ORIGINAL ARTICLE

Antioxidant activity and physicochemical stability of phytocosmetic formulations containing *Phyllanthus niruri* extract

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ABSTRACT

Antioxidants are an appreciable strategy to prevent skin photoaging as they inhibit reactive oxygen species formation under UV exposure. *Phyllanthus niruri* L. (Euphorbiaceae) is a medicinal plant utilized in treating urolithiasis and contains high amounts of secondary metabolites with antioxidant activity. The potential cosmetic usage of the dry extract of *P. niruri* in three cosmetic base formulations (non-ionic emulsion, anionic gel, and gel-cream were assessed by evaluating antioxidant activity and thermal stability. Antioxidant activity was assessed by the DPPH method and the preliminary stability test was performed in different storage temperature conditions (40.0±2.0, 20.0±5.0, 5.0±2.0 °C). Organoleptic (appearance, color, odor), sensory (consistency) and physical-chemical characteristics (pH) were evaluated after 30 days. Formulations containing the plant extract presented high antioxidant activity, whereas the non-ionic emulsion had the best performance. All formulations exhibited antioxidant activity stability when stored at low temperatures, however, medium and high temperatures caused a reduction of this parameter. Therefore, non-ionic emulsion was considered the ideal base formulation for antiaging purposes as long as stored at low temperature.
Keywords: Free radical scavenger, Phenolic compound, Semi-Solid Formulation, Cosmetic.

INTRODUCTION

Skin aging is a natural and complex biological process characterized by a reduction in the skin's antioxidant capacity over time. However, extrinsic factors, such as chronic exposure to ultraviolet (UV) radiation, are responsible for photoaging, a type of premature aging characterized by deep wrinkles, hyperpigmentation or hypopigmentation, reduced elasticity, and drier and rougher skin. UV radiation stimulates the production of reactive oxygen species (ROS) in the dermis, which may damage cell membranes, proteins, enzymes, and even DNA through a chain oxidation reaction that can induce cell death and skin cancer (Shanbhag et al. 2019).

Photoprotection and reducing sun exposure are well-accepted strategies to prevent premature aging (Kostyuk et al. 2018). Furthermore, antioxidant use helps prevent aging as it captures ROS and inhibits cell damage. As such, antioxidant phytocompounds are interesting active pharmaceutical ingredients in skincare products (Oliveira and Dario 2019).

Phyllanthus niruri L. (Euphorbiaceae) is a medicinal plant found in tropical and subtropical regions, especially Brazil, India, Africa, Malaysia, and Indonesia (Kumar et al. 2017). The species has several pharmacological properties, including antibacterial, anti-inflammatory, hepatoprotective, antiulcer, and antidiarrheal properties; however, it is mainly used in treating urolithiasis. These properties are due to its secondary metabolites, such as tannins, flavonoids, alkaloids, terpenes, coumarins, and lignans (Lee et al. 2016; Kaur et al. 2017). *P. emblica* L., known as emblica or amla, is a species of the same genus that has demonstrated antioxidant capacity as it has inhibited metalloproteinases related to skin aging, protecting fibroblasts from

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oxidative stress and stimulating their proliferation as well as increasing the production of Type I collagen (Chaikul et al. 2021).

Moreover, as most *P. niruri* compounds have antioxidant activity, this study aimed to evaluate this extract as a candidate for antiaging products.

MATERIAL AND METHODS

Chemicals

Disodium EDTA; a mixture of cetearyl alcohol, polysorbate 60, PEG-150 stearate, and steareth-20 (Polawax® NF); dimethicone; butylated hydroxytoluene; ethylhexyll stearate; C10-30 alkyl acrylate crosspolymer (Carbopol® 920); hydroxyethylcellulose; glycerin; methylparaben; propylparaben; aminomethyl propanol (AMP® 95); propylene glycol; and caprylic capric triglyceride were used without further purification. The solvents ethanol (Prolink) and methanol (Anidrol) were PA grade. Gallic acid (purity > 98.0%) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich. *P. niruri* was purchased from SantosFlora Comércio de Ervas Ltda., corresponding to Lot 1608175268. This research was recorded (A45E2DF) in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen).

Phyllanthus niruri extract

The dried *P. niruri* aerial plant was pulverized in a knife mill and sieved in 20 mesh sieves (850 µm). Extraction was performed through ultrasound at 22 °C for 30 min using a proportion drug to extractor liquid (ethanol 50% v/v) ratio of 1:10 (mass of the plant drug versus liquid extractor). Subsequently, the ethanol was removed in a rotary evaporator under reduced pressure at 40 °C. The remaining aqueous extract was dried and stored in a desiccator at 22 °C. Thin layer chromatography profile of dried *P. niruri extract* was performed by our group as shown at Carmagnani et al. (2020).

Preparation of cosmetic formulations

The dried *P. niruri* extract was incorporated at 2.5% (w/w) into three cosmetic bases: non-ionic emulsion, anionic gel, and gel-cream (Table 1). The percentage of flavonoids in the extract, previously shown at Carmagnani et al. (2020), was used to define the concentration of the extract in formulations.

Preliminary stability test

Formulations were subjected to a preliminary stability test to evaluate the occurrence of physical and organoleptic characteristics (color,

Table 1. Composition of base cosmetic formulations.

Ingredients	INCI*	Non-ionic emulsion	Anionic gel	Gel-cream
		% (w/w)		
EDTA	*Disodium EDTA*	0,1	0,1	0,1
-		-	-	-
Deionized water	*Aqua*	qsp 100	qsp 100	qsp 100
Polawax® NF	*Cetearyl Alcohol (and) Polysorbate 60 (and) PEG-150 Stearate (and) Steareth-20*	15	-	15
Dimethicone	*Dimethicone*	2	-	-
Butylated hydroxytoluene (BHT)	*BHT*	0,05	-	0,2
Ethylhexyll Stearate	*Ethylhexyll Stearate*	2	-	-
Carbopol® 920	*C10-30 Alkyl Acrylate Crosspolymer*	-	0,8	-
Hydroxyethylcellulose	*Hydroxyethylcellulose*	-	-	0,5
Glycerin	*Glycerin*	-	5	-
Methylparaben	*Methylparaben*	0,2	0,15	0,2
Propylparaben	*Propylparaben*	0,1	-	0,2
AMP 95	*Aminomethyl Propanol*	-	qs pH 6,5-7,0	-
Propylene Glycol	*Propylene Glycol*	3,0	-	8
Caprylic Capric Triglyceride	*Caprylic Capric Triglyceride*	-	-	10

odor, and aspect), sensory, pH value alterations, and antioxidant activity. The formulations were subjected to different storage temperature conditions (40.0±2.0, 5.0±2.0, and 20.0± 5.0 °C) for 30 days. Furthermore, pH values were measured through the direct immersion of an electrode in the formulations using a potentiometer, and antioxidant activity was determined using a DPPH assay.

Evaluation of antioxidant activity

Antioxidant activity of all formulations, including blank (no added extract), was assessed using the DPPH method. First, 0.2 g of each formulation was added to 5 ml of methanol PA and vortexed. Subsequently, the formulations were centrifuged at 600 ×*g* for 15 min. About 2.5 ml of the supernatant was transferred to a 10 ml volumetric flask and its volume was completed with ethanol PA. An aliquot of 500 µl of these solutions was added to 2.5 ml of 100 µM DPPH ethanolic solution. The reaction proceeded for 30 min in the dark, and the spectrophotometric reading was performed at 517 nm using absolute ethanol as a reading blank. Negative control was performed by adding 0.5 ml of ethanol PA and 2.5 ml of DPPH solution. The solutions were prepared in triplicate (Dario et al. 2013). The percentage of free radical scavenging (% FRS) was calculated following Equation 1.

$$\% \text{ FRS} = \frac{Abs_C - Abs_S}{Abs_C} \qquad \text{(Eq. 1)}$$

where: Abs_C: Negative control absorbance; Abs_S: Sample absorbance.

The antioxidant activity was expressed in gallic acid equivalents (GAE). A calibration curve was constructed from serial dilutions of reference standard gallic acid (1.5 - 12.0 µg/ml) in methanol PA. An aliquot of 500 µl of these solutions was added to 2.5 ml of 100 µM DPPH ethanolic solution, and the protocol proceeded as described above. The antioxidant capacity was determined by comparing the % FRS of each formulation to the gallic acid calibration curve and reported in mg GAE/g formulation.

Statistical analysis

Statistically significant differences in antioxidant activity during preliminary stability test were verified using a paired *t*-test comparing initial and final values (after 30 days of stability test) (α = 0.05).

RESULTS AND DISCUSSION

Formulations based on emulsions, gels, and gel-creams are the most common vehicles employed in cosmetics due to their excellent sensorial characteristics and acceptance by consumers (Soares et al. 2021). Emulsions are composed of two immiscible phases (aqueous and oily) stabilized by surfactants whose charge (anionic, cationic, non-ionic or amphoteric) determines the type of emulsion. This type of formulation has moisturizing and emollient properties. Gels are semisolid dosage forms, usually water-based, thickened by a gelling agent. They are cosmetically elegant, nongreasy and provide a cooling sensation (Mayba and Gooderham 2018). By the other hand, gel-creams are emulsions thickened by a hydrophilic colloidal consistency agent (Ferreira et al. 2018).

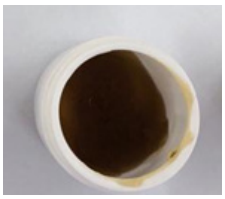
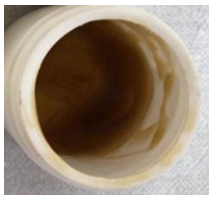
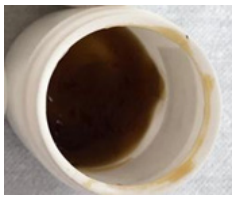
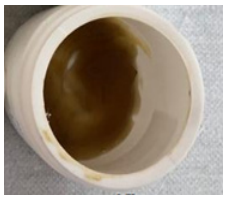
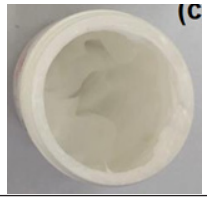
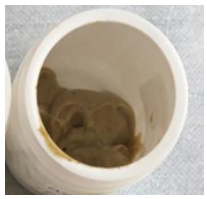
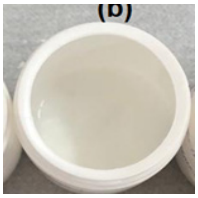
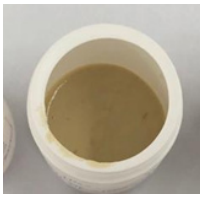
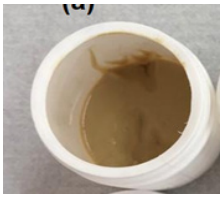
The organoleptic and sensory characteristics of a product determine its acceptance by the consumer as the analysis of these characteristics guarantees that any changes in the product do not alter its efficacy, safety or acceptability. Evaluating these characteristics provides parameters to estimate the state of the formulation under study through comparative analysis (i.e., the state of the formulation before and after going through certain processes). Parameters such as appearance, color, and odor are usually evaluated macroscopically (Rosário et al. 2021).

In general, the preliminary stability test showed no profound alterations in the formulations (Table 2). One of the alterations was the milder odor of the anionic gel containing the plant extract when stored at a high temperature (40.0±2.0 °C). This may be due to the evaporation of ethanol from the sample or various reactions, such as oxidation, hydrolysis, or interactions with the packaging material, that can reduce the number of substances in the extract (Casteli et al. 2008).

The non-ionic emulsion changed from a viscous to a firmer appearance, possibly owing to the loss of water from the formulation through evaporation (Pianovski et al. 2008). When opening the pots, small droplets of water were seen on the lids. This phenomenon was probably caused by the insufficient concentration of humectants, which can prevent water loss (Souza and Ferreira 2010). The addition of glycerin, for example, could solve instability.

The pH of the formulation is an important parameter because it evidences alterations and reactions that can modify the pH of the formulation. The pH of healthy skin is slightly acidic (4.5–6.0). This acidity is important for maintaining the homeostasis of the skin's barrier function (physical and chemical protection) and protecting against microorganisms, especially those aliens to the human microbiota

Table 2. Aspect, color and pH values of formulations subjected to preliminary stability test.

Formulation		20.0±5.0 ºC	40.0±2.0 ºC	5.0±2.0 ºC
Anionic gel base				
pH	5,78	5,1	6,2	5,18
Anionic gel + *P. niruri* extract				
pH	6,28	6,52	5,46	6,35
Non-ionic emulsion base				
pH	5,59	4,31	4,62	5,74
Non-ionic emulsion + *P. niruri* extract				
pH	7,2	5,03	5,62	6,6
Gel-cream base				
pH	5,57	6,99	4,86	4,41
Gel-cream + *P. niruri* extract				
pH	5,93	5,22	5,27	5,26

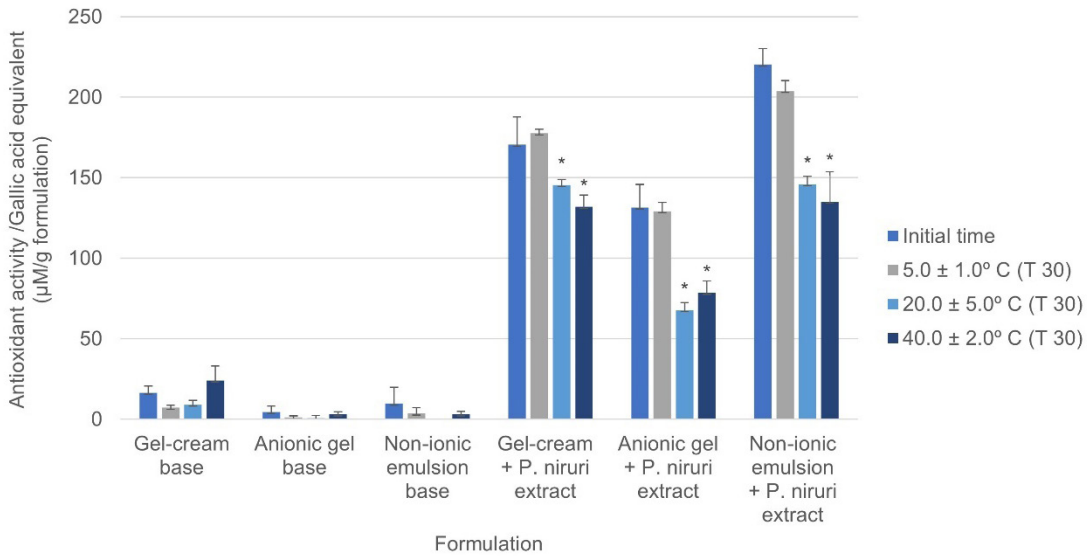


Figure 1. Antioxidant activity of the formulations during preliminary stability test. The formulations that contain an asterisk (*) have statistically different antioxidant activity ($p \leq 0.05$) compared to the initial time (t_0). The values are expressed by the average antioxidant activity (triplicate) and error bars represent the standard deviation.

(such as bacteria and fungi) (Leonardi et al. 2002) To maintain healthy skin, topical formulations must maintain an acidic pH (~5 is desirable). Formulations with pH < 5 help maintain skin homeostasis, thus preventing aggressive agents, such as microorganisms, as well as dermatitis, acne, and eczema (indicating unhealthy skin) (Das and Wong 2020).

The formulations in our study showed changes in pH as they were stored at different temperatures (Table 2). Monitoring pH in cosmetic formulations are essential as organoleptic characteristics (appearance, color, and odor) and the effectiveness of the active ingredient can be changed as a result of the pH changes. Additionally, phytocosmetics containing polyphenols (tannins, coumarins, and flavonoids) have increased stability at a slightly acidic pH (Anvisa 2021).

Carbomer gels, such as Carbopol®, are stable at a pH of 5.5–7.3, presenting the ideal viscosity. The gel-cream and non-ionic emulsion must have a pH of 5.5–6.5. Therefore, the gel remained at an ideal pH during the preliminary stability test, whereas the non-ionic emulsion and gel-cream showed a pH (4.31–6.99) outside the established ideal range but within the pH range biocompatible with the skin (Aulton 2005).

The antioxidant activity of the formulations and extract was expressed in gallic acid equivalents based on a calibration curve obtained for this primary reference standard (Figure 1). The base formulations (gel-cream, non-ionic emulsion, and anionic gel) presented extremely low antioxidant activity as the dry *P. niruri* extract was not incorporated. The antioxidant activity of the gel-cream base was higher than the other formulations, possibly due to its increased concentration of BHT, which is an antioxidant. Formulations containing the dry extract showed considerable antioxidant activity. Owabhel and Eboh (2022) demonstrated that DPPH scavenging of *Phyllanthus niruri* methanolic extract was slightly smaller than gallic acid, but the nitric oxide scavenging was higher. This result can be explained by the presence of phenolics, such as rutin, quercetin, niruriflavone, and other potent antioxidants phytocompounds (Bagalkotkar et al. 2006, Ferrante et al. 2020). The addition of the *P. niruri* extract caused an increase of 936, 2863, and 2161% in gel-cream, anionic gel and non-ionic emulsion, respectively. The non-ionic emulsion showed superior values of antioxidant activity before the stability test (t_0).

No statistical difference was observed for formulations containing the extract when stored at low temperatures (5.0±1.0 °C), while all formulations stored in an oven (40.0±2.0 °C) and at room temperature (20.0±5.0 °C) showed significant differences. These data indicate that storage at increased temperatures can cause alterations in the formulation, such as the formation of degradation products and loss of activity of the active pharmaceutical ingredient; therefore, low-temperature storage is recommended (Lange et al. 2009).

Temperature directly influences the conservation of plant drugs. In general, the plants

must remain at room temperature (less than 15 and up to 30 °C) without the incidence of light and protected from humidity. High temperatures (above 50 °C) can degrade plant compounds, causing the loss of pharmacological activity through the formation of other compounds (Oliveira 2014).

Our findings show that *P. niruri* extract, rarely used for cosmetics, has excellent potential for antiaging products due to its high antioxidant activity. The base cosmetic formulation directly influenced the physical and chemical stability of the formulations, and non-ionic emulsion was most suitable for incorporating the dry *P. niruri* extract since it is stored at low temperature conditions.

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AUTHORS' CONTRIBUTION

Conceptualization, F.S.*M.G. and M.F.D.*; methodology, M.F.D.; validation, F.S.*M.G. and M.F.D.*; formal analysis, M.F.D.; investigation, H.J.C. and G.B.M.; resources, F.S.*M.G. and M.F.D.*; data curation, M.F.D.; Writing - original draft, H.J.C., G.B.M. and R.V.G.; Writing - review & editing, M.F.D. and P.A.B.; visualization, R.V.G.; supervision, M.F.D.; project administration, M.F.D.; funding acquisition, M.F.D. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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