2±4.4 |
| EAFC | 90.9±7.1 | 3.6±4.4 |
| GFC | 51.7±5.6 | 3.7±1.3 |
| Cream at 5°C (1st) | 82.5±2.1 | 16.3±0.5 |
| Cream at 5°C (15th) | 79.7±3.1 | - |
| Cream at 25°C (1st) | 80.3±2.0 | - |
| Cream at 25°C (15th) | 78.5±2.6 | - |
| Cream at 45°C (1st) | 81.4±2.9 | - |
| Cream at 45°C (15th) | 83.1±3.0 | - |

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MFC=methanolic fraction from coffee; HFC=hexane fraction from coffee; CFC=chloroform fraction from coffee; EAFC= ethyl acetate fraction from coffee; GFC=glycolic fraction from coffee. (-) = undetermined. The cream was stocked at 5, 25 and 45°C and analyzed in the first (1st) and fifth (15th) days. In the •DPPH scavenging assay, samples were at 150 µg/mL. The total phenolic content was expressed as µg of quercetin equivalent per mg of sample. Pearson correlation between antioxidant activity and phenolic content was r = 0.00816 and p <0.98776.

In this study, it was possible to develop an antioxidant emulsion containing extracts from *Coffea arabica*. The cosmetic cream was very stable according to preliminary stability tests besides of its excellent antioxidant activity. Therefore, this emulsion is a potential product to be employed for the prevention of UV radiation-induced and physiological aging.

## IV. ACKNOWLEDGMENT

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