Research, Society and Development, v. 11, n. 6, e5011628542, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i6.28542

Computational study of the main flavonoids from *Chrysobalanus icaco* L. against NADPH-oxidase and *in vitro* Antioxidant Activity

Estudo computacional dos principais flavonoides obtidos de *Chrysobalanus icaco* L. contra NADPH-oxidase e avaliação *in vitro* da atividade antioxidante

Estudio computacional de los principales flavonoides obtenidos de *Chrysobalanus icaco* L. frente a NADPH-oxidasa y evaluación *in vitro* de la actividad antioxidante

Received: 03/27/2022 | Reviewed: 04/07/2022 | Accept: 04/15/2022 | Published: 04/19/2022

Lucas Villar Pedrosa da Silva Pantoja
ORCID: https://orcid.org/0000-0002-2717-5500
Universidade Federal do Pará, Brazil
E-mail: lucas-villar@outlook.com
Surianne Samantha Amorim Trindade
ORCID: https://orcid.org/0000-0002-8400-6711
Universidade Federal do Pará, Brazil
E-mail: suriannetrind@gmail.com
Agnaldo da Silva Carneiro
ORCID: https://orcid.org/0000-0002-6531-5148
Universidade Federal do Pará, Brazil
E-mail: agsicar@yahoo.com.br
João Paulo Bastos Silva
ORCID: https://orcid.org/0000-0003-3859-7474
Universidade Federal do Pará, Brazil
E-mail: joao.bastos@ics.ufpa.br
Thiago Portal da Paixão
ORCID: https://orcid.org/0000-0002-7751-1592
Universidade Federal do Pará, Brazil
E-mail: thiagoportal@ymail.com
Camila Fernanda Rodrigues Romeiro
ORCID: https://orcid.org/0000-0002-0770-9181
Universidade Federal do Pará, Brazil
E-mail: rodriguescamilafarma@gmail.com
Cleiane Santana Pinheiro de Moraes
ORCID: https://orcid.org/0000-0001-5752-7768
Universidade Federal do Pará, Brazil
E-mail: cleianedemoraes@gmail.com
Ana Carla Godinho Pinto
ORCID: https://orcid.org/0000-0001-7498-8865
Universidade Federal do Pará, Brazil
E-mail: anacarla_pinto@hotmail.com
Nádia Rezende Barbosa Raposo
ORCID: https://orcid.org/0000-0001-5271-1048
Universidade Federal de Juiz de Fora, Brazil
E-mail: nadia.barbosa@ufjf.br
Marcieni Ataíde de Andrade
ORCID: https://orcid.org/0000-0001-5875-695X
Universidade Federal do Pará, Brazil
E-mail: marcieni@ufpa.br

Abstract
The generation of free radicals is a physiological event resulting mainly from the cellular respiration process and the overactivation of the NOX leads to an excess production of ROS that is associated with oxidative stress. *Chrysobalanus icaco*, a medicinal plant that belongs to the Chrysobalanaceae family, possesses a high number of polyphenols, including phenolic acids and flavonoids. Due to its phytochemical composition, this study aimed to evaluate the antioxidant potential of the hydroalcoholic extract from the leaves of *Chrysobalanus icaco* (HECi) and the inhibitory potential of its main flavonoids against NOX. The *in silico* predictions of absorption, distribution, metabolism, excretion, and toxicity (ADMET), drug-likeness properties, and molecular docking with the enzyme NOX (PDB code 2CDU) were also performed to support the experimental results. The phytochemical screening of the

HECi showed the presence of phenols and flavonoids. HECi performed an excellent antioxidant activity (IC50 = 8.1 μg/mL), probably due to its rich phenolic (220.11 ± 0.4 mg GAE/g) and flavonoid (110.98 ± 0.37 mg QE/g) constitution. The ADMET prediction indicated that myricetin and quercetin had the best pharmacokinetic parameters. The molecular docking results showed that myricetin and especially quercetin had strong docking score on NOX (ΔG = –8.1 kcal/mol and ΔG = –8.3 kcal/mol, respectively). Frontier Orbital's analyzes (HOMO and LUMO) suggested that quercetin has better antioxidant properties than myricetin. Our results demonstrate for the first time the *in silico* action of quercetin against NOX, as well as reiterate the antioxidant potential of *C. icaco*.
Keywords: *Chrysobalanus icaco*; Flavonoids; Antioxidant activity; I*n silico* study.

Resumo

A geração de radicais livres é um evento fisiológico resultante principalmente do processo de respiração celular e a superativação da NOX leva a um excesso de produção de EROs que está associado ao estresse oxidativo. *Chrysobalanus icaco*, uma planta medicinal pertencente à família Chrysobalanaceae, possui uma grande quantidade de polifenóis, incluindo ácidos fenólicos e flavonóides. Por isso, este trabalho teve como objetivo avaliar o potencial antioxidante do extrato hidroalcoólico das folhas de *Chrysobalanus icaco* (HECi) e o potencial inibitório de seus principais flavonoides frente à NOX. As previsões *in silico* de absorção, distribuição, metabolismo, excreção e toxicidade (ADMET), *druglikeness* e docagem molecular com a enzima NOX (código PDB 2CDU) também foram realizadas para explicar os resultados experimentais. A triagem fitoquímica do HECi mostrou a presença de fenois e flavonoides. O HECi apresentou excelente atividade antioxidante (IC50 = 8,1 μg/mL), provavelmente devido à sua rica constituição fenólica (220,11 ± 0,4 mg GAE/g) e de flavonoides (110,98 ± 0,37 mg QE/g). A previsão ADMET indicou que a miricetina e a quercetina apresentaram os melhores parâmetros farmacocinéticos. Os resultados de docagem molecular mostraram que a miricetina e especialmente a quercetina ligaram-se fortemente à NOX (ΔG = –8,1 kcal/mol e ΔG = –8,3 kcal/mol, respectivamente). As análises dos orbitais de fronteira (HOMO e LUMO) sugeriram que a quercetina tem melhores propriedades antioxidantes do que a miricetina. Nossos resultados demonstram pela primeira vez a ação *in silico* da quercetina contra a NOX, bem como reiteram o potencial antioxidante de *C. icaco*.
Palavras-chave: *Chrysobalanus icaco*; Flavonoides; Atividade antioxidante; Estudo *in silico*.

Resumen

La abundante constitución fenólica de *Chrysobalanus icaco* despierta interés en su uso como antioxidante, ya que los compuestos fenólicos reducen el daño oxidativo. Este estudio tuvo como objetivo evaluar el potencial antioxidante del extracto hidroalcohólico de las hojas de *Chrysobalanus icaco* (HECi) y el potencial inhibidor de sus principales flavonoides sobre la NADPH-oxidasa (NOX). Así, se realizaron los siguientes métodos: prospección fitoquímica, perfil cromatográfico, cuantificación de fenoles y flavonoides y ensayo de secuestro de DPPH. Los estudios *in silico* realizados para los principales flavonoides presentes en *C. icaco* evaluaron los parámetros ADMET, simulaciones de acoplamiento molecular (código NOX - PDB: 2CDU) y orbitales límite (HOMO y LUMO). El cribado fitoquímico de HECi mostró la presencia de fenoles y flavonoides. HECi mostró una excelente actividad antioxidante (IC50 = 8,1 μg/mL), probablemente debido a su rica constitución fenólica (220,11 ± 0,4 mg GAE/g) y flavonoides (110,98 ± 0,37 mg QE/g). La predicción de ADMET indicó que la miricetina y la quercetina tenían los mejores parámetros farmacocinéticos. Los resultados del acoplamiento molecular mostraron que miricetina y especialmente quercetina se unían fuertemente al NO (ΔG = –8,1 kcal/mol y ΔG = –8,3 kcal/mol, respectivamente). La evaluación de los orbitales límite (HOMO y LUMO) sugirió que la quercetina tiene mejores propiedades antioxidantes que la miricetina. Nuestros resultados demuestran por primera vez la acción *in silico* de la quercetina contra la NOX, así como reiteran el potencial antioxidante de *C. icaco*.
Palabras clave: *Chrysobalanus icaco*; Flavonoides; Actividad antioxidante; Estudio *in silico*.

1. Introduction

The generation of free radicals is a physiological and natural event resulting mainly from the cellular respiration process. NADPH Oxidases (NOX) is a family of electron transporting membrane proteins responsible for the first step in reactive oxygen species (ROS) production, through the addition of an electron to the O_2, which results in the formation of superoxide anion ($O2^{\bullet-}$), in addition of production of other oxygen-derived radicals (Tarafdar and Pula, 2018). Although the human body possesses intrinsic enzymatic mechanisms to neutralize or scavenge the effects of these ROS, they could accumulate and trigger cellular damage. ROS plays pivotal roles in physiological processes at low concentrations, however, the increase in production associated with ineffective mechanisms to scavenging results in oxidative stress, which is correlated as a contributor or an event of aging and several diseases, such as inflammatory diseases, cardiovascular diseases,

neurodegenerative diseases and cancer (Roy et al., 2017; Liguori et al., 2018; Arfin et al., 2021; Izzo et al., 2021).

The overactivation of the NOX leads to an excess production of ROS that is associated with oxidative stress (Chocry and Leloup, 2020). Thus, the inactivation of NOX represents an interesting and promising therapeutic target to avoid or reduce the hazardous effects of oxidative stress in different pathologies (McCann and Roulston, 2013; Joshi and Khan, 2020). In this regard, the intake of antioxidants through diet or supplementation became a valuable tool because many plant-derived natural antioxidants possess, in addition to the antioxidant effect, other biological properties, including anti-inflammatory, anti-cancer, and anti-atherosclerotic (Ganesan & Xu, 2017; Hano & Tungmunnithum, 2020). Particularly, polyphenols are interesting plant-derived secondary metabolites that may modulate NOX (Yousefian et al., 2019).

Chrysobalanus icaco, a medicinal plant that belongs to the Chrysobalanaceae family, possesses a high number of polyphenols, including phenolic acids, flavonoids, and tannins (Silva et al., 2017; Onilude et al., 2020). The biological effects of the species are directly associated with the high content of polyphenol, mainly the flavonoids (Castilho et al., 2005; Chaudhuri et al., 2002; Fernandes et al., 2003). Flavonoids have been commonly identified in polar extracts of *C. icaco*, including quercetin, myricetin, and their derivatives (Barbosa et al., 2006; Silva et al., 2017). Flavonoids are secondary metabolites that act as antioxidants due to their oxidation-reduction properties and metal chelators and free radical scavengers, which can play an important role in free radicals stabilization and reduction of oxidative stress effects (Agati et al., 2020). In biological systems, this activity is also correlated with the inhibition of NOX, xanthine oxidase, and phospholipase enzymes, and stimulation of enzymes with antioxidant activity such as catalase and superoxide dismutase (Wen et al., 2021).

In this sense, current research has drawn attention to the search for new NOX inhibitors. Conventional methods employed to evaluate natural NOX inhibitors demand a lot of time, cost, and a long process while *in silico* methods are alternative and reliable approaches that can be used to evaluate polyphenols before *in vitro* and *in vivo* tests (Susanti, 2019). Thus, this study aimed to evaluate the antioxidant potential of the hydroalcoholic extract from the leaves of *Chrysobalanus icaco* (HECi) and the inhibitory potential of its main flavonoids against NOX. The *in silico* predictions of absorption, distribution, metabolism, excretion, and toxicity (ADMET), druglikeness properties, and molecular docking with the enzyme NOX (PDB code 2CDU) were also performed to support the experimental results.

2. Methodology

This study is an experimental work that, as a complement, also aimed to evaluate the physical-chemical, pharmacokinetic, biological activities and toxicological parameters of the selected flavonoids present in the hydroalcoholic extract of *C. icaco*, following the methodology proposed by Pereira et al., 2018.

2.1 Chemicals

All the chemicals to phytochemical screnning and antioxidant activity were obtained by Sigma-Aldrich (St. Louis, MO, USA). All the solvents were purchased from Merck (Darmstadt, Germany).

2.2 Plant material

Chrysobalanus icaco L. was collected in Belém, Pará State, Brazil, in May 2018. Plant samples were identified by a taxonomist at the Herbarium of the Museu Paraense Emílio Goeldi with voucher specimens' numbers MG236136.

2.3 Preparation of herbal drug

The aerial parts of the plant material were selected, washed in running water. Then, the material was dried at room temperature for 24 hours and in an oven with forced air flow (40ºC) for 48 hours. The dried material was pulverized in a knife mill, weighed and stored properly.

2.4 Preparation of plant extract

The hydroalcoholic extract of *Chrysobalanus icaco* (HECi) was obtained by maceration. Briefly, the herbal drug (1,300 g) remained in contact with a hydroethanolic mixture (70%, v/v; 3.9 L) for 7 days. After that, the extract was filtered and the residue was subjected to remaceration for another 7 days. The extracts were combined and evaporated under reduced pressure in a rotary evaporator. Then, the crude extract was lyophilized and stored for further use.

2.5 Phytochemical screening

Qualitative phytochemical tests were carried out in order to confirm the presence of saponins, steroids and triterpenes, phenols and flavonoids in the HECi, according to previously reported methods described by Oliveira and co-workers (2017).

2.6 Antioxidant Activity Measurements

2.6.1 Total phenolic content (TPC)

Total phenol content (TPC) was measured by Folin Ciocalteu's (FC) assay (Singleton e Rossi, 1965; Singleton et al, 1999; Oda, 2014). In brief, 1 mL sample and 1 mL FC reagent were added to a glass tube. After 8 minutes of incubation, 1,5 mL 20% (w/v) Na_2CO_3 were also added. The volume was then made up to 10 mL using distilled water. The mixture was placed in the dark during 2 hours at room temperature so that the absorbance was measured at 760 nm. The TPC was calculated using a calibration curve of gallic acid (equation: $y = 0,0921x - 0,0011$; $R^2 = 0,9983$) and expressed as µg gallic acid equivalents/g of HECi. All determinations were carried out in triplicate.

2.6.2 Total flavonoid content (TFC)

Total flavonoid content (TFC) was determined using aluminium complexation reaction (Denni e Mammen, 2012). In brief, 2 mL of an $AlCl_3$ solution (2%, w/v) was added to 1 mL of HECi dissolved in methanol (2.5 µg/mL). The resulting mixture was vigorously shaken and could react for 60 min at room temperature. After incubation, the absorbance was measured at 425 nm. A blank solution consisted in the above-mentioned mixture, which distilled water replaces the same volume of $AlCl_3$. The TFC was calculated using a calibration curve of quercetin prepared under the same conditions as described above (equation: $y = 0,0774x + 0,0343$; $R^2 = 0,9992$) and expressed as µg quercetin equivalents/g of HECi. All determinations were carried out in triplicate.

2.6.3 DPPH• radical scavenging assay

The scavenging activity of HECi was measured conforming to the 1,1-diphenyl-2-picrylhydrazil free radical (DPPH) method, as reported previously (Qin et al., 2014) with minor modifications. Briefly, the samples (100 µL) at different extract concentrations (0.122 – 250 µg/ml) were mixed with DPPH• (100 µL at a concentration of 100 µM) that was dissolved in 95% ethanol. The mixture was then vortexed vigorously and kept in the dark during 30 min at room temperature. The absorbance was measured in a spectrophotometer at 517 nm. The scavenging activity was calculated using the following equation: Scavenging DPPH• activity (%) = $(A_0 – A_1)/A_0 \times 100$. Where A_0 represents the Absorbance of control reaction and A_1 was the

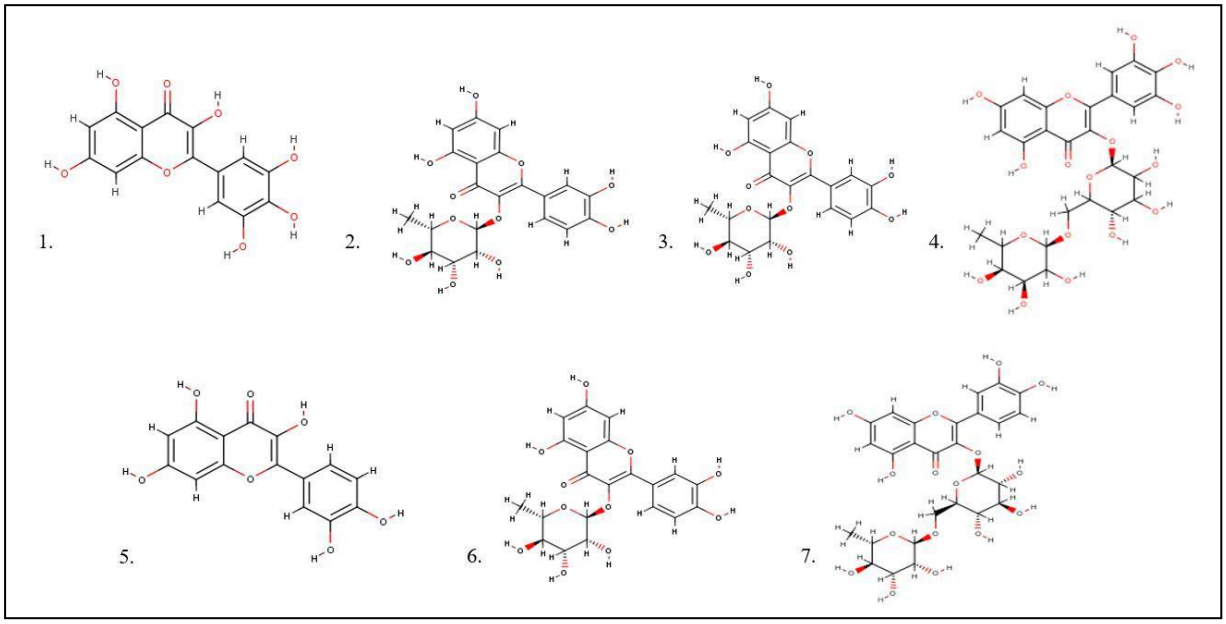
Absorbance of the HECi. The half maximal inhibitory concentration of DPPH• radicals (IC_{50}) was calculated from the equation of the linear dispersion graph obtained from a calibration curve of ascorbic acid (y = 28,85x + 6,7658; R^2 = 0,9927. All tests were performed in triplicate.

2.7 *In silico* analyses

2.7.1 Selection of phytochemical compounds

From previously published works reported in the literature, a total of 7 flavonoids identified in the hydroalcoholic extract of *C. icaco* were selected to integrate this research (Onilude et al., 2021; Wagner et al., 2006). Their chemical structures were obtained from PubChem NCBI (https://www.ncbi.nlm.nih.gov/pccompound) and these flavonoids were drawn via Marvin Sketch software version 21.20, ChemAxon (https://www.chemaxon.com). Their chemical structures are listed in the Figure 1.

Figure 1. Flavonoids presents in *C. icaco* leaves.



Legend: (1) Myricetin; (2) Myricitrin; (3) Myricetin 3-O-glucoronide; (4) Myricetin 3-O-rutinoside; (5) Quercetin; (6) Quercitrin; (7) Rutin. Source: Authors.

2.7.2 ADMET analysis

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the selected flavonoids were estimated using SwissADME (Daina et al., 2017) and pkCSM tool (Pires et al., 2015).

2.7.3 Druglikeness analysis

The drug-likeness of the compounds was calculated using swissadme (Daina et al., 2017). The ligands were subjected to Lipinski screening. Only ligands that were able to satisfy Lipinski filter were chosen for docking simulation.

2.7.4 Molecular docking

After the ADMET and druglikeness screening process, only the compounds with better properties were submitted to molecular docking study using the Autodock 4.2.6/Vina program (Trott & Olson, 2009) to evaluate their binding interaction into the active pocket of NADPH oxidase. The 3D-structure of this enzyme was acquired from the Protein Data Bank with

PDB code: 2CDU (Lountos et al., 2006). The receptor structure was prepared using Pymol program (Schrödinger & DeLano, 2020) to remove water molecules and co-crystallized ligands (ADP and FAD). Polar hydrogens and Gasteiger charges were added by AutoDock Tools (ADT) v1.5.6 (Morris et al., 1998) to generate the PDBQT format. The crystallographic inhibitor ADP was used to support grid box selection and for protocol validation (Table 1).

Table 1. Parameters used in the molecular docking.

Receptor	Ligand	Control molecule	Grid Box coordinates	Grid Size	Reference
NO	Adenosine-5'-diphosphate	Dextromethorphan (DEX)	1.687x	30 x	(Costa et al., 2018)
PDB code: 2CDU			9.885y	14 y	
			54.962z	32 z	

Source: Authors.

The 3D-structure of the selected compounds were obtained from Avogadro software (Hanwell et al., 2012) and saved as a PDB format. Then, these three-dimensional structures were converted into PDBQT using ADT. After all, the docking simulations were performed and receptor ligand interactions were visualized by Pymol program and Discovery Studio Visualizer (Biovia, Dassault Systèmes, 2021), considering a maximum cutoff value of 5.5Å for the distance between interactions, as described by Salamanca Viloria et al., 2017.

2.7.5 Frontier Orbitals

To assess the energy values of frontier orbitals HOMO and LUMO we used the Gaussian 9 program, through the Hartree-Fock method in the 6-31G** basis set. The GAP energy was calculated as: Gap = E_{LUMO} - E_{HOMO}.

2.8 Statistical analysis

Statistical analyses were performed using Microsoft® Excel® software 2019. The data were expressed as mean ± SD and analyzed using the student's t-test.

3. Results and Discussion

3.1 Phytochemical screening

Phytochemical screening of HECi was performed by colorimetric and precipitation reactions to identify the main classes of secondary metabolites. Through this process, the presence of chemical constituints already reported in the literature for *C. icaco*, such as saponins, steroids and triterpenes, phenols and flavonoids were identified in the HECi (Barbosa et al., 2013; Castilho & Kaplan, 2011). According to the literature, flavonoids and terpenes are the main compounds present in Chrysobalanaceae species (Feitosa et al., 2012). A previously published study by our research group showed the presence of the flavonoids myricetin and rutin in the hydroalcoholic extract of *C. icaco* (Silva et al, 2017). Furthermore, the work by Barbosa et al. (2006) also indicates the presence of myricetin and quercetin derivatives in fractions obtained from the hydroalcoholic extract of *C. icaco*.

3.2 Antioxidant Activity Measurements

3.2.1 Total phenol and flavonoid content (TPC and TFC)

Polyphenols are substances from secondary plant metabolism that provide essential benefits for plant growth and reproduction. Furthermore, they form under stressful conditions such as infections, injuries and UV radiation (Pandey & Rizvi, 2009). Among the phenolic compounds, flavonoids stand out as the main class of metabolites with a wide range of biological activities: anti-inflammatory, antitumor, antimicrobial, antioxidant, etc. (Hritcu et al., 2017). The presence of these substances and their importance in the phytochemical constitution of the plant can give *C. icaco* some of its pharmacological actions.

TPC was analyzed (Table 2) measured as total phenolic content (220.11 ± 0.40 mg GAE/g) and total flavonoid content (110.98 ± 0.37 mg QE/g of dry extract). These results suggest that approximately half of the phenols present in *C. icaco* are of the flavonoid type. It is well described that flavonoids perform antioxidant activity because they possibly have redox properties through the absorption and neutralization of free radicals or the decomposition of peroxides (Wang et al., 2017).

3.2.2 DPPH• radical scavenging assay

The methodology based on DPPH• sequestration is widely used to detect antioxidant capacity of plant extracts (Felhi et al., 2016). Thus, the analysis of the hydroalcoholic extract of *Chrysobalanus icaco* against the DPPH• radical revealed its ability to scavenge free radicals, which evidences the plant's antioxidant activity. The results obtained from the phytochemical screening and quantification of phenols and flavonoids already indicated that, probably, HECi would present antioxidant activity, since phenolic compounds, especially flavonoids, can eliminate a wide range of reactive oxygen species (ROS) for its classic hydrogen-donating antioxidant activity, as well as the inhibition of ROS formation (Khlebnikov et al., 2007; Williams et al., 2004).

The free radical scavenging activity of HECi (R^2 = 0.9912), which was obtained from a dose-response curve and showed a strong antioxidant activity in a dose-dependent manner, had a half maximal inhibitory concentration (IC_{50}) of 8.56 µg/mL, which was statistically significant compared to the ascorbic acid standard (IC_{50} = 1.5 µg / mL). This can be related to the various antioxidant constituents in HECi such as phenols, flavonoids and terpenes. The presence of flavonoids such as myricetin, rutin, quercitrin and myricitrin in *C. icaco* leaves greatly contributes to the plant's antioxidant potential (Araújo-Filho et al., 2016). Table 2 demonstrates our results for the sequestration of radical DPPH•.

Table 2. Antioxidant activity of Hidroalcoholic extract of *Chrysobalanus icaco*.

Sample	TPC (mg GAE/g of dry material)	TFC (mg QE/g of dry material)	DPPH• radical (IC_{50})
HECi	220,11 ± 0,4	110,98 ± 0,37	8,1 ± 0,21µg/mL
Ascorbic acid	-	-	1,5 ± 0,14 µg/mL

The values represent the mean ± SEM (n = 3). GAE: gallic acid equivalent; QE: quercetin equivalent. Source: Authors.

The fact that most compounds identified in HECi are well-known flavonoids as antioxidants may explain the great pharmacological potential of *C. icaco*. Furthermore, an essential factor that must be considered is that the phytochemical refinement of the extract seeking to obtain fractions rich in flavonoids could show even more promising results. Finally, our results, when compared to other study models that also assess the antioxidant activity of medicinal plants, clarify the large antioxidant capability of *C. icaco*.

3.3 *In silico* analyses

3.3.1 ADMET profile

In order to predict the pharmacokinectic profile of the selected flavonoids, we performed an *in silico* screening of their kinectics and toxicity properties using the online pkCSM and SwissAdme tool. The following ADME parameters were evaluated: aqueous solubility, human intestinal absorption (HIA), plasma protein binding (PPB), blood-brain barrier (BBB) penetration, central nervous system (CNS) penetration, cytochrome P450 (CYP450) substrates and inhibitors and total clearance. To evaluate toxicity properties, we conducted these parameters: AMES test, Oral Rat Acute Toxicity (LD50), Oral Rat Chronic Toxicity (LOAEL), Hepatotoxicity, Skin Sensitisation (Table 3).

Table 3. Pharmacokinetic predictions of the flavonoids present in *Chrysobalanus icaco* leaves.

ADMET properties	1	2	3	4	5	6	7
1. Absorption							
Aqueous solubility (LogS)	-2.915	-2.892	-2.894	-2.892	-2.925	-2.903	-2.892
HIA (% Absorbed)	65.93	43.334	7.537	14.071	77.207	52.709	23.446
2. Distribution							
PPB (%)	96,78	65,37	64,1	46,74	93,24	64,95	43,9
BBB (Log BB)	-1.493	-1.811	-2.018	-2.215	-1.098	-1.495	-1.899
CNS (Log PS)	-3.709	-1.811	-4.794	-5.397	-3.065	-4.156	-5.178
3. Metabolism							
CYP450 2D6 Substrate	No	No	No	No	No	No	No
CYP450 3A4 Substrate	Weakly	Weakly	Weakly	Weakly	Weakly	Weakly	Weakly
CYP450 1A2 Inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP450 2C9 Inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP450 2D6 Inhibitor	No	No	No	No	No	No	No
CYP450 2C19 Inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP450 3A4 Inhibitor	No	No	No	No	No	No	No
4. Excretion							
Total Clearance (mL/min/Kg)	7.716	5.262	2.284	1.251	8.284	6.373	1.502
5. Toxicity							
AMES toxicity	No	No	No	No	No	No	No
Oral Rat Acute Toxicity (LD50 = mol/kg)	2.497	2.537	2.492	2.484	2.471	2.586	2.491
Oral Rat Chronic Toxicity (LOAEL Log mg/kg_bw/day)	2.718	3.386	4.211	3.919	2.612	3.022	3.673
Hepatotoxicity	No	No	No	No	No	No	No
Skin Sensitisation	No	No	No	No	No	No	No

Legend: (1) Myricetin; (2) Myricitrin; (3) Myricetin 3-O-glucoronide; (4) Myricetin 3-O-rutinoside; (5) Quercetin; (6) Quercitrin; (7) Rutin. LogS: < -10 insoluble; < -6 poorly; < -4 moderately < -2 soluble; < 0 very. HIA: 0-20% low; 20-70% moderate; > 70% high. PPB: > 90% high; < 90% moderate to low absorption. LogBB: > 0.3 readily cross the blood-brain barrier; < -0.1 poorly distributed to the brain. Log PS: > -2 penetrate the Central Nervous System (CNS); < -3 unable to penetrate the CNS. Total Clearance: > 15 high; 5-15 moderate; < 5 low. Source: Daina et al., 2017; Pires et al., 2015). Source: Authors.

According to Table 3, only myricetin (1) and quercetin (5) showed good ADME properties. These two flavonoids are, in fact, flavanols that exhibit high intestinal absorption (65.93% to myricetin and 77.207% to quercetin). Further, the aqueous solubility is one of the most important factors to drug's bioavailability because poor aqueous solubility can limit drug absorption (Devadasu et al., 2018). Regarding this topic, all the compounds showed high solubility in water. Even though, perhaps for low lipophilicity, glycosides flavonoids (2, 3, 4, 6 and 7) showed low rates of intestinal absorption. It is possible that the sugar moiety of these compounds could limit their pharmacokinetics properties.

In reference of distribution, myricetin (1) and quercetin (5) were shown to be strongly bound to plasma proteins. This could be a point for a possible drug interaction, as other drugs that also bind to plasma proteins may become more available and, therefore, more susceptible to toxic events. The other compounds are weakly bound to plasma proteins. Regarding BBB and CNS penetration, none of the compounds have the ability to penetrate greatly to both areas, which suggests that these phytochemicals should have less probability for neurotoxicity.

Regarding metabolism via the CYP 450 enzyme system, since all the molecules are of medium to high polarity, they have been shown to bind weakly to CYP 3A4, which allows us to deduce that phase 1 biotransformation is an unlikely event to occur. In addition, perhaps for their structural similarity, our compounds showed the same inhibition profile: both can inhibit CYP 3A4, CYP 2C9 and CYP 2C9 isoforms. Although this may be a critical point for drug interaction, it should be noted that these molecules have low lipophilicity, meaning that there is less force by reversible binding at the active sites of enzymes (Ramos et al. al., 2020).

The assessment for excretion parameters showed that myricetin, myrictrin and quercitrin have a moderate clearance time, suggesting that these molecules have characteristics for immediate action. The other compounds, possibly because they are very large molecules, showed a low depuration time. In the toxicity evaluation, all the flavonoids were considered non-toxic and non-mutagen.

3.3.2 Druglikeness

Druglikeness prediction is a great tool to support the early stages of drug discovery, because most of drug candidates fails for pharmacokinectics reasons (Bickerton et al., 2012; Siramshetty et al., 2015). The Lipinski Rule, also known as Rule of five, establishes that a drug may not be available for oral via unless it does not break two or more parameters (Lipinski, 2004). Our results (Table 4) reveal that only myricetin (1) and quercetin (5) did not present significant violations for Lipinski filter, which means that these flavonoids have druglikeness features (Lipinski, 2004; Al-Nour et al., 2019). On the other hands, all the glycosidic flavonoids (compounds 2, 3, 4, 6 and 7) did not demonstrate druglikeness properties. This may be associated with the fact that glycosidic chains are quite polar and, therefore, limit the absorption of these compounds (Xiao, 2015).

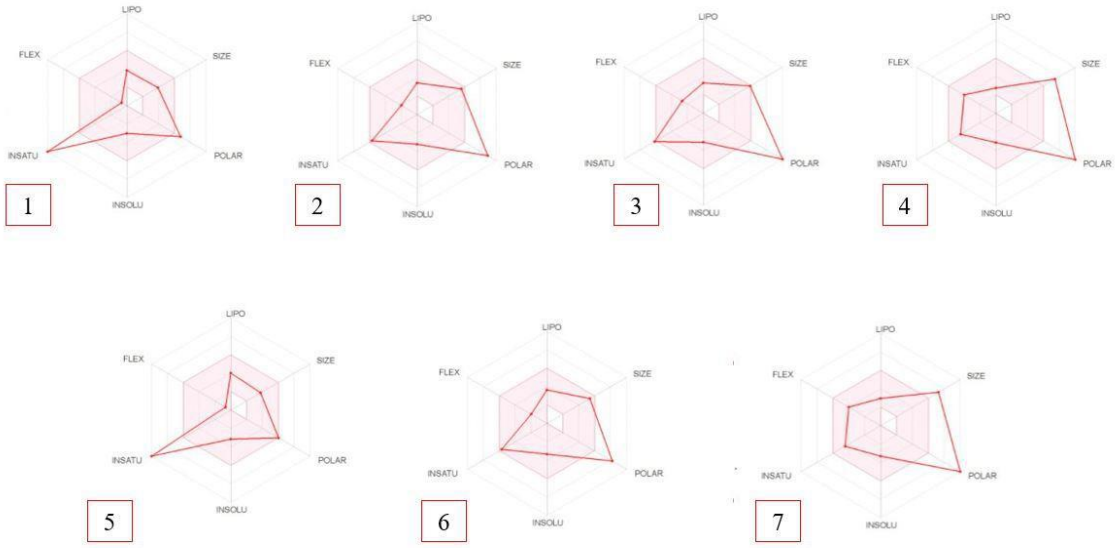
Table 4. Druglikeness prediction of the flavonoids presents in *Chrysobalanus icaco* leaves.

Items	1	2	3	4	5	6	7
Druglikeness	Yes	No	No	No	Yes	No	No
miLogP ≤ 5	0.79	-0.23	-0.86	-1.86	1.23	0.16	-1.12
MW (g/mol) ≤ 500	318.24	464.38	494.36	626.52	302.24	448.38	610.52
nON ≤ 10	8	12	14	17	7	11	16
nOHNH ≤ 5	6	8	9	11	5	7	10
Violations	1	2	2	3	0	2	3

Legend: (1) Myricetin; (2) Myricitrin; (3) Myricetin 3-O-glucoronide; (4) Myricetin 3-O-rutinoside; (5) Quercetin; (6) Quercitrin; (7) Rutin. Molecular Mass - up to 500 g/mol; Log P - up to 5; HBA (Hydrogen Bond Acceptors) - up to 10; HBD (Number of Hydrogen Bonding Donors) - up to 5. Source: Daina et al., 2017. Source: Authors.

The pink area in the radar plot indicates the zone in which a good drug for oral absorption should fit. All glycosidic compounds significantly extrapolate this area, chiefly in the polar region (Figure 2). In general, the most abundant forms of flavanoids is as O-glycosides (Xiao, 2015). In the scientific literature it is well reported that O-glycosidic bonds are quite unstable, which allows their rupture (Simone Badal Mccreath & Rupika Delgoda, 2016; Xiao, 2015). Furthermore, a lot of researchers have showed that flavonoid glycosides are quite likely hydrolyzed in the gastrointestinal tract (Riva et al., 2020), either by the action of the body's own hydrolases, such as β-glucosidase and α-L-rhamnosidase (Valentová et al., 2014; Bang et al., 2015) or by GUT microbiota (Xiao & Hogger, 2013). Beyond that, some studies have demonstrated that flavonoid glycosides could increase the biovailability of its aglycon forms to produce effects *in vivo* (Walle et al., 2005).

Figure 2. Bioavailability radar of the selected flavonoids.



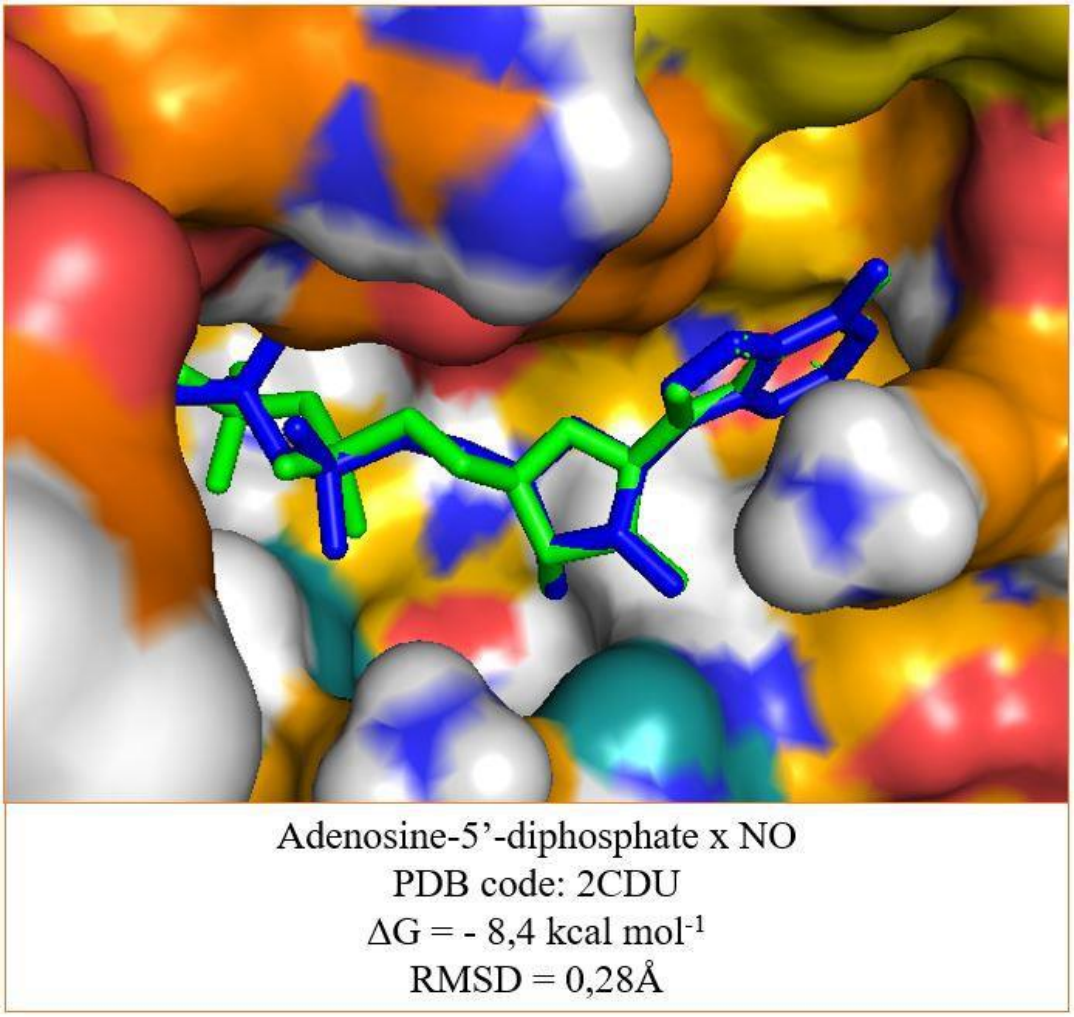
Legend: (1) Myricetin; (2) Myricitrin; (3) Myricetin 3-O-glucoronide; (4) Myricetin 3-O-rutinoside; (5) Quercetin; (6) Quercitrin; (7) Rutin. Source: Authors.

3.3.3 Molecular docking

Our experimental results reveal a great potention of *C. icaco* for antioxidant activity. To reinforce these results, we performed a molecular docking to better understand one of the possible mechanisms behind it. Then, we selected the NADPH enzyme (PDB: 2CDU) for its key role in generating ROS (Bedard & Krause, 2007). According to literature, this enzyme is a major source of superoxide anion (O_2^-), which is the precursor of most other ROS (Rahmani et al., 2019). Therefore, molecules capable of inhibiting the NADPH oxidase (NOX) contribute significantly to the balance of oxidative stress. This way, the best molecules for ADMET and druglikeness properties (myricetin and quercetin) were chosen to be evaluated against NOX. It is important emphasize that no *in silico* results has been reported with myricetin and quercetin acting on this enzyme.

In order to validate our protocol, we analyzed the root-mean-square deviation (RMSD) because is reported that this value must be less than 2.0 Å (Hevener et al., 2009; Sherman et al., 2006). Then, NOX receptor's own ligand was subjected to docking and, through the Pymol program, the overlapping of crystallographic poses was measured (Figure 3). This result confirms that our protocol can be applied for the next molecular docking analyses.

Figure 3. Validation protocol for molecular docking.



Source: Authors.

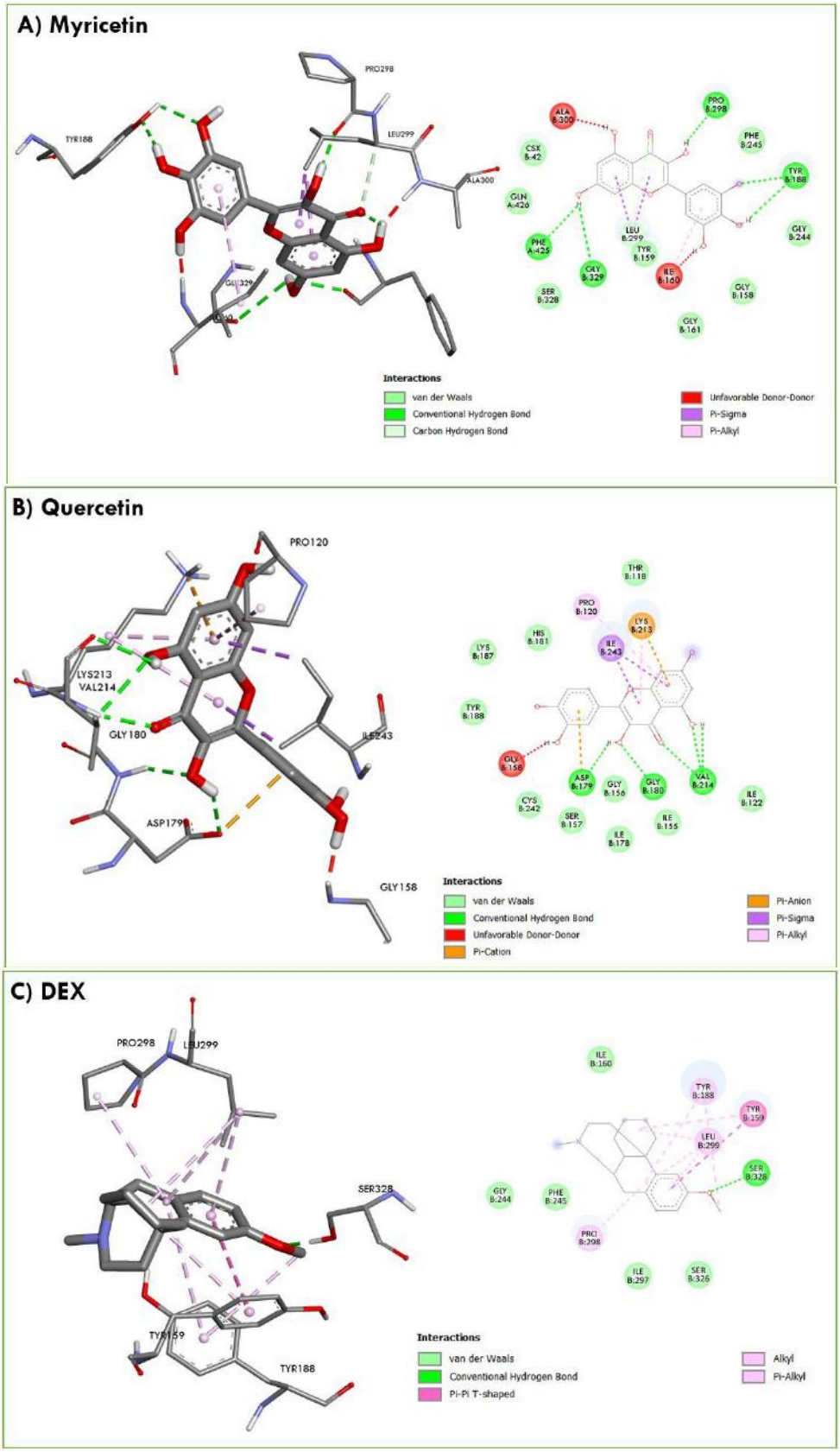
The reference inhibitor, Dextromethorphan (DEX), was also docked and exhibited a good binding energy (-8.0 Kcal/mol), although it mostly interacts via hydrophobic pi-alkyl bonds, especially with residues Tyr 188, Pro 298 and Leu 299 in a long distance from the aminoacids residues. Our molecules, myricetin and especially quercetin, performed an excellent binding energy on NO (-8.1 Kcal/mol and -8.3 Kcal/mol, respectively). These results are even better than those obtained for the control, DEX (Table 5 and Figure 4).

Table 5. Docking score and interaction details of myricetin, quercetin and DEX on NADPH-oxidase.

Compounds	**Binding Energy (BE – Kcal/mol)**	**Interactions details**		
		Residues	**Distance (Å)**	**Interaction**
Myricetin	- 8.1	Iso 160	5.21	Pi-alkyl
			3.33	Hydrophobic
		Tyr 188	5.27	Hidrogen bond
		Pro 298	2.99	Hidrogen bond
		Leu 299	4.53	Pi-sigma
			4.58	van der Waals
		Ala 300	3.37	Hydrophobic
		Gly 329	2.92	Hidrogen bond
		Phe 425	4.33	Hidrogen bond
Quercetin	- 8.3	Pro 120	4.13	Pi-cátion
		Gly 158	2.71	Hydrophobic
		Asp 179	3.19	Hidrogen bond
			4.19	Pi-cátion
		Gly 180	4.15	Hidrogen bond
		Lys 213	3.39	Pi-cátion
		Val 214	3.18	Hidrogen bond
			3.4	
			3.53	
		Ile 243	3.77	Pi-sigma
			4.09	
DEX	- 8.0	Tyr 159	4.28	Pi-pi
		Tyr 188	5.3	Pi-alkyl
		Pro 298	5.81	
		Leu 299	5.19	
		Ser	4.27	Hidrogen bond

Source: Authors.

Figure 4. Interactions of Myricetin, quercetin e control DEX with the NADPH-oxidase.



Source: Authors.

Other *in silico* studies performed with this same type of NADPH oxidase showed binding energies ranging from 6.2669 Kcal/mol to 10.62 Kcal/mol (Costa et al., 2018; Farouk et al., 2021; Herrera-Calderon et al., 2021). Among these results, the best ones were performed by molecules extracted from essential oils, which may represent a practical-experimental limitation, given the difficulty of extracting such compounds (Arce et al., 2006). Furthermore, an important parameter to be considered in molecular docking analyses is the distance between ligand-receptor interactions, since the closer these interactions are, the stronger they are. The analysis of our results showed strong intermolecular interactions, such as conventional Hydrogen bonds, Pi-pi, Pi-cations and Pi-sigma, which indicates the potentiality of these two flavonoids. Myricetin was shown to interact in a zone further away from the enzyme's active site, making strong interactions with residues Ile (3.3 Å, hydrophobic interaction), Pro 298 (2.98 Å, Hydrogen bond) and Gly 329 (2.92 Å, Hydrogen bond). Myricetin was also shown to bind weaklier to two important active site residues Ile 160 (5.21 Å, Pi-alkyl interaction; 3.33 Å, hydrophobic interaction) and Tyr 188 (5.27 Å, Hydrogen bond). The fact that myricetin interacted in a more distant zone than would be considered ideal, does not nullify it as potential inhibitor. In fact, proteins are complex and highly dynamic that can assume different conformations. This allows a disturbance, even occurring at a certain distance from the active site, may reduce its functioning. Furthermore, our docking results showed that all compounds interact with important residues of the enzyme's catalytic site at a maximum cutoff value of 5.5Å (Salamanca Viloria et al., 2017).

Quercetin, in turn, exhibited the best binding energy among the compounds analysed and showed strong interactions with important residues from the catalytic site, such as Gly 158 (2.71 Å, hydrophobic interaction), Asp 179 (3.19 Å, Hydrogen bond; 4.24, Pi-cation interaction), Gly 180 (4.15 A, Hidrogen bond) and Val 214 (3.18 Å, 3.4 Å and 3.53 Å by Hidrogen bond). Experimental models have already reported that, *in vivo*, quercetin seems to act by suppressing the generation of ROS from NOX (Sánchez et al., 2006; Luo et al., 2019). Thus, our results show, for the first time, that the interactions between quercetin and NOX are energetically viable and based on strong intermolecular interactions.

3.3.4 Frontier Orbitals

The evaluation of HOMO and LUMO orbitals is an important parameter that consolidates the antioxidant potential of a molecule. The electron donating capacity of a compound is correlated with the HOMO energies (Braga et al., 2011; Xue et al., 2014). Then, the greater the energy of this orbital, the greater the ability to donate electrons. On the other hand, the energy difference (GAP) between LUMO and HOMO is another important parameter for evaluation, as it reveals the stability and reactivity of the molecule. Thus, the higher the HOMO and the lower the GAP of a compound, the greater its antioxidant activity (Wang et al., 2015). The calculated frontier orbital energies for myricetin and quercetin are present in Table 6.

Table 6. Frontier orbitals properties for quercetin and myricetin.

Compounds	HOMO (eV)	LUMO (eV)	GAP (eV)
Myricetin	-7,41	-1,38	6,02
Quercetin	-7,04	-1,62	5,42
DEX (control)	-5,29	-0,20	5,09

Source: Authors.

From Table 6, quercetin presents a higher HOMO orbital energy and low GAP energy than myricetin. This agrees with our results obtained by molecular docking, which showed that quercetin is a better antioxidant. So, this flavonoid and its O-glycoside forms most likely contribute significantly to the antioxidant potential of *C. icaco*.

4. Conclusion

In the present study, *Chrysobalanus icaco* proved to be a plant rich in flavonoid derivatives, the major constituents of the hydroalcoholic extract. Among these stand out myricetin, quercetin and their O-glycosylated derivatives. Due to this rich phenolic composition, *C. icaco* presented a high antioxidant power and its main flavonoid aglycones, myricetin and especially quercetin, performed an excellent inhibitory profile against the enzyme NADPH oxidase, the main ROS generating enzyme. Our findings demonstrate for the first time that quercetin can strongly inhibit NOX. Furthermore, through the study of molecular orbitals, we identified that quercetin also had excellent antioxidant properties that confirm results already reported in the literature for this purpose.

Therefore, these findings may stimulate the development of more *in silico* research aimed at studying the mechanisms by which flavanoids exert antioxidant activity. In this aspect, further investigations will be needed to reveal the behavior of *C. icaco* constituents under different experimental models. From our experimental and *in silico* results, we conclude that *C. icaco* can be a potential resource for the future development of an herbal medicine with strong antioxidant activity to be used by the pharmaceutical industry.

Acknowledgments

The authors would like to thank the Fundação Amazônia de Amparo a Estudos e Pesquisas – FAPESPA (Belém, Brazil) for all the support to carry out this work.

References

Agati, G., Brunetti, C., Fini, A., Gori, A., Guidi, L., Landi, M., Sebastiani, F., & Tattini, M. (2020). Are Flavonoids Effective Antioxidants in Plants? Twenty Years of Our Investigation. *Antioxidants*, *9*(11), 1098. https://doi.org/10.3390/antiox9111098.

Al-Nour, M. Y., Ibrahim, M. M., & Elsaman, T. (2019). Ellagic Acid, Kaempferol, and Quercetin from Acacia nilotica: Promising Combined Drug With Multiple Mechanisms of Action. *Current Pharmacology Reports*, *5*(4), 255–280. https://doi.org/10.1007/s40495-019-00181-w.

Araújo-Filho, H. G., Dias, J. D. S., Quintans-Júnior, L. J., Santos, M. R. V., White, P. A. S., Barreto, R. S. S., Barreto, A. S., Estevam, C. S., Araujo, S. S., Almeida, J. R. G. S., Menezes, I. R. A., Coutinho, H. D. M., & Quintans, J. S. S. (2016). Phytochemical screening and analgesic profile of the lyophilized aqueous extract obtained from Chrysobalanus icaco leaves in experimental protocols. *Pharmaceutical Biology*, *54*(12), 3055–3062. https://doi.org/10.1080/13880209.2016.1204618.

Arce, A., Marchiaro, A., Rodríguez, O., & Soto, A. (2006). Essential oil terpenless by extraction using organic solvents or ionic liquids. *AIChE Journal*, *52*(6), 2089–2097. https://doi.org/10.1002/aic.10844.

Arfin, S., Jha, N. K., Jha, S. K., Kesari, K. K., Ruokolainen, J., Roychoudhury, S., Rathi, B., & Kumar, D. (2021). Oxidative Stress in Cancer Cell Metabolism. *Antioxidants*, *10*(5), 642. https://doi.org/10.3390/antiox10050642.

Bang, S.-H., Hyun, Y.-J., Shim, J., Hong, S.-W., & Kim, D.-H. (2015). Metabolism of Rutin and Poncirin by Human Intestinal Microbiota and Cloning of Their Metabolizing α-L-Rhamnosidase from Bifidobacterium dentium. *Journal of Microbiology and Biotechnology*, *25*(1), 18–25. https://doi.org/10.4014/jmb.1404.04060.

Barbosa, A. P. de O., Silveira, G. de O., de Menezes, I. A. C., Rezende Neto, J. M., Bitencurt, J. L. C., Estavam, C. dos S., de Lima, A. do C. B., Thomazzi, S. M., Guimarães, A. G., Quintans-Junior, L. J., & dos Santos, M. R. V. (2013). Antidiabetic Effect of theChrysobalanus icacoL. Aqueous Extract in Rats. *Journal of Medicinal Food*, *16*(6), 538–543. https://doi.org/10.1089/jmf.2012.0084.

Barbosa, W. L. R., Peres, A., Gallori, S., & Vincieri, F. F. (2006). Determination of myricetin derivatives in Chrysobalanus icaco L. (Chrysobalanaceae). *Revista Brasileira de Farmacognosia*, *16*(3), 333–337. https://doi.org/10.1590/s0102-695x2006000300009.

Bedard, K., & Krause, K.-H. (2007). The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. *Physiological Reviews*, *87*(1), 245–313. https://doi.org/10.1152/physrev.00044.2005.

Bickerton, G. R., Paolini, G. V., Besnard, J., Muresan, S., & Hopkins, A. L. (2012). Quantifying the chemical beauty of drugs. *Nature Chemistry*, *4*(2), 90–98. https://doi.org/10.1038/nchem.1243.

Biovia, Dassault Systèmes. (2021). Discovery studio visualizer. *San Diego, CA, USA*, *936*.

Braga, R. P., Moraes, F. K. C., Manhaes, M. M., Andrade, M. A., Baetas, A. C., Almeida, E. D., Fontes, E. A., Silva, M. V. S., Silva, M. N., Arruda, M. S. P., & Borges, R. S. (2011). An Electronic Study of Tocopherol-Ring Regioisomers as Antioxidants. *Journal of Computational and Theoretical Nanoscience*, *8*(10), 2061–2065. https://doi.org/10.1166/jctn.2011.1926.

Castilho, R. O., de Oliveira, R. R., & Kaplan, M. A. C. (2005). Licanolide, a new triterpene lactone from Licania tomentosa. *Fitoterapia*, *76*(6), 562–566. https://doi.org/10.1016/j.fitote.2005.04.018.

Castilho, R. O., & Kaplan, M. A. C. (2011). Phytochemical study and antimicrobial activity of Chrysobalanus icaco. *Chemistry of Natural Compounds*, *47*(3), 436–437. https://doi.org/10.1007/s10600-011-9953-x.

Chaudhuri, S., Badisa, R. B., Pilarinou, E., & Walker, E. (2002). Licamichauxiioic-A and -B Acids - TwoEnt-Kaurene Diterpenoids fromLicania Michauxii. *Natural Product Letters*, *16*(1), 39–45. https://doi.org/10.1080/1057563029001/4836.

Chocry, M., & Leloup, L. (2020). The NADPH Oxidase Family and Its Inhibitors. *Antioxidants & Redox Signaling*, *33*(5), 332–353. https://doi.org/10.1089/ars.2019.7915.

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7(1). https://doi.org/10.1038/srep42717.

Devadasu, V. R., Deb, P. K., Maheshwari, R., Sharma, P., & Tekade, R. K. (2018). Physicochemical, Pharmaceutical, and Biological Considerations in GIT Absorption of Drugs. *Dosage Form Design Considerations*, 149–178. https://doi.org/10.1016/b978-0-12-814423-7.00005-8.

Farouk, A., Mohsen, M., Ali, H., Shaaban, H., & Albaridi, N. (2021). Antioxidant Activity and Molecular Docking Study of Volatile Constituents from Different Aromatic Lamiaceous Plants Cultivated in Madinah Monawara, Saudi Arabia. *Molecules*, *26*(14), 4145. https://doi.org/10.3390/molecules26144145.

Feitosa, E. A., Xavier, H. S., & Randau, K. P. (2012). Chrysobalanaceae: traditional uses, phytochemistry and pharmacology. *Revista Brasileira de Farmacognosia*, *22*(5), 1181–1186. https://doi.org/10.1590/s0102-695x2012005000080.

Felhi, S., Baccouch, N., Ben Salah, H., Smaoui, S., Allouche, N., Gharsallah, N., & Kadri, A. (2016). Nutritional constituents, phytochemical profiles, in vitro antioxidant and antimicrobial properties, and gas chromatography–mass spectrometry analysis of various solvent extracts from grape seeds (Vitis vinifera L.). *Food Science and Biotechnology*, *25*(6), 1537–1544. https://doi.org/10.1007/s10068-016-0238-9.

Fernandes, J., Castilho, R. O., da Costa, M. R., Wagner-Souza, K., Coelho Kaplan, M. A., & Gattass, C. R. (2003). Pentacyclic triterpenes from Chrysobalanaceae species: cytotoxicity on multidrug resistant and sensitive leukemia cell lines. *Cancer Letters*, *190*(2), 165–169. https://doi.org/10.1016/s0304-3835(02)00593-1.

Ganesan, K., & Xu, B. (2017). A Critical Review on Polyphenols and Health Benefits of Black Soybeans. *Nutrients*, *9*(5), 455. https://doi.org/10.3390/nu9050455.

Hano, C., & Tungmunnithum, D. (2020). Plant Polyphenols, More than Just Simple Natural Antioxidants: Oxidative Stress, Aging and Age-Related Diseases. *Medicines*, *7*(5), 26. https://doi.org/10.3390/medicines7050026.

Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E., & Hutchison, G. R. (2012). Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics*, *4*(1). https://doi.org/10.1186/1758-2946-4-17.

Herrera-Calderon, O., Chacaltana-Ramos, L. J., Huayanca-Gutiérrez, I. C., Algarni, M. A., Alqarni, M., & Batiha, G. E.-S. (2021). Chemical Constituents, In Vitro Antioxidant Activity and In Silico Study on NADPH Oxidase of Allium sativum L. (Garlic) Essential Oil. *Antioxidants*, *10*(11), 1844. https://doi.org/10.3390/antiox10111844.

Hevener, K. E., Zhao, W., Ball, D. M., Babaoglu, K., Qi, J., White, S. W., & Lee, R. E. (2009). Validation of Molecular Docking Programs for Virtual Screening against Dihydropteroate Synthase. *Journal of Chemical Information and Modeling*, *49*(2), 444–460. https://doi.org/10.1021/ci800293n.

Hritcu, L., Ionita, R., Postu, P. A., Gupta, G. K., Turkez, H., Lima, T. C., Carvalho, C. U. S., & de Sousa, D. P. (2017). Antidepressant Flavonoids and Their Relationship with Oxidative Stress. *Oxidative Medicine and Cellular Longevity*, *2017*, 1–18. https://doi.org/10.1155/2017/5762172.

Izzo, C., Vitillo, P., Di Pietro, P., Visco, V., Strianese, A., Virtuoso, N., Ciccarelli, M., Galasso, G., Carrizzo, A., & Vecchione, C. (2021). The Role of Oxidative Stress in Cardiovascular Aging and Cardiovascular Diseases. *Life*, *11*(1), 60. https://doi.org/10.3390/life11010060.

Joshi, S., & Khan, S. R. (2019). NADPH oxidase: a therapeutic target for hyperoxaluria-induced oxidative stress – an update. *Future Medicinal Chemistry*, *11*(23), 2975–2978. https://doi.org/10.4155/fmc-2019-0275.

Khlebnikov, A. I., Schepetkin, I. A., Domina, N. G., Kirpotina, L. N., & Quinn, M. T. (2007). Improved quantitative structure–activity relationship models to predict antioxidant activity of flavonoids in chemical, enzymatic, and cellular systems. *Bioorganic & Medicinal Chemistry*, *15*(4), 1749–1770. https://doi.org/10.1016/j.bmc.2006.11.037.

Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing*, *89*(3), 217–233. https://doi.org/10.1016/j.fbp.2010.04.008.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, *Volume 13*, 757–772. https://doi.org/10.2147/cia.s158513.

Lipinski, C. A. (2004). Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies*, *1*(4), 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007.

Lountos, G. T., Jiang, R., Wellborn, W. B., Thaler, T. L., Bommarius, A. S., & Orville, A. M. (2006). The Crystal Structure of NAD(P)H Oxidase from Lactobacillus sanfranciscensis: Insights into the Conversion of O2 into Two Water Molecules by the Flavoenzyme,. *Biochemistry*, *45*(32), 9648–9659. https://doi.org/10.1021/bi060692p.

Luo, M., Tian, R., Yang, Z., Peng, Y.-Y., & Lu, N. (2019). Quercetin suppressed NADPH oxidase-derived oxidative stress via heme oxygenase-1 induction in macrophages. *Archives of Biochemistry and Biophysics*, *671*, 69–76. https://doi.org/10.1016/j.abb.2019.06.007.

Mammen, D., & Daniel, M. (2012). A critical evaluation on the reliability of two aluminum chloride chelation methods for quantification of flavonoids. *Food Chemistry*, *135*(3), 1365–1368. https://doi.org/10.1016/j.foodchem.2012.05.109.

McCann, S., & Roulston, C. (2013). NADPH Oxidase as a Therapeutic Target for Neuroprotection against Ischaemic Stroke: Future Perspectives. *Brain Sciences*, *3*(4), 561–598. https://doi.org/10.3390/brainsci3020561.

Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K., & Olson, A. J. (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry*, *19*(14), 1639–1662. https://doi.org/10.1002/(sici)1096-987x(19981115)19:14<1639::aid-jcc10>3.0.co;2-b.

Oda, F. B. (2014). *Avaliação da utilização de subprodutos de Coffea arabica L. para fins cosméticos* (p. 52) [Undergraduate Dissertation].

Oliveira, M., Eloir Paulo Schenkel, & Palazzo, C. (2017). *Farmacognosia do produto natural ao medicamento*. Porto Alegre, Rs Artmed.

Onilude, H. A., Kazeem, M. I., & Adu, O. B. (2021). Chrysobalanus icaco: A review of its phytochemistry and pharmacology. *Journal of Integrative Medicine*, *19*(1), 13–19. https://doi.org/10.1016/j.joim.2020.10.001.

Pandey, K. B., & Rizvi, S. I. (2009). Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. *Oxidative Medicine and Cellular Longevity*, *2*(5), 270–278. https://doi.org/10.4161/oxim.2.5.9498.

Pereira, A. S., Shitsuka, D. M., Pereira, F. J., & Shitsuka, R. (2018). *Metodologia da pesquisa científica*. Santa Maria: UFSM, NTE. https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf.

Pires, D., Blundell, T., & Ascher, D. (2015). *pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures*. ACS Publications. https://dx.doi.org/10.1021%2Facs.jmedchem.5b00104.

Qin, L., Wang, W., You, S., Dong, J., Zhou, Y., & Wang, J. (2014). In vitro antioxidant activity and in vivo antifatigue effect of layered double hydroxide nanoparticles as delivery vehicles for folic acid. *International Journal of Nanomedicine*, *9*, 5701. https://doi.org/10.2147/ijn.s74306.

Rahmani, H., Ghavamipour, F., & Sajedi, R. H. (2019). Bioluminescence Detection of Superoxide Anion Using Aequorin. *Analytical Chemistry*, *91*(20), 12768–12774. https://doi.org/10.1021/acs.analchem.9b02293.

Ramos, L. V. R., Barros, A. S. M., & Dolabela, M. F. (2020). Análise in silico de metabólitos ativos isolados de Libidibia ferrea Martius. *Research, Society and Development*, *9*(12), e7991210910. https://doi.org/10.33448/rsd-v9i12.10910.

Riva, A., Kolimár, D., Spittler, A., Wisgrill, L., Herbold, C. W., Abrankó, L., & Berry, D. (2020). Conversion of Rutin, a Prevalent Dietary Flavonol, by the Human Gut Microbiota. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.585428.

Roy, J., Galano, J., Durand, T., Le Guennec, J., & Chung-Yung Lee, J. (2017). Physiological role of reactive oxygen species as promoters of natural defenses. *The FASEB Journal*, *31*(9), 3729–3745. https://doi.org/10.1096/fj.201700170r.

Salamanca Viloria, J., Allega, M. F., Lambrughi, M., & Papaleo, E. (2017). An optimal distance cutoff for contact-based Protein Structure Networks using side-chain centers of mass. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-01498-6.

Sánchez, M., Galisteo, M., Vera, R., Villar, I. C., Zarzuelo, A., Tamargo, J., Pérez-Vizcaíno, F., & Duarte, J. (2006). Quercetin downregulates NADPH oxidase, increases eNOS activity and prevents endothelial dysfunction in spontaneously hypertensive rats. *Journal of Hypertension*, *24*(1), 75–84. https://doi.org/10.1097/01.hjh.0000198029.22472.d9.

Schrödinger, L., & DeLano, W. (2020). *PyMOL | www.pymol.org*. Www.pymol.org. http://www.pymol.org/pymol.

Sherman, W., Beard, H. S., & Farid, R. (2006). Use of an Induced Fit Receptor Structure in Virtual Screening. *Chemical Biology Drug Design*, *67*(1), 83–84. https://doi.org/10.1111/j.1747-0285.2005.00327.x.

Silva, J. B., Peres, A. R. N., Paixão, T., Silva, A. B., Baetas, A., Barbosa, W. R., Monteiro, M. & Andrade, M.(2017). Antifungal activity of hydroalcoholic extract of Chrysobalanus icaco against oral clinical isolates of Candida Species. *Pharmacognosy Research*, *9*(1), 96. https://doi.org/10.4103/0974-8490.199772.

Simone Badal Mccreath, & Rupika Delgoda. (2016). *Pharmacognosy : fundamentals, applications and strategies*. Academic Press.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Oxidants and Antioxidants Part A*, *299*, 152–178. https://doi.org/10.1016/s0076-6879(99)99017-1.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158.

Siramshetty, V. B., Nickel, J., Omieczynski, C., Gohlke, B.-O., Drwal, M. N., & Preissner, R. (2015). WITHDRAWN—a resource for withdrawn and discontinued drugs. *Nucleic Acids Research*, *44*(D1), D1080–D1086. https://doi.org/10.1093/nar/gkv1192.

Susanti, H. (2019). Total phenolic content and antioxidant activities of binahong (Anredera cordifolia.). *Jurnal Kedokteran Dan Kesehatan Indonesia*, *10*(2), 171–175. https://doi.org/10.20885/jkki.vol10.iss2.art9.

Tarafdar, A., & Pula, G. (2018). The Role of NADPH Oxidases and Oxidative Stress in Neurodegenerative Disorders. *International Journal of Molecular Sciences*, *19*(12), 3824. https://doi.org/10.3390/ijms19123824.

Thilakarathna, S., & Rupasinghe, H. (2013). Flavonoid Bioavailability and Attempts for Bioavailability Enhancement. *Nutrients*, *5*(9), 3367–3387.

https://doi.org/10.3390/nu5093367.

Trott, O., & Olson, A. J. (2009). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), NA-NA. https://doi.org/10.1002/jcc.21334.

Valentová, K., Vrba, J., Bancířová, M., Ulrichová, J., & Křen, V. (2014). Isoquercitrin: Pharmacology, toxicology, and metabolism. *Food and Chemical Toxicology*, *68*, 267–282. https://doi.org/10.1016/j.fct.2014.03.018.

Walle, T., Browning, A. M., Steed, L. L., Reed, S. G., & Walle, U. K. (2005). Flavonoid Glucosides Are Hydrolyzed and Thus Activated in the Oral Cavity in Humans. *The Journal of Nutrition*, *135*(1), 48–52. https://doi.org/10.1093/jn/135.1.48.

Wang, G., Xue, Y., An, L., Zheng, Y., Dou, Y., Zhang, L., & Liu, Y. (2015). Theoretical study on the structural and antioxidant properties of some recently synthesised 2,4,5-trimethoxy chalcones. *Food Chemistry*, *171*, 89–97. https://doi.org/10.1016/j.foodchem.2014.08.106.

Wang, X., Chen, Y., Wang, Q., Sun, L., Li, G., Zhang, C., Huang, J., Chen, L., & Zhai, H. (2017). Support for Natural Small-Molecule Phenols as Anxiolytics. *Molecules*, *22*(12), 2138. https://doi.org/10.3390/molecules22122138.

Wen, K., Fang, X., Yang, J., Yao, Y., Nandakumar, K. S., Salem, M. L., & Cheng, K. (2021). Recent Research on Flavonoids and their Biomedical Applications. *Current Medicinal Chemistry*, *28*(5), 1042–1066. https://doi.org/10.2174/0929867327666200713184138.

Williams, R. J., Spencer, J. P. E., & Rice-Evans, C. (2004). Flavonoids: antioxidants or signalling molecules? *Free Radical Biology and Medicine*, *36*(7), 838–849. https://doi.org/10.1016/j.freeradbiomed.2004.01.001.

Xiao, J. (2015). Dietary Flavonoid Aglycones and Their Glycosides: Which Show Better Biological Significance? *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2015.1032400.

Xiao, J., & Hogger, P. (2013). Metabolism of Dietary Flavonoids in Liver Microsomes. *Current Drug Metabolism*, *14*(4), 381–391. https://doi.org/10.2174/1389200211314040003.

Xue, Y., Zheng, Y., An, L., Dou, Y., & Liu, Y. (2014). Density functional theory study of the structure–antioxidant activity of polyphenolic deoxybenzoins. *Food Chemistry*, *151*, 198–206. https://doi.org/10.1016/j.foodchem.2013.11.064.

Yousefian, M., Shakour, N., Hosseinzadeh, H., Hayes, A. W., Hadizadeh, F., & Karimi, G. (2019). The natural phenolic compounds as modulators of NADPH oxidases in hypertension. *Phytomedicine*, *55*, 200–213. https://doi.org/10.1016/j.phymed.2018.08.002.