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# Cecropia Pachystachya Loefl. Extract: A Promising Source Of Bioactive Anti-Photoaging Agents For Cosmetics

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***Abstract* — Plant-based cosmetics became a modern trend owing to their richness in phytoconstituents and active ingredients that act as anti-aging molecules. *Cecropia pachystachya* is an example of this potential with extensive application in traditional medicine. This study evaluated the potential anti-photoaging of the dried extract from *C. pachystachya* leaves (HECP). We determined the total phenolic content (52.39 ± 0.9 mg.g$^{-1}$), antioxidant activity ($IC_{50}$ 3.07 ± 0.15 µg.mL$^{-1}$), photoprotection, enzymatic inhibition (elastase, collagenase, tyrosinase), cell viability - HaCaT (non-cytotoxic effect < 250 µg.mL$^{-1}$) and acute toxicity. The results showed that HECP reduced skin photoaging and prevented the degradation of elastin and collagen ($IC_{50}$ = 15.97 µg.mL$^{-1}$ to elastase, 38.96 to collagenase). HECP exhibited moderate toxicity in the brine shrimp assay ($LD_{50}$ = 187.81 ± 1.27 µg.mL$^{-1}$) and a non-toxic effect in the *Daphnia magna* assay. *Cecropia pachystachya* extract is a promising source of bioactive compounds used as anti-aging agents in cosmetics.**

***Keywords* — *Cecropia pachystachya; anti-aging; antioxidant activity; enzymatic inhibition; photoprotection; medicinal plant.***

## I. Introduction

The use of cosmetics is ancient, but a recent growing awareness of the importance of skin and the interest in a healthy and young appearance has made the skincare industry one of the fastest-growing markets [1]. Simultaneously, plant-based cosmetics became a modern trend owing to their richness in phytoconstituents and active ingredients [2]. In this scenario, Cecropia pachystachya, popularly known in Brazil as embauba, would be a potential source of phytocompounds.

Skin aging is a complex process related to endogenous and exogenous factors [3]. Moreover, it is hard to modify endogenous factors, so controlling exogenous factors is primordial, including ultraviolet radiation – UVR, toxins, and pathogens [4]. Excessive UVR promotes the accumulation of reactive oxygen species (ROS), causing oxidative damage [5]. The oxidative stress leads to unhealthy and aged skin, causing wrinkles, roughness, dryness, elasticity loss, and uneven pigmentation [6].

Sunlight directly stimulates melanin production to protect against the harmful effects of UVR, resulting in hyperpigmentation in some areas of the human body [7]. Tyrosinase is an essential enzyme related to this process, and the downregulation of its activity was associated with a decrease in melanin production [8]. UVR also induces the overexpression of collagenase and elastase. These enzymes are responsible for the degradation of collagen and elastin [9]. Together tyrosinase, collagenase, and elastase are essential to skin aging [8].

For the reasons above, this study aimed to evaluate the potential anti-photoaging of the dry hydroethanolic extract from C. pachystachya leaves (HECP). The assays we performed herein screened the total phenolic content (TPC); 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity; sun protection factor (SPF); enzymatic (elastase, collagenase, tyrosinase) activities; keratinocyte viability - HaCaT; and acute toxicity (Artemia salina and Daphnia magna).

## II. Results and Discussion

### A. Total phenolic content

The TPC of HECP was 52.39 ± 0.9 mg.g$^{-1}$. Polyphenols can absorb UVR and prevent its absorption into the skin, reducing the generation of free radicals and lipid peroxidation and protecting the tissue against photo-oxidative damage [10]. Phenolic compounds may exert antioxidant activity by reducing the accumulation of reactive oxygen species (ROS) in the skin. ROS is an inducing agent for collagenase, metalloproteinases, and elastase expression, accelerating dermal disruption [11].

### B. DPPH radical scavenging activity

The inhibitory concentration (IC) of HECP that blocked 50% of DPPH was 3.07 ± 0.15 µg.mL$^{-1}$, while resveratrol was 8.45 ± 0.44 µg.mL$^{-1}$. This activity was already reported before [12,13]. Altogether, there is evidence that C. pachystachya displays an optimum antioxidant capacity ($IC_{50}$ < 15 µg.mL$^{-1}$), even from different extract fractions, and our results come in the

same line. UVA radiation exerts a phototoxic effect by producing free radicals and ROS that damage DNA, proteins, and cell membranes. Despite the capacity of the human body to protect itself through its enzymatic and non-enzymatic natural antioxidants, this capacity is not enough in case of chronic or excessive exposure to UVRs. Sunscreens enriched with exogen antioxidants can provide a larger spectrum of protection, decreasing the damage caused by ROS [14].

### C. Photoprotection assay

The HECP showed less photoprotective activities than resveratrol, used as a reference compound (Table 1). We also evaluated UVA protection since this radiation can reach the basal layer. It is also responsible for the tanning reaction and new melanogenesis. The critical wavelength (λc) is related to the degree of UVA protection. Sunscreens with λc over 400 nm are considered broad-spectrum. To the best of our knowledge, it is the first time that the photoprotection capacity of C.

TABLE I. PHOTOPROTECTION CAPACITY OF CECROPIA PACHYSTACHYA AND RESVERATROL

| *Extract* | *SPF* | *UVAPF* | *λc(nm)* | *UVA/UVB* |
|---|---|---|---|---|
| *Cecropia pachystachya* | 2 ± 0.1* | 1 ± 0.2 | 370 | 0.590 |
| Resveratrol | 7 ± 0.2 | 2 ± 0.1 | 361 | 0.460 |

*SPF: sunscreen protection factor (UVB); UVAPF: UVA protection efficacy; λc: critical wavelength. The verification of the validity of the results was obtained using the Cosmetics Europe Reference Sunscreen S2. Data are expressed as the mean of 27 measures for the lotion containing 10% of the corresponding extract. *p<0.05 vs control group.*

### D. Enzymatic inhibitory activity

The HECP showed a high inhibitory enzymatic potential with $IC_{50}$ = 15.97 µg.mL$^{-1}$ to elastase and 38.96 µg.mL$^{-1}$ to collagenase. UVR affects the expression of the enzymes from the matrix-metalloprotease family via direct activation of transduction pathways [1]. A previous study [11] compared the properties of hydroethanolic (HE) and ethanolic (EE) extracts from C. pachystachya leaves. Table 2 summarizes the inhibitory effect of HECP on tyrosinase activity. The results suggest that HECP can reduce skin photoaging, preventing the degradation of collagen and elastin, arising as a potential ingredient for phytocosmetics. It would provide supplementary skin protection if added to sunscreen, for example.

TABLE II. ENZYMATIC INHIBITORY ACTIVITY OF TYROSINASE BY CECROPIA PACHYSTACHYA AND THE REFERENCE COMPOUNDS.

| **Extract /reference** | **Inhibitory Activity (IA)** | | |
|---|---|---|---|
| | ***IA 60 min (%)*** | ***IA 120 min (%)*** | ***IA50 (µg mL-1)*** |
| *Cecropia pachystachya* | 29.3 ± 1.3 | 22.1 ± 0.5 | 285.7± 3.2* |
| Resveratrol | 75.5 ± 2.3 | 50.5 ± 2.3 | 85.9 ± 5.3** |
| Kojic acid | 77.3± 3.1 | 52.8± 1.1 | 8.1± 0.6* |

*Data were expressed as mean ± SD (n=3). *p<0.05 vs control group and **between controls groups (one-way ANOVA following Tukey post hoc test).*

### E. Cell viability assay

HaCaT is an immortalized cell line. Its phenotype is comparable to that of ordinary human keratinocytes [8]. The result of the cell viability assay is in Fig. 1. There was a significant decrease in cell viability at the high HECP concentrations of 1,000 (24%) and 500 µg.mL$^{-1}$ (50%). All the other concentrations did not show cytotoxicity. Since the results were not dose-dependent, it was not possible to calculate the $IC_{50}$. Fernandes and coworkers [11] also evaluated the cell viability of extracts of C. pachystachya in dermal fibroblasts (BALBc/3T3) and human embryonic kidney cells (HEK-293). Their results indicated that both fractions did not present cytotoxicity, at least at the concentrations tested, and notably stimulated the fibroblasts' growth.

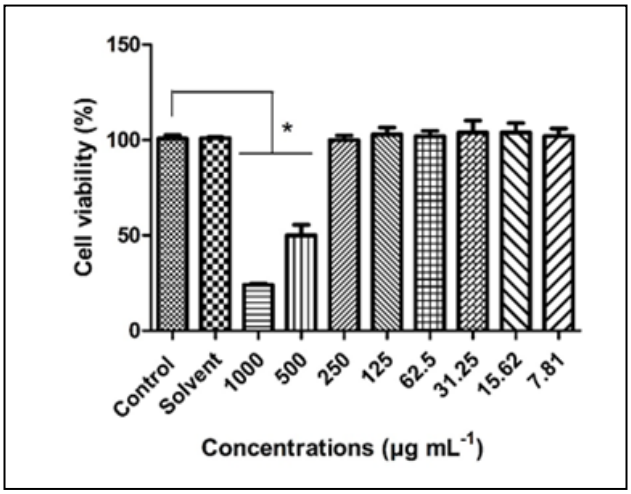


Fig. 1. *HaCaT cells viability treated with different concentrations of HECP. Data expressed as mean ± SD (n=5). * p<0.05 vs control group (one-way ANOVA following Bonferroni post hoc test).*

### F. Acute toxicity assays

The brine shrimp lethality evaluates acute toxicity. The HECP showed a moderately toxic effect [15]. It is worth noting that the dose that causes 50% of death found for HECP ($LD_{50}$ = 187.81 ± 1.27 µg.mL$^{-1}$) is well

above the concentration range that had the biological properties. Daphnia magna assay determines the effective concentration that causes 50% of death ($EC_{50}$). Zucker [16] categorized the $EC_{50}$ into five ranges according to toxicity. The HECP was in the last category ($EC_{50}$ 102.67 ± 1.08 mg.mL$^{-1}$) with a practically non-toxic effect. This assay is also helpful for monitoring the toxicity of compounds in freshwater environments. Many substances added to phytocosmetics may leach into water and affect organisms such as Daphnia magna [17]. According to our findings, HECP may be ecologically sustainable.

## III. Experimental

### A. Plant material

The leaves C. pachystachya Loefl. were collected in Juiz de Fora, Minas Gerais, Brazil, in September 2017. This species is listed in the Herbarium Leopoldo Krieger of Universidade Federal de Juiz de Fora under number 46591.

### B. Preparation of the hydroethanolic extract of Cecropia pachystachya

To prepare the HECP, 20 g of dried leaves were macerated in ethanol 70% [72 h; room temperature (RT)]. The residue was removed by filtration, the extract was evaporated under low pressure, and then lyophilized (ALPHA 1 – 4 LD plus, Christ, Germany) under 1.8-mbar pressure at -14°C. The final yield was 9.1%.

### C. Total phenolic content

TPC was determined by a spectrophotometric method [18], with few modifications. Briefly, we added HECP (10 mg.mL$^{-1}$, 50 µL), Folin–Ciocalteu reagent (250 µL), sodium carbonate (20%, 500 µL), and water (4.2 mL). This reaction occurred in the dark (RT; 30 min.). The absorbance was read on a spectrophotometer (Multiskan GO, Thermo Scientific, USA) at λ = 765 nm. Gallic acid (GA; Sigma-Aldrich, USA) was used to construct a standard curve to determine the extract's phenolic content (25–700 µg.mL$^{-1}$, ethanolic solutions). Tests were performed in triplicate, and results were expressed in milligrams of GA equivalents (GAE) per gram of the HECP (mg.g$^{-1}$).

### D. Antioxidant activity

The scavenging activity of the HECP was measured according to the DPPH method [19], with minor modifications. Briefly, different extract concentrations (0.98–250 µg mL$^{-1}$, 50 µL) were added to a 96-well microplate and mixed with DPPH solution (50 µM, 150 µL). The reaction mixture was kept in the dark (RT; 30 min.). Then, the absorbance was measured (λ = 510 nm) against the negative control (ethanol). Resveratrol (Sigma-Aldrich, USA) was used as the positive control at the same concentrations. Inhibition of DPPH radical was calculated using (1) where A0 and As are the values for the absorbance of the negative control and the absorbance of the sample, respectively. The $IC_{50}$ value was calculated from the straight-line equation of the linear dispersion graph and represents the extract concentration that inhibits 50% of DPPH radical. All tests were performed in triplicate.

$$IC50\ (\%) = \frac{100 \text{ x } (A0 - As)}{A0} \quad (1)$$

### E. Photoprotection assay

The photoprotection capacity of HECP was measured according to Polonini et al. [20]. The tests were conducted in a UV-2000S Ultraviolet Transmittance Analyzer (Labsphere, USA). Analysis was performed on polymethylmethacrylate (PMMA) plates (50 × 50 mm) Helioplate™ HD6 (HelioScreen, France), with a roughened surface on one side (Sa ≈ 6 µm). The determination of the sunscreen protection factor (SPF) was given by diffuse transmittance spectrophotometry. UVB and UVA protection efficacy, UVA/UVB ratio, and critical wavelength were calculated using equations described previously [21]. Data were expressed as the mean of 27 measures for the lotion containing 10% of HECP.

### F. Tyrosinase inhibition

Tyrosinase inhibition was performed according to a previous protocol [22], with minor modifications. Different extract concentrations (100, 80, 40, 20, and 10 µg.mL$^{-1}$; 60 µL) were added to a 96-well microplate, then we added aliquots of mushroom tyrosinase (125 U mL$^{-1}$, 10 µL, Sigma-Aldrich, USA) and phosphate buffer solution (pH 6.8, 70 µL). We used 60 µL of resveratrol (same concentration of the sample) and kojic acid (concentrations of 10, 5, 2.5, 1.25, and 0.625 µg.mL$^{-1}$, Sigma-Aldrich, USA) as positive controls and 60 µL of 2.5% DMSO as the negative control. Absorbances were read at λ = 510 nm (T0). Then, the microplates were incubated at 30 ± 1 °C for 60 min, and the absorbances were measured again (T1). An additional incubation for 60 min at 30 ± 1 °C was carried out, then a new spectrophotometric reading was taken (T2). The inhibitory percentage at the two-time points (T1 and T2) was obtained according to (2). Where IA (%) is inhibitory activity; C = negative control absorbance; S = sample or positive control absorbance (absorbance at time T1 or T2 minus the absorbance at time T0). The analytical curve was plotted between tyrosinase inhibition activity percentages at each time point and the concentrations of the extract/positive control. The inhibitory activity at 50% (IA50, in µg.mL$^{-1}$) was calculated using the equation of the straight line.

$$IA\ (\%) = \frac{100 \text{ x } (C - S)}{C} \quad (2)$$

### G. Collagenase inhibition

In a 96-well plate, the control and control blank wells received 10 µL of DMSO, while the sample (HECP) and sample blank wells received 10 µL of the HECP at a concentration of 20 fold greater than the desired final concentration. Then, 10 µL of collagenase enzyme (10 mg.mL$^{-1}$) were added. Followed by 80 µL of tricine buffer (pH = 7.5; 50 mM tricine, 400 mM

NaCl, and 10 mM $CaCl_2$) to all wells. The plate was incubated (RT; 15 min.) and then we added 100 µL of 1.6 mM FALGPA substrate - N-[3- (2-Furyl) Acryloyl-Leu-Gly-Pro-Ala]. Absorbance (λ = 340 nm) was read immediately after adding the substrate and after incubation (30 min.; RT; in the dark) [23]. The percentage of inhibition was calculated according to (3)

$$\% Inhib = 100 - [\frac{(\text{Abs.final s} - \text{Abs.initial s})}{(Abs.final\ cont - Abs.initial\ cont)}] \quad (3)$$

Where: s = sample and cont = control. The $IC_{50}$ value was calculated from the dose-response curve. Statistical analysis was performed using the ANOVA test followed by the Tuckey test for significance analysis. Differences were considered significant if $p < 0.05$.

### H. *Elastase inhibition*

The determination of the inhibitory activity of the HECP on the elastase enzyme was based on the method of Bieth [24] with some modifications. In a 96-well microplate were added Tris HCl buffer (pH = 8, 100 Mm, 200 µL), N-Succinyl-Ala-Ala-Ala-p nitroanilide (4.4 mM, 13 µL) substrate solution, and 20 µL of the solubilized extract in buffer, which was evaluated at different concentrations (10 to 40 $\mu g.mL^{-1}$). After homogenization, 7 µL of elastase enzyme (0.03 $IU.mL^{-1}$) was added to the wells. Fifteen consecutive readings were performed (λ = 410 nm). Tests were performed in triplicate. Curves referring to time × substrate concentration were constructed to calculate the percentage of inhibition. With the angular coefficient, the percentage of inhibition was determined according to (4):

$$\% I = 100 - [\frac{(100 \text{ x CAa})}{CAc}] \quad (4)$$

Where: CAa and CAc refer to the slope of the straight line obtained by the sample and control determinations, respectively. The $IC_{50}$ value was calculated from the dose-response curve. Statistical analysis was performed using the ANOVA test followed by the Tuckey test for significance analysis.

### I. *Cell viability assay*

HaCaT cells were cultured in Dulbecco's Modified Eagle`s Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 $U.mL^{-1}$ penicillin, 100 $\mu g.mL^{-1}$ streptomycin, and 10 mM HEPES (37 °C; 5 % $CO_2$ humidified atmosphere; pH=7.4). The cell viability was determined by MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] [25]. HaCaT cells were seeded in 96-well plates ($5 \times 10^4$ $cells.mL^{-1}$) in 100 µL of medium per well. After 24 h of incubation, the culture medium was replaced by a fresh medium with the treatments. Sextuplicate wells were treated with HECP at concentrations ranging from 500 – 7.81 $\mu g.mL^{-1}$. The plates were incubated (37 °C; 5 % $CO_2$). A control experiment was performed under the same conditions but without cell treatment. After 24 h, the medium was removed and 200 µL of DMEM with 50 µL of MTT (5 $mg.mL^{-1}$) dye solution was added, followed by incubation for 3h at 37 °C. The precipitated formazan was dissolved in DMSO, and the absorbance was measured (λ = 570 nm). Cell viability (%) was expressed as a percentage of the absorbance of non-treated control cells. The $IC_{50}$ value was calculated using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA).

### J. *Brine shrimp lethality assay*

The brine shrimp lethality assay was performed according to the protocol described previously [26], with some modifications. Brine shrimp cysts were obtained from Maramar Aquacultura (Cabo Frio, Rio de Janeiro, Brazil), incubated in artificial seawater (pH 8–9), and exposed to a 60-W lamp. After 48 h of incubation (RT 22 –29 °C), active nauplii free from eggshells (10 units) was collected and added to each set of wells containing ethanol extract dissolved in 2.5% DMSO and made up to 5 mL total volume using artificial seawater. The HECP was tested in triplicate at 1,000 – 10 µg mL-1. In addition, thymol and 2.5% DMSO were used as positive and negative controls, respectively. After 24 h, the number of survivors was counted, and the percentage of deaths was calculated. The lethal concentration of 50 % ($LC_{50}$ value) and the standard error were calculated by Probit analysis [27].

### K. *. Daphnia magna lethality assay*

The Daphtoxkit F magna (Microbio Tests Inc., Belgium) was used to conduct the acute toxicity test with Daphnia magna. Organisms with 2-26 h of life after hatching were used, and all procedures were performed following the manufacturer's guidelines. In this test, five HECP concentrations were assessed (0.1 to 40 $mg.mL^{-1}$). Controls were also prepared by using Tween 80:DMSO (1:1 v/v) in the same concentrations as used for dilution and with only the standard water available for solutions in the kit. Twenty-five microcrustaceans were added to each concentration. The experiments were incubated protected from light (48 h, 22 °C ± 0.2 °C), and results were expressed as median effective concentration (Associação Brasileira de Normas Técnicas (ABNT)[28].

## IV. Conclusion

Cecropia pachystachya emerged as a promising source of bioactive compounds. The multifunctional activities make HECP a potential anti-aging ingredient for phytocosmetics. It performed high antioxidant activity and inhibited enzymes of cosmetics interest, besides exhibiting low toxicity and eco-friendly proprieties. Despite these results, further biological and phytochemical studies are necessary to identify the metabolites responsible for the observed biological activities.

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