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In Vitro and In Silico Antioxidant Activity and Molecular Characterization of *Bauhinia ungulata* L. Essential Oil

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Bauhinia ungulata is an antioxidant medicinal plant that has been manipuled in Brazil to lower glycemic index as well is for alternative treatment for diabetes. Therefore, the present hearch has aimed to investigates the antioxidant effects of the essential oil of *Bauhinia ungulata* L. (EOBU) collected in Amazon region better specified in Boa Vista, Roraima, Brazil, located in the Amazon region. Gas chromatography had been used to characterize the components, and antioxidant assays such as DPPH, TAC, reducing power, Fe^{2+} chelation, and total phenols had also been performed. The major constituents had molecularly anchored with the human catalase (CAT) enzyme, and maltol has showed as a positive control. Among the 25 revealed components, the main ones have been α-bisabolol (27.2%), β-Caryophyllene (12.5%) and Epi-γ-eudesmol (13.6%). The EOBU has comproved a TAC value of 618.79 mg of ascorbic acid equivalent, free radical scavenging capacity (DPPH) around 53.7% and 65.27%, Fe^{2+} chelation capacity of 161 ± 6 and 126.7 ± 39.6, for 0.1 mg.mL^{-1} and 0.5 mg.mL^{-1}, respectively. The power around the EOBU has appeared percentages equals to 28.66%, 44.6%, and 77.03% in the concentrations tested. As well as, 96.5% of total phenols. The compounds α-bisabolol (−5.7 ± 0.4 Kcal.mol^{-1}) and β-caryophyllene (−6.1 ± 0.5 Kcal.mol^{-1}) have showed good interaction with CAT compared to Maltol (−4.4 ± 0.4 Kcal.mol^{-1}). The present work has demonstrated that EOBU functions as a potent antioxidant, capable of scavenging free radicals and reducing oxidative stress damage.

Introduction

Reactive oxygen species (ROS), such as singlet oxygen (O_2), hydroxyl radical (OH^-), superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2), as well as reactive nitrogen species (RNS), including nitric oxide (NO), nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), nitroxyl anion (NO^-), nitroxyl cation (NO^+), and peroxynitrite ($ONOO^-$), are products of biological reactions or exogenous factors.[1] The disproportionate production of ROS/RNS compared to the capacities of the antioxidant system can lead to elevated levels of free radicals. These harmful molecules can damage crucial biological components such as DNA, proteins, lipids, and sugars, negatively impacting cell and tissue health, often leading to neurodegenerative and cardiovascular diseases.[2,3]

It is possible on many situations, those damages can still be reversible and prevented, primarily through antioxidant mechanisms such as the action of natural antioxidants like vitamin C or through enzymes such as Catalase (CAT), responsible for converting H_2O_2 into O_2 and H_2O, superoxide dismutase (SOD), and glutathione peroxidase (GPx). The malfunctioning of these enzymes is often associated with the development and progression of chronic diseases such as Alzheimer's, Parkinson's, and cancer.[4,5,6]

Having this thought in mind, natural products and molecules have emerged in recent years as potential protagonists in preventing and protecting against reactive species originating from cellular oxidative stress, especially due to their antioxidant properties.[7,8] Among these natural products, essential oils have been gained increasing attention in research groups due to their low toxicity and hydrophobic nature, properties that allow for greater bioavailability and better absorption compared to other extracts. Many of them are already documented in the literature as potent antioxidants, such as citronella (*Cymbopogon nardus*),[9] cinnamon (*Cinnamomum zeylanicum*),[10,11] ginger (*Zingiber officinale*),[12] and basil (*Origanum majorana*).

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Bauhinia ungulata is a medicinal plant species found in the Brazilian Amazon forest. Traditional communities, such as the Tabeba indigenous people in Ceará state, use *B. ungulata* leaf tea for its beneficial properties, such as hypoglycemic action.[13,14] Studies have revealed that *B. ungulata* possesses antioxidant, antimicrobial, larvicidal, anti-inflammatory, cytotoxic, antiproliferative, and antiacetylcholinesterase properties, as well as hypoglycemic effects.[15,16] Plants of the *Bauhinia* genus exhibit various phytochemicals with antioxidant activities described in the literature, characterized by high levels of phenolic compounds and flavonoids.[17,18]

Although the mechanisms behind the antioxidant activity of this species remain obscure, the antioxidant effects of *Bauhinia ungulata* essential oil (EOBU) have not been addressed. With this premise, work aims to trace the different bioactive compounds in EOBU collected during the rainy season in Boa Vista, Roraima – Brazil. Antioxidant properties of major compounds, as well as the interactions between them and the human antioxidant enzyme CAT, were determined in vitro and in silico, respectively.

Results and Discussion

The studies on natural products have been impelled by their wide range of health properties and traditional use backgrounds. Essential oil formulations may increase the bioavailability of their antioxidant components, making them a potential treatment for diseases involving oxidative stress. In this sense, the present study analyzed the bioactive components of EOBU from the Brazilian Amazon, thus determining its chemical composition and antioxidant activity both in vitro and in silico.

Performance and Chemical composition of EOBU

After extracting the EOBU by hydrodistillation, it was possible to obtain the oil yield through the following equation 1. The EOBU showed a yield of approximately 0.07%.

$$\text{Yield } (\%) = \{[(\text{MOE1} + \text{MOE2} + \text{MOE3})/3]/1000\} \times 100\% \quad (1)$$

The yield of EOBU extraction has been higher than the work carried out by Gramosa (2010) and collaborators carried out in the Northeast of Ceará, which obtained 0.007%[19] and Mesquita et al., (2016) who obtained 0.02%.[20] Such yield variations can be attributed to seasonal changes that affect essential oil content in plants. Several studies have demonstrated variations in essential oil yields during different weather of the year.[21,22] In addition, it has been reported that essential oils can change their yield depending on the conditions under which the experiments were carried out, such as part of the studied plant, climatic conditions, extraction methods, type, and time of collection.[23]

After obtaining the EOBU, the chromatographic analysis was performed. Analysis of the chemical composition revealed that EOBU is composed of (84%) sesquiterpenes, (5.5%) monoterpenes, and (10.4%) unidentified substances. The major constituents of EOBU were α-bisabolol (27.2%), followed by Epi-γ-eudesmol (13.6%) and β-caryophyllene with (12.5%). The α-bisabolol compounds and their derivatives (α-bisabolol oxide A and α-bisabolol oxide B) accounted for more than 31% of the total chemical composition of the EOBU (Table 1) and the Figure 1 presents the major constituents.

Previous studies have found a similar sesquiterpene composition to this study. However, the percentage of significant components varied in different studies. Gramosa and coworkers (2010) study revealed other primary substances, for example, spathulenol with a content of (47.7%) and caryophyllene oxide with (18.3%).[19] Besides, α-bisabolol and its derivatives were not identified. In the present study, more than 31% of the chemical composition of EOBU was represented by α-bisabolol and its derivatives, with α-bisabolol being the majority at 27.2%. Thus, we can observe a difference in the chemical composition of EOBU concerning studies reported in the literature. It is fundamental to consider that abiotic and biotic factors may influence this variation in the concentration of volatile substances produced and released from plants.[23]

Antioxidant activity

To determine the ability of EOBU for donate electrons in an acidic medium, the total antioxidant capacity (TAC) test has performed. The values were expressed in the equivalent of ascorbic acid per gram (AAE.g^{-1}). The EOBU showed a total antioxidant activity of 618.79 mg of ascorbic acid equivalent, indicating a positive correlation between the 0.1 mg.mL^{-1} concentration of EOBU used and the level of antioxidant activity. Other studies with the ethyl acetate extract from the stem of this plant also has demonstrated its efficiency in reducing Mo (VI) to Mo (V), in addition, it had presented antioxidant activity, with 1.70 mg equivalent of the antioxidant activity of butylated hydroxytoluene (BHT) per milligram of *B. ungulata* extract, therefore it is being showied greater activity than the BHT that had been used as a positive control before.[24]

Figure 2 presents the EOBU has reducing power in absorbance value and reduction percentage, respectively. It has been watched that the reducing power of the oil raised as its concentration level. The concentrations of 0.05 mg.mL^{-1}, 0.1 mg.mL^{-1}, and 0.5 mg.mL^{-1} showed percentage values of reducing potential of 28.6%, 44.6%, and 77%, respectively.

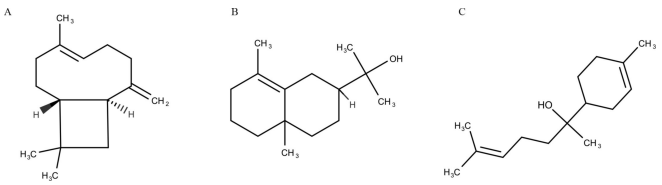

Figure 1. Main constituents of Essential oil of *B. ungulata* (EOBU): β-caryophyllene (A), Epi-γ-eudesmol (B) and α-bisabolol (C).

Table 1. The phytochemicals in EOBU and Maltol (β-caryophyllene and α-bisabolol) were obtained from PubChem with the respective descriptors: α-bisabolol (CID: 1549992), β-caryophyllene Kovats Index.

*RT (min)	EOBU (%)	**IK Calculated	Substance Suggested
7.155	0.5	918	α-Thujene
7.53	3.3	928	α-Pinene
8.025	0.3	940	Camphene
9.002	0.6	964	β-Pinene
10.97	0.1	1013	Eucalyptol
13.92	0.7	1086	Linalool
25.957	0.7	1385	Copaene
26.643	1.0	1402	β-Elemene
27.787	**12.5**	**1430**	**β-Caryophyllene**
29.155	4.5	1464	α-Humulene
30.27	3.4	1492	γ-Muurolene
30.893	2.2	1507	γ-Elemene
30.997	2.0	1510	Valencene
31.227	1.1	1516	α-Cadinene
31.95	0.8	1534	δ-Cadinene
33.228	1.0	1565	Cubenol
34.035	1.5	1585	Spathulenol
34.223	4.3	1590	Caryophyllene Oxide
35.205	0.8	1614	Nerolidol
35.952	1.6	1633	Aromadendrene
36.89	1.8	1656	α-Bisabolol Oxide A
37.702	2.6	1676	α-Bisabolol Oxide B
38.085	**27.2**	**1686**	**α-Bisabolol**
39.393	1.4	1718	Farnesol 2E-6Z
41.267	**13.6**	**1765**	**Epi-γ-eudesmol**
Others	10.4	–	–
Identified	89.6	–	–
Total	100	–	–

* Retention time (RT), Retention index (RI) or Kovats index (IK) calculated. Identification confirmed by GC-MS.

Another important antioxidant mechanism is deactivating oxidative species. This characteristic is defined as a redox reaction in which a reactive species is reduced while another molecule is oxidized. Here, the *Phlomis bourgaei* reducing power was used as the wavelength reference to the spectrophotometer absorption analysis.[25] The iron chelating activity of EOBU was also evaluated (Figure 3). Both concentrations exhibited iron-chelating activity in a non-concentration-dependent manner, in which EOBU concentrations of g.mL^{-1} showed values of 90.33 % and 91 %, respectively.

Metal ions are critical initiators of lipid peroxidation and the catalysis of these metal ions is associated with diseases of global relevance, such as rheumatoid arthritis and Alzheimer's[26]. Foods contain ferrous ions that can trigger lipid peroxidation using the Fenton reaction. Furthermore, these ions can speed up peroxidation by breaking down lipid hydroperoxides into peroxyl and alkoxyl radicals and these kidnap hydrogens, causing a lipid peroxidation chain reaction.[25] The EOBU showed Fe^{2+} chelating capacity, which implies an excellent binding activity to this metal by reducing the excess of Fe^{2+} in the organism and, consequently, decreasing the toxic effects of lipid peroxidation. Previous studies has indicated that the synthetic molecule β-D-fucopyranoside, structurally is derivided from α-bisabolol, exhibits iron-chelating activityn and this one process may be related to the molecular structure of α-bisabolol in the synthetic molecule. However, further analysis is required to confirm this[27]. As α-bisabolol is the main component of EOBU, it could be responsible for the observed iron chelation characteristic. Species belonging to the same genus as EOBU, for example *B. forficata*, has also apresented a high capacity for chelating iron (25 to 300 μg.mL^{-1}) in a concentration-dependent way.[28]

Moreover, we has tested the EOBU performance in the DPPH inhibitory activity. We have noticed that DPPH is a stable nitrogen-centered free radical used to test the scavenging activity of compounds, which its naturally color is violet and has

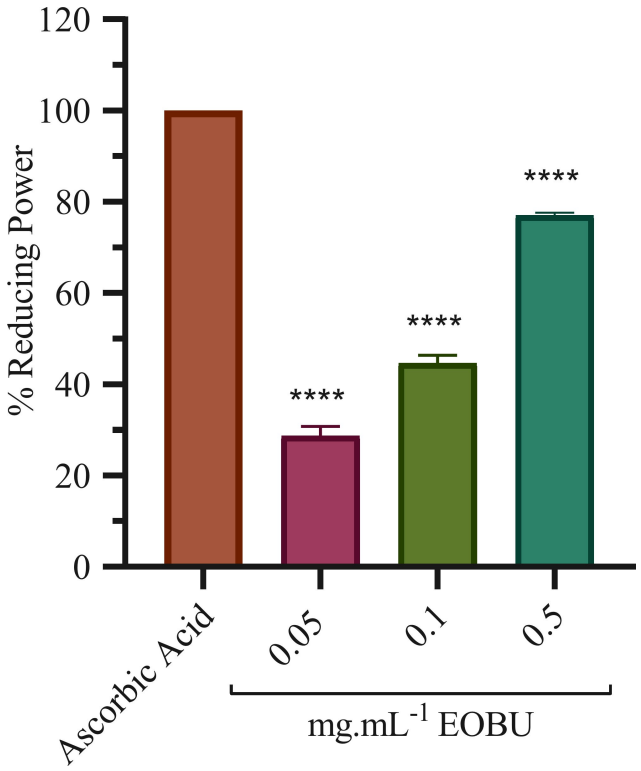

Figure 2. Reducing power of Essential oil of *B. ungulata* (EOBU). Data are plotted as % of the mean ± SD (Standard Derivation of Mean). The reducing power of EOBU at different concentrations demonstrated concentration-dependent reducing power, where all concentrations were significantly different from ascorbic acid (****$p < 0.0001$).

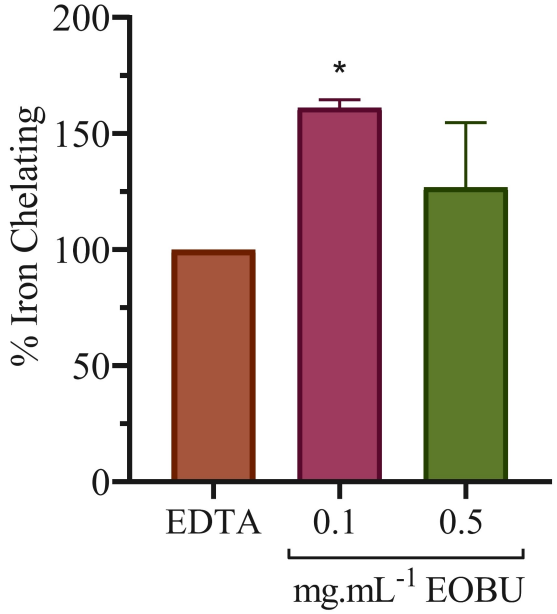

Figure 3. Iron chelation of Essential oil of *B. ungulata* (EOBU). Data are plotted as % of mean ± SD (Standard Derivation of Mean). EOBU showed significantly different iron chelating activity compared to standard EDTA (*$p < 0.05$).

changes when DPPH had been reduced to yellowish (2,2-diphenyl-1-picrylhydrazyl)[29]. EOBU analysis has showed different levels of DPPH radical scavenging activity at concentrations of 0.25 $mg.mL^{-1}$, 0.5 $mg.mL^{-1}$, and 5 $mg.mL^{-1}$. The elimination of the DPPH radical occurs according to the increase in EOBU concentrations with scavenging percentages of 53.7%, 65.2%, and 103.9%, respectively (Figure 4, $p < 0.05$).

EOBU showed DPPH inhibitory activity and these results are consistent with previous research in which the genus *Bauhinia* was studied, where the stratum of *B. variegata* showed 50.51% inhibition of the DPPH radical at a concentration of 20 $\mu g.mL^{-1}$, showing that this genus has good potential for scavenging this free radical.[30] In Santos et al., (2014), leaves and branch extracts of *B. purpurea* also showed DPPH radical scavenging activity, with IC_{50} to 195.80 ± 5.66 $\mu g.mL^{-1}$ and 38.29 ± 2.07 $\mu g.mL^{-1}$ respectively, using default to Quercetin.[31] Other studies have shown good percentages of antioxidant activity against DPPH for fractions of *B. pulchella* extracts, where values of 50.12% and 60.13% were obtained at a concentration of 250 $\mu g.mL^{-1}$.[32]

The genus *Bauhinia* has been known to contain numerous phenolic compounds. Phenolic compounds are a group of phytochemicals produced through secondary metabolism in various plants. Phenolic compounds contain aromatic rings that prevent the oxidation of biomolecules such as proteins, amino acids, carbohydrates, nucleic acids, and lipids. The more hydroxyl groups attached to the aromatic ring, the better the

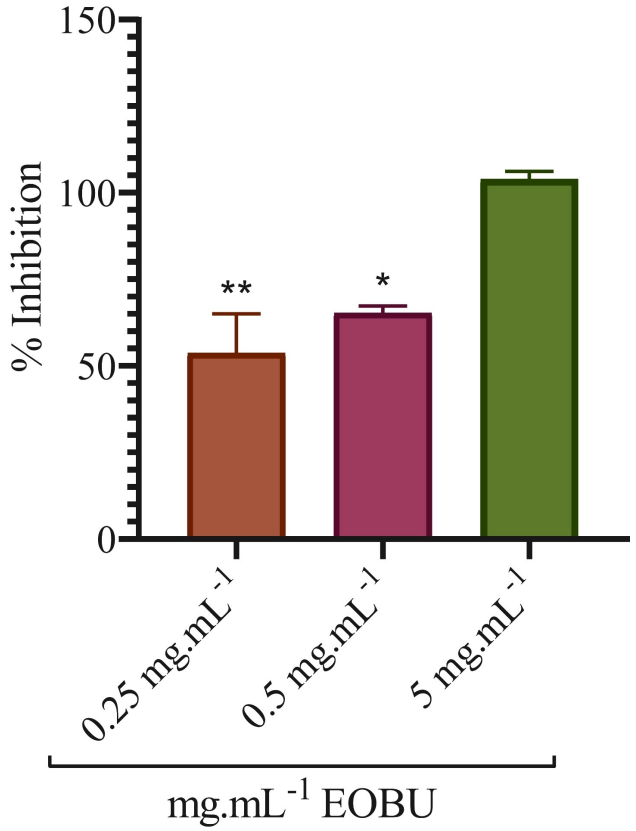

Figure 4. DPPH free radical scavenging capacity of Essential oil of *B. ungulata* (EOBU). Data are plotted as mean ± SD (Standard Derivation of Mean). EOBU showed DPPH free radical inhibitory activity at the concentrations tested, showing significant differences when compared to concentrations of 5 $mg.mL^{-1}$, *$p < 0.05$; **$p < 0.01$ and Ascorbic acid was used as a standard.

antioxidant properties.[33] Besides, these compounds have been able to modulate the biochemistry of the human body, making them of great interest for fighting against diseases related to oxidative stress.[34,35]

Based on the absorbance values of the methanolic extract, the phenolic content of the EOBU was compared with the standard solution of gallic acid. The EOBU presented a total phenolic content of 96.5%, similar to gallic acid, which presented a variation of 99%. Our outcomes have pointed to EOBU as an excellent natural source of phenolic compounds. That is an interesting result compared to the previously shown, whose the stem extracts of *B. ungulata* presented only 46.04% phenolic content.[25] *Bauhinia racemosa* leaves extract had a total phenolic content concentration of 695.1 ± 3.56 mg.g^{-1},[36] which has been similar to our studies findings, indicating that Bauhinia species have good phenol content.

The antioxidant capacity of other plants from the Bauhinia genus has already been analyzed. For instance, *B. racemosa* extract exhibited a 201 ± 3.6 TE.g^{-1} value.[15] The unique chemical profilein EOBU from the Brazilian Amazon may be related to this distinct biological activity. The literature shows that sesquiterpenes are the significant constituents identified in EOBU, which was also confirmed in this study. Besides, these compounds can regulate the oxidative state by exhibiting antioxidant actions.[37]

Therefore, the present study clearly indicates the antioxidant properties of EOBU. However, more detailed work is needed on the activity of the main isolated compounds on the enzymes that make up the cellular antioxidant machinery and their in vitro antioxidant properties. Even so, the elucidation of the activities of the isolated molecules on the antioxidant potential may provide new pharmacological alternatives to combat the increase in free radicals and the exacerbated production of reactive species.

In Silico Analysis

The sesquiterpenes α-bisabolol, Epi-γ-eudesmol, and β-caryophyllene are present at high levels in EOBU. The antioxidant activity of α-bisabolol can be supported by its role in increasing the activity of SOD and CAT enzymes. Also, cells treated with α-bisabolol showed a decrease in ROS levels and an increase in the reduced glutathione (GSH) depletion.[38] β-caryophyllene is a potent molecule with antioxidant action by restoring antioxidant enzymes (SOD and CAT), improving GSH levels and inhibiting lipid peroxidation.[39,40]

Thus, the volatile constituents with the highest concentration in EOBU, α-bisabolol and β-caryophyllene were anchored in the human CAT enzyme binding pockets (Figure 5). Maltol was used as a positive control, as it increases CAT activity and is already established in the literature.[41] α-Bisabolol showed good interaction with CAT, where it obtained a mean value of −5.7 ± 0.4 Kcal.mol^{-1} and β-caryophyllene with a mean value of −6.1 ± 0.5 Kcal.mol^{-1}. The Maltol control showed an average value of −4.4 ± 0.4 Kcal.mol^{-1}. Both α-bisabolol and β-caryophyllene had lower binding energies than Maltol, showing significant

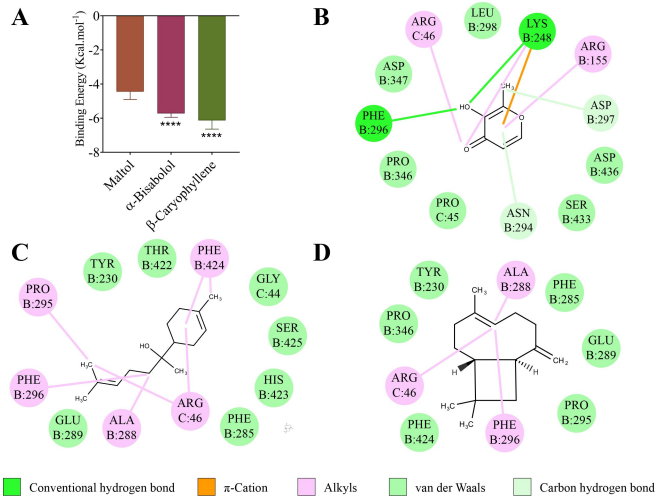

Figure 5. Interactions of phytochemicals and control with human catalase (CAT) enzyme. (A) α-bisabolol and β-caryophyllene showed better protein affinities when compared to Maltol control (****p < 0.0001). (B) The binding profile between Maltol and CAT. (C) Binding of α-bisabolol to CAT. (D) β-caryophyllene binding to CAT. Data are plotted as mean ± SD (Standard Derivation of Mean).

phytochemical differences compared to the positive control (Figure 5A, p < 0.0001).

In the maltol binding profile, the residues PHE B: 296 and ARG C: 46 were common in establishing interactions between the three molecules analyzed in the CAT (Table 2). EOBU components exhibited interactions with the amino acids Arginine, Phenylalanine, Asparagine, Lysine, and Proline. The atomic distances of interactions range from 1.88 to 5.37 Å. Various types of bonds were present at EOBU interaction with the amino acids, including hydrogen bonds, carbon-hydrogen bonds, alkyl bonds, Pi-alkyl bonds, and Van der Waals bonds. In contrast, only Maltol showed hydrogen bonds (Figure 5B), and only β-caryophyllene showed Van der Waals-type bonding forces.

In the present study, we have combined previous data showing the impacts of *B. ungulata* components on CAT enzyme activity and studied molecule-protein interactions in silico. At α-bisabolol and β-caryophyllene have showed higher structural affinities with CAT, by in silico analysis. As mentioned before, Maltol was chosen as a positive control because it has been reported by some researchers. It promoted an increase in the molecular and cellular activity of CAT when join links to the Heme group.[43] Although molecular anchoring occurred in other portions of the protein, we visualized better binding energy between α-bisabolol and β-caryophyllene with CAT, even better than in maltol and CAT anchoring. We have hypothesize that the antioxidant effects of EOBU may also occur directly on cells by protein stimulation, such as CAT.

Conclusions

The results of this study indicate that EOBU is a promising source of bioactive compounds, in particular, sesquiterpenes,

Table 2. Mapping of phytochemical interactions and control with human catalase protein (CAT).

Molecule	Amino acids residues and Chains	Distance (Å)
Maltol	ARG B: 155	5.32 Å
	PHE B: 296	2.10 Å
	ASP B: 297	3.27 Å
	LYS B: 348	1.88 Å, 4.06 Å
	ARG C: 46	and 3.99 Å
		4.23 Å
α-Bisabolol	ALA B: 288	4.01 Å
	PRO B: 295	4.83 Å
	PHE B: 296	4.97 Å
	PHE B: 424	5.21 Å and 4.64 Å
	ARG C: 46	5.16 Å and 4.61 Å
β-Caryophyllene	ARG C: 46	5.18 Å
	ALA B: 288	5.37 Å
	PHE B: 296	5.17 Å

such as α-bisabolol. The high antioxidant activity of EOBU may be linked to specific chemical constituents, particularly sesquiterpenes, and the remarkable abundance of total phenols. In addition, EOBU can neutralize oxidative damage due to its property of iron chelation, neutralization of free radicals, reducing action, and its major constituents interactions with the CAT enzyme. Our studies encourage further researches into the effects of primary EOBU components on the human antioxidant system, as, to the best of our knowledge. No studies have been reporting the antioxidant capacity of essential oil with the species in this research so far. Finally, such properties of EOBU can benefit the development of new drugs designed to reduce oxidative stress in diseases related to this mechanism.

Experimental Section

Obtaining leaves of Bauhinia ungulata

Fresh leaves of *B. ungulata* had been collected during the rainy season (from July to September) in the municipality of Boa Vista, Roraima, Brazil, with the following georeferences were located at 2°51′11.03976 N 60° N latitude and 60° 38′26 de longitude W and taken to the laboratory of the NPPGCT Graduate Program at the Federal University of Roraima. One specimen was duly identified by Dra. Amélia Tuler and has been listed heritage in the UFRR Herbarium, with number 9685 and registered with SISGEN number A789974.

Essential oil extraction

The chosen plant material was cleaned using distilled water and then transferred to a round bottom flask. It was then subjected to hydrodistillation in a Clevenger apparatus with a double Spell condenser for 2 hours continuously for obtaing the EOBU. The fresh leaves had been cut into pieces of about 1 cm^{-1} and transferred to a round bottom flask in the proportion of 1000 g and 6 L of distilled water. The flask was heated on a heating mantle at 100 ± 2 °C. The hydrolate was removed from the EOBU by adding anhydrous sodium sulfate and stored in a freezer at −20 ± 2 °C.[20]

Chromatography

Gas chromatography coupled with mass spectrometry (GC-MS)

The analysis was performed on a GC-MS (QP2010) ULTRA (Shimadzu) gas chromatograph equipped with a Rxi-1 ms (30 m 0.25 mm×0.25 μm) Column (Restek) in temperature variations of 50 °C (3 min), increasing 3 °C.min^{-1}, up to 230 °C. At 1 μL of EOBU sample was diluted at 1% in chloroform that also was injected with an initial temperature of 250 °C, together a split mode ratio (1:10), GC-MS Interface at 250 °C and an electronic ionization MS detector operated with ionization energy of 70 eV at 250 °C. The Helium gas was used as a carrier with a flow rate of 2.0 mL.min^{-1}. Data was acquired using the GC-MS Solution Software (Shimadzu) of each EOBU, where were analyzed and compared to spectra from the spectral component libraries (NIST11) collection of the National Institute of Standards and Technology (NIST).

Gas chromatography with flame ionization detector (GC-FID)

The HP 7820 A Gas Chromatograph (Agilent), equipped with a capillary column measuring 30 m×0.32 mm×0.25 μm (Agilent), was used to identify the constituents of the oil at 50 °C (0 min), 3 °C.min^{-1}. After it was up to 230 °C. At 1 μL of EOBU sample diluted at 1% in chloroform was injected, with an initial temperature of 250 °C in Split ratio (1:30). The FID detector, with a temperature of 250 °C and it was also carrier gas, H_2, at 3 mL.min^{-1}. Data Acquisition Software was chosed: with EZChrom Elite Compact (Agilent). The quantitative analysis was accomplished using standard areas from the chromatograms obtained by GC-FID.

In vitro antioxidant activity of EOBU

Total antioxidant capacity test (TAC)

These assays comprises the reduction of Mo (VI) to Mo (V) by sulfated polysaccharides, followed by a green phosphate/Mo (V) complex formation at acid pH. An aliquot of EOBU (1 mg.mL^{-1}) was added to a reagent solution composed of sulfuric acid (0.6 mg.mL^{-1}), also potassium phosphate (28 mmol.L^{-1}) and ammonium molybdate (4 mmol.L^{-1}).[42] The solution was stirred in a vortex mixer for 90 minutes at 100 °C. After cooling, the absorbance of each sample was determined at 695 nm by using a spectrophotometer. TAC was expressed as an ascorbic acid equivalent.

Reducing power test

EOBU at concentrations from 0.05 to 0.5 mg.mL^{-1} were incubated with 0.2 mol.L^{-1} phosphate buffer (pH 6.6) and 1% potassium ferricyanide (m/v) for 20 minutes at 50 °C. After the incubation period, 10% (m/v) trichloroacetic acid was added to terminate the reactions, followed by adding 0.1% (m/v) iron chloride. Then, the samples were analyzed in a spectrophotometer at 700 nm. Ascorbic acid was considered the standard for calculations, and the results were expressed as a percentage of reducing power compared to ascorbic acid.[43]

Iron ion chelating capacity test (Fe^{2+})

The iron chelation test was performed according to the methodology by Costa et al., (2010).[42] The reaction mixture, containing samples of $FeCl_2$ (0.05 mL, 2 mM) and ferrozine (0.2 mL, 5 mM), was shaken well and incubated for 10 min at room temperature. The reading was performed in a spectrophotometer at 562 nm, and EDTA was used as a standard. EOBU were used at concentrations from 0.5 and 0.1 mg.mL^{-1}. The results have been expressed according to equation 2.

$$\% \text{ de Chelation} = ([\text{Acontrol} - \text{Assemple}]/\text{Acontrol}) \times 100 \quad (2)$$

Elimination capacity of 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The ability of EOBU to scavenge free radicals was analyzed by Brand-Williams and coworkers' method.[44] EOBU at 0.25 mg.mL^{-1}, 0.5 mg.mL$^{-1,}$ and 5 mg.mL^{-1} was added to 2.0 mL of a 0.1 mmol.L^{-1} DPPH ethanolic solution. After 30 min of incubation at room temperature, the absorbance was measured at 517 nm. The results were expressed as a percentage of radical scavenging according to equation 3.

$$\text{Elimination of radicals DPPH } (\%) = [1 - (\text{As}/\text{A0})] \times 100 \quad (3)$$

Phenolic constitution

The dosage of EOBU phenolic compounds was determined by the spectrophotometric method of Folin-Ciocalteau.[45] The experiments were carried out at room temperature under dark conditions. Phenolic compounds reduce the Folin-Ciocalteau reagent, changing the color from yellow to green. Samples were read by spectrophotometry at 765 nm. Gallic acid was used as a positive control.

In Silico Analysis

Molecular docking

The phytochemicals in EOBU and Maltol (β-caryophyllene and α-bisabolol) were obtained from PubChem with the respective descriptors: α-bisabolol (CID: 1549992), β-caryophyllene (CID: 5281515) and maltol (CID: 8369).[46] The three-dimensional structure of the human CAT was obtained from the Protein Data Bank (ID: 1TGU), with a resolution of 2.80 Å. In order to define binding sites suitable for molecular docking, GRaSP software was utilized. This program relies on machine learning to identify these sites by evaluating the accessibility of residues to solvent, as well as considering the physicochemical characteristics and established interactions.[47] Molecular docking tests were performed using Autodock Vina software.[48] The defined grid box is sized 50x60x50 with a spacing of 0.375 Å, with the center of mass as 40.583, 39.019, and 24.834. Molecular interactions between ligands and amino acids were demonstrated and analyzed using the BIOVIA Discovery Studio viewer.

Statistical Analysis

The outcomes were expressed in graphics as mean and standard deviation. Statistical analysis was performed by Student's Test T and ANOVA followed by the Tukey-Kramer test and Bonferroni ($p < 0.05$) using GraphPad Prism 8 Software.

Author Contributions

S.R.N.A.M.: Research, Conceptualization, Methodology, Data curation, Formal analysis, Writing - original draft, Written - review and edition review. **A.J.S. and L.A.L.P**: Formal analysis, Writing - original draft, Writing - review and editing. **A.C.G.R.M.**: Formal analysis, Data curation, Writing - review and revision. edition. **I.C.B.**: Research, Methodology, Conceptualization, Data curation, Relevant results from AutoDock Vina. Molecule Poses Best Vine Score Major Hydrophobic Interactions Major H Bonds Total Per Molecular Fit of EOBU. **P.G.**: Resources, Writing - proofreading, editing, supervision, validation. **R.M.G.M.**: Written - revision, investigation, editing, and validation. **A.M.H.M**. and **W.S.P**. Contribution to experimental antioxidant analysis. **A.A.M.F.**: Project management, Supervision, Conceptualization, Data curation, Formal analysis, Validation, original draft, Written review, edition, and acquisition of financing.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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