

Potencial antioxidante e toxicológico do extrato hidroalcoólico da casca do Ipê-amarelo
Antioxidant and toxicological potential of the Golden trumpet hydroalcoholic stem bark
extract

Potencial antioxidante y toxicológico del extracto hidroalcohólico de corteza de Lapacho
amarillo

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Resumo

Handroanthus chrysotrichus é uma árvore da família Bignoniaceae, conhecida como ipê-amarelo e distribui-se pelo Nordeste, Sudeste e Sul do Brasil. Suas flores, caule e casca são usadas para fins medicinais no tratamento de doenças relacionadas ao sistema cardiovascular e imunológico. Esse estudo tem por objetivos avaliar o perfil fitoquímico, espectro de atividade biológica, capacidade antioxidante e potencial toxicológico do extrato da casca de *H. chrysotrichus*. O extrato hidroetanólico foi obtido por percolação e liofilizado. Os compostos presentes no extrato foram analisados por métodos colorimétricos e GC-MS. A avaliação do espectro de atividade biológica foi realizada *in silico*. O poder antioxidante foi determinado pela investigação da capacidade antioxidante total, capacidade quelante de ferro, ensaios DPPH[•] e ABTS^{•+}, e teste de degradação da desoxirribose. A capacidade de inibição da lipoperoxidação induzida por Fe⁺ foi avaliada em cérebros e fígados de camundongos. Náuplios de *Artemia salina* foram utilizados para avaliação da dose letal mediana. A toxicidade foi avaliada por simulação computacional e *in vitro* em linfócitos humanos. Como resultados, os métodos colorimétricos sugerem altos níveis de polifenóis e os dados de GC-MS indicaram a ocorrência de α -curcumeno, β -bisaboleno, 4-(4-metilfenil) pentanal, ácido pentanóico e acetato de isoamil no extrato da casca. Simulações computacionais apontaram atividades biológicas que estão de acordo com seu uso tradicional. A casca do extrato exibiu atividade antioxidante em diversos ensaios e foi efetiva em proteger cérebros e fígados de camundongos da lipoperoxidação induzida por Fe⁺. A casca de *H. chrysotrichus* demonstrou uma toxicidade média em *A. salina* com potencial presença de compostos bioativos. Em geral, os compostos apresentaram baixa probabilidade de toxicidade nas previsões *in silico*. Não houve citotoxicidade e genotoxicidade nos ensaios realizados com linfócitos humanos. Os resultados indicam que a casca de *H. chrysotrichus* possui compostos com espectro de atividade biológica e baixo potencial

toxicológico. Além disso, mostra capacidade antioxidante e ação protetora contra a peroxidação lipídica. Os dados apresentados apoiam o uso medicinal do ipê-amarelo e apontam o mesmo como um extrato promissor para avaliações *in vivo*.

Palavras-chave: Fitoquímica; Terpenos; Cromatografia; Medicina tradicional; Tabebuia.

Abstract

Handroanthus chrysotrichus is a tree of the Bignoniaceae family known as golden trumpet that is distributed throughout Northeast, Southeast and South Brazil. Its flowers, stem and bark are used for medicinal purposes in the treatment of cardiovascular and immune system diseases. This study aims to evaluate the phytochemical profile, biological activity spectrum, antioxidant capacity and toxicological potential of *H. chrysotrichus* stem bark extract. Hydroethanolic extract was obtained by percolation and lyophilized. Compounds present in the extract were analyzed by colorimetric methods and by GC-MS. Evaluation of the biological activity spectrum was performed *in silico*. Antioxidant power was determined by investigation of total antioxidant capacity, iron chelating capacity, DPPH[•] and ABTS^{•+} assays, and deoxyribose degradation test. The ability to inhibit Fe⁺ induced lipoperoxidation was evaluated in mouse brains and livers. Nauplii of *Artemia salina* were used to evaluate the median lethal dose. Toxicity was assessed by computer simulation, and *in vitro* in human lymphocytes. As a result, colorimetric methods suggest high levels of polyphenols and GC-MS data indicated the occurrence of α -curcumene, β -bisabolene, 4- (4-methylphenyl) pentanal, pentanoic acid and isoamyl acetate. Computer simulations have pointed biological activities that are in accordance with their traditional use. The *H. chrysotrichus* stem bark extract exhibited antioxidant activity in several assays and was effective in protecting mouse brains and livers from Fe⁺ induced lipoperoxidation. *H. chrysotrichus* stem bark extract showed medium toxicity in *A. salina* with potential presence of bioactive compounds. In general, the compounds showed low probability of toxicity *in silico* predictions. There was no cytotoxicity and genotoxicity in human lymphocyte evaluation. The results indicate that *H. chrysotrichus* stem bark extract has compounds with biological activity spectrum and low toxicological potential. It also shows antioxidant capacity and protective action against lipid peroxidation. The data presented support the medicinal use of golden trumpet and point to it as a promising extract for *in vivo* evaluations.

Keywords: Phytochemistry; Terpenes; Chromatography; Traditional medicine; Tabebuia.

Resumen

Handroanthus chrysotrichus es un árbol de la familia Bignoniaceae conocido como lapacho amarillo (ipe-amarelo en Brasil) y se distribuye por todo el noreste, sudeste y sur de Brasil. Sus flores, tallo y corteza se utilizan con fines medicinales en el tratamiento de enfermedades relacionadas con el sistema cardiovascular y sistema inmune. Este estudio tiene como objetivo evaluar el perfil fitoquímico, el espectro de actividad biológica, la capacidad antioxidante y el potencial toxicológico del extracto de

corteza de *H. chrysotrichus*. El extracto hidroetanólico se obtuvo por percolación y se liofilizó. Los compuestos presentes en el extracto se analizaron por métodos colorimétricos y GC-MS. La evaluación del espectro de actividad biológica se realizó *in silico*. El poder antioxidante se determinó mediante la investigación de la capacidad antioxidante total, la capacidad quelante de hierro, los ensayos DPPH[•] y ABTS^{•+}, y la prueba de degradación de desoxirribosa. La capacidad de inhibir la lipoperoxidación inducida por Fe⁺ se evaluó en cerebros e hígados de ratones. Se utilizaron nauplios de *Artemia salina* para evaluar la dosis letal media (DL₅₀). La toxicidad se evaluó mediante simulación por computadora y también *in vitro* en linfocitos humanos. Como resultados los métodos colorimétricos sugieren altos niveles de polifenoles y los datos de GC-MS indicaron la presencia de α -curcumeno, β -bisaboleno, ácido 4- (4-metilfenil) pentanal, ácido pentanoico y acetato de isoamilo en el extracto. Las simulaciones por computadora han señalado actividades biológicas que están de acuerdo con su uso tradicional. El extracto exhibió actividad antioxidante en varios ensayos y fue eficaz para proteger los cerebros e hígados de ratones de la lipoperoxidación inducida por Fe⁺. El extracto de la corteza de *H. chrysotrichus* mostró una toxicidad media en *Artemia* con posible presencia de compuestos bioactivos. En general, los compuestos mostraron baja probabilidad de toxicidad en predicciones *in silico*. No hubo citotoxicidad ni genotoxicidad en los ensayos con linfocitos humanos. Los resultados indican que el extracto de la corteza de *H. chrysotrichus* tiene compuestos con espectro de actividad biológica y bajo potencial toxicológico. También muestra capacidad antioxidante y acción protectora contra la peroxidación lipídica. Los datos presentados respaldan el uso medicinal del lapacho amarillo y lo señalan como un extracto prometedor para evaluaciones *in vivo*.

Palabras clave: Fitoquímica; Terpenos; Cromatografía; Medicina tradicional; Tabebuia.

1. Introduction

The family Bignoniaceae is the major group of Angiosperms plants and has been used for its beneficial health properties. Several parts of Bignoniaceae species as leaves, fruits, roots, sap, flowers and bark are traditionally used for treatment of diabetes, high blood pressure, asthma, cancer, uterine infection and others diseases. In all these uses, different extractive methods are employed including decoction, maceration, infusion, poultice, syrup and tincture (Bolson et al., 2015; Ribeiro et al., 2017).

In Brazil, *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos, known as golden trumpet, is a native tree of Bignoniaceae family and occurs in Northeast, Southeast and South of country (Jardim Botânico do Rio de Janeiro, 2018). Its flowers, stem and bark are used in popular medicine to treat cardiovascular and immunological system diseases, allergic process and poisoning by insect bite and snakebite (Bolson et al., 2015; Ribeiro et al., 2017). *H.*

chrysotrichus was previously identified as *Tabebuia chrysotricha*. However, according to “The Plant List” (www.theplantlist.org), currently both names are synonymous.

In this way, the scientific literature reports many therapeutics properties for *Handroanthus*. For example, species *H. impetiginosus* showed analgesic, anti-inflammatory and antiulcerogenic effects in animal models (Lee et al., 2012; Twardowschy et al., 2008). *H. impetiginosus* has presented antibacterial and antioxidant activity (Park et al., 2003; Park et al., 2006). *H. chrysanthus* demonstrated immunostimulant activity (Perez et al., 2004) and *T. aurea* decreases inflammatory, myotoxic and hemorrhagic activities induced by snake venom (Reis et al., 2014). These studies point confirmations that family Bignoniaceae is a promising group with pharmacological activities.

Thus, considering the *H. chrysotrichus* empirical use, this study aimed: a) to quantify the total polyphenols and flavonoids contents; b) to identify the phytochemical major compounds in bark extract; c) to evaluate the biological activity spectrum of identified major compounds; d) to determine the antioxidant capacity of extract; d) to verify the median lethal dose, cytotoxicity and genotoxicity for crude extract and major compounds.

2. Materials and Methods

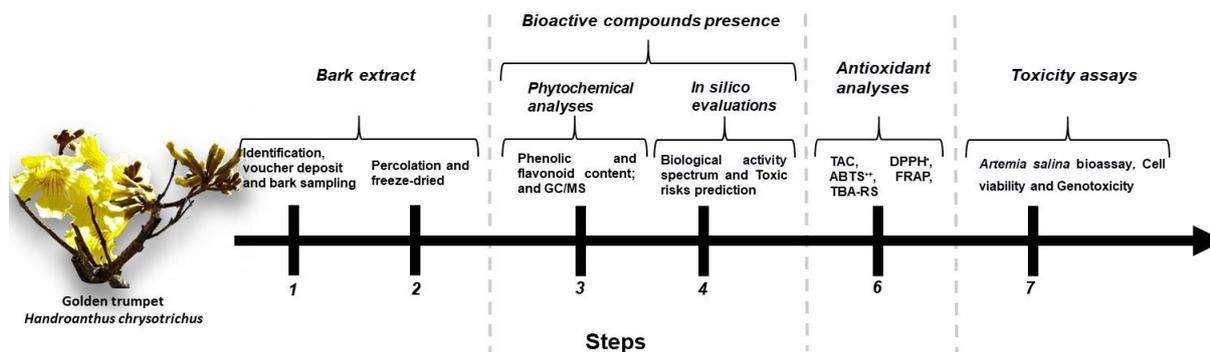
Chemicals

Ethanol, methanol, acetic acid, ascorbic acid, gallic acid, iron (III) chloride anhydrous and ferrous sulfate were purchased from Merck (Darmstadt, Germany). Quercetin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 2-deoxy-D-ribose, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis (3-ethylbenzothiazoline-6- sulfonic acid) diammonium salt (ABTS), and histopaque® were acquired from Sigma Chemical Co. (St. Louis, MO, USA).

Plant Material and Extract preparation

H. chrysotrichus bark was collected at 29°49'49.2"S 57°06'07.1"W geographical coordinates in summer 2017. After botanical identification, a voucher specimen was deposited at institutional herbarium (protocol number 142/2017). The plant material was subjected to drying at 40°C for five days and reduced to powder. Subsequently, samples of the powdered material were used in the percolation technique. Percolation was carried out by two hours in a glass column using hydroethanolic solution (70%) in a ratio of 1:10 (w/v). After that, the *H. chrysotrichus* bark extract (HCBE) was freeze-dried for later use. The experimental design is shown in Figure 1.

Figure 1. Experimental design of this study.



Phytochemical Evaluation

Total phenolic and flavonoid content of HCBE was measured according Nurmi et al. (1996) and Choi et al. (2002), respectively. A standard curve of gallic acid and quercetin was used to determine the polyphenols and flavonoids equivalents content. GC-MS analyses were performed according Soares et al. (2017), with some modifications. Briefly, the oven temperature program was as follows: initial oven temperature was held at 50°C for 5 min, and then increased to 150°C at a rate of 10°C. min⁻¹ for 10 min, after was increased to 200°C at a rate of 10°C. min⁻¹ and detained for 01 min and finally increased to 280°C at a rate of 10°C. min⁻¹ and held for 10 min. Ion source and transfer line temperatures were 280°C. Compounds were identified by comparing mass spectra with data from the NIST library that are available in the instrument.

In silico Evaluations of GC-MS Identified Compounds

Biological activity spectrum

The computational Pass (Prediction of Activity Spectra for Substances, available in <http://www.pharmaexpert.ru/PASSonline/predict.php>) was applied on five major compounds identified in HCBE (Drwal & Griffith, 2013) to search potential pharmacological actions. Results were expressed in percentage of probable activity (Pa) and probable inactivity (Pi). Pa and Pi values vary from 0.000 to 1.000 and it was considered significant activity with Pa > Pi and Pa > 0.700.

Toxic risks prediction

A computational simulation experiment was performed to estimate possible toxicity risks of five major compounds from HCBE. For this five online computer program were

employed: ACD/Labs (Toronto, Canada), admetSAR server (Cheng et al., 2012), pkCSM platform (Pires et al., 2015), PreADMET web-based (<https://preadmet.bmdrc.kr/>) and OSIRIS Properties Explorer (<http://www.organic-chemistry.org/prog/peo/>). The toxic risks assessed were expressed in a flexible manner: (+) low potential, (++) medium risk, (+++) high risk and non-detected risk (ND).

HCBE Antioxidant Analyses

Total antioxidant capacity (TAC), DPPH[•] and ABTS^{•+} scavenger assay

TAC of HCBE was measured through spectrophotometric method proposed by Prieto et al. (1999). HCBE activity against DPPH[•] and ABTS^{•+} radicals were performed in accordance with Choi et al. (2002) and Re et al. (1999), respectively. Results were expressed as inhibitory concentration of 50% (IC₅₀), based on percentage of radical inhibition in relation to the control without extract.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was measured for HCBE by spectrophotometric method (Benzie & Strain, 1996) and determined by plotting in a standard curve produced by the addition of ferrous sulfate to the FRAP reagent. Results were expressed as IC₅₀, based on the extract ability to reduce Fe³⁺ to Fe²⁺.

Thiobarbituric acid reactive substances (TBA-RS) assay

TBA-RS was measured according Ohkawa et al. (1979) and used as an index of 2-deoxy-D-ribose degradation, and lipid peroxidation marker in brain and liver of Swiss albino mice, as described below.

a) Deoxyribose degradation assay

The deoxyribose degradation assay was performed according Puntel et al. (2005) with modifications. Briefly, the reaction medium was prepared containing: HCBE (concentrations 0 – 1000 µg. mL⁻¹), 2mM deoxyribose, 0.05mM and FeSO₄. After incubation at 37°C for 60 min, the reaction was stopped with 2% of trichloroacetic acid (TCA). Color reaction started with addition of 0.4mL of TBA and allowed to incubate for 30 min at 100°C. Standard curves of malondialdehyde (MDA) were performed to determine the MDA generated by the deoxyribose degradation, and the values were expressed as percentage of control (blank).

b) Analyses with brain and liver of Swiss albino mice

These biological tissues were donated from another projects where animals were maintained and used in accordance with guidelines of the Committee on Care and Use

of Experimental Animal Resources (009/2016). Mouse of controls groups (3 months, 30-35g) were euthanized by decapitation and the brain and liver were quickly removed, homogenized in NaCl (150 mM) and kept in ice. After homogenization, samples were centrifuged at 2000g at 4°C for 10 min to yield a low speed supernatant fraction (S1). The obtained S1 was mixed with ferrous sulfate (FeSO₄) in concentrations of 0.01mM, with or without HCBE (concentrations 0 – 1000 µg. mL⁻¹). A standard curve of MDA was constructed to determine TBA-RS content. Results are expressed as IC₅₀, based on the extract ability to inhibit lipid peroxidation of tissue, corrected by mg of tissue.

Median Lethal Dose Determination (LD50) and Toxicity assays

HCBE LD₅₀ was evaluated according Meyer et al. (1982), with some modifications. Briefly, *A. salina* cysts were induced to hatch in aerating solution (saline water 3%) for 24 h. The nauplii were collected and transferred individually to a 96-well plate containing different HCBE concentrations (50, 100, 300, 600 and 900 µg. mL⁻¹) or control saline solution. The mortality was analyzed after 24 h. Assays were performed thrice in triplicate, with n=180 nauplii in each assay. The median lethal dose (LD₅₀) was the required concentration to kill 50% of nauplii. It was considered LD₅₀ < 1000 µg. mL⁻¹ as toxic and a possible presence of bioactive compounds.

For toxicity evaluation, bioassays in peripheral blood mononuclear cells (PBMC) were performed. Briefly, samples of human blood (10mL) were collected from healthy adult volunteers by venous puncture in heparinized tubes and incubated for four hours with HCBE (10 – 500 µg. mL⁻¹ concentrations) or hydrogen peroxide (H₂O₂ 5 mM, positive control). After incubation time, PBMC were separated with histopaque® (1:1) and submitted the following analyzes:

a) Cell viability

Cell viability was tested with trypan blue (0.2%) dye exclusion test. Peripheral blood mononuclear cells (PBMC) were counted in a Neubauer chamber. Stained cells and cells that have undergone the balloon effect were considered dead.

b) Genotoxicity

Genotoxicity was evaluated in PBMC through comet assay according to Singh et al. (1988). After mounting slides and performing electrophoresis, the lengths of tails were measured under fluorescence microscopy after adding ethidium bromide on the slides. One hundred cells from each of the three replicate slides were analyzed. Cells were visually scored according to tail length and receive scores from 0 (no migration) to 4

(maximal migration). Analyses was based on the reading damage index, which represented the sum of cells identified in each class multiplied by the class.

Statistical Analyses

Data were analyzed by two-way ANOVA followed by Tukey's multiple comparisons Test. Cell viability assays were interpreted by one-way ANOVA followed by Bonferroni's *post hoc* Test. Genotoxicity were analyzed by nonparametric Kruskal-Wallis with Dunn's Test. Values of $p < 0.05$ were considered significant. Data are presented as mean and standard deviation (SD). IC_{50} and LD_{50} determinations were performed using a logarithmic regression curve.

3. Results

Phytochemical Evaluation

Data showed that HCBE presents high levels of polyphenols (154.3 mg. g^{-1} gallic acid equivalents), and flavonoids (0.5 mg. g^{-1} of quercetin equivalents). Furthermore, GC/MS analyses identified the five most representative volatile compounds: α -curcumene, β -bisabolene, 4-(4-Methylphenyl) pentanal, pentanoic acid and isoamyl acetate respectively (Figure 2 and Table 1).

Figure 2. GC/MS analyses from *Handroanthus chrysotrichus* bark extract. Five identified volatile compounds are (1) pentanoic acid, (2) isoamyl acetate, (3) 4-(4-methylphenyl) pentanal, (4) α -curcumene, and (5) β -bisabolene.

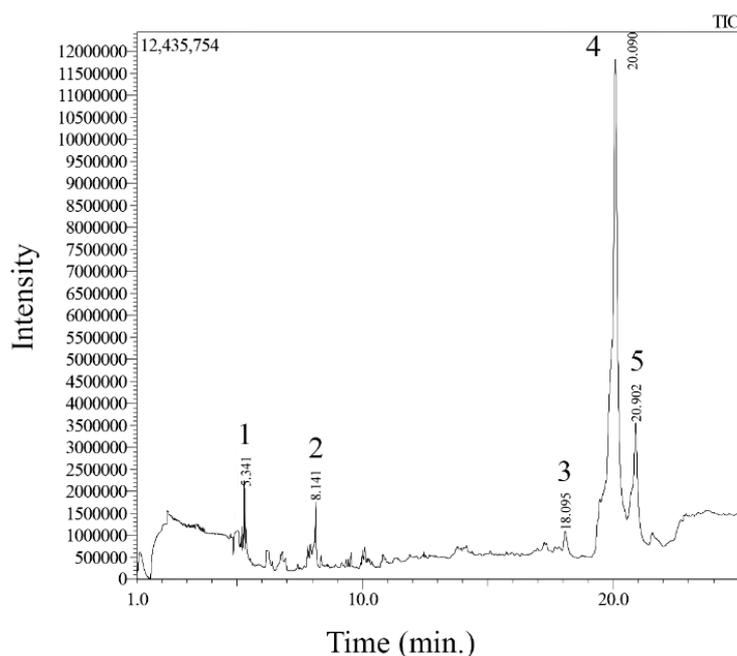


Table 1. Volatile compounds identified from *Handroanthus chrysotrichus* hydroethanolic extract.

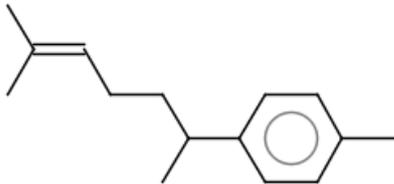
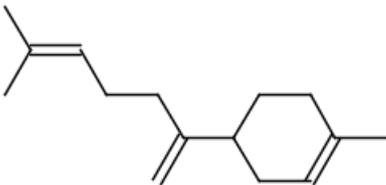
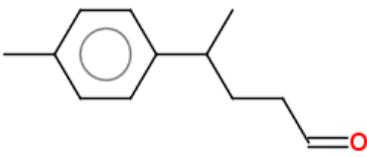
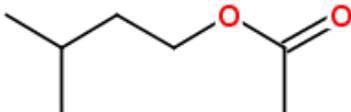
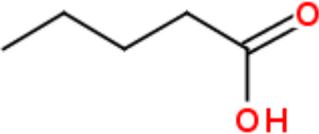
Volatile compounds	RT*	RI**	Area	
			(.10 ⁵)	%
Pentanoic acid	5.341	875	72	2.67
Isoamyl acetate	8.141	820	58	2.14
4-(4-methylphenyl) pentanal	18.095	1429	75	2.79
α -curcumene	20.090	1524	2007	73.74
β -bisabolene	20.902	1500	508	18.66

* Retention Time; ** Retention Index

In silico Evaluations HCBE Major Compounds

The possible biological activity spectrum related to the five HCBE major compounds was evaluated by online platform PASS (Table 2). We selected the predicted properties with values of Pa > 0.7. Our data indicate that α -curcumene, β -bisabolene, 4-(4-methylphenyl) pentanal, pentanoic acid and isoamyl acetate, respectively, presented properties in common as mucobembranous protector and antieczematic. Others frequently properties in compounds are its carminative and fibrinolytic potential.

Table 2. Pharmacological activities predicted for *Handroanthus chrisotrichus* compounds.

<i>Phytoconstituents</i>	<i>Main predicted properties by PASS online</i>	<i>Pa[#]</i>	<i>Pi[#]</i>
<p><i>alpha-Curcumene</i></p> 	Mucomembranous protector	0.942	0.004
	Antieczematic	0.872	0.007
	Carminative	0.783	0.004
	Fibrinolytic	0.728	0.014
	Gastrin inhibitor	0.715	0.004
	Cholesterol antagonist	0.717	0.007
<p><i>beta-Bisabolene</i></p> 	Carminative	0.895	0.002
	Antieczematic	0.868	0.008
	Antineoplastic	0.856	0.006
	Mucomembranous protector	0.787	0.022
	Antiinflammatory	0.726	0.013
<p><i>4-(4-Methylphenyl) pentanal</i></p> 	Immunosuppressant	0.722	0.014
	Antieczematic	0.770	0.025
	Adenomatous polyposis treatment	0.718	0.006
	Carminative	0.711	0.006
	Fibrinolytic	0.708	0.02
<p><i>Isoamyl Acetate</i></p> 	Mucomembranous protector	0.710	0.052
	Phobic disorders treatment	0.947	0.003
	Anesthetic general	0.876	0.004
	Antiseborrheic	0.858	0.009
	Mucomembranous protector	0.837	0.011
	Fibrinolytic	0.733	0.013
	Antieczematic	0.709	0.043
<p><i>Pentanoic Acid</i></p> 	Mucomembranous protector	0.933	0.004
	Antieczematic	0.920	0.004
	Mucositis treatment	0.874	0.008
	Antiseborrheic	0.866	0.008
	Preneoplastic conditions	0.821	0.003
	Adenomatous polyposis treatment	0.819	0.002
	Antimutagenic	0.783	0.004
	Fibrinolytic	0.780	0.005
	Antiinflammatory intestinal	0.727	0.002
Gastrin inhibitor	0.720	0.004	
Anesthetic general	0.706	0.006	

Probable activity; Pi = Probable inactivity. Pa > 0.700 = probable activity greater than 70%. The PASS prediction results were interpreted and used as follows: (i) only activities with Pa > Pi are considered as possible for a particular compound; (ii) if Pa > 0.7, the chance to find the activity experimentally is high.

The toxic risk predictions performed (mutagenic, carcinogenic, cardiotoxic, hepatotoxic, skin sensitization and reproductive toxicity) for α -curcumene, β -bisabolene, 4-(4-methylphenyl) pentanal, pentanoic acid and isoamyl acetate were assessed by five online platforms and the results are present in Table 3. In general, the compounds displayed low toxicity probability. However, β -bisabolene and α -curcumene showed medium and high carcinogenic potential. For the α -curcumene, 4-(4-methylphenyl) pentanal and isoamyl acetate, the results showed a skin sensitization probability on two of the platforms employed. In the other evaluated parameters, isoamyl acetate and pentanoic acid demonstrated high risks to reproductive system. Moreover, theoretical toxicity of compounds in the others tools employed suggesting a low toxicity risk of the compounds.

Table 3. Toxicity prediction for the five major *Handroanthus chrysotrichus* compounds obtained via computer simulation.

Toxic risk by ACD/Labs ¹ ; admetSAR ² ; pkCSM ³ ; PreADMET ⁴ ; OSIRIS Property Explorer ^{5a}						
Phytoconstituents ¹	Mutagenic	Carcinogenic ²	Cardiotoxic ³	Hepatotoxicity ⁴	Skin Sensitization ⁵	Reproductive system toxicity
<i>Alpha-Curcumene</i>	ND ¹		(+) ¹			
	ND ²	(++) ²	(+) ²	ND ²	(+) ³	
	ND ³	(+++) ⁴	ND ³	ND ³	(+++) ⁵	ND ⁵
	(+) ⁴		(++) ⁴			
	ND ⁵					
<i>Beta-Bisabolene</i>	ND ¹		(+) ¹			
	ND ²	(++) ²	ND ²	ND ²	(+) ³	
	ND ³	(++) ⁴	ND ³	ND ³	ND ⁵	ND ⁵
	(+) ⁴		(++) ⁴			
	ND ⁵					
<i>4-(4-Methylphenyl)-pentanal</i>	ND ¹		ND ¹			
	ND ²	ND ²	ND ²	ND ²	(+) ³	
	ND ³	ND ⁴	ND ³	ND ³	(+++) ⁵	ND ⁵
	(+) ⁴		(++) ⁴			
	(+++) ⁵					
<i>Isoamyl Acetate</i>	ND ¹		ND ¹			
	ND ²	ND ³	ND ²	ND ³	(+) ³	
	ND ³	(++) ⁴	ND ³	ND ³	(++) ⁵	(+++) ⁵
	(+) ⁴		(+) ⁴			
	ND ⁵					
<i>Pentanoic Acid</i>	ND ¹		ND ¹			
	ND ²	ND ²	ND ²	ND ²	ND ⁴	
	ND ³	(++) ⁴	ND ³	ND ³	ND ⁵	(+++) ⁵
	(+) ⁴		(+) ⁴			
	ND ⁵					

The scale of toxicity risk ranges from low (+), medium (++) and high (+++) and non-detected (ND).

HCBE antioxidant analyses

HCBE presented antioxidant potential in all performed tests (Table 4). The IC₅₀ in TAC assay was 45.1 ± 1.55 µg. mL⁻¹. In DPPH[•] and ABTS^{•+} scavengers assay the IC₅₀ was 543.15 ± 6.29 and 50.6 ± 3 µg. mL⁻¹, respectively. FRAP presented IC₅₀ of 20.05 ± 0.2 µg. mL⁻¹. Finally, extract decreased the deoxyribose degradation induced by Fenton reaction at IC₅₀ of 91.9 ± 5.56 µg. mL⁻¹.

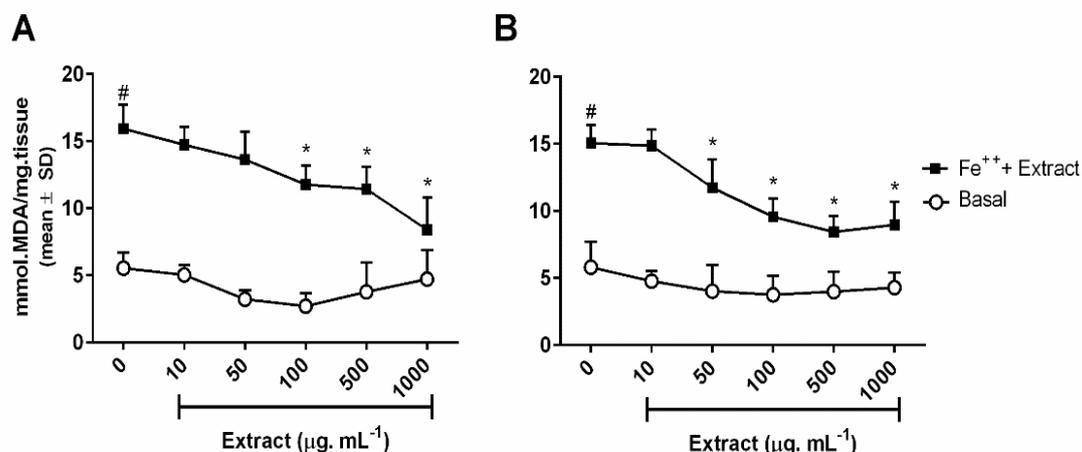
Table 4. IC₅₀ values to different antioxidant assays of *Handroanthus chrysotrichus*.

Test	<i>H. chrysotrichus</i> IC ₅₀ (µg. mL ⁻¹)
TAC	45.1 ± 1.55
DPPH [•] scavenger	543.15 ± 6.29
ABTS ^{•+} scavenger	50.6 ± 3
FRAP	20.05 ± 0.2
Deoxyribose assay	91.9 ± 6.56

Values are expressed as mean ± SD (n=3).

Analyses with tissues of Swiss albino mice exposed to Fe⁺⁺ showed the protective capacity of HCBE from 100 and 50 µg. mL⁻¹ for brain and liver, respectively (Figure 3). In this context, the extract decreased TBA-RS levels for tissue reported.

Figure 3. Effects of *Handroanthus chrysotrichus* bark extract on TBA-RS levels from brain (A) and liver (B) tissues Fe⁺-induced (#p ≤ 0.05 compared to baseline; * p ≤ 0.05 compared to concentration 0 of the same group).



HCBE LD50 and Toxicity assays

HCBE presents a LD₅₀ of 276 µg. mL⁻¹ (R² = 0.9912) to brine shrimp in *A. salina* bioassay. No toxicity of the HCBE was observed for the cell viability and comet assay (Table 5). Both results showed that HCBE did not present toxicity in PBMC in all tested concentrations compared to controls, including concentrations approaching double the LD₅₀ found to *A. salina* (500 µg. mL⁻¹). Thus, there were not decrease in viable cells and significant increase in cells damage index (p < 0.05).

Table 5. Number of cells with comet, distribution of damage classes and damage index.

	CA	Viability (%)	Comet class (mean ± SD)					DI
			0	1	2	3	4	
Saline	100	93.7 ± 1.8	67 ± 4.2	30 ± 2.8	2.5 ± 2	0.5 ± 0.7	0	36.5 ± 4.9
H ₂ O ₂ 5 mM	100	84.9 ± 4.4*	32 ± 5.6	17.5 ± 3.5	18 ± 7	21 ± 8.4	11.5 ± 3	162.5 ± 6*
10 µg. mL ⁻¹	100	94.3 ± 1.3	65.5 ± 19	26.5 ± 14.8	7.5 ± 4.9	0.5 ± 0.7	0	43 ± 22.5
50 µg. mL ⁻¹	100	95.8 ± 0.3	66 ± 4.2	24 ± 8.4	5.5 ± 2.1	4 ± 1.4	0.5 ± 0.7	49 ± 2.8
100 µg. mL ⁻¹	100	92 ± 1.9	62.5 ± 12	22 ± 5.6	9.5 ± 3.5	3 ± 1.4	3 ± 1	62 ± 22
500 µg. mL ⁻¹	100	90.4 ± 0.1	52.5 ± 3.5	36	10 ± 1.4	1.5 ± 2.1	0	60.5 ± 9

CA: Total cells analyzed. DI: Damage index. * indicates significant difference compared to the negative control.

4. Discussion

This study aimed to evaluate the toxicological and antioxidant potential of HCBE *in silico* and *in vitro*. The extract showed five major compounds identified with pharmacological potential and low toxicity risks predictions *in silico*. In addition, HCBE presented antioxidant activity, protected brain and liver of mice against oxidative damage and no present cell toxicity *in vitro*. These data may justify its use in folk medicine, and it proves to be a promising extract for *in vivo* testing.

Initially, *A. salina* assays resulted medium toxicity. It indicate bioactive compounds presence, besides a positive correlation with cytotoxicity in cancer cell lines (McLaughlin, 1991). In agreement GC/MS analyses suggested the presence of substances as β -bisabolene which it was reported tumor-specific pro-apoptotic properties (Yeo et al., 2016). Although tests of α -curcumene in human colon cancer cell line did not inhibit cell proliferation (Tyagi et al., 2015).

Major compounds found α -curcumene and β -bisabolene are terpenes also presents in bark of *H. heptaphyllus* (Garcez et al., 2007) and act among other things against insects protecting the plant (Júnior, 2003). These insecticidal functions promoted the mortality of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* larvae (AlShebly et al., 2017). *H. impetiginosus* bark also presented larvicidal activity in three mosquitos species and *H. serratifolius* steam had antileishmanial activity (Costa et al., 2017; Kim et al., 2013).

Other compounds are ester isoamyl acetate, well known in the food industry (Torres et al., 2010); and the short-chain fatty acid (SCFA) pentanoic acid, a superior class of cellulosic biofuels. This SCFA may be produced by intestinal bacteria (Topping, 1996) and induces thymic stromal lymphopoietin production (Mizuno et al., 2017). Thymic stromal lymphopoietin is an IL-7-like cytokine mainly derived from fibroblasts and epithelial cells and is related to allergic inflammation (Liu, 2006; Sims et al., 2000). Nevertheless, 4-(4-Methylphenyl) pentanal is not target of many studies.

Throughout chromatography analyzes β -lapachone and lapachol was expected in extract studied, since they are cited in literature as major compound in *H. chrysotricus* (Grazziotin et al., 1992), *H. impetiginosus* (Castellanos et al., 2009; Ferreira et al., 1989) and others Bignoniaceae plants. In addition, lapachol is soluble in ethanol (Kiage-mokua et al., 2012), being consistent with method here applied, but this compound was not detected by High Performance Liquid Chromatography (Supplementary material). Thus, it seems to be absent in the *H. chrysotricus* bark analyzed. These substantial differences between specimen may be due

to environmental conditions, because lapachol presence may vary in the same specie according rainfall, temperature and the soil content (Wright & Setzer, 2013).

The biological activity spectrum *in silico* of isolated compounds (Table 2) showed compatible action with popular practice. In this line, Salgueiro et al. (2018) suggest that *in silico* investigations can be used as a complementary route after the ethnopharmacological research. Indeed, *in silico* studies may be useful to point out several possibilities for conducting *in vitro* and *in vivo* studies (Salgueiro et al., 2018). Brazilian ethnopharmacological investigations indicated therapeutic use of genus *Handroanthus* as anti-inflammatory and for cancer treatment, back pain, stomachache, gastritis, ulcer and allergy (Bieski et al., 2015; Ribeiro et al., 2017). According to Bolson et al. (2015) *H. chrysotrichus* also may be used for cardiovascular system diseases, immunological and poisoning. Moreover, other *in silico* analyses were made, and the results showed low toxicity probability in most of the analyzed criteria (Table 3). Nevertheless, care should be taken with carcinogenic potential and topical use of the extract, due skin sensitization of some compounds.

The biological activity spectrum *in silico* no showed, but the HCBE had significant antioxidant activity (Table 4). These important effect may reduce oxidative damage induced by several diseases (Andréia Caroline Fernandes Salgueiro et al., 2016). The absence of antioxidant activities in *in silico* investigations can be explained by synergism among different molecules in a crude plant extract (Salgueiro et al., 2018). In the context of computational evaluations, the interaction between compounds cannot be evaluated (Salgueiro et al., 2018). The extract exhibited better activities in FRAP, TAC and ABTS^{•+} scavenger. Antioxidant analyses as ferric reducing antioxidant power assay, FRAP, propose the affinity of HCBE with Fe ions. We know that iron is an essential element for the body and may induce oxidative stress via Fenton reaction (Benzie & Strain, 1996; Halliwell & Gutteridge, 2007). In addition, HCBE decreased Fe⁺-induced damage to brain and liver tissues of mice (Figure 3). TAC and ABTS^{•+} are based on the reduction of molybdenum and ABTS radicals, respectively. ABTS^{•+} assay is a simple method applicable to the extracts antioxidant study, but this radical is not found in biologicals systems (Magalhães et al., 2008; Prieto et al., 1999; Re et al., 1999). Therefore, these antioxidants properties may be linked to polyphenols found in the colorimetric phytochemical analyses. In fact, polyphenols have a high reactivity and ability to stabilize unpaired electron, including to chelate transition metal ions (Vertuani et al., 2004). On the other hand, antioxidant activity against DPPH[•] showed high IC₅₀, compatible data with bark extract of *T. rosea* (Jimenez-Gonzalez et al., 2018), suggesting that bark extracts these trees have low efficiency in this test. The extract here analyzed not indicated reaction with nitric oxide (NO[•])

(data not show), a pro-inflammatory mediator that plays an important role in the regulation of immune functions, neurotransmission, and vasodilation (Sharma, Al-Omran, & Parvathy, 2007).

Despite knowledge about vegetal extracts are necessary, because shows potential actions and may value the plant, we considered important to perform *in vitro* assays evaluating the cell responses (Fraga et al., 2020; Lima et al., 2020). There was not changes of viability and genotoxicity in cells exposed to HCBE (Table 5). These data are according to Boriollo et al. (2017), which suggested absence of genotoxicity of the *H. impetiginosa* bark. In addition, confirm the low toxicity found in *in silico* analyses.

Therefore, the species studied showed low toxic potential and exhibited a biological activity spectrum, including the antioxidant capacity and the protective action in liver and brain of mice. Literature suggested that its major compounds presents biological act. These data support point the HCBE as a promising extract for *in vivo* evaluations.

5. Conclusion

The present study observes different parameters of golden trumpet *Handroanthus chrysotrichus*. We identify five major volatile compounds from hydroalcoholic stem bark extract: α -curcumene, β -bisabolene, 4-(4-Methylphenyl) pentanal, pentanoic acid and isoamyl acetate. These compounds present pharmacological activities predicted which are compatible with popular practice. On other hand, β -bisabolene and α -curcumene show carcinogenic potential toxic risk predictions.

The results also verify high levels of polyphenols and show that *H. chrysotrichus* bark has antioxidant effect in different systems. Despite the extract had presented toxicity in *Artemia saturoioides* bioassay, there was not cell toxicity. Finally, our tests indicate *H. chrysotrichus* as a potential agent for *in vivo* testing, especially for the treatment of diseases in which the bark is used and to ensure safety the medicinal use of this plant.

Referências

AlShebly, M. M., AlQahtani, F. S., Govindarajan, M., Gopinath, K., Vijayan, P., & Benelli, G. (2017). Toxicity of α -curcumene and β -bisabolol from *Hedychium larsenii* (Zingiberaceae) essential oil on malaria, chikungunya and Japanese encephalitis mosquito vectors. *Ecotoxicology and Environmental Safety*, 137(2017), 149–157. <https://doi.org/10.1016/j.ecoenv.2016.11.028>

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>

Bieski, I. G. C., Leonti, M., Arnason, J. T., Ferrier, J., Rapinski, M., Violante, I. M. P., Balogun, S. O., Pereira, J. F. C. A., Figueiredo, R. D. C. F., Lopes, C. R. A. S., Silva, D. R. da, Pacini, A., Albuquerque, U. P., & Oliveira, D. T. (2015). Ethnobotanical study of medicinal plants by population of Valley of Juruena Region , Legal Amazon , Mato Grosso , Brazil. *Journal of Ethnopharmacology*, 173, 383–423. <https://doi.org/10.1016/j.jep.2015.07.025>

Bolson, M., Hefler, S. R., Dall’Oglio Chaves, E. I., Gasparotto Junior, A., & Cardozo Junior, E. L. (2015). Ethno-medicinal study of plants used for treatment of human ailments, with residents of the surrounding region of forest fragments of Paraná, Brazil. *Journal of Ethnopharmacology*, 161, 1–10. <https://doi.org/10.1016/j.jep.2014.11.045>

Boriollo, M. F. G., Silva, T. A., Rodrigues-Netto, M. F., Silva, J. J., Marques, M. B., Dias, C. T. S., Höfling, J. F., Resck, M. C. C., Oliveira, N. M. S., Boriollo, M. F. G., Silva, T. A., Rodrigues-Netto, M. F., Silva, J. J., Marques, M. B., Dias, C. T. S., Höfling, J. F., Resck, M. C. C., & Oliveira, N. M. S. (2017). Reduction of doxorubicin-induced genotoxicity by *Handroanthus impetiginosus* in mouse bone marrow revealed by micronucleus assay. *Brazilian Journal of Biology*, 78(1), 1–12. <https://doi.org/10.1590/1519-6984.18515>

Castellanos, J. R. G., Prieto, J. M., & Heinrich, M. (2009). Red Lapacho (*Tabebuia impetiginosa*) — A global ethnopharmacological commodity? *Journal of Ethnopharmacology*, 121, 1–13. <https://doi.org/10.1016/j.jep.2008.10.004>

Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., Lee, P. W., & Tang, Y. (2012). admetSAR: A Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties. *Journal of Chemical Information and Modeling*, 52(11), 3099–3105. <https://doi.org/10.1021/ci300367a>

Choi, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ahn, H. J., Lee, M. Y., Park, S. H., & Kim, S. K. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science*, 163(6), 1161–1168. [https://doi.org/10.1016/S0168-9452\(02\)00332-1](https://doi.org/10.1016/S0168-9452(02)00332-1)

Costa, E. V. S., Brígido, H. P. C., Silva, J. V. da S. e, Coelho-Ferreira, M. R., Brandão, G. C., & Dolabela, M. F. (2017). Antileishmanial Activity of *Handroanthus serratifolius* (Vahl) S. Grose (Bignoniaceae). *Evidence-Based Complementary and Alternative Medicine*, 2017, 1–6. <https://doi.org/10.1155/2017/8074275>

Drwal, M. N., & Griffith, R. (2013). Combination of ligand- and structure-based methods in virtual screening. *Drug Discovery Today. Technologies*, 10(3), e395-401. <https://doi.org/10.1016/j.ddtec.2013.02.002>

Ferreira, D. T., Oliveira, A. B. de, & Castro, C. R. de. (1989). Constituintes químicos da *Tabebuia impetiginosa* (MART) Standl. Bignoniaceae. *Semina: Exact and Technological Sciences*, 10(4), 272–273.

Fraga, L. N., Oliveira, A. K. de S., Aragão, B. P., Silva, A. M. de O. e, Wartha, E. R. S. de A., Bacci, L., Lobato, L. P., & Carvalho, I. M. M. de. (2020). Physico-chemical characterization of the pulp and peel of Brazilian Pitomba (*Talisia esculenta* (A. St.-Hill.) Radlk). *Research, Society and Development*, 9(1), 1–14.

Garcez, F. R., Garcez, W. S., Mahmoud, T. S., Figueiredo, P. de O., & Resende, U. M. (2007). Novos constituintes químicos das cascas do caule de *Tabebuia heptaphylla*. *Quimica Nova*, 30(8), 1887–1891. <https://doi.org/10.1590/S0100-40422007000800017>

Grazziotin, J. D., Schapoval, E. E. S., Chaves, C. G., Gleye, J., & Henriques, A. T. (1992). Phytochemical and analgesic investigation of *Tabebuia chrysotricha*. *Journal of Ethnopharmacology*, 36(3), 249–251. [https://doi.org/10.1016/0378-8741\(92\)90051-R](https://doi.org/10.1016/0378-8741(92)90051-R)

Halliwell, B., & Gutteridge, J. M. C. (2007). *Free radicals in biology and medicine* (4^a). Oxford University Press Inc.

Jardim Botânico do Rio de Janeiro. (2018). *Flora do Brasil 2020 em construção. Handroanthus Chrysotrichus*. <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB114078>

Jimenez-Gonzalez, F. J., Vélez-Gómez, J. M., Melchor-Moncada, J. J., Veloza, L. A., & Sepúlveda-Arias, J. C. (2018). Antioxidant, anti-inflammatory, and antiproliferative activity of extracts obtained from *Tabebuia Rosea* (Bertol.) DC. *Pharmacognosy Magazine*, 14(55), 25–31.

Júnior, C. V. (2003). Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. *Quimica Nova*, 26(3), 390–400.

Kiage-mokua, B. N., Roos, N., & Schrezenmeir, J. (2012). Lapacho Tea (*Tabebuia impetiginosa*) Extract Inhibits Pancreatic Lipase and Delays Postprandial Triglyceride Increase in Rats †. *Phytotherapy Research*, 26, 1878–1883.

Kim, M.-G., Jeon, J. H., & Lee, H.-S. (2013). Larvicidal activity of the active constituent isolated from *Tabebuia avellanadae* bark and structurally related derivatives against three mosquito species. *Journal of Agricultural and Food Chemistry*, 61(45), 10741–10745. <https://doi.org/10.1021/jf403679h>

Lee, M. H., Choi, H. M., Hahm, D. H., Her, E., Yang, H. I., Yoo, M. C., & Kim, K. S. (2012). Analgesic and anti-inflammatory effects in animal models of an ethanolic extract of Taheebo, the inner bark of *Tabebuia avellanadae*. *Molecular Medicine Reports*, 6(4), 791–796. <https://doi.org/10.3892/mmr.2012.989>

Lima, A. R. N., Macedo, R. G., Batista, G. G., Câmara, G. B., Lima, R. de F., & Oliveira, T. K. B. de. (2020). Antimicrobial and anti-inflammatory activity of *Anadenanthera colubrina* (Vell.) Brenan. *Research, Society and Development*, 9(1), 1–12.

Liu, Y.-J. (2006). Thymic stromal lymphopoietin: master switch for allergic inflammation. *The Journal of Experimental Medicine*, 203(2), 269–273. <https://doi.org/10.1084/jem.20051745>

Magalhães, L. M., Segundo, M. A., Reis, S., & Lima, J. L. F. C. (2008). Methodological aspects about in vitro evaluation of antioxidant properties. *Analytica Chimica Acta*, 613(1), 1–19.

<https://doi.org/10.1016/J.ACA.2008.02.047>

McLaughlin, J. L. (1991). Crown gall tumors on potato discs and brine shrimp lethality: two simple bioassays for higher plant screening and fractions. In P.M.DEY & J. B. HARBONE (Eds.), *Methods in Plant Biochemistry* (1st ed., pp. 1–32). Academic Press.

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., & McLaughlin, J. (1982). Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Medica*, 45(05), 31–34. <https://doi.org/10.1055/s-2007-971236>

Mizuno, N., Abe, K., Morishita, Y., Yamashita, S., Segawa, R., Dong, J., Moriya, T., Hiratsuka, M., & Hirasawa, N. (2017). Pentanoic acid induces thymic stromal lymphopoietin production through G q/11 and Rho-associated protein kinase signaling pathway in keratinocytes. *International Immunopharmacology*, 50, 216–223. <https://doi.org/10.1016/j.intimp.2017.06.024>

Nurmi, K., Ossipov, V., Haukioja, E., & Pihlaja, K. (1996). Variation of total phenolic content and individual low-molecular-weight phenolics in foliage of mountain birch trees (*Betula pubescens* ssp.tortuosa). *Journal of Chemical Ecology*, 22(11), 2023–2040. <https://doi.org/10.1007/BF02040093>

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2), 351–358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)

OSIRIS Properties Explorer. (n.d.). Retrieved September 16, 2018, from <https://www.organic-chemistry.org/prog/peo>

Park, B.-S., Lee, K.-G., Shibamoto, T., Lee, S.-E., & Takeoka, G. R. (2003). Antioxidant Activity and Characterization of Volatile Constituents of Taheebo (*Tabebuia impetiginosa* Martius ex DC). *Journal of Agricultural and Food Chemistry*, 51, 295–300.

Park, B., Lee, H., Lee, S., Piao, X., Takeoka, G. R., Wong, R. Y., Ahn, Y., & Kim, J. (2006). Antibacterial activity of *Tabebuia impetiginosa* Martius ex DC (Taheebo) against *Helicobacter pylori*. *Journal of Ethnopharmacology*, 105, 255–262. <https://doi.org/10.1016/j.jep.2005.11.005>

Perez, J. E., Isaza, G., Bueno, J. G., Arango, M. C., Hincapié, B. L., Nieto, A. M., & Londoño, D. P. (2004). Efecto de los extractos de *Phenax rugosus*, *Tabebuia chrysantha*, *Althernantera williamsii* y *Solanum dolichosepalum* sobre el leucograma y la producción de anticuerpos en ratas. *REVISTA MÉDICA DE RISARALDA*, 10(2), 13–21.

Pires, D. E. V, Blundell, T. L., & Ascher, D. B. (2015). pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *Journal of Medicinal Chemistry*, 58(9), 4066–4072. <https://doi.org/10.1021/acs.jmedchem.5b00104>

PreADMET web-based, Online. (n.d.). Retrieved September 16, 2018, from <https://preadmet.bmdrc.kr/>

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant

capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. *Anal Biochem*, 269, 337–341.

Puntel, R. L., Nogueira, C. W., & Rocha, J. B. T. (2005). Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain in vitro. *Neurochemical Research*, 30(2), 225–235. <https://doi.org/10.1007/s11064-004-2445-7>

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant Activity Applying an Improved Abts Radical Cation Decolorization Assay. *Free Radical Biology and Medicine*, 26(9), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)

Reis, F. P., Senna Bonfa, I. M., Cavalcante, R. B., Okoba, D., De Souza Vasconcelos, S. B., Candeloro, L., De Oliveira Filiu, W. F., Duenhas Monreal, A. C., Da Silva, V. J., Santa Rita, P. H., Carollo, C. A., & Toffoli-Kadri, M. C. (2014). *Tabebuia aurea* decreases inflammatory, myotoxic and hemorrhagic activities induced by the venom of *Bothrops neuwiedi*. *Journal of Ethnopharmacology*, 158(PART A), 352–357. <https://doi.org/10.1016/j.jep.2014.10.045>

Ribeiro, R. V., Bieski, I. G. C., Balogun, S. O., & Martins, D. T. de O. (2017). Ethnobotanical study of medicinal plants used by Ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. *Journal of Ethnopharmacology*, 205(April), 69–102. <https://doi.org/10.1016/j.jep.2017.04.023>

Salgueiro, A.C.F., Folmer, V., Bassante, F. E. M., Cardoso, M. H. S., da Rosa, H. S., & Puntel, G. O. (2018). Predictive antidiabetic activities of plants used by persons with Diabetes mellitus. *Complementary Therapies in Medicine*, 41, 1–9. <https://doi.org/10.1016/j.ctim.2018.08.009>

Salgueiro, A. C. F., Folmer, V., da Silva, M. P., Mendez, A. S. L., Zemolin, A. P. P., Posser, T., Franco, J. L., Puntel, R. L., Puntel, G. O., Puntel, R. L., Puntel, G. O. (2016). Effects of *Bauhinia forficata* Tea on Oxidative Stress and Liver Damage in Diabetic Mice. *Oxidative Medicine and Cellular Longevity*, 2016, 1–9. <https://doi.org/10.1155/2016/8902954>

Sharma, J. N., Al-Omran, & Parvathy, S. S. (2007). Review Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*, 15, 252–259. <https://doi.org/10.1007/s10787-007-0013-x>

Sims, J. E., Williams, D. E., Morrissey, P. J., Garka, K., Foxworthe, D., Price, V., Friend, S. L., Farr, A., Bedell, M. A., Jenkins, N. A., Copeland, N. G., Grabstein, K., & Paxton, R. J. (2000). Molecular cloning and biological characterization of a novel murine lymphoid growth factor. *The Journal of Experimental Medicine*, 192(5), 671–680. <http://www.ncbi.nlm.nih.gov/pubmed/10974033>

Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*, 175(1), 184–191. [https://doi.org/10.1016/0014-4827\(88\)90265-0](https://doi.org/10.1016/0014-4827(88)90265-0)

Soares, J. J., Rodrigues, D. T., Gonçalves, M. B., Lemos, M. C., Gallarreta, M. S., Bianchini, M. C., Gayer, M. C., Puntel, R. L., Roehrs, R., & Denardin, E. L. G. (2017). Paraquat exposure-induced Parkinson's disease-like symptoms and oxidative stress in *Drosophila melanogaster*: Neuroprotective effect of *Bougainvillea glabra* Choisy. *Biomedicine and Pharmacotherapy*,

95(July), 245–251. <https://doi.org/10.1016/j.biopha.2017.08.073>

Topping, D. L. (1996). Short-chain fatty acids produced by intestinal bacteria. *Asia Pacific Journal of Clinical Nutrition*, 5, 15–19.

Torres, S., Pandey, A., & Castro, G. R. (2010). Banana flavor: Insights into isoamyl acetate production. *Cell*, 549(1), 776–802.

Twardowschy, A., Freitas, C. S., Baggio, C. H., Mayer, B., dos Santos, A. C., Pizzolatti, M. G., Zacarias, A. A., dos Santos, E. P., Otuki, M. F., & Marques, M. C. A. (2008). Antiulcerogenic activity of bark extract of *Tabebuia avellanedae*, Lorentz ex Griseb. *Journal of Ethnopharmacology*, 118(3), 455–459. <https://doi.org/10.1016/j.jep.2008.05.013>

Tyagi, A. K., Prasad, S., Yuan, W., Li, S., & Aggarwal, B. B. (2015). Identification of a novel compound (β -sesquiphellandrene) from turmeric (*Curcuma longa*) with anticancer potential: Comparison with curcumin. *Investigational New Drugs*, 33(6), 1175–1186. <https://doi.org/10.1007/s10637-015-0296-5>

Vertuani, S., Angusti, A., & Manfredini, S. (2004). The Antioxidants and Pro-Antioxidants Network: An Overview. *Current Pharmaceutical Design*, 10(14), 1677–1694. <https://doi.org/10.2174/1381612043384655>

Wright, C. R., & Setzer, W. N. (2013). Volatile components of organ pipe cactus , *Stenocereus thurberi* Engelm., growing in the Organ Pipe Cactus National Monument and the Arizona-Sonora Desert Museum. *American Journal of Essential Oils and Natural Products*, 1(3), 19–22. <https://doi.org/10.1086/497361>

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