

# Effects of a Brazilian herbal compound as a cosmetic eyecare for periorbital hyperchromia (“dark circles”)

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## Summary

**Background** Evidence suggests that periorbital hyperchromia (dark circles) occurs mainly as a consequence of postinflammatory hemodynamic congestion producing a typical bruising aspect on the lower eyelids.

**Aims** To evaluate the clinical effects of *Pfaffia paniculata*/*Ptychopetalum olacoides* B./*Lilium candidum* L.-associated compound (PPLAC) on periorbital hyperchromia and to study *in vitro* its underlying anti-inflammatory and antioxidant mechanisms.

**Methods** Twenty-one volunteers presenting with periorbital hyperchromia received a serum sample containing 5.0% PPLAC, which was applied topically in the periorbital area twice a day for 28 days. Skin color was measured using variations in the individual typological angle ( $\Delta ITA^0$ ) and skin luminance ( $\Delta L^*$ ) calculated in the area around the eyes and in the adjacent area. Colorimetric readings were taken at the onset and end of the 28-day treatment. Volunteers were also asked to fill out a questionnaire concerning the improvement in “dark circles.” The anti-inflammatory and antioxidant effects of PPLAC were measured by quantification of prostaglandin E<sub>2</sub>, leukotriene B<sub>4</sub>, histamine, and superoxide dismutase levels using an *in vitro* model of human skin culture.

**Results** Topical application of PPLAC led to a significant improvement in skin luminance and tone in the periorbital area, which was demonstrated by increased values of  $ITA^0$  and  $L^*$  in about 90% of volunteers. In addition, subjects reported reduced intensity and improved appearance of “dark circles.” A dose-dependent decreased production of inflammatory mediators, concomitant to increased antioxidant enzyme levels, was observed in our *in vitro* studies, under basal and lipopolysaccharide-stimulated conditions.

**Conclusions** Although the precise mechanisms related to PPLAC remain to be clarified, our results indicate that the reduction in the inflammatory process as well as the antioxidant protection against deleterious elements may be considered as an integral approach to preserve the integrity of vascular endothelium, preventing the hemodynamic congestion that culminates in the formation of “dark circles” around the eyes.

**Keywords:** periorbital hyperchromia, eyecare, anti-inflammatory, antioxidant, dark circles

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## Introduction

Periorbital hyperchromia (dark circles) is an esthetic facial concern characterized by alterations in the color of the upper and lower eyelids. The terminology “dark

circles” is not a medical concept; however, it is widely used by both patients and dermatologists to indicate hyperchromic macules and patches around the eyes.<sup>1</sup> In contrast to the high frequency of its occurrence, there are few published studies about the pathogenesis and treatment of periorbital hyperchromia.

It has been reported that darkening around the eyes is caused by a process of dermal melanization and/or postinflammatory hemodynamic congestion.<sup>1–3</sup> Melanin and hemoglobin are the dominant chromophores of human skin and their local accumulation promotes changes in skin color.<sup>4</sup> The dark rings formed from melanin pigmentation are associated with certain ethnic groups and are frequently seen in multiple members of the same family.<sup>3</sup> Nevertheless, histological characteristics of periorbital biopsies revealed a poor correlation between improvement in “dark circles” and reduced melanosis in the skin.<sup>5,6</sup>

Evidence suggests that an important mechanism involved in the development of “dark circles” is related to sluggish blood flow.<sup>1–3,7</sup> It is known that the skin of eyelids is the thinnest in the body, and conditions such as congestion, hyperemia, or any other circulatory alterations are readily reflected at the surface.<sup>3</sup> Both congenital and environmental factors, including ultraviolet radiation, chronological aging, physical and emotional stress, allergic and atopic reactions, exogenous or even unbalanced endogenous estrogen are involved in the cutaneous disturbance, and all these variables lead to the release of inflammatory mediators, affecting vascular permeability.<sup>2,8</sup> Keratinocytes, which comprise the vast majority of epidermal cells, are largely responsible for the release of these mediators, as they are the first target of noxious stimuli and the first line of defense of the skin immune system. Keratinocyte-derived mediators are pivotal in modulating cellular communication that enables dermal fibroblast and endothelial cells lining the cutaneous vasculature to participate in immune and inflammatory responses.<sup>8–13</sup> The local micro-inflammatory process, once installed, compromises the hemodynamic balance, capillary integrity, and lymphatic draining.<sup>14–17</sup> Due to blood extravasation, the successive enzymatic conversion of hemoglobin produces pigments, such as hemosiderin, methemoglobin, and hemein, creating a typical bruising aspect (bluish-purple, brownish-blue, and/or brownish-purple tones).<sup>7,18–21</sup>

The absence of statistical evaluation of the frequency of this periorbital occurrence in the literature is counterbalanced by the amount of launchings and advertising on cosmetics marketed as adequate to treat it, which reflects the importance of studying this phenomenon. In this study, we have demonstrated the effects of *Pfaffia*

*paniculata*/*Ptychopetalum olacoides*/*Lilium candidum*-associated compound (PPLAC) as dermocosmetically active in the clinical improvement in periorbital hyperchromia. The instrumental evaluation, using a spectrophotometric colorimeter, showed significant improvements in skin tone and luminance in the periorbital area after the topical application of PPLAC for 28 days. These clinical observations were also confirmed by the volunteers reporting reduced intensity and improved appearance of “dark circles.” In addition, considering the contribution of microinflammatory process on hemodynamic stasis, *in vitro* studies were performed to investigate the anti-inflammatory and antioxidant effects of this compound in human keratinocyte culture. We observed a significant dose-dependent decrease in the production of prostaglandin E<sub>2</sub>, leukotriene B<sub>4</sub>, and histamine in the groups incubated with PPLAC, under basal and lipopolysaccharide (LPS)-stimulated conditions. Similarly, in both these conditions, PPLAC also stimulated the synthesis of the antioxidant enzyme superoxide dismutase (SOD).

## Materials and methods

### Plant material

*Pfaffia paniculata*/*Ptychopetalum olacoides* B./*Lilium candidum* L.-associated compound (PPLAC) (Bioskinup Contour) was manufactured and provided by Chemunion Química Ltda (Sorocaba, Brazil). The INCI name of this compound is water (and) butylene glycol (and) PEG-40 hydrogenated castor oil (and) *pfaffia paniculata* root extract (and) *ptychopetalum olacoides* bark/stem extract (and) *lilium candidum* flower extract. PPLAC is standardized in total saponins (5.5–10.0%) and total flavonoids (0.5–2.5%). PPLAC is recognized through the order of patent PI-0605812-4, deposited in the INPI (National Institute of Industrial Property) on December 22, 2006.

### Cell culture and treatment protocol

Normal human epidermal keratinocytes were obtained from a commercial supplier (Cell Applications, Inc.<sup>TM</sup>, San Diego, CA, USA) and subcultured at 37 °C in a humidified incubator with 5% CO<sub>2</sub>. After confluence, cells were seeding into 24-well culture plates (1 × 10<sup>5</sup> cells per well) and incubated at concentrations of 5.0, 2.5, and 1.25 mg/mL PPLAC dissolved in culture medium. Selection of these doses was based on previous results of cytotoxicity assays (data not shown). Bacterial LPS (50 µg/mL) (Sigma, St Louis, MO, USA) was also applied in the cell culture to stimulate an inflammatory stress.<sup>22</sup>

### *In vitro* quantification of inflammatory mediators and antioxidant enzyme

Cell-free supernatants and lysates were collected 48 h after the onset of incubation of keratinocytes and both inflammatory mediators [prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and leukotriene B<sub>4</sub> (LTB<sub>4</sub> and histamine)] and the antioxidant enzyme SOD were quantified using colorimetric competitive enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA; UsBiological, Swampscott, MA, USA; Cayman, Ann Arbor, MI, USA). Each experiment was conducted in triplicate of three independent experiments.

### Clinical trials

Twenty-one healthy female volunteers aged 20–55 years with skin types I–IV according to Fitzpatrick, neither pregnant nor nursing, diagnosed with a long-term presence of periorbital hyperchromia and regular users of facial and eyecare cosmetics were selected for this study. Additional requirements were absence of skin disease, no medical treatment during the study, no use of retinoic acid, skin peels, or similar procedures within 6 months prior to the onset of the study, nonexposure to intensive sun or any methods of tanning and non-occurrence of unusual emotional or physical stress. No changes in contraceptive method or dietary and cosmetic habits were allowed during the study. Noncompliance with these criteria resulted in volunteer exclusion. Each volunteer was used as her own control, as they were instructed to only replace their eyecare cosmetic in use by the serum sample containing 5.0% PPLAC. To keep with the routine of cosmetic application of each volunteer, the serum was applied to whole face and neck twice a day for 28 days. After the period, volunteers were asked to fill out a binary questionnaire concerning the improvement in the intensity and appearance of “dark circles.” Skin color and melanin index measurements were performed under controlled environmental conditions using a spectrophotometric colorimeter adapted for reflectance (Color Guide Sphere; Byk Gardner, Geretsried, Germany) and a mexameter (Courage and Khazaka, Koln, Germany). Experimental sites, for measurements with the probes, were delimited in the infra-orbital and adjacent (cheeks) areas, with the purpose of taking the measurements in the same sites, before (D1/T0) and after the 28-day treatment (D28). In addition, external influences were assessed through comparative measurements taken at the same time in a nontreated area (forearms). Skin tone was calculated

using the individual typological angle (ITA<sup>0</sup>) according to Chardon's formula:  $ITA^0 = [\arctan(L^* - 50)/b^*] \times 180/3.14$ .<sup>23</sup> The higher the ITA<sup>0</sup> and L\*, the lighter was the skin. This color assessment is based on the CIELab (Commission Internationale de l'Eclairage) system color and includes the parameter L\* (skin luminance) as the summary on a light–dark scale and b\* (chromametric coordinate) as the summary on a blue–yellow scale.<sup>24</sup> Results were expressed individually as variations in both skin tone ( $\Delta ITA^0$ ) and luminosity ( $\Delta L^*$ ). The improvement in periorbital hyperchromia was evaluated by comparing the color of the skin before (D1/T0) and after the 28-day treatment (D28). This study was approved by the Ethics committee.

### Statistical analysis

For *in vitro* studies, a parametric method, the one-way analysis of variance (ANOVA) followed by the Tukey test, was used to compare data among all groups. A one-sample *t*-test was used for clinical trial analysis. A value of  $P < 0.05$  was considered statistically significant.

## Results and discussion

The physiopathology of periorbital hyperchromia is not clearly defined; however, blood flow stagnation seems to be a determinant factor involved in the development of this process.<sup>2,3</sup> This concept is supported by the fact that in the last years, cosmetic companies have been presenting preparations for “dark circles” containing mainly ingredients for stimulating local blood flow.<sup>3,21</sup>

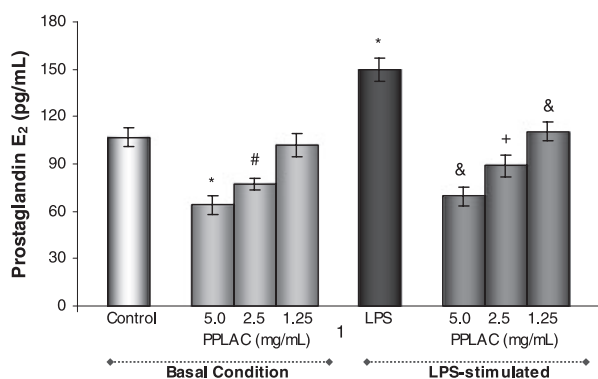
The microvascular network is directly connected to cell metabolism, supplying oxygen and nutrients to skin tissues and removing unwanted products from metabolic activity.<sup>15,16</sup> Therefore, a good cutaneous circulation is essential for maintaining healthy skin function, and it has a strong relationship with skin color.<sup>23–26</sup> The responsiveness of the skin to the noxious actions of different environmental and endogenous factors promotes changes in vascular integrity that lead to the accumulation of fluid, hydrophylic solutes, and hemoglobin chromophores within the tissue space.<sup>25,27,28</sup> These physiopathological conditions occur as a consequence of the release of adhesion molecules, pro-inflammatory cytokines, and autacoids produced by epidermal cells, which culminate in later skin color changes.<sup>8,11,15,16,29</sup> Once these substances are produced, they can diffuse readily across cell membranes reaching endothelial cell receptors and exerting their vasoactive effects.<sup>12,16,30</sup> Histamine and the eicosanoids, PGE<sub>2</sub> and LTB<sub>4</sub>, produced mainly by keratinocytes, have

been shown to be the key mediators of these responses through their potent vasodilator and chemotactic actions.<sup>10,27,30–33</sup>

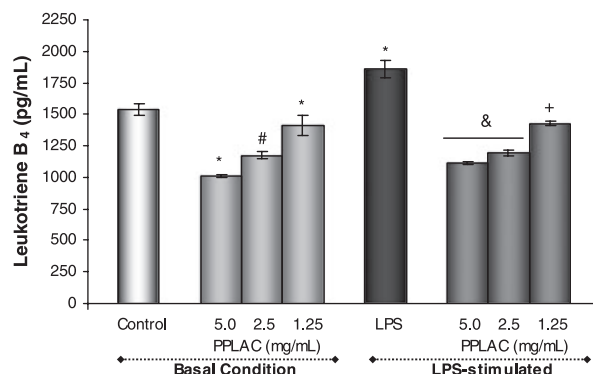
In this study, experiments were performed to investigate the effects of PPLAC in the release of inflammatory mediators using an *in vitro* model of human skin culture. Our findings demonstrate that incubation of keratinocytes with PPLAC, at concentrations of 5.0–1.25 mg/mL, promoted significant decreases in the synthesis of inflammatory mediators under basal conditions and, more importantly, prevented increases in such factors caused by LPS stimulation ( $P < 0.001$ ) (Figures 1–3). In these culture systems, a pattern of dose–response was established and the optimal biological effects were elicited by the concentration of 5.0 mg/mL, which reduced the levels of PGE<sub>2</sub>, LTB<sub>4</sub>, and histamine up to 2.1-, 1.7-, and 2.3-fold, respectively, in relation to LPS-stimulated controls. Antioxidant activity was drastically reduced in LPS-stimulated cultures reaching levels 2.2-fold lower than in normal controls. PPLAC treatment significantly increased SOD activity to levels above control in both basal condition and LPS-stimulated cultures (Figure 4).

Color of human skin is an important index in dermatology and cosmetology.<sup>34</sup> Chromametric coordinates of the CIELab system and ITA<sup>0</sup> constitute a precise noninvasive method widely used to assay the colorimetric patterns of color changes in the skin, including tanning, erythema and bruises.<sup>19,34</sup> As the

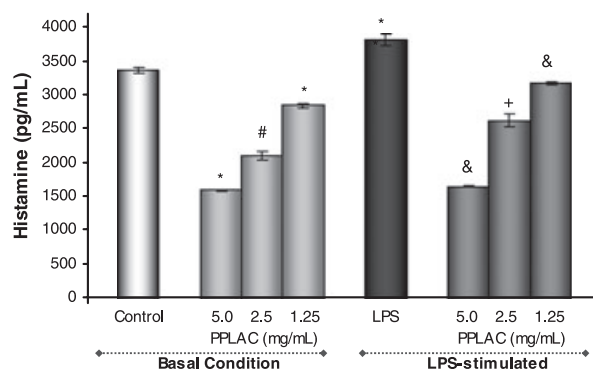
microinflammatory process is an important triggering factor for the darkening of the periorbital area, as it culminates in hemostasis and local deposition of hemoglobin metabolites, skin color could be effectively evaluated using ITA<sup>0</sup> and L\* measurements.<sup>3,24</sup> To investigate the dermocosmetic properties of PPLAC, clinical experiments were conducted aiming at its



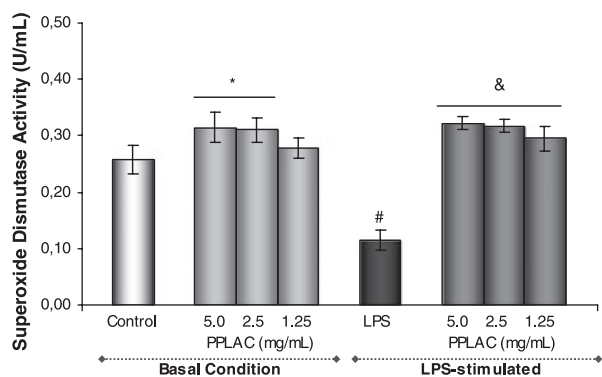
**Figure 1** Effects of *Pfaffia paniculata*/*Ptychopetalum olacoides* B./*Lilium candidum* L.-associated compound (PPLAC) on prostaglandin E<sub>2</sub> production by human keratinocytes after 48 h of incubation, under basal and lipopolysaccharide (LPS)-stimulated conditions. Data are presented as mean ± SD of three independent experiments ( $n = 6$ ). \* $P < 0.001$  in relation to control;  $P < 0.001$  in relation to control, and  $P < 0.05$  in relation to PPLAC 5.0 mg/mL; # $P < 0.001$  in relation to LPS; + $P < 0.001$  in relation to LPS, to LPS + PPLAC 5.0 mg/mL and LPS + PPLAC 1.25 mg/mL (ANOVA, Tukey).



**Figure 2** Effects of *Pfaffia paniculata*/*Ptychopetalum olacoides* B./*Lilium candidum* L.-associated compound (PPLAC) on leukotriene B<sub>4</sub> production by human keratinocytes after 48 h of incubation, under basal and lipopolysaccharide (LPS)-stimulated conditions. Data are presented as mean ± SD of three independent experiments ( $n = 6$ ). \* $P < 0.001$  in relation to control;  $P < 0.001$  in relation to control, to PPLAC 5.0 mg/mL and  $P < 0.01$  in relation to PPLAC 1.25 mg/mL; & $P < 0.001$  in relation to LPS; + $P < 0.001$  in relation to LPS and to LPS + PPLAC 2.5 mg/mL (ANOVA, Tukey).



**Figure 3** Effects of *Pfaffia paniculata*/*Ptychopetalum olacoides* B./*Lilium candidum* L.-associated compound (PPLAC) on histamine production by human keratinocytes after 48 h of incubation, under basal and lipopolysaccharide (LPS)-stimulated conditions. Data are presented as mean ± SD of three independent experiments ( $n = 6$ ). \* $P < 0.001$  in relation to control;  $P < 0.001$  in relation to control, to PPLAC 5.0 mg/mL and to PPLAC 1.25 mg/mL; & $P < 0.001$  in relation to LPS; + $P < 0.001$  in relation to LPS, to LPS + PPLAC 5.0 mg/mL and to LPS + PPLAC 1.25 mg/mL (ANOVA, Tukey).

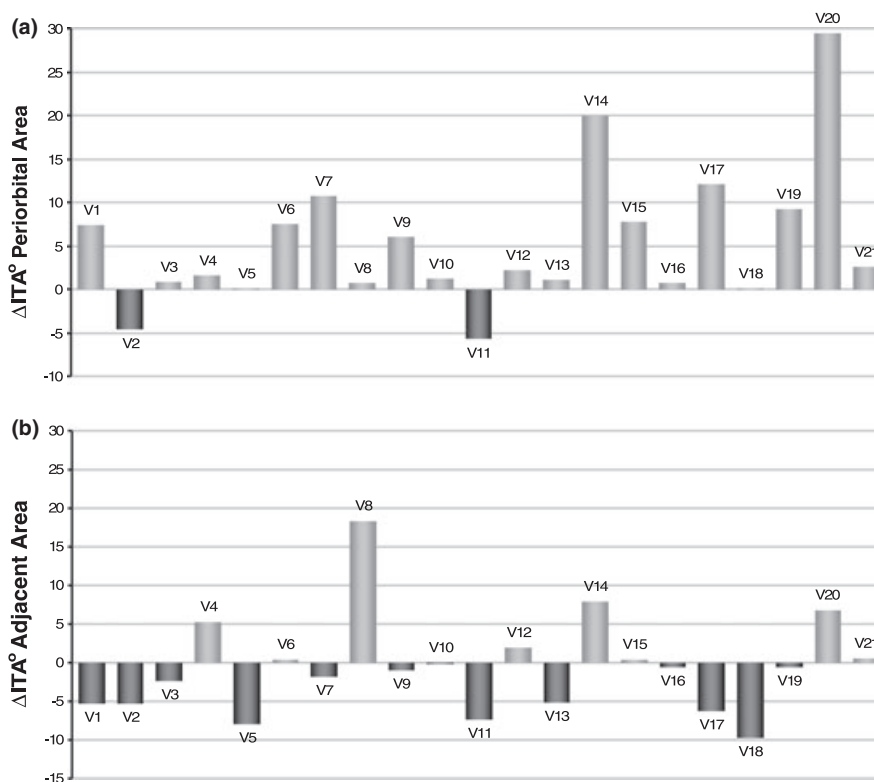


**Figure 4** Effects of *Pfaffia paniculata*/*Ptychopetalum olacoides* B./*Lilium candidum* L.-associated compound (PPLAC) on superoxide dismutase activity in human keratinocyte culture after 48 h of incubation, under basal and lipopolysaccharide (LPS)-stimulated conditions. Data are presented as mean  $\pm$  SD of three independent experiments ( $n = 6$ ). \* $P < 0.01$  in relation to control;  $P < 0.001$  in relation to control; & $P < 0.001$  in relation to LPS (ANOVA, Tukey).

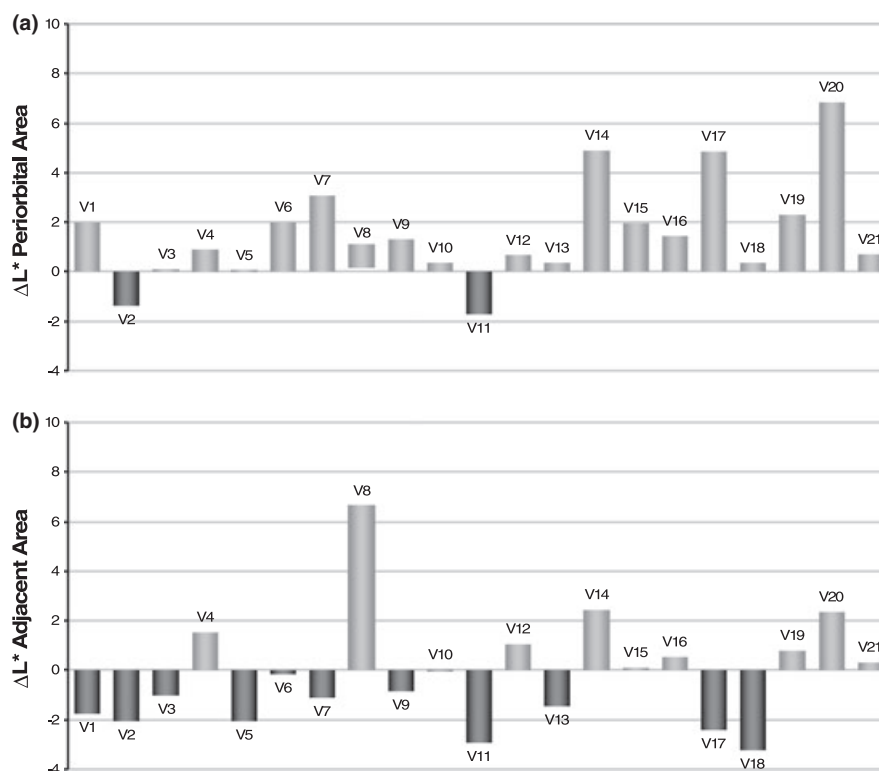
possible application in eyecare formulations. Our results demonstrated improvements in skin tone and luminance

in about 90% of volunteers, which were confirmed by significant increases in  $ITA^0$  and  $L^*$  values in the periorbital area after 28 days of treatment with a serum sample containing 5.0% PPLAC ( $P < 0.05$ ) (Figures 5 and 6). These clinical observations were corroborated by the subjects, who reported improvements in the intensity (81%) and appearance (76%) of “dark circles.” In addition, no significant increases in  $ITA^0$  and  $L^*$  parameters were observed in the adjacent area treated (cheeks), suggesting an effect of this compound on local hemodynamic recovery. In spite of the positive response on the attenuation of infraorbital skin color originated from hemodynamic stasis, no improvements were observed in mexametric measurements after PPLAC treatment of “dark circles” formed by melanin accumulation (data not shown), when compared with dermal melanization, anatomic structure, and genetic influence.<sup>1</sup>

A variety of mechanisms could be postulated to explain the beneficial effect of PPLAC on periorbital hyperchromia. *Pfaffia paniculata* (Mart.) Kuntze (Amaranthaceae), popularly known as “Brazilian ginseng” or “Suma,” is



**Figure 5** Clinical instrumental evaluation of skin tone after the application of a face fluid serum sample containing 5.0% *Pfaffia paniculata*/*Ptychopetalum olacoides* B./*Lilium candidum* L.-associated compound. The result of each volunteer was expressed individually as the variation in individual typological angle ( $\Delta ITA^0$ ) after 28 days of treatment, in the infraorbital area (a) ( $P < 0.05$ ) and in the adjacent area (b) ( $n = 21$ , one-sample  $t$ -test).



**Figure 6** Clinical instrumental evaluation of skin luminance ( $L^*$ ) after the application of a face fluid serum sample containing 5.0% *Pfaffia paniculata*/*Ptychopetalum olacoides* B./*Lilium candidum* L.-associated compound. The result of each volunteer was expressed individually as the variation in  $L^*$  ( $\Delta L^*$ ) after 28 days of treatment, in the infraorbital area (a) ( $P < 0.05$ ) and in the adjacent area (b) ( $n = 21$ , one-sample  $t$ -test).

one of the most employed species in Brazil due to its adaptogenic property to increase the response to stressful stimuli.<sup>35</sup> Studies using root extracts of *P. paniculata* showed important anti-inflammatory and analgesic effects in models of carrageenin-induced edema and ascitic tumors.<sup>36,37</sup> These biological effects are attributed to pfaffic acid and the saponins Pfaffosides, which have been shown to be the most bioactive secondary metabolites of *P. paniculata*.<sup>37–40</sup> Another ingredient of PPLAC is the Brazilian plant *Ptychopetalum olacoides* B. (Olacaceae) known as “Marapuama,” that is also traditionally used as an adaptogenic.<sup>35,41</sup> It was found that this plant presents a marked free radical scavenging activity, a reduction in lipid peroxidation, and increased activities of the antioxidant enzymes catalase and glutathione peroxidase.<sup>41–43</sup> Phytochemical trials of *P. olacoides* indicated the presence of fatty acids, saponins, flavonoids, sterols, and alkaloids.<sup>44</sup> The third PPLAC compound, the plant *Lilium candidum* L. (Liliaceae, “white lily” or “white Madonna lily”) is used in folk medicine throughout Europe for the treatment of skin burns, ulcers, swelling, and other cutaneous affections. Experimental studies demonstrated

anti-irritant, antioxidant, and anti-inflammatory properties of *L. candidum*, which is explained by the rich presence of steroidal saponins, organic acids, flavonoids, glycosides, and nitrogenous compounds isolated from its bulbs and flowers.<sup>45–47</sup>

Of particular interest, the phytochemical groups of saponins and flavonoids present in PPLAC are associated with a range of well-established biological effects, such as anti-inflammatory, anti-edematogenic, and antioxidant activities. The mechanisms involved are related to the down-regulation of the metabolic pathway of nitric oxide and arachidonic acid as well as eicosanoids and pro-inflammatory cytokine production.<sup>46,48–54</sup> In addition, both compounds present chelating and cytoprotective effects against deleterious actions of xenobiotics, including both the reduced activation and enhanced detoxification of mutagens and carcinogens.<sup>52,55–59</sup> These activities are directly connected with the prevention of inflammatory response and consequently tissue damage.

The reduction in micro-inflammatory response as well as the protection of cell cycle by deleterious elements demonstrated by PPLAC may be considered as an

integral approach to preserve the integrity of vascular endothelium, preventing the hemodynamic congestion that culminates in hyperchromia around the eyes.

## Conclusion

The present study demonstrates the effects of PPLAC in the clinical improvement in periorbital hyperchromia. Our results encourage the use of this compound as an adjuvant to eyecare dermocosmetic products with the purpose of reducing the local bruised appearance particularly originated from microvascular stasis. Considering the limitations of this study, such as the lack of placebo-controlled group, further studies are needed to confirm and define the exact mechanisms behind the clinical effects of PPLAC in reducing periorbital “dark circles.”

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