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Original Article

The application of the aqueous extract of milona leaves (*Cissampelos sympodialis* Eichl) in a cosmetic formulation

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Abstract

Background: In the light of the search for untried natural active ingredients to provide new possibilities and increased benefits with their application in the cosmetic industry, we have introduced the species *Cissampelos sympodialis* Eichl (Menispermaceae) into the field of cosmetics. Our research was focused on the aqueous extract of *Milona* leaves (*C. sympodial*) and its application in cosmetic formulations.

Materials and Methods: A phytochemical screening confirming the presence of some classes of secondary metabolites such as alkaloids, tannins, flavonoids, and saponins had previously been reported in the literature. Due to its medicinal potential, mentioned in the literature, we applied the aqueous extract of *C. sympodialis* leaves in the development of cosmetic formulations in view of its antioxidant and anti-inflammatory properties. On this basis, we consider the extract to be a promising active ingredient for skin and hair care. Therefore, the aim of the present study was to develop a cosmetic formulation (o/w), namely, a facial care cream containing the aqueous extract of *milona* leaves, and perform the stability study of the formulation.

Results: The stability study showed that the formulation presented no change in organoleptic characteristics nor in physical and chemical parameters. The mean pH value was 4.5 ± 0.2, and the mean viscosity values were 999 ± 0.3 cP, which remained unchanged throughout the study. Microbiological analysis showed no formation of bacteria or fungi in the formulation containing the aqueous extract of *C. sympodial*.

Conclusions: We concluded that the cosmetic formulation containing this innovative raw material presents the desired characteristics for a new product. Further research will be necessary to perform clinical tests with volunteers to verify the benefits for the skin and hair.

Keywords: *Cissampelos sympodialis* Eichl, Aqueous extract, Cosmetic formulation, Facial cream, *milona*, Skin

Background

Natural products are a great source of inspiration for research in various areas of knowledge, especially in the area of pharmacology, in the synthesis of new drugs.[1] This is also the case in the field of phytocosmetics; many plants are used in cosmetic formulations in the activation of various properties such as anti-oxidation, anti-aging, and skin and hair moisturizing.[2]

The search for natural active ingredients in the cosmetic area has become an increasing challenge. Based on strong consumer interest, we have developed a cosmetic formulation with the aqueous extract of a medicinal plant species, popularly known in Brazilian Portuguese as *Milona*, *Abuteira*, *Jarrinha*, and *Orelha-de-Onça*, whose scientific name is *Cissampelos sympodialis* Eichl. It belongs to the *Menispermaceae* family, which is composed of approximately 70 genera and 420 species, is represented in Brazil by about 15 genera and 108 species. The *Cissampelos* genus is composed of 19 species, 9 of which are found in Brazil. Of these, three occur in the state of Paraíba: *Cissampelos sympodialis*, *Cissampelos parreira* and *Cissampelos fasciculata*.[3-6]

Cissampelos sympodialis Eichl. is an endemic species found in the Brazilian Northeast and Southeast.[3,4] It is used by indigenous tribes and folk medicine to treat genito-urinary infections, inflammation, diseases in the respiratory tract, particularly asthma, and gastrointestinal disorders, such as diarrhea.[3-8]

This species has already been studied from the pharmacological and phytochemical points of view and has been found to have effective anti-inflammatory, immune-modulatory, anti-allergic, and anti-depressant action and effective in the treatment of gastric ulcers.[9-17] The *Menispermaceae* family is characterized by the presence of alkaloids. One thousand five hundred twenty-two such molecules have been isolated, particularly the bisbenzylisoquinoline alkaloids, as well as aporphine and protoberberine.[10] Phytochemical research into *C.*

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sympodialis has identified and characterized the structure of the following alkaloids: sympodialine, laurifoline,[18] milonine,[19] warifteine, methyl warifteine,[20] des-7'-o-methylroraimine, epi-des-7'-O-methylroraimine epimer[21] and sousin.[22]

One of the principal chemical constituents identified in the aqueous extract of *C. sympodialis* was a bisbenzylisoquinoline alkaloid, warifteine, together with its derivative methylwarifteine and milonine, which are considered to be the primary markers of the species and were responsible for several pharmacological actions of the plant leaf extract.[6,21]

With regard to combating or preventing skin aging, several medicinal plants, such as *Camellia sinensis* (antioxidant, anti-aging); *Aloe vera* (moisturizer, sunscreen, emollient); Cucumber extract (relief of swelling, anti-inflammatory effects), and *Rhodiola rosea* (anti-oxidative properties) have been used as natural sources in the development of cosmetic products.[23,24]

The literature has revealed that extrinsic and intrinsic factors cause skin aging. Aging is a process characterized by physiological changes caused by genetic and environmental factors, which the epidemiologist Christopher Wild defined as the exposome.[25-27] Intrinsic or chronological aging is caused by the decay of vital body functions, cellular turnover index, inadequate immune responses, and other normal body impairments.[28,29]

As a result of these changes throughout the cellular structure, the organism becomes vulnerable. Changes in the genetic transcription of various proteins, enzymes, and DNA molecules impair their functionality. Intrinsic aging is natural, predictable, and inevitable, being slower and more gradual than extrinsic aging. Intrinsic aging is proportionate to chronological age and does not cause such pronounced damage as extrinsic aging, caused by external aggressions such as (*a*) photo-aging exposure to ultraviolet rays, visible light and infrared radiation, (*b*) air pollution, (*c*) smoking, (*d*) lack of sleep, (*e*) inadequate lifestyle, (*f*) stress, (*g*) climate; (*h*) inadequate skincare, and (*j*) infections.[25-27]

The search for natural active ingredients that can reverse the effects of skin exposure has exercised the scientific community. Given the above, and taking into account its anti-inflammatory and antioxidant properties,[30] secondary metabolites of the *C. sympodial* species can promote anti-aging benefits for the skin due to their anti-inflammatory and antioxidant properties already mentioned in the literature.[5-11]

The aim of the present study was to incorporate different concentrations of the aqueous extract of *C. sympodialis* leaves in cosmetic face cream formulations (o/w). As a result of its natural chemical composition, this species has possible anti-aging properties when used in a cosmetic formulation. To obtain a face cream containing the aqueous extract of *C. sympodialis*, we used several raw materials such as emulsifiers, emollients, humectants, and preservatives in the formulations.

It is important to highlight that the use of this plant species in the field of phytocosmetics is unprecedented.

Objective

The objective of the present research was to develop an innovative cosmetic formulation with the aqueous extract of *milona* leaves. The study incorporated the aqueous extract of *C. sympodialis* into face cream (o/w) and examined the stability of the formulation with different concentrations of the extract.

Materials and Methods

Professor Fátima Agra had already performed the botanical identification of this species with an *exsicata* (code Agra 1456) deposited in the Lauro Pires Xavier Herbarium of the Federal University of Paraíba (UFPB), as mentioned in previous papers.[12]

Obtaining milona Extract

The leaves were dried (35º-38ºC) in an oven and were later crushed in a Harley mill. An aqueous extract of the *milona* leaves was then obtained by percolation. In order to preserve its composition, the sample was submitted to microbiological tests and found to be free of bacteria and yeasts, enabling it to be used in cosmetic applications as required by Brazilian legislation. The aqueous extract was obtained and applied in concentrations of 0.1-15.0% (w/w); pH was measured in triplicate using a pH meter (Quimis) and calibrated with pH 4.0 and 7.0 buffer solution by inserting an electrode into the aqueous extract of *C. sympodial*. The chemical composition of the *milona* leaves was confirmed by phytochemical screening; the following identification reagents were used for alkaloids: Dragendorff Reagent, Mayer's Reagent, Bouchardat Reagent, Bertrand Reagent, flavonoids, saponins, and tannins, followed by thin-layer chromatography analysis, using specified reagents for each of the constituent groups.[31]

Cosmetic Formulation

An emulsion is a system composed of a mixture of two immiscible liquids. The liquid in greater quantity is dispersive, and the liquid in smaller amount is the disperse, which is distributed in tiny droplets diffused in a suspension.[32-34] The change in free energy during the formation of the emulsion is usually positive and, for this reason, emulsions can be thermodynamically unstable.[32-34] When dealing with emulsions, it is necessary to distinguish each of the two phases clearly. The phase in which the emulsion is in droplets, finely divided, is called the dispersed or internal phase. The phase that forms the matrix in which these droplets are suspended is called the continuous or external phase. The process

of distinguishing between the different types of emulsion consists of noting which component is continuous and which one is dispersed. Thus, we can classify emulsions as oil in water (oil is the dispersed phase) or water in oil (water is the dispersed phase). These types of emulsion are conveniently abbreviated as o/w and w/o, respectively.[34-37] The aqueous extract developed in the present study was applied to formulate a facial cream (o/w). The emulsions were obtained with raw materials in maximum and minimum quantities represented in percentage by weight, based on the total weight of the composition: vehicle (0.0%-100%), thickeners (0.2%-0.7%), emollients (0.5%-5.0%); humectants (0.3%-4.0%); aqueous *milona* extract (0.1%-15.0%); chelating agent (0.01%-0.02%); antioxidant (0.01-0.02%); preservative (0.01%-0.1%) and fragrance (0.01%-0.5%).[35-37]

Microbiology Test

The microbiological test to check whether the aqueous extract of *milona* leaves (*C. sympodial*) and the facial cream containing was complete; this extract was free of microorganisms, such as bacteria and fungi. It was performed using the company's Biolaminotest microbiological control kit called IPEL [38] – specially developed for industrial verification of microbial contamination on surfaces, equipment, utensils, raw material, and finished products – following the manufacturer's directions. The slides contain specific media for the detection of microorganisms; one side contains specific media for the detection of total heterotrophic bacteria, and the other side contains specific media for the detection of yeasts and filamentous fungi. The result is readable within two to three days. Counting is performed by the direct counting method; the number of colonies on the surface of the blade is counted and compared with the standard table provided by the manufacturer. The number of colonies found must be multiplied by the relative exponential magnitude.[38]

Stability Analysis

The development of a cosmetic product requires knowledge of the formulation and its components as well as a physical-chemical stability study to ensure that the product has standard quality. For this purpose, the researcher needs to perform several stabilization tests. The stability study of this gel-cream formulation, containing the aqueous extract of *C. sympodial*, was evaluated according to ANVISA (Brazilian National Health Surveillance Agency)[39] standards, as well as parameters such as color, odor, appearance, pH value, viscosity, texture and possible signs of separation under the following temperature conditions: room temperature, exposure to sunlight, oven (50°C) and freezer (5°C), for a period of 45 days.[39] The readings were taken after samples had been selected from the stability test chambers and after they had reached room temperature.[39,40] The pH value of facial creams containing *C. sympodialis* extract was obtained by averaging three measurements using a pH meter (Quimis), calibrated with pH 4.0 and 7.0 buffer solution by inserting the electrode into the cream formulation. The viscosity was measured with a digital rotary viscometer–model NDJ-5S. In addition, we performed a centrifugation test for 60 minutes at 3000 rpm[39-41] to verify the stability of the cream formulation containing the extract.

Results and Discussion

The aqueous *milona* extract obtained had a pH of 4.6. This pH value was within the skin pH range.[42] Average skin pH value in women is approximately 5.5 and in men closer to 5. Moreover, the pH values of the skin vary according to the area used for the measurements and to various external factors.[42,43] The pH value of the aqueous extract developed in the present study therefore compatible with the pH value of our skin, which implies that its application in cosmetic formulations may help to maintain the pH balance of the skin. For this reason, it has the potential to be used in the treatment of skin disorders such as atopic dermatitis, acne, dry skin, and psoriasis, caused by alterations in the pH of the skin, as is known from the literature.[44] The pH value of the aqueous extract of *Cissampelos sympodialis* is thus compatible with topical application to human skin.

Preliminary phytochemical analysis by thin-layer chromatography screening of the aqueous extract revealed various groups of compounds (alkaloids, flavonoids, tannins, and saponins). According to the colors observed on the spot extract, these data were obtained to which each specific reagent was added. Results are summarized in Table 1. The composition of *milona* leaves, according to the literature, reveals the following class of chemical constituents: alkaloids, flavonoids, tannins, and saponins, which were confirmed by previous corroborating phytochemical studies.[6-10] *C. sympodialis* presented a class of chemical constituents such as alkaloids and flavonoids in phytochemical screening. These classes of secondary metabolites are generally of interest in cosmetic anti-aging formulations and thus show promise in phytocosmetics.

The Result of the Microbiological Test

The microbiological test performed showed that the aqueous *milona* extract and the derived cosmetic formulation under study presented no undesirable

Table 1. Phytochemical Screening of Aqueous Extract of *Cissampelos sympodialis* Leaves

Species	Alkaloids	Tannins	Flavonoids	Saponins
Cissampelos sympodialis leaves	+++*	++**	++**	+***

(+++)* strong concentration, (++) medium concentration, (+)*** lower concentration**

microorganisms such as opportunistic pathogens and saprophytes. Hence, the result is compliant with the requirements of the legislation on microbiological tests for cosmetic products in Brazil, according to ANVISA.[39,45,46] The formulation is therefore safe for the consumer.

The Results of the Stability Study

Stability is an essential requirement for any facial formulation. These tests were performed because there have been no previous studies with this extract in cosmetic applications. The formulation was subjected to accelerated stability tests under different temperature conditions (room, sunlight, refrigerator 5ºC, and oven 50ºC) for 45 days. In the stability study, organoleptic characteristics such as color and odor and physical and chemical parameters were checked. The stability study showed that the formulation presented no changes in organoleptic characteristics nor in physical and chemical parameters. The facial cream containing the aqueous extract of *C. sympodialis* presented a pleasant tactile characteristic, shiny aspect, easy application, good spreadability, and good absorption. It is vital in the stability study of a cosmetic formulation that, even under stress, the physical-chemical characteristics of the formulation should remain unchanged, guaranteeing a safe and stable product for the consumer.

All the stability tests were applied to different concentrations of the aqueous *milona* extract in the formulation of the face cream (o/w) attested to its stability. Regarding stability, emulsions (o/w), like the one developed in the present study, are more prone to cremation since oils are generally less dense than water. On the other hand, emulsions (w/o) tend to sediment due to the inverse ratio, and water tends to rise.[47] In effect, the stability of this formulation can be verified by the fact that the emulsion did not undergo a phase separation process such as coalescence, flocculation or cremation.[35-37] (Table 2). It is very important that the active components of the aqueous *milona* extract. The formulation components have a good level of stability, as demonstrated in the present study, to maximize the extract's effectiveness when applied to the skin.

Table 2. Stability Study of the Face Cream Containing the Aqueous Extract of *milona* Leaves (*Cissampelos sympodial*) in Greenhouse Conditions (50°C)

Specification	Time Zero	Week One	Week Two	Week Three	Week Four
Aspect	U	U	U	U	U
Color	U	U	U	SA	SA
Smell	U	U	U	SA	SA
pH	5.21	5.21	5.20	5.20	5.19
Spindler	1	1	1	1	1
Speed (rpm)	6	6	6	6	6
Viscosity (cP)	999	999	999	999	999

U: unaltered; SA: slightly altered.

Concerning the pH and viscosity value analysis, the mean pH value was 5.2 ± 0.2, and the mean viscosity values were 999 ± 0.3 cP, which remained unchanged throughout the study (Table 2). This result showed that the pH and viscosity values of the entire formulation were stable to temperature variations. The pH value of the face cream containing the aqueous extract of *C. sympodialis* demonstrated excellent results for the application of products in topical formulations; the value was 5.2 ± 0.2. The literature shows that low pH values in cosmetic formulations can cause skin irritation.[48,49] A stable pH value guarantees a formulation without instability effects between the formulation components and the aqueous extract of *C. sympodial*, promoting a good level of efficacy of the active ingredients on the skin. The centrifugation test performed on the face cream formulation containing the aqueous extract of *C. sympodialis* showed no phase separation. We know that an emulsion (o/w) tends to separate and stay in the upper space of emulsion,[33] but this effect was not observed during the present study.

Concerning the parameters analyzed in the present study, it is important to highlight the contribution of these tests, for they guide the development of a formulation, provide information for the improvement of formulas, estimate the expiry date and provide information for its confirmation. They also assist in monitoring the organoleptic stability and physical-chemical and microbiological parameters, producing information on the reliability and safety of products,[39-41] and help to select the formulations for the next steps of the research.

The accelerated stability study of the formulations was carried out as recommended by ANVISA. The analysis was undertaken based on the climatic parameters previously mentioned for 45 days, providing evidence that the facial moisturizing creams met the requirements of the accelerated stability study, with unchanged viscosity, almost constant pH, and stable organoleptic characteristics.

Conclusions

The present study confirmed the practical application of an aqueous extract of *C. sympodialis* in a facial cream, obtaining a stable emulsion within the analyzed parameters. It was possible to obtain a final product with acceptable quality for the following stages of research using a new active ingredient in varied concentrations,. We concluded that the cosmetic formulation containing this innovative raw material presents interesting characteristics for a new product. Further research is required in clinical tests with volunteers to study the benefits of the skin and hair. The next steps of the study will focus on conducting tests with volunteers to verify the possible benefits of *milona* extract about skin anti-aging and skin disease.

Competing Interests

None.

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