O MUNDO DA SAÚDE

Evaluation of the stability and antioxidant activity of formulations with *Mangifera indica* L. extract

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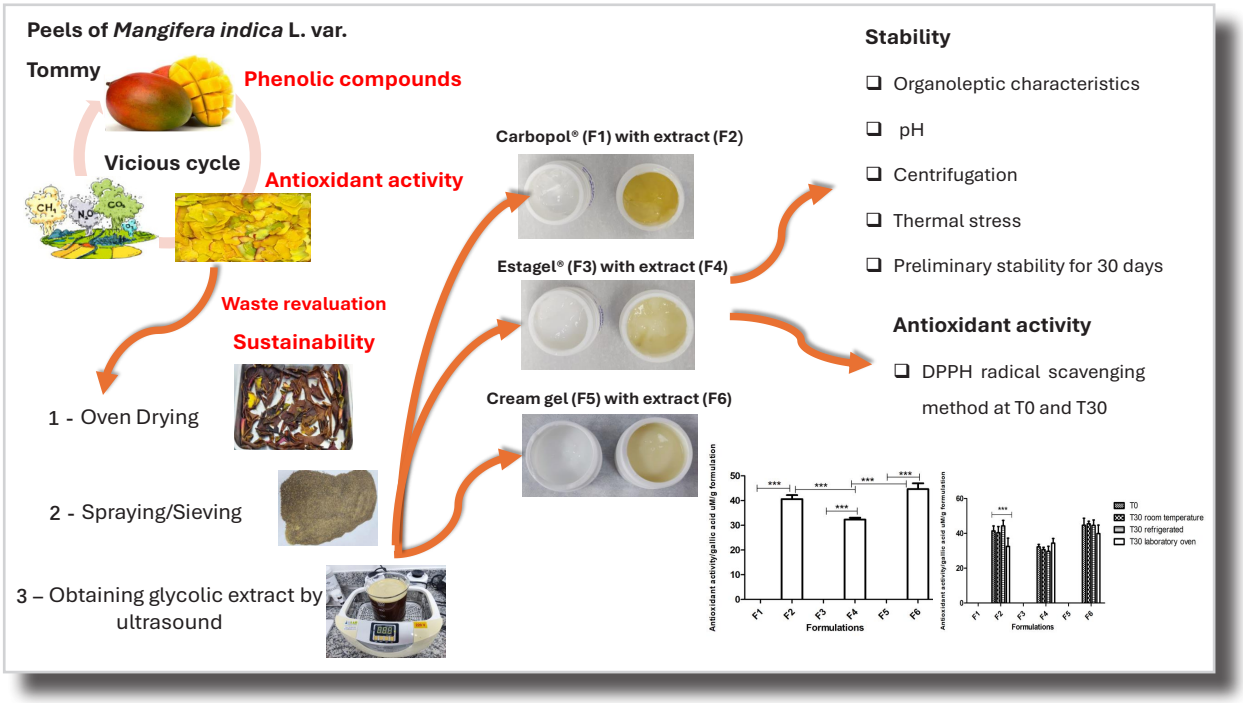
Graphic Abstract



Abstract

The high global production of *Mangifera indica* generates a considerable amount of waste, such as peels and seeds that are often discarded. The use of these by-products promotes a more sustainable approach, reducing environmental impacts and opening new perspectives in the phytocosmetics area. The peel presents secondary metabolites known mainly for their antioxidant properties, highlighting phenolic compounds. These antioxidants are capable of slowing down the rate of oxidation promoted by free radicals formed by external or pathophysiological factors. Thus, natural antioxidants extracted from plant species are increasingly being studied for application in the cosmetic and pharmaceutical industry. The phytocosmetic potential of the glycolic extract from the peel of *M. indica* L. var. Tommy Atkins in three galenic bases (Carbopol® gel, cream gel and Estagel® gel) was evaluated through antioxidant activity tests, the DPPH radical scavenging method, and stability studies. The formulations with the extract were stable and compatible for topical use, as there were no signs of instability such as changes in organoleptic characteristics and pH. Regarding antioxidant activity, formulations with the extract showed antioxidant potential, however the formulation with Carbopol® and gel-cream showed better performance compared to Estagel®. After 30 days of preliminary stability in different temperature conditions (40.0 ± 2<0°C, 20.0 ± 5.0°C, 5.0 ± 2.0°C) there was a reduction in antioxidant activity only in the gel of Carbopol® stored at high temperature, indicating the best form of storage. Therefore, the results suggest the promising incorporation of *M. indica* L. var. Tommy Atkins in cosmetic foundations.

Keywords: *Mangifera indica*. Antioxidant. Flavonoids. Phenolic compounds. Stability of Cosmetic Products.

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INTRODUCTION

Mangifera indica L., popularly known as mango, belongs to the Anacardiaceae family[1,2,3,4,5]. According to the Brazilian Institute of Geography and Statistics (IBGE), Brazil is the seventh world producer of *M. indica* L., in the Tommy Atkins and Palmer varieties, notably in the São Francisco Valley[3].

The increased global production and consumption of tropical and subtropical fruits rich in macronutrients and micronutrients has resulted in a large waste of high-value-added waste, including seeds and peels, reaching approximately 30% from harvest to consumption[1,2,6,7]. The Food and Agriculture Organization of the United Nations (FAO) pointed out that this waste was one of the causes of greenhouse gas emissions and atmospheric, aquatic and terrestrial pollution[4], which also affected the quality of food, thus generating a cycle vicious relationship between environmental impact and waste[2]. In the current scenario, the revaluation of agri-food waste can provide financial returns for cosmetic, pharmaceutical and food industries. According to FAO, appropriate measures must be taken to ensure sustainability.

The peel of *M. indica* L. var. Tommy Atkins presents a variety of compounds, such as total carbohydrates, proteins, amino acids, lipids, organic acids and dietary fiber, in addition to bioactive compounds such as phenolics (mangiferin (2-C-beta-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone), protocatechuic acids and flavonoids) and β-carotene[4,5,7,8,9,10,11,12]. These compounds are associated with various biological activities, such as antioxidant action, which helps combat oxidative stress, responsible for lipid peroxidation and oxidative damage to DNA and proteins. Peroxidation reduces membrane fluidity, impairing selectivity in ionic transport and signaling, also affecting cellular transport. Additionally, these compounds are beneficial in treating hyperpigmentation, acne, and aging[1,4,5,8,13,14].

Antioxidant activity may vary according to the structure and physicochemical properties of flavonoids. The intermediates formed by the action of phenolic antioxidants are relatively stable, due to the resonance of the aromatic ring present in the structure of these substances due to the donation of electrons by functional hydroxyl groups. Therefore, plant extracts can be advantageous because they contain a variety of these compounds, acting on different reactive oxygen species, and because they have a more powerful antioxidant effect than synthetic antioxidants combined[15,16,17,18,19].

Considering this scenario, the development of formulations with antioxidant potential and cosmetic application is promising. The objective of the work was to evaluate the potential cosmetic use of the glycolic extract from the peel of *M. indica* L. var. Tommy Atkins in formulations by evaluating their antioxidant activity and preliminary stability for 30 days.

METHODOLOGY

Preparation of Glycolic Extract From the Peel of *Mangifera Indica* L. Var. Tommy Atkins

The peels of *M. indica* L. var. Tommy Atkins were dried in an air circulation oven at 40°C for 4 days, then pulverized in a knife mill and sieved in 20 mesh sieves (850 µm). The extraction method was carried out using ultrasound, with indirect contact, frequency of 40 KHz and power of 123 W for a period of 60 minutes. The extracting solvent used was propylene glycol: water (70 : 30). The proportion of 1.0 g of mango peel was used for 10 mL of propylene glycol: water (70: 30)[20].

Preparation of Cosmetic Formulations

The glycolic extract from the peel of *M. indica* L. var. Tommy Atkins was incorporated into three cosmetic bases (Carbopol® gel, Estagel® 2.0 gel and cream gel) at a concentration of 5% (w/w) (Table 1).

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Table 1 - Quali-quantitative composition of cosmetic formulations.

Ingredients	INCI nomenclature*	F1	F2	F3	F4	F5	F6
		% (p/p)					
Disodium EDTA	*Disodium EDTA*	0.05	0.05	0.1	0.1	-	-
Carbopol® 980	*Carbomer*	1.0	1.0	-	-	-	-
Purified water	*Aqua*	qsp 100	qsp 100	qsp 100	qsp 100	qsp 100	qsp 100
Propylene glycol	*Propylene glycol*	5.0	5.0	-	-	5.0	5.0
Preservative solution	*Methylparaben, Propylparaben, Propylene Glycol*	0.5	0.5	0.2	0.2	0.8	0.8
AMP-95	*Aminomethylpropanol*	qs pH 6.5 – 7.0	qs pH 6.5 – 7.0	-	-	-	-
ESTAGEL® 2.0	*Polyacrylamide & C13-14 Isoparaffin & Laureth-7*	-	-	5.0	5.0	-	-
Capric/caprylic acid triglycerides	*Caprylic/Capric Triglyceride*	-	-	-	-	11.5	11.5
Polawax™ NF	*Cetearyl Alcohol and Polysorbate 60*	-	-	-	-	6.0	6.0
Aristoflex® AVC	*Ammonium Acryloyldimethyltaurate/VP Copolymer*	-	-	-	-	0.5	0.5
Glycolic extract from the peel of *M. indica* L. var. Tommy Atkins	-	-	5.0	-	5.0	-	5.0

**International Nomenclature of Cosmetic Ingredients.*

Caption: F1: Carbopol® gel base; F2: Carbopol® gel containing plant extract; F3: Estagel® gel base; F4: Estagel® gel containing plant extract; F5: Gel-cream base; F6: Gel-cream containing plant extract.

Centrifugation Test

The samples were centrifuged at 3,000 rpm for 30 minutes, 24 hours after handling. After the test, the formulations were macroscopically analyzed for appearance and classified as:

i. (N) Normal - no change in appearance.

ii. (M) Modified - phase separation, color or appearance change, cloudiness or precipitation[21,22].

Thermal Stress Test

Approximately 5 g of samples were subjected to heating in a thermostated bath in a controlled temperature range between 40.0 and 80.0º C, with a progression of 10.0º C every 30 minutes. After cooling to room temperature, the formulations were evaluated for possible changes in appearance, being classified as:

i. (N) Normal - no change in appearance.

ii. (M) Modified - changes in appearance, viscosity, color, odor, occurrence of turbidity or phase separation[23].

Preliminary Stability Assessment

The formulations were subjected to Preliminary Stability Assessment, with the aim of evaluating the occurrence of physical instabilities and changes in pH values. The formulations were subjected to different storage temperature conditions (40.0 ± 2.0 ºC, 5.0 ± 2.0 ºC, 20.0 ± 5.0 ºC) for 30 days[24,25]. Both organoleptic characteristics and pH values were evaluated at T0 and T_{30}[22,26].

Organoleptic Characteristics

The formulations were evaluated macroscopically by adding an aliquot of each to a watch glass, being analyzed on a white background, being classified as:

i. (N) Normal - no change in appearance.

ii. (M) Modified - changes in appearance, viscosity, color, odor, occurrence of turbidity or phase separation[27].

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Determining the PH

The pH was determined by dispersing the formulation in distilled water resulting in a concentration of 10% (w/v) with the aid of a magnetic stirrer for 15 minutes for homogenization. The results were determined in triplicate with a pH meter previously calibrated with pH 4, pH 7 and pH 10 buffer solutions. The results were expressed as mean ± standard deviation21. Values compatible with the pH of the skin (between 4.5 and 6.0) were used as stability criteria[26].

Evaluation of Antioxidant Activity

The antioxidant activity of the formulations was evaluated using the DPPH radical scavenging method (2,2-diphenyl-1-picrylhydrazyl) based on the ability of phenolic compounds to donate a proton to the DPPH radical, in order to stabilize the free radical[19]. The analytical curve was prepared with gallic acid solutions at different concentrations (2, 3.2, 6, 8, 10 and 12 µg/ml) in P.A. ethanol. The stock solution was obtained from the solubilization of 20 mg of gallic acid in a 1000 ml volumetric flask with P.A. ethanol. Subsequently, serial dilutions were prepared in a 10 ml volumetric flask. The reaction with the free radical DPPH was carried out by adding an aliquot of 500 µL of the diluted solutions to 2.5 ml of 100 µM ethanolic solution of the DPPH radical. After 30 min of reaction in the dark, spectrophotometric reading was carried out at 517nm using P.A. ethanol as reading blank. The negative control was prepared by adding 0.5 mL of PA ethanol to 2.5 mL of 100 µM DPPH radical solution. The percentage of DPPH free radical inhibition was calculated according to Equation 1[19] From the results obtained, an analytical curve was constructed relating the concentration of gallic acid (µg/ml) and the percentage of inhibition of the DPPH radical•

$$\% \text{ de inibição do radical DPPH}\bullet = \frac{Abs_A - Abs_{Am}}{Abs_A} \times 100 \quad \text{(Equation 1)}$$

Where: $\mathbf{Abs_A}$: Negative control absorbance; $\mathbf{Abs_{AM}}$: Sample absorbance.

To evaluate the samples, aliquots of 1.0 g of the formulations were dispersed in 5.0 ml of P.A. ethanol using a vortex. In order to ensure total dispersion, the formulations remained in an ultrasound bath for 10 min and were subsequently centrifuged at 3000 rpm for 15 min. The preparation of the glycolic extract sample was carried out by adding an aliquot of 0.2 g of extract to 5.0 ml of P.A. ethanol, following the same preparation described previously.

Exactly 2.5 ml of the glycolic extract supernatant and each diluted formulation were transferred to a volumetric flask, completing the volume to 10 ml with P.A. ethanol. Aliquots of 500 µL of these solutions were added to 2.5 ml of ethanolic radical solution DPPH 100 µM, following the methodology described previously. Antioxidant activity was expressed as gallic acid equivalent per gram of formulation or per gram of glycolic extract[15,28]. The analysis was performed in triplicate at T_0 and T_{30}.

Statistical Analysis

The results were subjected to statistical treatment, using analysis of variance (ANOVA) followed by the Tukey test, using the GraphPrisma® software. The results were considered statistically different when the p-value was less than or equal to 0.05 ($p \leq 0.05$).

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RESULTS

Cosmetic Formulations

The Carbopol® gel (F1) was presented as an anionic, colorless, odorless, homogeneous and stable aqueous gel at a pH between 5.5 and 7.3, in accordance with the physicochemical characteristics described in Batistuzzo[29]. After incorporation of the glycolic extract of *M. indica* L. var. Tommy Atkins (F2), demonstrated a gel consistency, homogeneous, yellowish in color and odorless.

The Estagel® gel (F3) appeared as a milky white, odorless, homogeneous gel, with a pH between 6 and 6.5 and a gel-cream consistency[30]. With the incorporation of the glycolic extract of *M. indica* L. var. Tommy Atkins (F4), presented a milky, homogeneous appearance, with a slightly yellowish color and odorless.

The cream-gel, that is, a gel that has an aqueous and oily portion[30] (F5) appeared milky white, odorless, with a more fluid consistency, with a pH between 5 and 6. When incorporated with the glycolic extract of M. indica L. var. Tommy Atkins (F6), had a milky appearance, homogeneous, slightly yellowish in color and odorless.

Centrifugation and Thermal Stress Test

After being subjected to the centrifugation process, all samples were stable, except for the gel-cream containing glycolic extract from the peel of *M. indica* L. var. Tommy Atkins (F6), which showed slight phase separation (Figure 1). In the thermal stress test, after visual evaluation of the samples at different temperatures, all showed no instability, indicating stability of the manipulated formulations.

Preliminary Stability Test

After preliminary stability of 30 days, the formulations did not show any indication of instability, such as changes in appearance, color and odor (Table 2).

The pH of the formulations was compatible with the pH of the skin, between 4.5 and 6.0, indicating stability under different storage conditions (refrigerated, room temperature and laboratory oven) after 30 days. There was a small change in the pH value (6.62-6.45) in the formulations with Carbopol® gel (F2) after stability testing under different storage conditions, but the pH remained acceptable. With Estagel® gel (F4), the variation was also small (5.56-5.23), remaining biocompatible with the skin's pH. In the case of the gel-cream (F6), there was a change in pH outside the product's stability range, but the formulation still remained within the biocompatible range (Table 3).

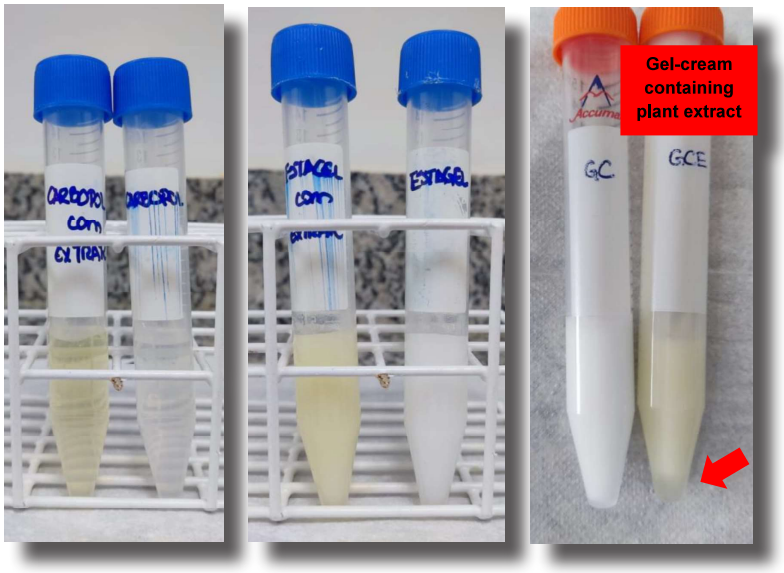

Figure 1 - Samples after centrifugation test.

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Table 2 - Aspect of the formulations during the preliminary stability study, under different storage conditions.

Formulations	T_0	T_{30} Laboratory Oven	T_{30} Room Temperature	T_{30} Refrigerated
F1				
F2				
F3				
F4				
F5				
F6				

Caption for Tables 2 and 3: F1: Carbopol® gel base; F2: Carbopol® gel containing plant extract; F3: Estagel® gel base; F4: Estage® gel containing plant extract; F5: Gel-cream base; F6: Gel-cream containing plant extract.

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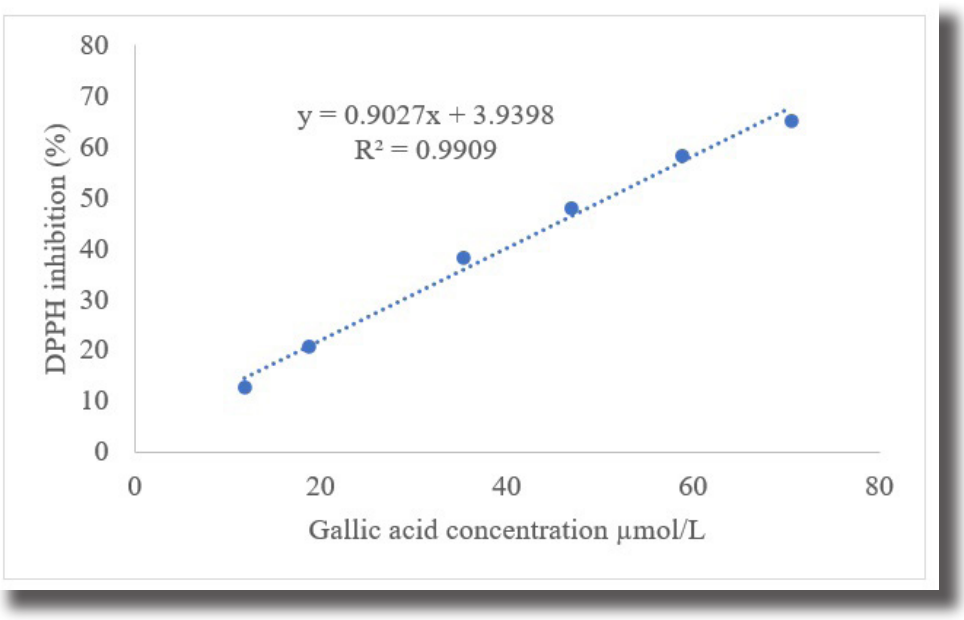
Table 3 - pH values (mean ± standard deviation) of the formulations at different temperature conditions at T_0 and T_{30}.

	T_0	T_{30} Refrigerated	T_{30} Room Temperature	T_{30} Laboratory Oven
F1	6.94 ± 0.03	6.63 ± 0.02	6.43 ± 0.04	6.56 ± 0.03
F2	6.63 ± 0.02	6.55 ± 0.00	6.55 ± 0.03	6.46 ± 0.04
F3	5.61 ± 0.09	5.5 ± 0.00	6.78 ± 0.00	6.33 ± 0.00
F4	5.57 ± 0.13	5.23 ± 0.01	5.01 ± 0.01	5.68 ± 0.11
F5	5.4 ± 0.08	4.9 ± 0.01	4.5 ± 0.01	5.56 ± 0.09
F6	5.46 ± 0.23	4.85 ± 0.02	4.48 ± 0.1	5.34 ± 0.18

Evaluation of Antioxidant Activity

From the analytical curve (Graph 1) prepared with gallic acid solutions, the straight-line equation expressed in Graph 1 below was obtained:



Graph 1 - Analytical curve with gallic acid.

Using the DPPH radical scavenging method, it was observed that the glycolic extract from the peel of *M. indica* L. var. Tommy Atkins showed antioxidant activity with a value of 248.93 ± 13.99 µM of gallic acid/g of extract.

The evaluation of the antioxidant activity of the formulations indicated high antioxidant potential in the glycolic extract of the peel of *M. indica* L. var. Tommy Atkins, since the bases used in the work did not demonstrate appreciable results, as can be seen in Figure 2. Carbopol® gel (F2) and cream gel (F6) showed better performance in terms of antioxidant activity when compared to Estagel® gel with extract (F4). It was not possible to assume that the cream gel (F6) showed greater activity than the Carbopol® gel (F2).

In the preliminary stability study, comparing

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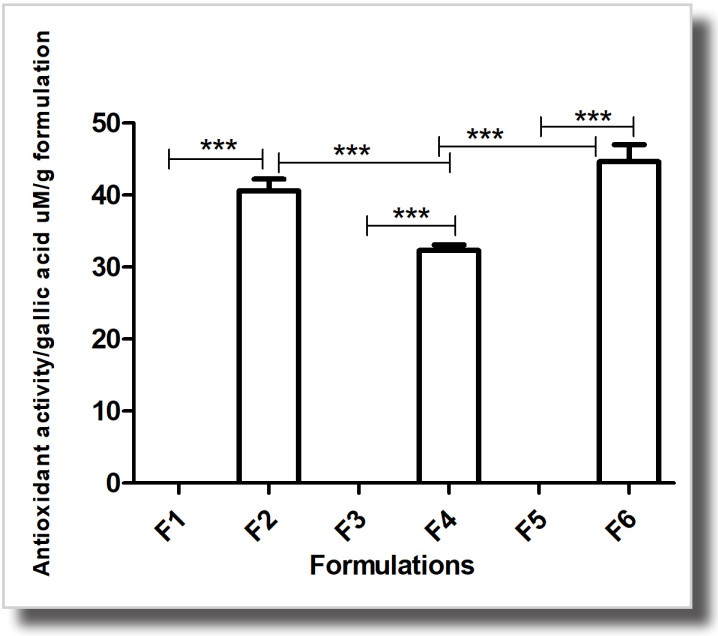
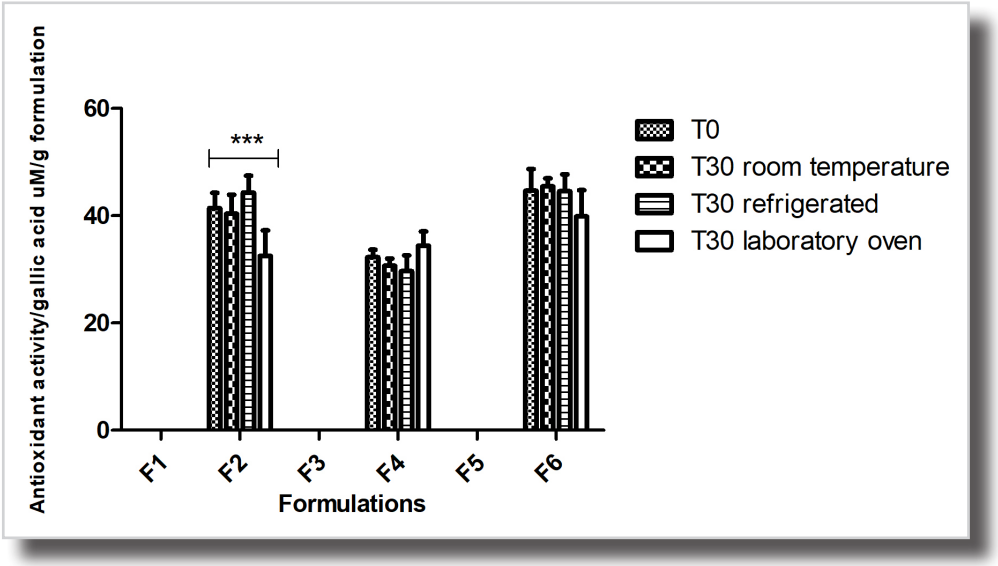
the formulations containing the glycolic extract under different storage conditions, statistically significant differences ($p \leq 0.05$) were observed only for the Carbopol® gel (F2). This indicates that this formulation lost antioxidant activity after being subjected to heating, probably due to the increase in temperature, which accelerates physical-chemical and chemical reactions. These reactions can result in changes in the antioxidant activity, viscosity, appearance, color and odor of the products[22], phenomena not detected in other formulations (Figure 3).



Caption for Figure 2: : F1: Carbopol® gel base; F2: Carbopol® gel containing plant extract; F3: Estagel® gel base; F4: Estagel® gel containing plant extract; F5: Gel-cream base; F6: Gel-cream containing plant extract. Results expressed as gallic acid equivalent per gram of formulation ± standard error. *** $p \leq 0.05$.

Figure 2 - Statistical analysis of the antioxidant activity of the formulations at T_0.



Caption for Figure 3: F1: Carbopol® gel base; F2: Carbopol® gel containing plant extract; F3: Estagel® gel base; F4: Estagel® gel containing plant extract; F5: Gel-cream base; F6: Gel-cream containing plant extract. Results expressed as gallic acid equivalent per gram of formulation ± standard error. *** $p \leq 0.05$.

Figure 3 - Statistical analysis of the antioxidant activity of formulations at T_{30} under different storage conditions.

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DISCUSSION

The increase in consumption and waste of tropical fruits has stimulated growing interest in the revaluation of these high-value by-products, such as phenolic compounds with high bioactive potential. *M. indica* var. Tommy Atkins was chosen with a view to reusing its peels, which have a high concentration of bioactive compounds, mainly mangiferin and flavonoids with antioxidant action, considering that the reuse of this high-value waste minimizes the environmental impacts generated by its disposal[4,31]. For the incorporation of plant extracts into bases for topical cosmetic purposes, the appropriate choice of the base and active ingredients is crucial, in order to guarantee the absorption and stability of the formulation, and, consequently, the expected efficacy of the product. Stability studies are fundamental requirements for the quality and safety of products before reaching consumers, such as centrifugation and thermal stress tests, in order to visualize possible instabilities, such as precipitation, phase separation, caking and coalescence[22].

In view of the stability, organoleptic characteristics and pH tests, it was notable that semi-solid cosmetic bases proved to be stable and compatible for topical use, being widely used to incorporate active ingredients due to their good sensorial characteristics and good acceptance by consumers[32], considering the pH stability of Carbopol® from 5.5 to 7.3[29], Estagel® from 2 to 11[33], and gel-cream from 5.5 to 6.5[32]. Glycolic extracts are frequently used for cosmetic purposes, as they have wide compatibility with galenic bases, in addition to guaranteeing a certain rheological characteristic to the formulations[15].

In the evaluation of antioxidant activity, a high antioxidant potential was found in the glycolic extract in the peels of *M. indica* var. Tommy Atkins, as well as after incorporation into galenic bases. The gel-cream with the glycolic extract (F6) showed antioxidant activity, however, a small phase separation was observed in the centrifugation test and pH outside the stability range of the formulation (between 5.5 and 6.5), requiring reformulation. According to Rowe[34], non-ionic emulsifiers, such as Polawax®, are incompatible with tannins and phenolic compounds, which may be the cause of the slight phase separation observed in the gel-cream with the glycolic extract (F6) after centrifugation. However, new tests and studies need to be carried out to confirm this proposition. Carbopol® gel (F2) was the formulation that performed best; however, it showed a loss of antioxidant activity after being subjected to the oven, and storage at low temperatures was recommended. Lange[21] evaluated a non-ionic based emulsion added with resveratrol and found that increasing the temperature (45 ºC for 15 days) can cause loss of antioxidant activity.

Antioxidant activity can vary according to the functional group and its arrangement around the fundamental nucleus, mainly in rings B and C. Functional hydroxyls can donate an electron and a hydrogen, stabilizing the structure of phenolic compounds through resonance. As most flavonoids are in glycosylated form, the position of their sugar also affects their antioxidant capacity[16]. The presence of a 3-ring system linked to a sugar via C-C-glucoside in the structure makes the molecule even more stable to chemical and enzymatic hydrolysis. On the other hand, the aglycone form has greater antioxidant capacity, however, its availability is limiting[9,17,31,35,36]. According to Souza[18], the method of drying the peels of *M. indica* L. var. Tommy Atkins can influence the antioxidant activity of the extract obtained by ultrasound using ethanol as a solvent. The evaluation of the extracts (500 mg/ml) by the DPPH radical scavenging method indicated that peels subjected to natural drying and oven drying showed 94.4% ± 0.4% and 72.4% ± 0.7% inhibition of DPPH radical, respectively, suggesting that increased temperature can degrade bioactive compounds with antioxidant properties present in the ethanolic extract, such as phenolic compounds. According to Araújo[37], flour extracts from freeze-dried peels of *M. indica* L. var. Tommy Atkins demonstrated significant

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antioxidant capacity against the DPPH radical, showing no statistically significant difference in relation to gallic acid.

The pharmaceutical, cosmetic and food industries show great interest in obtaining extracts and oils from raw materials of plant origin, as several studies have reported long-term harmful effects of synthetic antioxidants, such as carcinogenesis, being the natural ones a safer, sustainable, cheap and practical alternative[13]. Although little explored in the scientific literature, the bioactive potential of the Tommy Atkins variety of Mangifera indica mango for use in cosmetics offers promising perspectives. Rich in antioxidants, such as vitamin C, carotenoids and polyphenols, Tommy Atkins mango can help neutralize free radicals in the skin, responsible for oxidative stress and premature aging. Therefore, considering its compatibility with galenic bases and demonstrated antioxidant activity, the incorporation of *M. indica* L. var. Tommy Atkins proves to be an interesting choice in anti-aging cosmetic foundations and skin protectors[38].

CONCLUSION

The glycolic extract of the peels of *Mangifera indica* L. var. Tommy Atkins presented an antioxidant potential, particularly standing out in Carbopol® gel. This characteristic can be attributed to the presence of phenolic compounds, particularly flavonoids, present in the glycolic extract. The results obtained in the work indicate the promising investigation of bioactive compounds in by-products, such as the peels of *M. indica* L. var. Tommy Atkins for its incorporation into new phytocosmetic formulations, promoting a sustainable approach. Furthermore, Brazil, as one of the largest food producers in the world, has enormous potential to take advantage of these byproducts. The use of peels, which are often discarded, can contribute to reducing waste and adding value to agricultural products, promoting a more sustainable and innovative economy.

CRediT author statement

Methodology: Balista, PA; Dario, MF; Gonçalves, FSM. Validation: Ji, S; Balista, PA; Dario, MF; Gonçalves, FSM. Statistical Analysis: Gonçalves, FSM. Formal Analysis: Ji, S; Balista, PA; Dario, MF; Gonçalves, FSM. Investigation: Ji, S; Gonçalves, FSM. Writing-original draft preparation: Ji, S; Gonçalves, FSM. Writing-review and editing: Ji, S; Balista, PA; Dario, MF; Gonçalves, FSM. Visualization: Ji, S; Balista, PA; Dario, MF; Gonçalves, FSM. Supervision: Gonçalves, FSM. Project Administration: Gonçalves, FSM.

All authors have read and agreed with the published version of the manuscript.

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