MS EDITIONS

Blacpma

BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Evaluation of the pharmacognostic parameters, qualitative phytochemical profile and investigation of the toxicity of *Sacoglottis ceratocarpa* Ducke containing Bergenin, a gallic acid derivative

[Evaluación de los parámetros farmacognósticos, perfil fitoquímico cualitativo e investigación de la toxicidad de *Sacoglottis ceratocarpa* Ducke que contiene Bergenina, un derivado del ácido gálico]

Gustavo Rezende Bellei de Oliveira[1], Fabíola Dutra Rocha[2], Rita de Cássia Saraiva Nunomura[3], João Paulo Roberto[4], Francis José Zortéa Merino[1], Vinícius Bednarczuk de Oliveira[1], Katlin Suellen Rech[1], Emellie Cristine Alves[1], Aletheia dos Santos Furusho[5], Deise Prehs Montrucchio[1], Josiane de Fátima Gaspari Dias[1], Glaucio Valdameri[5], Isadora da Silva Zanzarini[5], Obdulio Gomes Miguel[1] & Marilis Dallarmi Miguel[1]

[1]Department of Pharmacy, Federal University of Paraná, Curitiba, Brazil
[2]Department of Pharmacy, Federal University of Juiz de Fora, Juiz de Fora, Brazil
[3]Department of Chemistry, Federal University of Amazonas, Manaus, Brazil
[4]Agronomist, Federal University of Paraná, Curitiba, Brazil
[5]Department of Clinical Analysis, Federal University of Paraná, Curitiba, Brazil

Reviewed by:
Akondi Butchi Raju
IBN-Sina National College for Medical Studies
Saudi Arabia

Onder Yumrutas
Adiyaman University
Turkey

Correspondence:
Gustavo Rezende Bellei DE OLIVEIRA
bellei10@yahoo.com.br

Section Biological activity

Received: 11 May 2022
Accepted: 11 January 2024
Accepted corrected: 4 June 2024
Published: 30 November 2024

Citation:
de Oliveira GRB, Rocha FD, Nunomura RCS, Roberto JP, Merino FJZ, de Oliveira VB, Rech KS, Alves EC, Furusho AS, Montrucchio DP, Dias JFG, Valdameri G, Zanzarini IS, Miguel OG, Miguel MD. Evaluation of the pharmacognostic parameters, qualitative phytochemical profile and investigation of the toxicity of *Sacoglottis ceratocarpa* Ducke containing Bergenin, a gallic acid derivative
Bol Latinoam Caribe Plant Med Aromat
23 (6): 1001 - 1021 (2024)
https://doi.org/10.37360/blacpma.24.23.6.62

Abstract: Trunk barks of *Sacoglottis ceratocarpa* Ducke (ScTB) are used in Amazonian folk medicine against hyperglycemia and dyslipidemia, although scientific information on efficacy and quality control is lacking. Thus, pharmacognostic parameters, qualitative phytochemical profile, antioxidant properties (DPPH, TEAC and FRAP), toxicity and cytotoxicity were evaluated. The purity tests in the quality control performed for the vegetable raw material showed compliance with the specifications in official compendiums. Tannins, catechins, terpenes and steroids were characterized. Bergenin, a gallic acid derivative, was isolated and identified. The ScTB was rich in total phenolic (0.420-0.562 g/g) and flavonoid (11.37-24.31 mg/g) content, and considerable antioxidant potential. ScTB did not demonstrate cytotoxicity for human erythrocytes and renal fibroblasts, nor toxicity for brine shrimp. The results demonstrated the importance of the pharmacognostic study for the immediate knowledge of the quality and qualitative chemical profile of the trunk barks, contributing to the safe and effective use of the species.

Keywords: Antioxidant properties; Humiriaceae; Phytochemical profile; Toxicity; Traditional use

Resumen: La corteza del tronco de *Sacoglottis ceratocarpa* Ducke (ScTB) se utiliza en la medicina popular Amazónica contra la hiperglucemia y la dislipidemia, aunque se carece de información científica sobre su eficacia y control de calidad. Así, se evaluaron parámetros farmacognósticos, perfil fitoquímico cualitativo, propiedades antioxidantes (DPPH, TEAC y FRAP), toxicidad y citotoxicidad. Las pruebas de pureza de la materia prima vegetal demostraron el cumplimiento de las especificaciones de los compendios oficiales. Se caracterizaron taninos, catequinas, terpenos y esteroides. Se aisló e identificó la bergenina, un derivado del ácido gálico. El ScTB fue rico en contenido fenólico total (0,420-0,562 g/g) y flavonoides (11,37-24,31 mg/g), y un considerable potencial antioxidante. ScTb no demostró citotoxicidad para células humanas, ni toxicidad para camarones en salmuera. Los resultados demostraron la importancia del estudio farmacognóstico para el conocimiento inmediato de la calidad y perfil químico cualitativo de las cortezas del tronco, contribuyendo al uso seguro y eficaz de la especie.

Palabras clave: Propiedades antioxidantes; Humiriaceae; Perfil fitoquímico; Toxicidad; Uso tradicional.

INTRODUCTION

Brazil stands out among the world's ecosystems due to its high biodiversity value. The abundance of plant species still unknown, mainly from the Amazon biome, represents a bioresource of important chemical substances that may have bioactivities of great medicinal interest (Arruda *et al*., 2018). Most species of this biome are used in different preparations based on the associated popular and traditional use for the treatment of various diseases (Schultes & Raffauf, 1990; Nunomura *et al*., 2009; Bento *et al*., 2014; Alade *et al*., 2017; De Oliveira *et al*., 2017). As a promising source of natural bioactive compounds, these species can serve as a model for the development of new therapeutic options (Nafiu *et al*., 2017; Kim *et al*., 2021).

It is known that the quality of bioactive chemical compounds present in plants used as natural medicine can vary greatly, due to biotic and abiotic influences. This may result in interference with therapeutic efficacy, as well as making the species toxic (Kim *et al*., 2021). For the establishment of a consistent phytochemical profile and proof of the safety and quality of medicinal plants, it is essential to standardize the preparation of the plant material through evaluation of some pharmacognostic parameters (Bessa *et al*., 2013; Chanda, 2014; Kim *et al*., 2021). This standardization is important to ensure the quality and identity of the species and to control the variability in the content of chemical constituents. Therefore, it is essential to establish specifications such as collection, purity and physical-chemical aspects of the plant material in order to ensure the correct, safe and effective use by the population (Chanda, 2014; Nafiu *et al*., 2017; Patnala & Kanfer, 2021).

Species of the Humiriaceae have aroused scientific interest due to the various extractive products used as natural medicine by different folks from tropical and neotropical regions (Bove & Melhem, 2000). Among the species that have aroused medicinal interest in popular medicine in the Brazilian Amazon are those of the *Sacoglottis* genus.

Sacoglottis ceratocarpa Ducke, or "achuá", is a small to large tree that reaches up to 30 meters in height, being native and endemic to the Brazilian Amazon. It is also found in Colombia, Bolivia, Peru and Venezuela (Cuatrecasas 1945; Ducke, 1945; Cuatrecasas, 1961; Woodson *et al*., 1975; Schultes, 1979; Carvalho & Holanda, 2018). The trunk barks of *S. ceratocarpa* (ScTB) are used by traditional communities in respiratory problems and to reduce serum levels of glucose and cholesterol. They are also used as a natural medicine due to their expectorant, antimicrobial, anti-inflammatory and obesity control properties, associated or not with other species of the genus (Schultes & Raffauf, 1990; Mohagheghzadeh *et al*., 2006; Bento *et al.,* 2014).

Although it is not a pharmacopoeial plant and does not have an official monograph, the ScTB is sold in popular fairs and herbalists in the Brazilian Amazon region. There is a lack of data and studies that prove the quality and efficacy for the safe and rational use of this species as a natural medicine. Furthermore, its chemical composition and toxicity are unknown. Thus, the present study aimed to define the pharmacognostic parameters, prepare and purify the plant extract from the ScTB and investigate qualitative phytochemical profile. As well evaluate the antioxidant properties and test the preliminary *in vivo* toxicity and *in vitro* cytotoxicity, in order to contribute to the immediate knowledge of the quality, qualitative chemical profile and toxicity for safe and effective use of this species.

MATERIALS AND METHODS

Plant material

Trunk barks of *Sacoglottis ceratocarpa* Ducke were collected from a specimen cataloged and identified in the herbarium of the "Instituto Nacional de Pesquisa da Amazônia (INPA)", voucher number 177668. The collection was carried out in june, at the "Reserva Florestal Adolpho Ducke", Manaus, Amazonas, Brazil (02°53`S and 59°58'W). Proof of collection and transport of botanical material number 52964 and authorization for activities with scientific purpose number 54035, issued by the Ministry of the Environment of Brazil through SISGEn, ICMBio and SISBio, were granted. The trunk barks collected were smooth and thin. They were dried in an drying oven for 48 hours at 35-40°C, crushed and pulverized to obtain the powder.

Pharmacognostic assays

The pharmacognostic assays were performed in triplicate according to the methods described by Brasil (2019).

Total ash

To determine the total ash content, the ScTB powder

(3 g) was incinerated using a temperature gradient from 200ºC to 600ºC for approximately 180 minutes. At the end of the process, the incineration product was left in a desiccator to cool and then the resulting mass of ash was recorded. The result was expressed as an average percentage of total ash (TA%) according to: TA% = [(fresh powder (g)/(incinerated powder (g)] x 100.

Moisture content

For this purpose, approximately 2 g of ScTB powder were weighed exactly. The containers with the powder were kept in an oven at 100-105ºC, firstly for 3 hours. After this, the containers were allowed to cool in a desiccator and then the masses were registered in an analytical balance. This process was repeated until constant weight. The moisture content (MC%) was calculated according to: MC% = [(fresh powder (g) – (dry powder (g))/fresh powder (g)] x 100.

Powder particle size

In this assay, exactly 25 g of ScTB powder were weighed and placed on top of a set of vibrating sieves, whose mesh openings ranged from 53 to 300 µm. A medium vibration was maintained for 25 min. The mass of powder retained in each mesh was weighed and the retention percentage (R%) was calculeted as: R% = [(powder on the sieve (g)/(powder on the sieve and collector (g)] x 100. The particle size of the powder (µm) was obtained through the linear regression between the percentages of passage and retention of the powder in the sieve.

Uncompacted bulk density

For the uncompacted bulk density assay, a graduated cylinder with a volume of 100 mL was used, which was filled to its total volume with the sieved ScTB powder. Uncompacted bulk density (g/mL) was calculeted as: UBD = [(graduated cylinder with powder (g) – (graduated cylinder without powder)/(total volume of the graduated cylinder)].

Ethanolic extract preparation

The ethanolic extract was prepared using a Soxhlet apparatus, modified by Carvalho *et. al*. (2009), patented by the National Institute of Industrial Property under No. 0601703-7 A2. The ScTB powder (500 g) and absolute ethanol (1.5 L) kept under reflux until exhaustively complete extraction (± 20 h). Then, the liquid extract was vacuum filtered, concentrated in a rotary evaporator and dried at room temperature, vielding the dry ethanolic extract named ESC.

Phytochemical prospection

Qualitative phytochemical profile

Thin-layer chromatography (TLC) phytochemical screening of ESC (10 mg/mL) was performed according to the methods described by Wagner & Bladt (1996). Hexane:chloroform (7:3), hexane:chloroform (3:7), Hexane:ethyl acetate (8:2), hexane:ethyl acetate (2:8), chloroform:ethyl acetate (7:3) , chloroform:ethyl acetate (3:7), chloroform:methanol (7:3), chloroform:methanol (3:7), ethyl acetate:methanol (7:3), ethyl acetate:methanol (3:7), butanol:acetic acid:water (4:1:1), ethyl acetate:formic acid:water (100:10:5) and ethyl acetate:formic acid:acetic acid:water (100:11:11:27), were used as mobile phases. Sulfuric vanillin, ceric sulfate, dragendorff reagent, diphenylboryloxyethylamine polyethylene glycol (NP-PEG) and ferric chloride were the and development solutions. Gallic acid, tannic acid, catechin, quercetin, rutin and bergenin were used as comparison standards.

Purification of ESC

A chromatography column was prepared from 5 g of the ESC solubilized in methanol and incorporated in silica gel 60 (10 g). Through this column, the sample was eluted by passing a solvent mixture initiated by hexane (100), to hexane:dichloromethane (95:5), dichloromethane (100), dichloromethane:ethyl acetate (90:10), ethyl acetate (100), ethyl acetate:methanol (90:10), and ending in methanol (100). The eluates collected in each gradient were allowed to evaporate at room temperature.

Chemical characterization of isolated compound

Samples eluted with dichloromethane:ethyl acetate (20:80), dichloromethane:ethyl acetate (10:90), ethyl acetate:methanol (40:60), and ethyl acetate:methanol (30:70) showed similarity on ccd, being gathered and yielding a slightly white crystalline powder. Thereafter, it was left to evaporate at room temperature, resulting in 165 mg of a crystallized material, which was identified by X-ray diffraction and Nuclear Magnetic Resonance ($^{13}C\{^{1}H\}$and ^{1}H NMR).

X-Ray Diffraction (XRD)

A fragment of crystallized material purified from the ESC was analyzed in mineral oil and transferred to a mesh-type micro-assembly. A fragment of crystallized material purified from the ESC was analyzed in a Bruker-D8 Venture diffractometer equipped with a Photon100 CMOS area detector, graphite monochromator and Kryoflex II device. The analysis was performed at 100 K using the Cu-kα radiation source (λ = 0.54178 Å). The intensities of the reflections, collected at room temperature, were measured by ω and φ scans. The results obtained were compared with data from the Cambridge Structural Database (CSD) database. The values referring to the crystal structure were made with the program ORTEP3 (Farrugia, 2012).

$^{13}C\{^1H\}$ and ^1H NMR

Crystal sample isolated from ESC (5 mg/mL) was solubilized in MeOD-d4 containing 0.1% (v/v) tetramethylsilane (TMS). The $^{13}C\{^1H\}$ and ^1H NMR spectra were acquired at room temperature 20°C (± 5°C) in a Bruker AVANCE III 400 NMR spectrometer, operated at 9.4 Tesla, observing the nucleus of $^{13}C\{^1H\}$ and ^1H at frequencies of 100.6 and 400.1 MHz, respectively. The device was equipped with a 5 mm direct detection multinuclear probe (X-core and 1H) with a field gradient on the Z axis. The chemical shifts of $^{13}C\{^1H\}$ and ^1H were expressed in parts per million (ppm) and referenced against the TMS signal, as an internal reference, at 0.00 ppm. The data obtained were compared with the literature data.

Total phenolic content (TPC)

The TPC was determined in triplicate according to the method described by De Oliveira *et al*., (2017). Methanolic ESC solutions (2.5 to 20 μg/mL) were mixed with Folin-Ciocalteu reagent/sodium carbonate solution (10% w/v) and left for 25 minutes at room temperature in the dark. Absorbances were recorded in a spectrophotometer at 760 nm. The results were expressed in grams equivalent in gallic acid (g GAE/g), tannic acid (g TAE/g) and catechin (g EC/g) per gram of sample. To build the analytical curves, standard solutions were used: gallic acid (15 to 150 μg/mL), tannic acid (40 to 180 μg/mL) and catechin (20 to 200 μg/mL).

Total flavonoids content (TFC)

The TFC was determined in triplicate according to the method described by De Oliveira *et al*. (2017). Methanolic solutions of ESC at 3 mg/mL were prepared by heating under reflux. This methanolic solution was diluted with deionized water and then clarified by partitioning with chloroform. The clarified hydromethanolic solution was centrifuged at 3,500 rpm for 10 minutes and left under refrigeration (2-8°C) for 24 hours. The mixture of different volumes (50 to 400 μL) this hydromethanolic solution with 48 μL of glacial acetic acid, 800 μL of pyridine-methanol (2:8), 200 μL of anhydrous aluminum chloride 8% and 2 mL of methanol was left in rest for 30 minutes, at room temperature in the dark. Absorbances were recorded in a spectrophotometer at 415 nm and 420 nm. The results were expressed in milligram equivalent in quercetin (mg EQ/g) and rutin (mg ER/g) per gram of sample. To build the analytical curves, standard solutions were used: quercetin (12.5 to 250 μg/mL) and rutin (50 to 750 μg/mL).

Antioxidant properties: DPPH radical reduction

The DPPH radical reduction assay was determined in triplicate using the method described by Rocha *et al*. (2010). 300 μM DPPH ethanolic solution was reacted for 30 min with ethanolic solutions of ESC (2.5 to 15 μg/mL), Bergenin (3.5 to 49 μg/mL), or standardized dry extract of *Ginkgo biloba* (5 to 75 μg/mL) used for comparison. After the reaction time, at room temperature, in the dark, the absorbances were recorded in a spectrophotometer at 517 nm. The percentages of antioxidant activity (AA%) were calculated according to: AA% = 100 – [(sample absorbance) – (blank absorbance)/(DPPH absorbance) x 100)]. The results were expressed in 50% effective concentration (EC_{50})

Trolox equivalente antioxidant capacity (TEAC)

The TEAC was determined in triplicate using the method described by Re *et al*. (1999). A volume of 9.82 mL of the aqueous solution of $ABTS^{\bullet+}$ was left to react with 0.176 mL of potassium persulfate ($K_2S_2O_8$: 2.45 mM), in the dark, for 16 hours, at room temperature, to generate the $ABTS^{\bullet+}$ radical (7 mM). Reaction kinetics after mixing 30 μL of methanolic solutions of ESC (2.5 to 10 μg/mL), Bergenin (7 to 21 μg/mL) or Trolox (0.75 to 6.0 μg/mL) with 2970 μL of $ABTS^{\bullet+}$ solution (7 mM), were measured for 5

minutes in 30-second periods. The absorbances of the samples were measured in a spectrophotometer at 734 nm. Radical inhibition percentages were calculated using an integrated area under absorbance decay curves (AUC) according to: I% = [($ABTS^{\bullet+}$ decay curve) - (sample decay curve)/($ABTS^{\bullet+}$ decay curve)] x 100. The TEAC value was calculated by the ratio of the slope coefficients of the linear regression of the samples to that of Trolox.

Ferric reducing antioxidant potential (FRAP)

For this assay the FRAP reagent was prepared by mixing 2.5 ml of TPTZ (10 mM) in 40 mM hydrochloric acid, 2.5 ml of 216 mM ferric chloride in deionized water and 25 mL of 0.3 M sodium acetate buffer, pH 3.6. The mixture was left in the dark for 30 minutes at 37°C to generate the Fe^{+3}-TPTZ radical (Pulido *et al*., 2000). The assay was performed by reacting methanolic solutions of ESC (0.5 to 3.5 µg/mL) or Bergenin (40 to 100 µg/mL) with the FRAP reagent for 30 minutes at 37°C in the dark. Absorbances were recorded in a spectrophotometer at 593 nm. Gallic acid (8.5 to 55 µg/mL), tannic acid (55 to 155 µg/mL), catechin (35 to 85 µg/mL), quercetin and rutin (15 to 120 µg/mL) were used for comparison. The results were expressed in equivalent concentration in micromol of ferrous ion (EC µmol Fe^{+2}/L). To build the analytical curves, standard solution was used: $FeSO_4$ (125 to 2000 µM).

In vivo* toxicity assay: *Brine shrimp lethality

The *in vivo* assay was conducted in triplicate according to the method described by Meyer *et al*. (1982), with modifications. The mixture of 4.5 mL of Instant Ocean Sea Salt® (35% w/v), pH 8-10 and 500 µL of the solutions in DMSO:H_20 (1:9) of Quinidine sulfate (comparison standard), ESC or Bergenin (10 to 1000 µg/mL) was incubated with 10 nauplli of *Artemia salina* Leach for 24 hours and allowed to rest, at ambient temperature and light. The average lethality percentages (L%) were calculated according to: L% = [(sample with dead nauplii) – (control with dead nauplii)/(100 – control with dead nauplii)] x 100. Results were expressed as mean lethal concentration 50% (LC_{50}).

In vitro* cytotoxicity assays: *Human erythrocytes cytotoxicity

The cytotoxicity assay was conducted in triplicate using the method described by Aslam *et al*. (2011), with modifications. The mixture of 180 µL of the 1% erythrocyte suspension (in phosphate buffered saline - PBS, pH 7.2-7.4) with 20 µL of the ESC or Bergenin solutions (10 to 1000 µg/mL) in DMSO:PBS (0.5:9.5) was incubated at 37°C (overnight). After that, the reaction mixtures were centrifuged at 3,000 rpm for 5 minutes and the supernatants were used to record the absorbances in a spectrophotometer at 540 nm. The 1% saponin solution was used as a positive control (100% hemolysis) and the negative control consisted of DMSO:PBS (0.5:9.5). The average of the percentages of hemolysis (H%) was calculated according to: H% = [(supernatants absorbance) – (negative control absorbance)/(positive control absorbance) – (negative control absorbance)] x 100. Results were expressed as mean percentage of hemolysis.

Human kidney fibroblastos cytotoxicity

The cytotoxicity assay was performed in HEK-293 cell line exhibiting epithelial morphology (isolated from the kidney of a human embryo). The MTT colorimetric method (Mosmann, 1983; Liu & Nair, 2010) was used to determine the viability of HEK-293 cells, with 90% confluence and at a density of 2x104 cells/well, against ESC or Bergenin solutions (10, 100 and 500 µg/mL) in culture medium (DMEM/FBS and 1% penicillin/streptomycin) for 72 h at 37°C and 5% CO_2. The absorbances of purple formazan crystals dissolved in a solution of DMSO/Ethanol (1:1) were registered in a microplate reader at 570 nm. The percentages of cell viability (CV%), compared to the test samples, were calculated as: CV% = (sample absorbance − sample blank absorbance)/(negative control absorbance − white absorbance negative control)] x 100. Results were expressed as the percentage of cell viability relative to control (0.05% DMSO v/v), considered 100%.

Statistical analysis

Results were expressed as the mean of three replicates ± standard deviation. Data comparisons between all groups were performed by ANOVA followed by Tukey's test using the GraphPad® prism program, version 5 (2007). The correlation between antioxidant properties and the content of phenolic compounds was evaluated by Pearson's coefficient (r). Statistical significance was assigned with a 95%

confidence interval (*p*<0.05) in all tests.

RESULTS

Pharmacognostic assays

The data for total ash, moisture content, powder particle size, uncompacted bulk density and extractive content (Table No. 1), are in compliance with monographs of plant species. The ScTB powder was classified as semi-fin with particles size of 189 ± 0.11 µm (Figure No. 1).

Table No. 1
Pharmacognostic assays

Sample	Total ash* (TA%)	Moisture content* (MC%)	Particle size (µm)	Uncompacted bulk density* (UBD)	Extractive content (%)
ScTB powder	5,62 ± 0,37	7,02 ± 0,12 (after 3h)	189 ± 0,11	0,515 ± 0,012	16,86 ± 0,22
	RV: 2 a 16	RV: 7 a 14	Semi-fine	RV: 0,1 a 0,7	(Yield: 84,29 g)

***Data represents the mean ± standard deviation of three independent experiments. RV: reference value (Amidon *et al*., 2017; Brasil, 2019)**

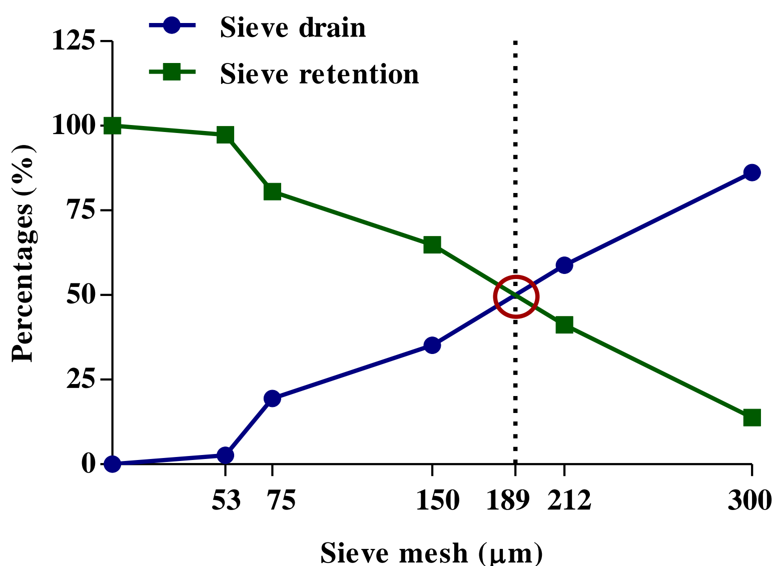

Figure No. 1
ScTB powder particle size
***Data represents the mean ± standard deviation of three independent experiments**

Phytochemical prospection

Qualitative phytochemical profile

Phytochemical screening characterized fatty acids, terpenes (mono, di and triterpenes), steroids, metabolites of phenolic origin such as glycosylated flavonoids, non-glycosylated flavonoids, flavone, flavonol, as well as coumarin, catechin, hydrolysable and condensed tannin (Figures No. 2, Figures No. 3 and Figures No. 4). Gallic acid and bergenin, a derivative of gallic acid, were identified by comparison with reference standard (Figure No. 2 and Figure No. 3).

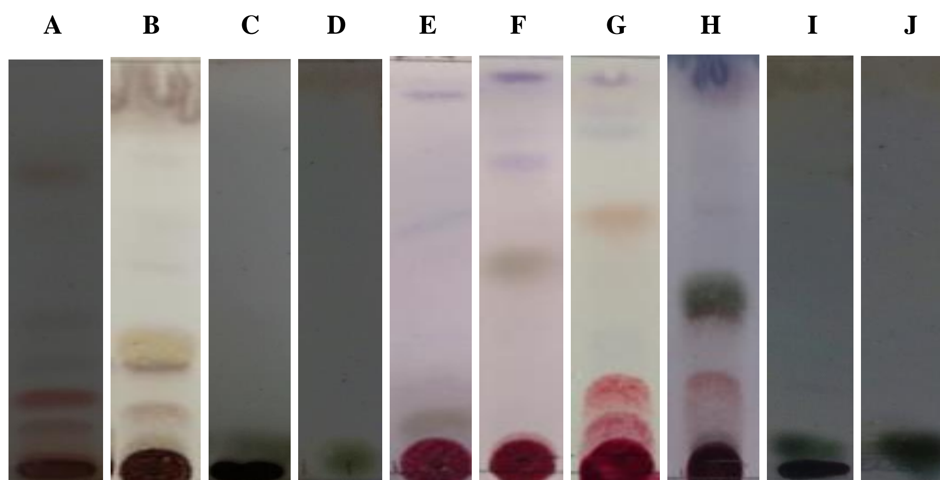

Figure No. 2
Phytochemical screening by TLC. Ceric sulfate development solution, identification of terpenes and steroids (A), fatty acids and flavonoids (B), bergenin (C), standard bergenin (D). Sulfuric vanillin development solution, identification of monoterpenes, diterpenes and triterpenes (E, F, G, H), bergenin (I) and standard bergenin (J)

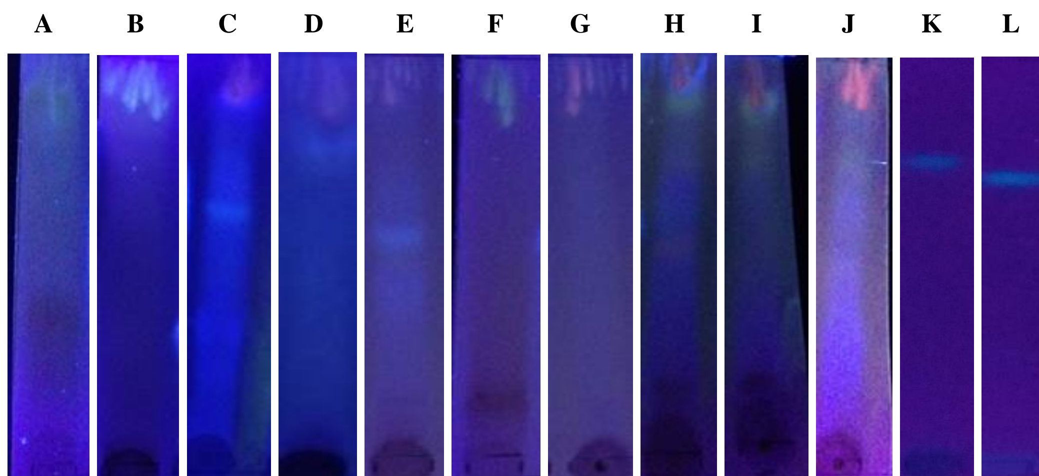

Figure No. 3
Phytochemical screening by TLC. NP-PEG development solution, identification of coumarin (A), non-glycosylated flavonoid (B), phenolic carboxylic acids and flavones (C, D and E), flavonol and glycosylated flavonoid (F), phenolic carboxylic acids and flavonol aglycone (G), phenolic carboxylic acids, non-glycosylated flavonoid and flavonol (H and I), flavones and flavonol aglycone (J), gallic acid (K) standard gallic acid (L)

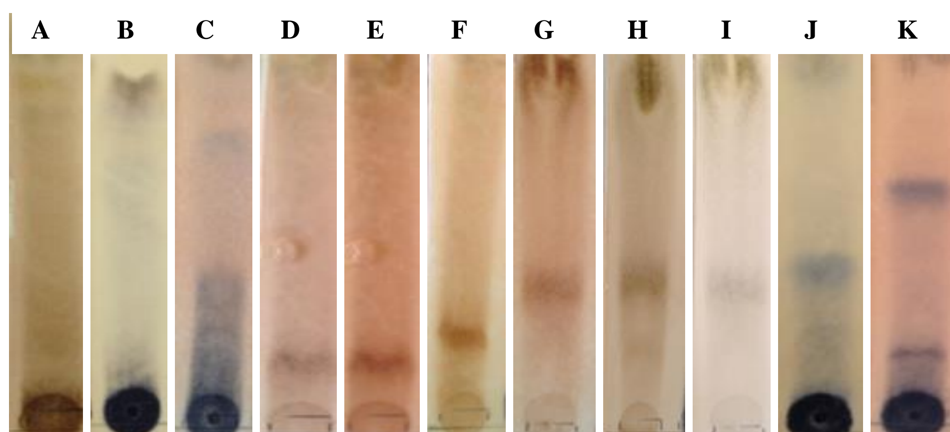

Figure No. 4
Phytochemical screening by TLC. Ferric chloride development solution, identification of catechin and flavonoids (A), trihydroxylated phenolics (B), hydrolyzable tannin and trihydroxylated phenolics derived from gallic acid (C), condensed tannin and non-glycosylated flavonoid (D), condensed tannin and catechin (E) , condensed tannin, glycosylated and non-glycosylated flanonoid (F), condensed tannin (G), catechin and glycosylated and non-glycosylated flavonoid (H), catechin and dihydroxylated phenolic derivatives tannic acid (I), hydrolysable tannin and flavonoids (J) and tannin hydrolysable and trihydroxylated phenolics (K)

Chemical characterization of isolated compound
X-Ray Diffraction (XRD)
Crystalline unit cell parameter data are in compliance with the Bergenin monohydrate structure described in the CSD database (Figure No. 5). Bergenin was crystallized in the orthorhombic space group $P2_12_12_1$ (Table No. 2).

Table No 2
Unit cell parameters of the dentified isolate

Crystal data	Experimental
$C_{14}H_{16}O_9.H_2O$	
Space group	Orthorhombic P ($P2_12_12_1$)
a/Å	7.48
b/Å	13.87
c/Å	14.13
$\alpha = \beta = \gamma$/ °	90
Crystal volume / Å3	1466
R_{exp}	3.1528
R_{wp}	7.4972
Adjustment quality, X^2	2.3779
Analysis temperature	200 K

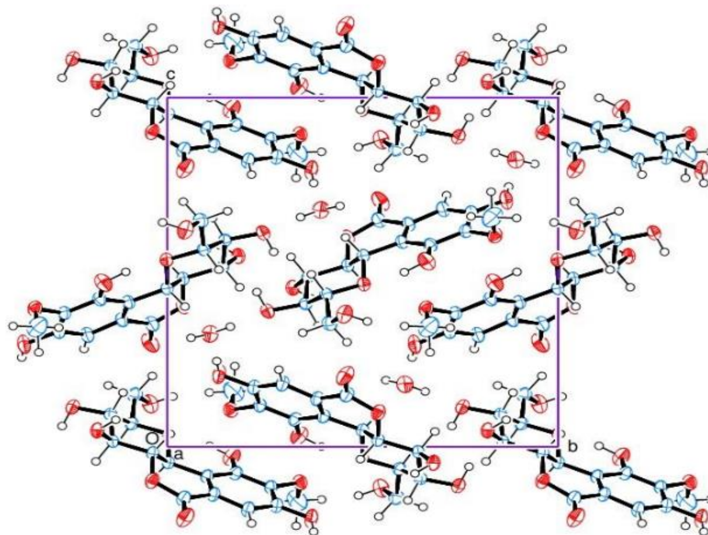

Figure No 5
ORTEP projection of the molecular structure of Bergenin monohydrate crystallized in the orthorhombic space group $P2_12_12_1$

$^{13}C\{^1H\}$and ^1H NMR

Structural elucidation of the isolated compound was performed by comparing the $^{13}C\{^1H\}$ and ^1H NMR data with the literature (Nunomura *et al.,* 2009). The data obtained showed compliance with that reported for Bergenin (Figure No. 6).

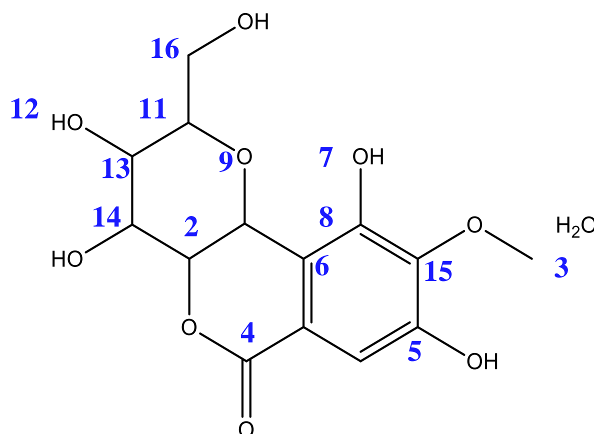

Figure No. 6
Structure of Bergenin monohydrate isolated from the trunk barks of *Sacoglottis ceratocarpa* Ducke

In the ^{1}H spectrum (400.13 MHz, MeOD-d_4) 11 signals were observed (Table No. 3). Singlet at δ 7.08 (^{1}H) is typical of hydrogen bonded to an aromatic ring. Signals between δ 3.45 (dd, 9.5; 7.6, 1H) and 4.07 (dd, 9.5; 10.04, 1H, H-14), are characteristic of hydrogen bonded to electronegative atoms (oxygen). The signal at δ 3.91 (3H; s) characterizes a methoxyl group. Doublet at δ 4.96 (1H, 10.0), suggests of a sugar in the molecule structure.

Table No 3
$^{13}C\{^{1}H\}$ and ^{1}H NMR data for bergenin

Position	δ_C [a]	δ_H [a]
1		
2	165,80	
3	119,42	
4	111,11	7,08 (*s*, 1H)
5	152,32	
6	142,31	
7	149,43	
8	117,33	
9	74,27	4,96 (*d*, 10,0, 1H)
10		
11	83,05	3,69 (*m*, 1H)
12	71,92	3,45 (*dd*, 9,5; 7,6, 1H)
13	75,65	3,72 (*dd*, 9,5, 7,6, 1H)
14	81,41	4,07 (dd, 10,4; 9,5, 1H)
15	60,95	3,91 (*s*, 3H, OMe)
16	62,70	3,68 (*m*, 1H)
		4,04 (*dd*, 11,7; 2,0, 1H)

[a] NMR experimente performed at 100.6 MHZ for $^{13}C\{^{1}H\}$ and 440.1 MHZ for ^{1}H on MeOD-d_4.
[b] (δ) Chemical shift in ppm

In the spectrum of $^{13}C\{1H\}$ (100.61 MHz, MeOD-d_4), 14 signals were observed (Table No. 3). The signal at δ 165.8 is characteristic of the presence of cyclic ester carbon (lactone). The signals at δ 111.1 (C-4), 119.4 (C-3), 117.3 (C-8), 142.3 (C-6), 149.4 (C-7) and 152.3 (C-5) were assigned to the pentasubstituted aromatic ring. The signal at δ 60.9 confirmed the presence of a methoxyl group in the molecule. Oxygen-linked carbon signals in the ranges δ 83.0 to 62.7 confirmed the presence of a glycosidic unit.

Total phenolic and flavonoid content

The ESC showed rich in total phenolic compounds (Figure No. 7A). The equivalent content of tannic acid (0.562 ± 0.017 g EAT/g) was higher than the equivalent content of catechin (0.464 ± 0.013 g EC/g) and gallic acid (0.420 ± 0.012 g EAG/g), ANOVA, $p<0.05$. The rutin equivalent glycosylated flavonoid content (24.31 ± 0.562 mg RE/g) was higher than the quercetin equivalent non-glycosylated flavonoid content (11.37 ± 0.265 mg QE/g), ANOVA, $p<0.05$, (Figure No. 7B).

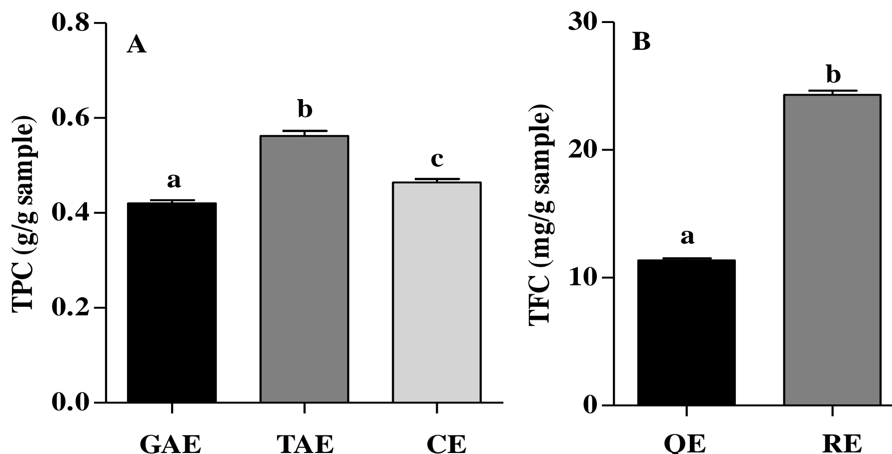

Figure No 7
Total phenolics and flavonoids content
***Data represents the mean ± standard deviation of three independent experiments. a *vs*. b and b *vs.* c (*p*<0.001); a *vs.* c (*p*<0.01). (ANOVA and Tukey, *p*<0.05). Total phenolic content (TPC). Total flavonoid content (TFC). Gallic acid equivalent (GAE). Tanic acid equivalent (TAE). Catechin equivalent (CE). Quercetin equivalent (QE). Rutin equivalent (RE)**

Antioxidant properties: DPPH radical reduction

In the DPPH radical reduction assay, the ESC (EC_{50} = 7.41 ± 0.22 µg/mL) and Bergenin (EC_{50} = 29.53 ± 0.21 µg/mL) showed higher antioxidant activity than standardized extract of *Ginkgo biloba* (EC_{50} = 40.63 ± 2.09 µg/mL), widely known for its antioxidant activity and content of phenolic metabolites (Figure No. 8).

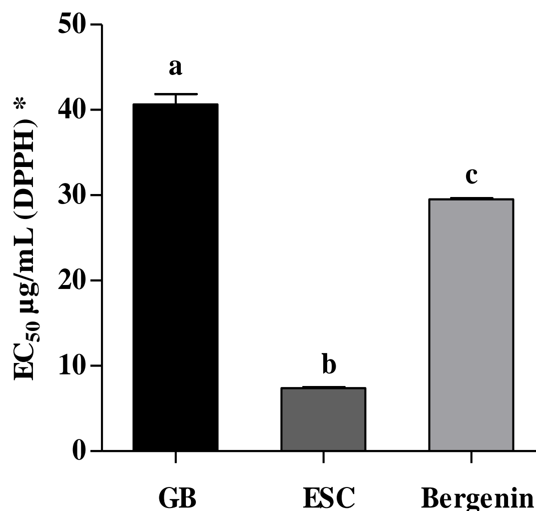

Figure No 8
Effect of ESC and Bergenin on DPPH radical reduction
***Data represents the mean ± standard deviation of three independent experiments. a *vs*. b; a *vs.* c; b *vs.* c (*p*<0.001). (ANOVA and Tukey, *p*<0.05). 50% effective concentration (EC_{50}). Standardized dry extract of *Ginkgo biloba* (GB). Ethanolic extract from the trunk barks of *Sacoglottis ceratocarpa* Ducke (ESC)**

Trolox equivalente antioxidant capacity (TEAC)

The reaction kinetics of ESC, Bergenin and Trolox are shown in Figure No. 9. Trolox caused an instantaneous drop in the absorbance of the ABTS+ radical, without prolonging the reaction (Figure No. 9A). The ESC reacted slowly, causing the initial drops in absorbances to be smaller. However, the reaction continued and extended over time (Figure No. 9B). For Bergenin, all reactions were slow, continuous and occurred gradually over time (Figure No. 9C). In this assay, the ESC (TEAC = 2.659 ± 0.102) and Bergenin (TEAC = 1.23 ± 0.045) showed higher antioxidant activity than Trolox (TEAC = 1.0 ± 0.045), ANOVA, $p<0.05$.

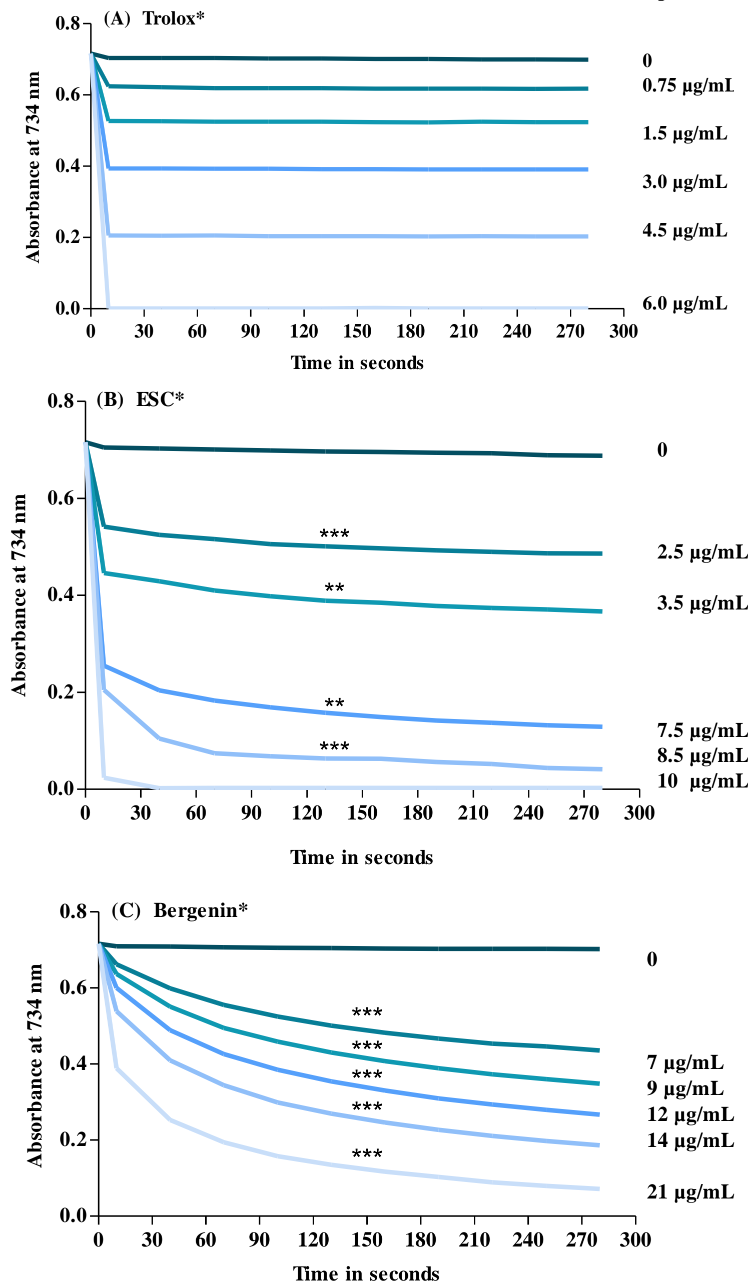

Figure No 9

Effect of ESC and Bergenin on ABTS•+ radical reduction kinetics

***Data expressed as mean ± standard deviation of three independent experiments. *** *vs.* Trolox (*p*<0.001) and ** *vs.* Trolox (*p*<0.01). Ethanolic extract from the trunk barks of *Sacoglottis ceratocarpa* Ducke (ESC)**

Ferric reducing antioxidant potential (FRAP)

The ESC and Bergenin were highly effective in reducing Fe^{+2} (Figure No. 10). The ESC (EC = 284.55 ± 0.13 µmol Fe^{+2}/L) and Bergenin (EC = 294.47 ± 1.69 µmol Fe^{+2}/L), with quercetin (EC = 276.50 ± 1.73 µmol Fe^{+2}/L), catechin (EC = 281.90 ± 6.92 µmol Fe^{+2}/L) and tannic acid (EC = 290.02 ± 0.81 µmol Fe^{+2}/L), showed equivalent concentration lower than rutin (EC = 378.01 ± 17.20 µmol Fe^{+2}/L) and gallic acid (EC = 395 .29 ± 1.41 µmol Fe^{+2}/L) and, consequently, higher antioxidant power of reduction of the FRAP reagent than these compounds (ANOVA, $p<0.05$).

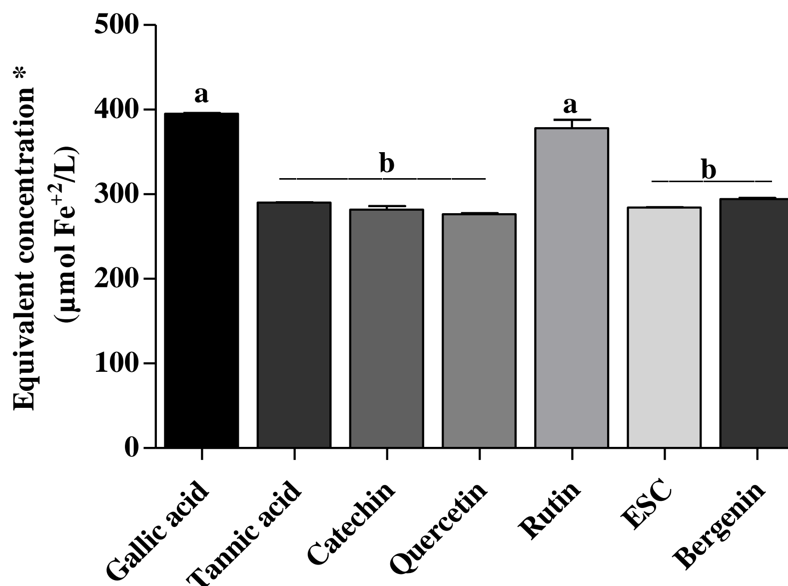

Figure No 10

Effect of ESC and Bergenin on FRAP reagent reduction

***Data represents the mean ± standard deviation of three independent experiments. a *vs*. b ($p<0.001$). (ANOVA and Tukey, $p<0.05$). Ethanolic extract from the trunk barks of *Sacoglottis ceratocarpa* Ducke (ESC)**

Pearson's coefficient (r)

The correlation between total phenolic content (GAE, TAE and EC) and the antioxidant properties (DPPH, TEAC and FRAP) showed r >0.9, ($p<0.001$), i.e., very strong and positive correlations (Table No. 4).

Table No. 4

Pearson`s coefficient

	Correlation (*r*)*		
	DPPH	**TEAC**	**FRAP**
GAE	0,983	0,990	0,981
TAE	0,987	0,993	0,983
CE	0,989	0,997	0,986

Gallic acid equivalent (GAE). Tannic acid equivalent (TAE). Catechin equivalent (CE). 2,2-Diphenyl-1-picrylhydrazyl (DPPH). Trolox equivalent antioxidant capacity (TEAC). Ferric ion reducing antioxidant power (FRAP). * $p<0.001$ (ANOVA, $p<0.05$)

In vivo toxicity assay: Brine shrimp lethality

In the *in vivo* toxicity assay the ESC and Bergenin showed LC_{50} > 1000 µg/mL and did not toxicity for *brine shrimp*, when compared to quinidine sulfate (LC_{50} = 165,91 ±18,91 µg/mL), ANOVA, $p<0.05$. Samples are toxic when LC_{50} < 1000 µg/mL (Meyer *et al*., 1982).

In vitro cytotoxicity assays: Human erythrocytes cytotoxicity

The ESC (H% = 1,60-3,27± 0,15) and Bergenin (H% = 0,53-5,80 ± 0,16) did not cytotoxic to human blood cells, when compared to positive control (100% hemolysis) and negative control (H% = 2.63 ± 0.130), ANOVA, $p<0.05$. Samples are cytotoxic when H% ≥ 10-49%, in all tested concentrations (Pagano & Faggio, 2015).

Human kidney fibroblastos cytotoxicity

The ESC and Bergenin did not cytotoxic to HEK-293 cells (Figure No. 11). The lowest concentration of the ESC (10 µg/mL) and the concentrations of 10 and 100 µg/mL of the Bergenin did not differ significantly from the control condition (ANOVA, $p<0,05$). Interestingly, a mild increase of the cell viability was observed in cells treated with the ESC at 500 µg/mL and the concentrations of 100 and 500 µg/mL with the Bergenin (ANOVA, $p>0,05$). In contrast, microscopic visualization performed prior the assay suggested a complete absence of effect on cell viability, since the confluence of cells were similar in all conditions tested (data not shown).

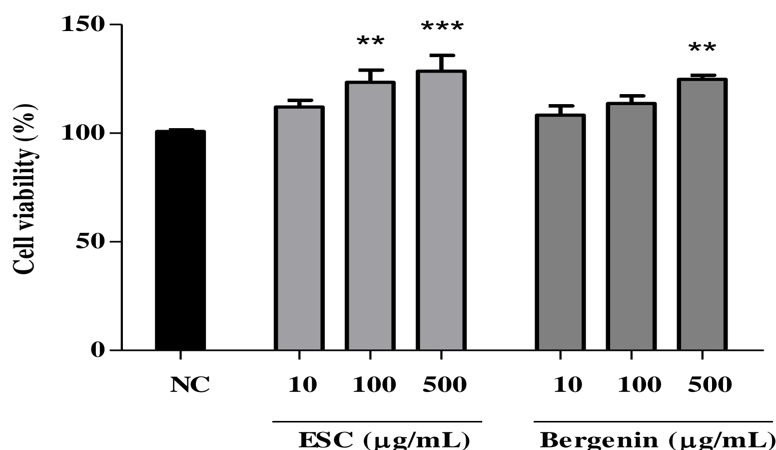

Figure No. 11
Effect of ESC and Bergenin on HEK-293 cells. Data represents the mean ± standard deviation of three independent experiments. Negative control (NC). ** *vs.* NC (*p*<0.01), * *vs.* NC (p<0.001). (ANOVA, *p*<0.05). Ethanolic extract from the trunk barks of *Sacoglottis ceratocarpa* Ducke (ESC)**

DISCUSSION

Studies of pharmacognostic parameters and qualitative chemical composition of non-validated medicinal species are important for knowledge of the use and proof of quality, safety and efficacy (Arruda *et al.,* 2018; Kim *et al*., 2021). In this work, the purity and physicochemical tests showed compliance with the specifications described in official compendiums (Brasil, 2019; Patnala & Kanfer, 2021). The collection, drying and pulverization conditions contributed to the quality of the vegetable raw material.

In the total ash test, the data showed a compliant content for plant materials of a compacted nature (Table No. 1) and showed greater purity for ScTB than other species of the *Sacoglottis* genus (TA% = 5.91-6.55), allowing the identification and differentiation between species (Politi, 2009; Alade *et al.,* 2017; Brasil, 2019; Liu, 2019). In this study, the total ash test was used to quantify non-volatile inorganic impurities (physiological ash) and external materials (non-physiological ash). As well as evaluating the identity and purity of the plant material (Passialis *et al*., 2008; Hytonen & Nurmi, 2015;

Trishala & Thangavelu, 2018; Brasil, 2019; Fedorov & Ryazanova, 2021).

Moisture content was determined to provide quantitative data of residual substances (water, essential oils and volatile substances) in ScTB powder after drying. In addition, for provide data on chemical and microbiological stability of plant material and degradation and decomposition of bioactive chemical compounds (Dias & Marenco, 2016; Malavasi *et al*., 2016; Hytönen *et al.,* 2018; Brasil, 2019). The assay time and moisture content presented for ScTB (Table No. 1) was lower than other Humiriaceae species (MC% = 7.38-8.16) (Politi, 2009; Alade *et al*., 2017). Normally, thinner trunk barks are less resistant to heat and their exchange, resisting the drying process for shorter time. In absolute terms, the moisture content is lower and, consequently, less heat is stored and less drying time is required (Gava *et al*., 1995; Loram-Lourenço *et al*., 2020; Ilek *et al*., 2021). Thus, the time and temperature used in this study were sufficient to release all the moisture retained in the ScTB powder, showed the good drying process of the trunk barks after collection.

The determination of the particles size of ScTB powder is essential in the process of standardization of the vegetable raw material, as it influences the evaluation of parameters such as total ash, moisture content and storage conditions. In addition, it is one of the first stages for obtaining the plant extract (Mozafar *et al.,* 1990; Makanjuola, 2017; Patnala & Kanfer, 2021). After linear regression analysis, the ScTB powder was classified as semifine (particle size: 189 ± 0.11 µm) (Brasil, 2019). Powders with particles < 180 µm are compact and reduce the contact area of the plant material with the extracting solvent, making the extraction process more difficult, resulting in lower yields. In addition, they absorb a greater quantity of moisture during storage, increasing the moisture content and total ash content by carrying, along with excess moisture, contamination (Mozafar *et al.,* 1990; Michelin *et al*., 2010; Cardoso *et al*., 2017; Patnala & Kanfer, 2021). Thus, the homogeneity of the particle size powder allows the standardization of the quality parameters of the vegetable raw material and efficient extractions.

The uncompacted bulk density is a parameter directly related to the thickness and compact nature of the vegetable raw material, but also to the drying and spraying process of the sample. Compact materials and high moisture content will consequently give rise to denser powders (Santomaso *et al*., 2003; Amidon *et al*., 2017). However, the bulk density obtained for the ScTB powder was medium density (Table No. 1). According to reference data for powders of vegetable origin, the fact that the ScTB powder has a low moisture content and particle sizes >180 µm, probably influenced the obtained density to be below the values considered of high density (0.7-1 g/mL). Semifine particles, in addition to resisting intra and interparticle cohesion forces, have larger dimensions, reducing the space and number of particles inside the measuring cylinder, reducing the density value of this powder (Amidon *et al*., 2017; Neikov & Yefimov, 2019; Barona *et al.,* 2021).

The ethanolic extract (ESC) was obtained with a modified Soxhlet apparatus. This extraction method is simple, fast, with a high extraction capacity and proved to be efficient with yield >16% (Table No. 1). High yields are common using modified Soxhlet apparatus. The Soxhlet reflux system combined with the amphiphilic characteristics of ethanol (extraction solvent) and standardized pharmacognostic parameters, can influence the integrity of cellular structures in plant material (Carvalho *et al*., 2009; Jahromi, 2019). Thus, several chemical compounds present in ScTB, such as high molecular weight antioxidant compounds, are easily transferred to the solvent and solubilized, obtaining an extract rich in plant biomass and antioxidant and biological potential (Cardoso *et al*., 2017; Hoyos-Martinez *et al*., 2019; Tanase *et al*., 2019; Fedorov & Ryazanova, 2021).

The Bergenin was isolated from the ESC extract. The Bergenin or 4-methoxy-2-[(1S, 2R, 3S, 4S, 5R)-3,4,5,6-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yl]-α-resorcylic δ-lactone monohydrate, ($C_{14}H_{16}O_9.H_2O$), is a pentahydroxylated 4-O-methylgallic acid C-glucoside (Figures No. 5 and Figures No. 6). The molecule is composed of three six-membered rings: an aromatic ring, a glucopyranose ring and an ringed δ lactone ring (Patel *et al*., 2012, Castro, 2013). Bergenin has been reported in genus of different families. In the Humiriaceae, it was described and reported in the *Endopleura* genus, in the species *Endopleura uchi* (Uber) Cuatrec endemic to the Brazilian Amazon. In the *S. ceratocarpa* it is reported for the first time, being possibly the major compound of the species

due to easily of isolation by the extraction technique used. Bergenin is a compound of pharmacological interest for having a variety of biological activities, such as antiulcerative, hepatoprotective, antiviral, antidiabetic, antimicrobial, anti-inflammatory, neuroprotective, enzyme inhibitor, in addition great antioxidant potential (Abreu *et al*., 2008; Rastogi & Rawat, 2008; Nunomura *et al*., 2009; Patel *et al*., 2012, Castro 2013).

Preliminary prospecting research of bioactive compounds performed by TLC is a fast, efficient and low-cost qualitative method. It is a widely used method for immediate quality control of plant raw materials and investigation studies of the chemical composition of non-validated medicinal species (Wagner & Bladt, 1996; Gatto *et al*., 2021; Patnala & Kanfer, 2021). In the ESC extract, TLC identified different natural compounds, predominantly phenolic compounds, such as flavonoids, tannins and catechins (Figures No. 1, Figures No. 2, Figures No. 3 and Figures No. 4). Polyphenolics are well described in some Humiriaceae species and are associated with antimicrobial, antiparasitic, anti-inflammatory, antiviral, antitumor and antidiabetic activities. The absence of nitrogen compounds and alkaloids in ScTB is a common profile in species of the *Sacoglottis* genus (Abreu *et al*., 2008; Ferreira *et al*., 2009; Nunomura *et al*., 2009; Politi *et al*., 2011; Abreu *et al*., 2013; Bento *et al*., 2014; Frausin *et al*., 2015; Silva & Teixeira, 2015; Hidalgo *et al*., 2016; Alade *et al*., 2017; De Oliveira *et al*., 2017).

Phenolic compounds are one of the principal and most diverse groups of antioxidant substances presents in plants. They are of great interest due to known biological and pharmacological properties (Dai & Mumper, 2010; Tanase *et al*., 2019). In this study, the phenolics were easily extracted with ethanol, resulting in high concentrations in the ESC. The contents in the three determinations were greater than 0.05 g/g of sample (Gatto *et al*., 2021). However, the tannin and catechin equivalent contents were higher than the gallic acid content (Figure No. 7A). Polyphenolics such as tannins, catechins and their polymers are easily found in the heartwood or close to organs and tissues for growth and nutrient exchange of the trunk barks. This is the form of protection and survival of the species, obtaining yields > 40%. In contrast, derivatives of gallic acid have different biosynthetic pathways, moving to different parts of the plant organism, obtaining low yields (Dai & Mumper, 2010; Okuda & Ito, 2011; Ashok & Upadhyaya, 2012; Hoyos-Martinez *et al.,* 2019). The data for the ESC were superior to those of other Humiriaceae species (contents: 0.250 to 0.400g/g), and the catechin content is reported for the first time in a species of the *Sacoglottis* genus (Politi, 2009; Politi *et al*., 2011; Silva & Teixeira, 2015; De Oliveira *et al*., 2017).

Flavonoids are polyphenolic derivatives that are concentrated in leaves, flowers and branches, contributing to lower yields in trunk barks (Stalikas, 2007; Huber & Rodriguez-Amaya, 2008; Stalikas, 2010). In ESC the flavonoid content varied in glycosylated and non-glycosylated (Figure No. 7B). However, the rutin content (glycosylated flavonoid) was higher than the quercetin content (non-glycosylated flavonoid). Non-glycosylated flavonoids are linked to cellulose, fibers and proteins in the trunk barks, extraction difficult and contributing to lower yields. In contrast, glycosylated flavonoids are found free in plant material because they have a glycosylated bond, contributing to higher yields (Dai & Mumper, 2010; Savi *et al*., 2017). Comparing with other Humiraceae species (contents: 1.35 to 2.13 mg/g), the flavonoid contents in ESC were 10 times higher than those reported (Politi, 2009; De Oliveira *et al*., 2017).

The antioxidant properties of *S. ceratocarpa* were evaluated, since most of the therapeutic effects observed for medicinal species and their natural compounds are related to the antioxidant potential (Abreu *et al*., 2008; Silva & Teixeira, 2015; De Oliveira *et al*., 2017). At low concentrations, ESC and Bergenin significantly neutralized the action of three distinct species of radicals through three different mechanisms (Figures No. 8, Figures No. 9 and Figures No. 10). The high content of phenolic compounds and polyhydroxylated derivatives present in ESC contributed to this antioxidant potential (r>0.9). The antioxidant activity shown in the tests in the DPPH and FRAP assays can be attributed to the presence of hydroxyls; or the double bonds in the heterocycle of the flavonoid molecule and derivatives, allowing the conjugation between rings A, B and C, or the degree of hydroxylation and position of the hydroxyl in the molecule, or the degree of conjugation of the phenolic compounds present in the ESC (Pulido *et al*., 2000; Dornas *et al*., 2007; Tian & Schaich, 2013; Santos-Sánchez *et al*., 2019; Tanase *et al*., 2019). For bergenin, free radical

attack is favored on the aromatic ring or on the methoxy group, i.e., the methoxy group is the target for radical attack (Patel *et al*., 2012; Castro, 2013).

Specifically, in the TEAC test (Figure No. 9), the ESC and Bergenin showed higher values than the Trolox, mainly ESC, due to the high content of polyphenolic compounds present, since two electrons can be transferred from each phenol group. The difference between reaction time and effectiveness presented in the TEAC assay probably shows a certain dependence on the size and molecular concentration of the bioactive compounds present in the ESC when reacting. Normally, high molecular weight and polyhydroxylated compounds such as flavonoids, tannins, catechins and derivatives have greater difficulty in approaching the radical and reacting. This is due to the decrease in diffusion, or excessive presence of hydroxyls, and the steric accessibility created by the radical. So, they need more time to reorient and engage. This is in contrast to monohydroxylated or simpler molecules, such as Trolox, which react in a matter of minutes by rapid electron transfer or generation of secondary species that easily approach the radical (Pulido *et al*., 2000; Tian & Schaich, 2013; Santos-Sánchez *et al*., 2019; Tanase *et al*., 2019). In the case of Bergenin, also it may be related to the presence and position of hydroxyls and methoxyl group in the molecule, since its molecule is pentahydroxylated (Patel *et al*., 2012; Castro, 2013).

The fact that ESC and Bergenin showed a prolongation of their activities on the $ABTS^{\bullet+}$ radical and at the same time increased their antioxidant capacity over the reaction time is advantageous, as it indicates that both have the ability to maintain their antioxidant activity for a long time longer, maintaing an adequate antioxidant status (Tian & Schaich, 2013). A greater antioxidant capacity over time may mean a more prolonged protective effect of these metabolites present in ESC, against oxidative damage in biological and physiological systems related to various diseases.

Information on the toxicity of plant extracts and isolated natural substances is important, since several medicinal species, whose use is poorly validated, are used based on popular knowledge. Thus, toxicity assays provide chemical quality and safety data of the use of these species by the population. In the *brine shrimp* lethality assay, the ESC and Bergenin were not toxic (LC_{50}>1000 µg/mL). The assay was conducted as a pre-screening for cytotoxicity assays, to define and estimate the concentrations/doses to be used. As well as a form of initial evaluation of the safety of the use of ScTB, being a useful alternative to models that use animals, as to predict the preliminary *in vivo* toxicity (Meyer *et al*., 1982; Hamidi *et al*., 2014; Gatto *et al*., 2021).

Cytotoxicity assays were used in this study to evaluate the biological effects caused by ESC and Bergenin in humans cells of different lineages. Erythrocytes and human embryo kidney cells are good models to be used in *in vitro* cytotoxicity assays, being suitable for measuring cell damage caused by plant extracts or natural molecules (Mosmann, 1983; Liu & Nair, 2010, Aslam *et al*., 2011). In the human erythrocyte cytotoxicity assay, ESC and Bergenin were not cytotoxic. In all concentrations the homolysis porcentage was > 10% (Pagano & Faggio, 2015). In the cytotoxicity assay by the MTT method, the samples were also not cytotoxic (Figure No. 11). The increase in the cell viability observed for ESC and Bergenin related to the interference on MTT method in presence of mitochondrial-targeted compounds. The antioxidant potential presented by the ESC and Bergenin explain this result (Figure No. 8, Figure No. 9 and Figure No. 10). In addition, polyhydroxylated phenolic compounds such as flavonoids, tannins and catechins, present in the ESC, have produced a direct effect on MTT reduction.

CONCLUSION

The pharmacognostic study accomplished in this work contributed to define the quality parameters, the qualitative chemical profile of the trunk barks of *S. ceratocarpa* and for the isolation and identification of bergenin, a gallic acid derivative. The species proved to be non-toxic and a promising source of natural antioxidant bioactive compounds, contributing to the knowledge of safe and effective use of the species. The observed antioxidant properties are probably related to the therapeutic effects of the species based on popular and traditional use, contributing to the future development of herbal medicines and serving as a model for new therapeutic options.

ACKNOWLEDGEMENTS

This work was carried out with the support of the Coordination of Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001.

REFERENCES

Abreu HA, Lago IAS, Souza GP, Piló-Veloso D, Duarte HA, Alcântara AF. 2008. Antioxidant activity of (+)-bergenin a phytoconstituent isolated from thebark of *Sacoglottis uchi* Huber (Humiriaceae). **Org Biomol Chem** 6: 2713 - 2718. **https://doi.org/10.1039/b804385j**

Abreu VGC, Corrêa GM, Lago IAS, Silva RR, Alcântara AF. 2013. Pentacyclic triterpenes and steroids from the stem bark of uchi (*Sacoglottis uchi*, Humiriaceae). **Acta Amazonica** 43: 525 - 528. **https://doi.org/10.1590/S0044-59672013000400015**

Alade GO, Moody JO, Awotona OR, Adesanya AS, Lai D, Proksch P. 2017. Spermicidal constituents of ethanolic extract of *Sacoglottis gabonensis* stem bark. **Folia Med** 59: 437 - 442. **https://doi.org/10.1515/folmed-2017-0047**

Amidon GE, Meyer PJ, Mudie DM. 2017. Chapter 10 - Particle, powder, and compact characterization. **Pharmaceut Theor Pract** 10: 271 - 293. **https://doi.org/10.1016/B978-0-12-802447-8.00010-8**

Arruda G, Périco LGV, Parpinelli R, Peretti RF, Scur MC, Fallador FAC. 2018. Phytochemical prospecting, antimicrobial activity, and acute toxicity of aqueous plant extract of two plant species *Allophylus edulis* (A. St. Hilaire, Cambessedes & A. Jussieu) RADLK ex WARM and *Matayba elaeagnoides* RADLK. **Int J New Technol Res** 5: 10 - 13. **https://doi.org/10.31871/IJNTR.5.2.7**

Aslam F, Riaz M, Zunair M, Rizwan K, Abbas M, Bukhari TH, Bukhari IH. 2011. Antioxidant, haemolytic activities and GC-MS profiling of *Carissa carandas* roots. **Int J Phytomed** 3: 567 - 578. **https://doi.org/55947135**

Ashok, PK, Upadhyaya K. 2012. Tannins are astringent. **J Pharmacogn Phytochem** 1: 45 - 50. **https://doi.org/10.22271/phyto**

Barona D, Shamsaddini A, Aisenstat M, Thalberg K, Lechuga-Ballesteros D, Damadzadeh B, Vehring R. 2021. Modulated uniaxial compression analysis of respirable pharmaceutical powders. **KONA Powder Part J** 38: 209 - 225. **https://doi.org/10.14356/kona.2021014 https://doi.org/10.14356/kona.2021014**

Bento JF, Noleto GR, Petkowicz CLO. 2014. Isolation of an arabinogalactan from *Endopleura uchi* bark decoctionand its effect on HeLa cells. **Carbohyd Polymer** 101: 871 - 877. **https://doi.org/10.1016/j.carbpol.2013.10.014**

Bessa NGF, Borges JCM, Beserra FP, Carvalho RHA, Pereira MAB, Fagundes R. Campos SL, Ribeiro LU**,** Quirino MS**,** Chagas Junior AF**,** Alves A. 2013. Preliminary phytochemical screening of native Cerrado plants of medicinal popular use by the rural community of the Vale Verde settlement – Tocantins. **Rev Bras Plant Med** 15: 692 - 707. **https://doi.org/10.1590/S1516-05722013000500010**

Bove CP, Melhem TS. 2000. Humiriaceae. **Pollen Spore Flora World** 22: 1 - 35. **https://doi.org/10.1007/978-3-030-82359-7_6**

Brasil. 2019. **Farmacopeia Brasileira.** Agência Nacional de Vigilância Sanitária. Anvisa, Brasília, Brasil.

Cardoso IC, Pereira HMG, Itappin MRR, Behrrens MD. 2017. Influência da técnica de extração e do tamanho de partícula do material vegetal no teor de compostos fenólicos totais da tintura das folhas de *Alpinia zerumbet.* **Revista Fitos** 1-126: 62 - 68. **https://10.5935/2446-4775.20170016**

Carvalho, JLS, Cunico MM, Dias JFG, Miguel MD, Miguel OG. 2009. Termoestabilidade de processos extrativos de *Nasturtium officinale* R. Br., (Brassicaceae) por sistema Soxhlet modificado. **Quim Nova** 32: 1031 - 1035. **https://doi.org/10.1590/S0100-40422009000400034**

Carvalho FA, Holanda ASS. 2018. Flora of the canga of the Serra dos Carajás, Pará, Brazil: Humiriaceae. **Rodriguésia** 69: 1143 - 1145. **https://doi.org/10.1590/2175-7860201869322**

Castro GF. 2013. **Estudo espectroscopico experimental e teórico da acetilbergenina.** Tesis, Universidade do Estado do Amazonas, Brasil.

Chanda S. 2014. Importance of pharmacognostic study of medicinal plants: An overview. **J Pharmacogn Phytochem** 2: 69 - 73. **https://doi.org/10.22271/phyto**

Cuatrecasas J. 1945. Taxonomic revision of Humiriaceae: *Sacoglottis ceratocarpa* Ducke. **Bol Tecn Inst Agron Norte** 4: 13. **https://doi.org/10.3897/phytokeys.124.34679**

Cuatrecasas J. 1961. A taxonomic revision of the Humiriaceae. **Systematic Plant Studies** 35: 25 - 214.

Dai J, Mumper RJ. 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties.

Molecules 15: 7313 - 7352. **https://doi.org/10.3390/molecules15107313**

De Oliveira GRB, Simão AA, Pereira LLS, Rocha FD, Raposo NRB, Oliveira VR, Pereira TV, Do Carmo HP, Oliveira TC, Sousa OV De Araújo ALA, Yamamoto CH. 2017. Stem bark extracts of *Endopleura uchi* (Huber) Cuatrec: Inhibition of pancreatic lipase and antioxidant activity. **J Med Plant Res** 11: 472 - 479. **https://doi.org/10.5897/JMPR2016.6271**

Dias DP, Marenco RA. 2016. Tree growth, wood and bark water content of 28 Amazonian tree species in response to variations in rainfall and wood density. **iForest** 9: 445 - 451. **https://doi.org/10.3832/ifor1676-008**

Dornas WC, Oliveira TT, Rodrigues-Das-Dores, RG, Santos AF, Nagem TJ. 2007. Flavonóides: potencial terapêutico no estresse oxidativo, **Rev Cienc Farm Bas Aplic** 28: 241 - 249.

Ducke A. 1945. Forest trees and climbers of the Brazilian Amazon. **Bol Tecn Inst Agron Norte** 4: 1 - 34.

Farrugia L. 2012. WinGX and ORTEP for Windows: An update, **J App Cryst** 45: 849 - 854. **https://doi.org/10.1107/S0021889812029111**

Fedorov VS, Ryazanova TV. 2021. Bark of Siberian conifers: Composition, use and processing to extract tannin. **Forests** 12: 1043. **https://doi.org/10.3390/f12081043**

Ferreira LCO, Lima NB, Araujo RNM, Monteiro HO, Damasceno TS, Lopes Junior ML, Ripardo Filho HS, Guilhon GMSP, Santos LS, Muller AH. 2009. **Friedelan triterpenes obtained of *Endopleura uchi* leaves.** 32 Annual meeting of the Brazilian Chemical Society Chemists for an emergent power, Brazil.

Frausin G, Hidalgo AF, Lima RBS, Kinupp VF, Ming LC, Pohlit AM, Miliken W. 2015. An ethnobotanical study of anti-malarial plants among indigenous people on the upper Negro River in the Brazilian Amazon. **J Ethnopharmacol** 174: 238 - 252. **https://doi.org/10.1016/j.jep.2015.07.033**

Gatto LJ, De Oliveira GRB, Rech KS, Moura PF, Gribner C, Merino FJZ, Ávila S, Dias JFG, Miguel OG, Miguel MD. 2021. Inhibition of α-glucosidase, pancreatic lipase, and ntioxidant property of *Myrcia hatschbachii* D. Legrand containing gallic and ellagic acids. **Bol Latinoam Caribe Plant Med Aromat** 20: 226 - 243. **https://doi.org/10.37360/blacpma.21.20.3.18**

Gava JL, Ometto ML, Nibe TM, Seixas F. 1995. Influência da espessura da casca de *Eucalyptus torelliana* e *Eucalyptus tereticornis* sobre a variação de temperatura do câmbio durante ocorrência de um incêndio florestal. **IPEF** 48: 126 - 132. **https://www.ipef.br/publicacoes/scientia/nr48-49/cap13.pdf**

Hamidi MR, Jovanova B, Panovska TK. 2014. Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L.) model. **Macedonia Pharm Bull** 60: 9 - 18. **https://doi.org/10.33320/10.33320/maced.pharm.bull.2021.67.01.005**

Hidalgo PSP, Nunomura RCS, Nunomura SM, Pinto ACS, Tadei WP. 2016. Larvicidal activities against *Aedes aegypti* and *Culex quinquefasciatus* of some extracts from Amazon edible fruits. **J Mosq Res** 6: 1 - 5. **https://doi.org/10.5376/jmr.2016.06.0028**

Hoyos-Martinez PL, Merle J, Labidi JEl, Bouhtoury FC. 2019. Tannins extraction: A key point for their valorization and cleaner production. **J Clean Prod** 206: 1138 - 1155. **https://doi.org/10.1016/j.jclepro.2018.09.243**

Huber LS, Rodriguez-Amaya DB. 2008. Flavonoids and flavones: the Brazilian sources and factors that influence the composition in food. **Alim Nutr** 19: 97 - 108.

Hytönen J, Beuker E, Viherä-Aarnio A**.** 2018. Clonal variation in basic density, moisture content and heating value of wood, bark and branches in hybrid aspen. **Silva Fennica** 52: 1 - 15. **https://doi.org/10.14214/sf.9938**

Hytonen J, Nurmi J. 2015. Heating Value and ash contente of intensivel Y managed Stands. **Wood Res** 60: 71 - 82.

Ilek A, Siegert CM, Wade A. 2021. Hygroscopic contributions to bark water storage and controls exerted by internal bark structure over water vapor absorption. **Trees** 35: 831 - 843. **https://doi.org/10.1007/s00468-021-02084-0**

Jahromi SG. 2019. Extraction techniques of phenolic compounds from plants. **IntechOpen** **https://doi.org/10.5772/intechopen.84705**

Kim LJ, Ohashi M, Zhang Z, Tan D, Asay M, Cascio D, Rodriguez JA, Tang Y, Nelson HM. 2021. Prospecting for natural products by genome mining and microcrystal electron diffraction. **Nat Chem Biol** 17: 872 - 877. **https://doi.org/10.1038/s41589-021-00834-2**

Loram-Lourenço L, Farnese FDS, Sousa LFD, Alves RDFB, Andrade MCPD, Almeida SEDS, Moura LMDF,

Costa AC, Silva FG, Galmés J, Cochard H, Franco AC, Menezes-Silva PE. 2020. A structure shaped by fire, but also water: Ecological consequences of the variability in bark properties across 31 species from the Brazilian Cerrado**. Front Plant Sci** 10: 1718. **https://doi.org/10.3389/fpls.2019.01718**

Liu Y, Nair MG. 2010. An efficient and economical MTT assay for determining the antioxidant activity of plant natural product extracts and pure compounds. **J Nat Prod** 73: 1193 - 1195. **https://doi.org/10.1021/np1000945**

Liu K. 2019. Effects of sample size, dry ashing temperature and duration on determination of ash content in algae and other biomass. **Algal Res** 40: 101486. **https://doi.org/10.1016/j.algal.2019.101486**

Malavasi UC, Davis AS, Malavasi MM. 2016. Estimating water in living woody bark – A review. **CERNE** 22: 415 - 422. **https://doi.org/10.1590/01047760201622032169**

Makanjuola AS. 2017. Influence of particle size and extraction solvent on antioxidant properties of extracts of tea, ginger, and tea–ginger blend. **Food Sci Nutr** 5: 1179 - 1185. **https://doi.org/10.1002/fsn3.509**

Meyer BN, Ferrigni NA, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. 1982. *Brine Shrimp*: A convenient general bioassay for active plant constituents. **Planta Med** 45: 31 - 34. **https://doi.org/10.1055/s-2007-971236**

Michelin DC, Finati SCG, Sacramento LVS, Vilegas W, Salgado HRN. 2010. Controle de qualidade da raiz de *Operculina macrocarpa* (Linn) Urb., Convolvulaceae. **Braz J Pharmacogn** 20: 18 - 22. **https://doi.org/10.1590/S0102-695X201000010005**

Mohagheghzadeh A, Faridi P, Shams-Ardakani M, Ghasem Y. 2006. Medicinal smokes. **J Ethnopharmacol** 108: 161 - 184. **https://doi.org/10.1016/j.jep.2006.09.005**

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. **J lmmunol Methods** 65: 55 - 63. **https://doi.org/10.1016/0022-1759(83)90303-4**

Mozafar A, Oertli JJ, Ambuehl B. 1990. Particle size and element concentration in ground plant material. **Commun Soil Sci Plant Anal** 21: 1619 - 1632. **https://doi.org/10.1080/00103629009368327**

Nafiu MO, Hamid AA, Muritala HF, Adeyemi SB. 2017. **Preparation, standardization, and quality control of medicinal plants in Africa**. Medicinal spices and vegetables from Africa. Academic Press. **https://doi.org/10.1016/B978-0-12-809286-6.00007-8**

Neikov OD, Naboychenko SS, Yefimov NA. 2019. **Powder characterization and esting.** Handbook of non-ferrous metal powders. Elsevier. **https://doi.org/10.1016/B978-0-08-100543-9.00001-4**

Nunomura RCS, Oliveira VG, Silva SL, Nunomura SM. 2009. Characterization of bergenin in *Endopleura uchi* bark and its anti-inflammatory activity. **J Braz Chem Soc** 6: 1060 - 1064. **https://doi.org/10.1590/S0103-50532009000600009**

Okuda T, Ito H. 2011. Tannins of constant structure in medicinal and food plants - hydrolyzable tannins and polyphenols related to tannins. **Molecules** 16: 2191 - 2217. **https://doi.org/10.3390/molecules16032191**

Pagano M, Faggio C. 2015. The use of erythrocyte fragility to assess xenobiotic cytotoxicity. **Cell Biochem Funct** 33: 351 - 355. **https://doi.org/10.1002/cbf.3135**

Passialis C, Voulgaridis E, Adamopoulos S, Matsouka M. 2008. Extractives, acidity, buffering capacity, ash and inorganic elements of black locustwood and bark of different clones and origin. **Holz Roh Werkst** 66: 395 - 400. **https://doi.org/10.1007/s00107-008-0254-4**

Patel DK, Patel K, Kumar R, Gadewar M, Tahilyani V. 2012. Pharmacological and analytical aspects of bergenin: a concise report. **Asian Pac J Trop Dis** 2: 163 - 167. **https://doi.org/10.1016/S2222-1808(12)60037-1**

Patnala S, Kanfer I. 2021. **Quality control, extratction methods, and standardization: Interface between traditional use and scientific investigation**. Herbal medicine in andrology. Academic Press. **https://doi.org/10.1016/B978-0-12-815565-3.00006-0**

Politi FAZ. 2009. **Estudos farmacognósticos e avaliação de atividades biológicas de extratos obtidos das cascas pulverizadas de *Endopleura uchi* (Huber).** Tesis, Universidade Estadual Paulista, Brasil.

Politi FAZ, De Mello JCO, Migliato KF, Nepomuceno ALA, Moreira RRD, Pietro RCLR. 2011. Antimicrobial, cytotoxic and antioxidant activities and determination of the total tannin content of bark extracts *Endopleura uchi.* **Int J Mol Sci** 12: 2757 - 2768. **https://doi.org/10.3390/ijms12042757**

Pulido R, Bravo L, Saura-Calixto F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified

ferric reducing/antioxidant power assay. **J Agric Food Chem** 48: 3396 - 3402. **https://doi.org/10.1021/jf9913458**

Rastogi S, Rawat AKS. 2008. A comprehensive review on bergenin, a potential hepatoprotective and antioxidative phytoconstituent. **Kerba Polonica** 54: 66 - 79.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang, M, Rice-Evans CA. 1999. Antioxidant activity applying an improved ABTS radical cation descolorization assay. **Free Radic Biol Med** 26: 1231 - 1237. **https://doi.org/10.1016/s0891-5849(98)00315-3**

Rocha FD, Yano M, Da Cunha MR, Gabriel FT, Cordeiro RSB, Menezes FS, Kaplan MAC. 2010. Brazilian Bromeliaceae species: isolation of arylpropanoid acid derivatives and antiradical potential. **Rev Bras Farmacogn** 20: 240 - 245. **https://doi.org/10.1590/S0102-695X2010000200017**

Santomaso AC, Lazzaro P, Canu P. 2003. Powder flowability and density ratios: the impacto of granule. **Chem Eng Sci** 58: 2857 - 2874. **https://doi.org/10.1016/S009-2509(03)00137-4**

Santos-Sánchez NF, Salas-Coronado R, Villanueva-Cañongo C, Hernández-Carlos B. 2019. **Antioxidant compounds and their antioxidant mechanism.** IntechOpen. **https://doi.org/10.5772/intechopen.85270**

Savi PRS, Santos L, Gonçalves AM, Biesek S, Lima CP. 2017. Analysis of total flavonoids present in some of the most consumed conventional and organic fruits and vegetables in southern Brazil. **Demetra** 12: 275 - 287. **https://doi.org/10.12957/demetra.2017.22391**

Schultes RE. 1979. De plantis toxicariis e mundo novo tropicale commentationes. XXI. Interesting native uses of the Humiriaceae in the northwest Amazon. **J Ethnopharmacol** 1: 89 - 94. **https://doi.org/10.1016/0378-8741(79)90019-9**

Schultes RE, Raffauf RF. 1990. **The healing forest: Medicinal and toxic plants of the northwest Amazonia**. Dioscorides Press, Portland, USA. **https://doi.org/10.1017/S0376892900032641**

Silva LR, Teixeira R. 2015. Phenolic profile and biological potential of *Endopleura uchi* extracts. **Asian Pac J Trop Med** 8: 889 - 897. **https://doi.org/10.1016/j.apjtm.2015.10.013**

Stalikas CD. 2007. Extraction, separation, and detection methods for phenolic acids and flavonoids. **J Sep Sci**. 30(18): 3268 – 3295. https://doi.org/10.1002/jssc.200700261

Stalikas CD. 2010. Phenolic acids and flavonoids: occurrence and analytical methods. **Methods Mol Biol**. 610:65-90. https://doi.org/10.1007/978-1-60327-029-8_5

Tanase C, Cosarcã S, Muntean DL. 2019. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. **Molecules** 24: 1182. **https://doi.org/10.3390/molecules24061182**

Tian, X., Schaich, K.M. 2013. Effects of molecular structure on kinetics and dynamics of the trolox equivalent antioxidant capacity assay with $ABTS^{+\bullet}$. **J Agric Food Chem** 61: 5511 - 5519. **https://doi.org/10.1021/jf4010725**

Trishala AL, Lakshmi Thangavelu. 2018. Physicochemical profile of *Acacia Catechu* bark extract – An *in vitro* study. **Int J Pharm Phytopharmacol Res** 8: 84 - 87. **https://doi.org/10.5958/0976-5506.2019.04129.9**

Wagner H, Bladt S. 1996. **Plant drug analysis: A thin layer chromatography atlas**. Springer-Verlag, Berlin, Germany. **https://doi.org/10.1007/978-3-642-00574-9**

Woodson R.E, Schery RW, Gentry AH. 1975. Flora of Panama. Part VI. Family 87A. Humiriaceae. **Ann Missouri Bot Gard** 62: 35 - 44. **https://doi.org/10.2307/2395048**