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Original Article

FORMULATION OF AN ANTIOXIDANT HYDROGEL FACE MASK USING AN ORGANIC, SUSTAINABLY SOURCED, STANDARDISED WATER EXTRACT OF YERBA MATE (*ILEX PARAGUARIENSIS*) AS ACTIVE INGREDIENT.

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Abstract

Background: The use of natural products in cosmetics has become increasingly popular in recent years. One such natural product that has shown promise as a cosmetic ingredient is *Ilex paraguariensis*, also known as yerba mate.

Aim: to formulate an antioxidant hydrogel face mask using an organic, sustainably sourced, standardised water extract of yerba mate (*Ilex paraguariensis*) as active ingredient.

Methods: Organic yerba mate was water-extracted at 40°C and 80°C. Theobromine, theophylline, caffeine, and various phenolic acids were quantified using RP-HPLC-UV analysis. Extracts were then evaluated for antioxidant activity (DPPH assay). A commercially available mask-making machines for home use was used to produce hydrogel masks based on marine collagen and these were analysed for active ingredient distribution (homogeneity).

Results: We did not find differences between 40°C and 80°C water extracts of yerba mate. The content in phytochemicals ranged 0.02-1 mg/mL. The extract equivalence in chlorogenic acid (CA) according to the DPPH assay was 0.25 mg_{CA} / $mg_{extract}$. The hydrogel facial mask was successfully formulated with 0.1 mg/mL of standardised yerba mate extract. The content in phytochemicals within the gel varied most for caffeic acid and theobromine but less for theophylline, caffeine, chlorogenic acid, ferulic acid, rutin, quercitrin, and quercetin.

Conclusion: Face mask “home” machines can deliver hydrogel face masks based on marine collagen that incorporate yerba mate (*Ilex paraguariensis*) water extracts as active ingredients without detriment to the consistency, texture, and sensory characteristics, with relatively homogenous concentrations of the active principles.

INTRODUCTION

The use of natural cosmetic products has become increasingly popular in recent years due to the growing awareness of the potential harmful effects of synthetic ingredients. Natural products are derived from plants, animals, and minerals and are often considered to be safer and more environmentally friendly than synthetic ingredients. Due to their natural properties, they are also believed to be more effective in treating various skin conditions (Sahota, 2014).

One such natural product that has shown promise as a cosmetic ingredient is *Ilex paraguariensis*, also known as yerba mate (Figure 1). This plant is native to central and southern regions of South America and is traditionally used as a beverage. Its active constituents include polyphenols, flavonoids quercetin and rutin, xanthine such as caffeine, theobromine, and theophylline (Schinella et al., 2014); and minerals such as magnesium, manganese, and potassium salts. There is ample scientific evidence of its "anti-inflammatory" (Schinella et al., 2014), "antioxidant" (Schinella et al., 2000), and "anti-ageing" (Niraula et al., 2018) properties in both *in vitro* and *in vivo* models. Moreover, *Ilex paraguariensis* leaf extract can help to protect the skin from UV-induced skin epithelium protein damage by increasing metalloproteinases and myeloperoxidase activities (Cuelho, et al., 2018).

These bioactivities make it a promising active ingredient in skincare, hair care products, pre, and post-sun care products. Indeed, Several *Ilex* spp. cosmetic ingredients are now included in the International Nomenclature of Cosmetic Ingredients (INCI) and the leaves extracts of *I. paraguariensis* can be specifically used as a cosmetic ingredient in Europe with the approved functions of hair conditioning, perfuming, skin conditioning (European Commission, 2023).

Figure 1: Yerba Mate (*Ilex paraguariensis*) (Credits: Dick Culbert from Gibsons, B.C., Canada)(CC-BY 4.0).

Hydrogel-based commercial products are already very well established in the cosmetic market (Mitura, Sionkowska, & Jaiswal, 2020), and semiautomated machines for home use are readily available. Recently they are gaining attention for their potential biomedical applications (Cascone, S. & Lamberti, G. 2020). Hence, the current study is an attempt to formulate an ecological, herbal facial mask using *Ilex paraguariensis* leaves as the active principle.

METHODS

Plant materials

The Commercial, organic yerba mate (Net weight 500 g) was purchased from Kraus (Misiones, Argentina) (Batch number 475; expiry date: June 25). The plant is an organic product of Argentina; It contains whole leaves (with stems) dried with hot air (100% free from smoke) and stored for 12 months. This product is certified by the Organico Argentina by the Organizacion International Agropecuaria (OIA), fair trade by IMO

Fair for Life, the U.S Department of Agriculture (USDA) and the EU Ecological Agriculture. The product is also registered in the German Bio-Siegel database and Canada Organic Regime (Kraus, 2020).

Phytochemical Reference Standards

Theobromine (99%), theophylline (99%), ferulic acid (99%), chlorogenic acid (ε 95%), caffeine (ε 98%), quercetin (ε 95, HPLC), rutin (ε 95, HPLC) were purchased from Sigma Aldrich (Gillingham, United Kingdom); caffeic acid (ε 98%, HPLC) was obtained from Koch-Light Laboratories Ltd (Colnbrook Bucks England); Quercitrin was purchased from L-Light & Co Ltd (Colnbrook, England). All were stored at 4°C.

All standards were prepared as 1 mg /1 mL (70% acetonitrile and 30% water), except rutin which was prepared in 1 mg / 10 mL (90% water and 10% acetonitrile). They were ultrasonicated (Emerson, Branson Bransonic® M Mechanical Bath 2800) at ambient temperature for 2-4 minutes, until fully dissolved. The solvent mixture was prepared as the following: Solvent A (0.05% TFA in 1 L H_2O), Solvent B (0.05% TFA in 1 L MeOH)

The cocktail was prepared as 1 mL of each of the nine standards (9 mL) with a high concentration of 0.1111 mg/mL, then underwent a serial dilution to yield the following concentrations 0.0555, 0.0277, 0.011, 0.0055 mg/mL.

Chemicals and Reagents

Methanol (ε 99.9%), acetonitrile (ε 99.9%) and trifluoroacetic acid (TFA) (ε 99%, HPLC) were supplied by Sigma Aldrich (Gillingham, United Kingdom). Distilled water was purchased from Fischer Chemical (UK). All solvents utilised for the HPLC analysis were of HPLC gradient grade.

Plant Extraction

The dry herbal product was ground into powder using a domestic grinder machine (Krups, Germany). The extraction of the plant was as follows: 1 g of dry herbal product was weighed on an analytical balance scale (Kern- ABT 100-5NM). 1 g of plant material was added to a beaker and then hot purified water (40°C or 80°C) for 10 minutes and subsequently filtered using Whatman™ qualitative filter (Healthcare Life Science, UK).

After sample filtration, 1.5 mL of each sample was taken using 2mL syringe (BD Emerald, UK) and a sterile PES Syringe Filter (PTFE 0.2 µm) (Fisher, UK) was placed in the syringe to filtrate the sample from potential residues then placed in 1.5 mL HPLC vials (Agilent, UK). Extract samples were prepared freshly every day just before use to avoid microbial growth.

Formulation of facial mask

The mask preparation was made using a Silvercrest Professional Care face mask maker machine (Bochum, Germany). Two main ingredients are required for the formulation: 120 mL of yerba mate infusion and one marine collagen tablet (1 g) (SGM 110 A1) (Kompernass; Bochum, Germany). The herbal infusion was made by adding 120 mL water (40°C) to 12 g of the dry herbal product in a 150 mL conical flask then stirred for one minute and left on the bench to settle. The infusion was then filtered three times using filter paper. The mask mould was slightly tilted to ensure the ideal distribution of the liquid. The mask was later left for 10-15 min until it showed a consistent texture. The collagen is available in tablets and is composed of citric acid, sodium bicarbonate, *Zea mays* starch, PEG-6M and collagen (Kompernass Handels GmbH, Germany).

For HPLC-UV quantitative analysis, samples were taken from five different areas of the facial mask (Top, Right, Centre, Left and bottom) with a cylindric puncher (R= 2.5 mm, area approximately 19.635 mm^2). Samples were directly put in a 20 mL vial with 3 mL of 50%aq MeOH, closed with screw top to avoid evaporation and sonicated in MeOH 50%aq (Emerson, Branson Bransonic® M Mechanical Bath 2800) at ambient temperature for 10 minutes, then filtered using 2 mL syringe (BD Emerald) and Fisherbrand™ Sterile PES Syringe Filter (PTFE 0.2 µm) to remove any residues. The addition of MeOH to the extracting solvent was to extract the phytochemicals whilst avoiding the dissolution of the gel matrix and potential contamination of the HPLC column.

High-Performance Liquid Chromatography analyses

The Agilent 1260 Series Gradient HPLC System (Waldbronn, Germany) was used, and composed of a 1260 Infinity II Quaternary Pump, preparative autosampler, High-performance Degasser, Bio-Inert Fraction Collector, multiple wavelength detector and thermostat column compartment. This system was supplied with the Agilent Open Lab software. Chromatographic separation was performed on an Ascentis® Express C18 HPLC column (25 cm x 4.6 mm, 5 µm) from Supelco® Analytical (Sigma Aldrich, Gillingham, United Kingdom) at a controlled temperature of 35°C. For the aqueous extract and reference standards, the gradient mode with mobile phase 0.05% TFA in water (Solvent A) and 0.05% TFA in methanol (Solvent B) was used as the following method for preliminary identification:0-5 min, 90% A, 10% B; 5-45 min, 80% A, 20% B; 45- 47 min, 50% A, 50% B; 47-49 min, 100% B; 49-53 min, 100% B; 53-55 min, 90% A, 20% B.

For quantitative analysis, the gradient mode with mobile phase including solvent A and solvent B respectively was performed as the following method: 0-5 min, 90% A, 10% B; 5-45 min 80% A, 20% B; 45-55 min 50% A, 50% B; 55-80 min 20% A, 80% B; 80-82 min 20% A and 80% B; 82-84 min 100% B; 84-88 min 90% A, 10% B; 88-90 min, 90% A, 10% B. The flow rate remained constant at 0.8 mL/min and the UV/Vis detector was set at 254, 275, 330 and 360 nm wavelength. The injection volume of all the extracts and reference standards including the cocktails was 10 µL.

Antioxidant activity

The antioxidant activity of *Ilex paraguariensis* was evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. A 0.2 mM stock solution of DPPH was prepared (8 mg in 100 mL MeOH) and kept at room temperature for 30 minutes in the dark. The absorbance of the reaction mixture was measured at 517 nm using an Epoch Microplate spectrophotometer (Agilent BioTek). Data was collected using Gen5™ Data Analysis Software. A serial dilution was prepared for both the standard and the 40°C aqueous extract (25, 12.5, 6.25 and 3.12 mg/mL). The absorbance of DPPH radical without antioxidant was also measured and used as blank. The ability of the plant to quench the stable free radical DPPH was monitored at 517 nm. The test was performed in triplicate. The percentage of DPPH reduction was calculated by applying the following equation:

% Inhibition: [1- (A1 / A0)] x 100

where A1 is the absorbance of crude extract and A0 is the absorbance of the DPPH solution. The results were also expressed as the concentration required to decrease the DPPH by 50% (IC50). Chlorogenic acid was used as the positive control for the antioxidant activity.

Statistics

Peak integration data from HPLC analyses were collected from the Openlab® (Agilent, UK). Data analysis and curve fittings were performed using Microsoft® Excel (Microsoft Corporation, USA).

RESULTS

HPLC-UV analysis

To standardise the water extract of *I. paraguariensis*, a total of nine bioactive ingredients belonging to three different groups were chosen: theobromine, theophylline, and caffeine (xanthine alkaloids, detection at 275 nm); chlorogenic acid, caffeic acid, and ferulic acid (phenolic acids, detection at 330 nm); rutin, quercitrin, and quercetin (flavonoids, detection at 254 nm). Their elution order is shown in Figure 2.

The identification of phytochemicals within the aqueous extract (Figure 3) was done by comparing the retention time (Rt) and UV spectrum of the standards with those of the aqueous extract (Table 1). There are slight differences in the Rt of the reference standards when injected as a cocktail and the corresponding phytochemicals in the herbal extract due to the “matrix effects”.

The quantification of the phytochemicals using a calibration curve gave similar results for the two temperatures of extraction used (Table 1). Therefore, we decided to use the extract at 40 ºC as it is less energy-consuming thus reducing the carbon footprint of the cosmetic formulation.

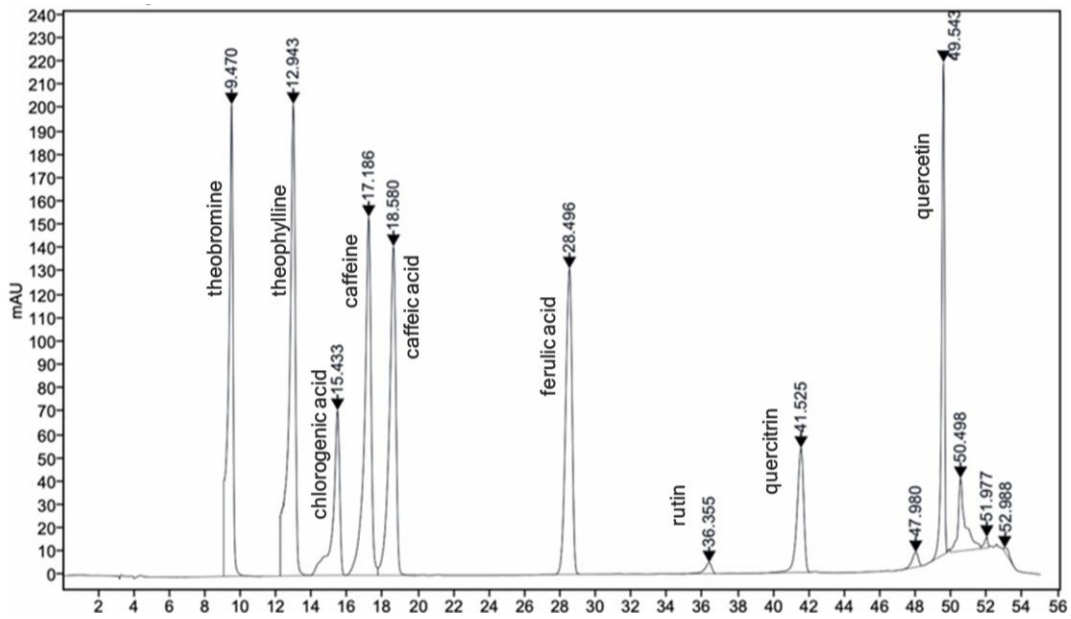

Figure 2. *HPLC-UV (275 nm)* chromatogram of the phytochemical standards.

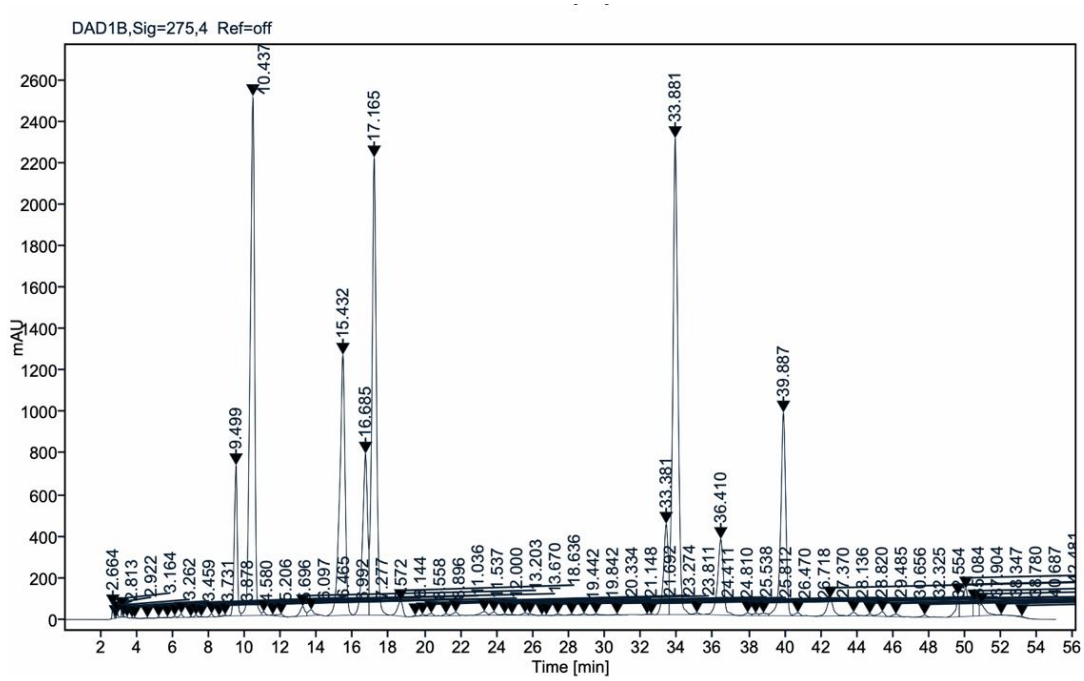

Figure 3. HPLC-UV (275 nm) chromatogram of Ilex paraguariensis aqueous (40°C) extract.

Table 1. Identification and concentration of phytochemicals in the yerba mate extracts.

Active principle	Retention times (min)			Concentration in water extract (mg/mL)	
	Std Only	Std Cocktail	Mate Extract	40°C	80°C
Theobromine	9.42	9.47	9.50	0.110	0.0950
Theophylline	12.9	12.9	13.0	0.0160	0.0140
Chlorogenic acid	15.4	15.4	15.4	1.06	0.981
Caffeine	17.1	17.2	17.2	1.00	0.922
Caffeic acid	18.5	18.6	18.6	0.0491	0.0460
Ferulic acid	28.4	28.5	28.5	0.00203	0.00192
Rutin	36.4	36.4	36.4	0.110	0.0961
Quercitrin	41.4	41.5	41.5	0.0960	0.0890
Quercetin	49.5	49.5	49.5	0.0230	0.0212

The facial mask's total weight was typically 65 g (± 1.3 g). The extract conferred the product an appealing natural golden colour. The extract did not alter the consistency and sensory characteristics of collagen hydrogel as compared with a blank formulation. The average concentration of each metabolite in different zones of the mask (Figure 4) was calculated and taken as 100%, then each zone value expressed as % of the average as a means to normalise the values (Table 2).

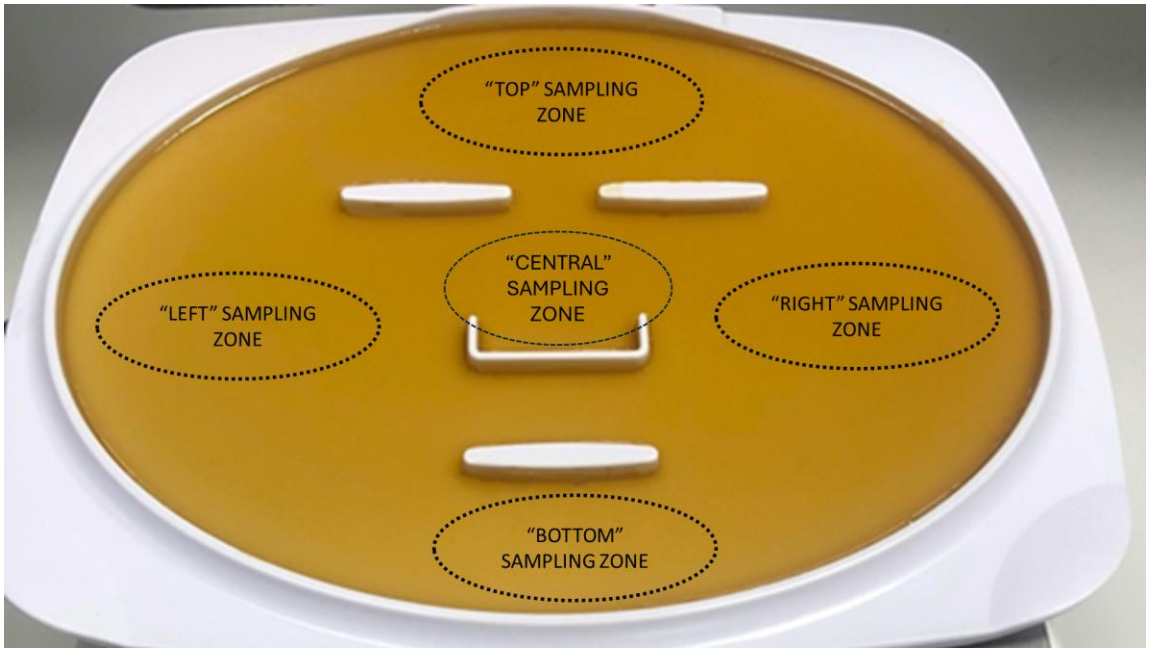

Figure 4. Hydrogel facial mask formulated with 0.1 mg/mL of standardised yerba mate extract showing the sampling zones.

Table 2. Heat map of the normalised concentration of each standardised phytochemical within different parts of the formulated product. Variability is expressed as the standard deviation (SD) of the normalised values.

	Top	Right	Centre	Left	Bottom	SD
Theobromine	200%	200%	100%	100%	100%	0.490
Theophylline	100%	125%	108%	92%	75%	0.166
Caffeine	109%	118%	105%	91%	82%	0.129
Chlorogenic acid	71%	124%	110%	107%	90%	0.182
Caffeic acid	50%	150%	100%	100%	50%	0.374
Ferulic acid	100%	117%	100%	83%	83%	0.127
Rutin	100%	100%	100%	100%	75%	0.100
Quercitrin	100%	100%	100%	100%	100%	0.000
Quercetin	111%	117%	106%	94%	89%	0.104

Radical scavenging activity

The antioxidant activity of the aqueous extract (40°C) was evaluated using DPPH radical assay. The calibration curve of Absorbance 517nm vs. micromolar DPPH is shown in Figure 5. The standardised yerba mate extract reduced 50% the absorbance of the DPPH radical at 20.7 µg/mL whilst chlorogenic acid (antioxidant of reference) did it at 5.14 µg/mL. Therefore, the extract equivalence in chlorogenic acid (CA) is 248 mg_{CA} / $g_{extract}$.

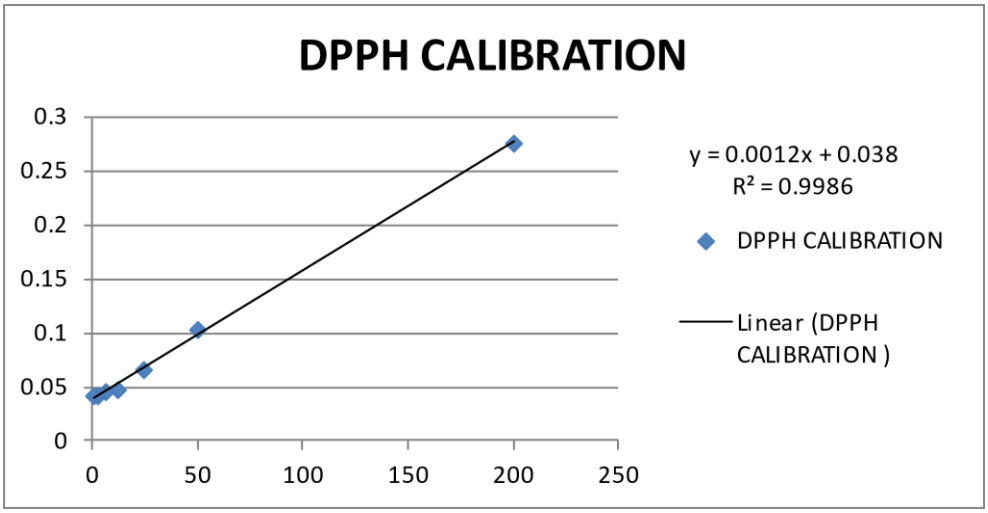

Figure 5. Calibration curve for absorbance (517nm) vs. concentration DPPH (µM).

DISCUSSION:

Consumers increasingly require remedies for various skin disorders that are free of side effects. Natural plant ingredients succeeded in producing cosmetic creations with minimum adverse effects. Facial hydrogel masks are highly regarded in the cosmetic field, thus the increased usage of herbal ingredients in personal care products requires studying new plant compounds and biological functions on the skin, therefore creating innovation and variation of cosmetic products to both industry and customers (Nilforoushzadeh et al, 2018).

Based on the reference standards and optimisation of the aqueous extract readings, the presence of aimed standards was present in the extract and varied in concentrations. The total content of polyphenols and flavonoids of a commercial brand of yerba mate was analysed in two aqueous extractions at 40°C and 80°C. There are no significant differences in the content of different polyphenols using any of the temperatures, therefore we opted to use the lower temperature in the spirit of keeping the carbon footprint of the cosmetic as low as possible (Sahota, 2014).

Sampling different areas of the face mask showed that theobromine and caffeic acid concentrations were the most variable across the product, followed by theophylline and chlorogenic acid whilst the rest appeared more homogenous. The reason why that the higher variability for theophylline and chlorogenic acid is not clear and would require further experiments. In the case of caffeic acid, we may hypothesise some losses in contact with the air (oxidation) but no clear reason can be put forward in the case of the xanthine alkaloid. The distribution of each phytochemical could also depend on different face mask machines, chemical stability in collagen, and noon homogeneous thickness of the gel. All these factors warrant further research.

The antioxidant activity of the extract is consistent with previous data published using slightly different methods and reference antioxidant 248 mg CA/g vs. 200 mg GAE/g (Dudonné, et al., 2009). The presence of well-known antioxidants such as phenolic acids (chlorogenic acid, caffeic acid, and ferulic acid) alone or in combination with quinic acids and flavonoids (rutin, quercitrin, and quercetin) warrants a high radical scavenging activity. Mate is also renowned for its high content of xanthine alkaloids rivalling coffee, tea, chocolate, and soft and energy drinks. Although many of the physiological effects of these alkaloids are well known their antioxidant properties are not yet fully understood. Research on the model of alkaloid caffeine has suggested protective effects against oxidative stress. Although it is inert to hydrogen/electron scavengers such as ABTS·+ and DPPH, caffeine has been proposed as an excellent HO· scavenger (Petrucci, Curulli, and Marrosu, 2017).

This product has been ecologically formulated at a low temperature (40°C) and uses purified water, Ilex organic materials and collagen as the only ingredients, so it is 100% natural. It is easily made with consumer tools to make homemade facial masks; Future efforts will look into replacing the collagen with cellulose, xanthan or other algae polysaccharides acting as gel-forming thickeners to qualify for a “vegan formulation” (Mitura, Sionkowska, & Jaiswal, 2020).

CONCLUSION

Face masks “home” machines can deliver hydrogel face masks based on marine collagen incorporating yerba mate (*Ilex paraguariensis*) water extracts as active ingredients without detriment of the consistency, texture and sensory characteristics. Treatments with such products could benefit from the cosmeceutical functionality of the active principles (theobromine, theophylline, caffeine, chlorogenic acid, caffeic acid, ferulic acid, rutin, quercitrin, and quercetin).

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