Chapter

Borututu (*Cochlospermum angolense welw*) Extracts with Antioxidant Activity: Pressurized Liquid Extraction Used

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Abstract

Borututu (*Cochlospermum angolense welw*) is a plant native to Africa. The infusion obtained from borututu roots by decoction with water has been traditionally consumed by many African communities for the treatment of malaria due to its hepatoprotective properties. The search for ecological alternatives to obtain functional borututu extracts based on the application of techniques linked to new technologies, such as pressurized liquid extraction (PLE), was studied in comparison to the most commonly applied conventional solid-liquid extraction processes. Using PLE was used to evaluate different green/ecological solvents (water, ethanol, and ethyl acetate), providing lower consumption, different extraction temperatures (50–200°C), short extraction times (10 minutes), obtaining extraction yields with high concentrations of total phenolics compounds (TPC) for root (57–21%) and flower (47–16%) extracts. Generating greater antioxidant capacity at a temperature of 150°C with water in both root extracts (1413, 3645 μmol/g) and flower extracts (2029–4457 μmol/g) obtained by the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) method, surpassing extracts obtained by the traditional infusion method (1179–1698 μmol/g) root and (1225–3097 μmol/g) flowers. The borututu plant has demonstrated potential for obtaining bioactive extracts for the production of functional ingredients on an industrial scale.

Keywords: borututu, *Cochlospermum angolense welw*, pressurized liquids extraction, antioxidant activity, ecological solvents and phenolics compounds

1. Introduction

In traditional medicine across many cultures, plants are considered the primary source of remedies, and for this reason, they have aroused interest in studying their

chemical composition, bioactive properties, and the mechanisms by which they affect health. Many of these plants are widely consumed as infusions (hot herbal water) as dietary supplements [1].

In some rural communities, traditional therapeutic practices have been carried out based on beliefs, in an empirical way, which is transmitted between generations, providing greater demand for products of natural origin. This interest is due to the wide variety of chemical compounds distributed across various parts of plants (seeds, floral buds, fruits, leaves, stems, and roots) [2]. Among these, the borututu plant tree reaches 6 m in height and 20 cm in diameter, belongs to the Bixaceae family, genus *Cochlospermeaceae*, which has great potential as a source of bioactive extracts commonly known as Borututu or Brututu (*Cochlospermum angolense welw) C. angolense* is an African tropical tree native to Angola [3–5]. There are also authors who also refer to its presence in some places in the Democratic Republic of the Congo [6] and on a smaller scale in some areas in southern Guinea [7].

Borututu (*Cochlospermum angolense welw*) has been traditionally used by different communities in Africa as a natural remedy against various pathologies, generating benefits for health when incorporated as a food in the diet, as it has been described in the literature as a source of bioactive compounds with a potential effect on health, among them, with antioxidant capacity. The consumption of this plant has been carried out by obtaining extracts through infusion for traditional therapeutic purposes due to its medicinal and nutritional properties. **Figure 1** shows some of the parts of the plant (root, leaves, flowers, fruit) [8].

Borututu roots are currently marketed in Europe primarily by herbalists and commercial establishments in Portugal, packaged as tea or nutritional supplements due to nutritional and medicinal properties, described by several authors [6, 9–14], with claims regarding antioxidant, antimalarial, antimicrobial, and hepatoprotective or neuroprotective and antitumor activity, beneficial health properties, related to its consumption. Likewise, Presber [15] demonstrates antiplasmodic activity of extracts of the root husk of the species *C. angolense in vitro*, such as influenza virus inhibition and the treatment of diseases caused by plasmodium [15] and worms [16]. Malaria, a disease caused by plasmodium in Africa, particularly in Angola, is the cause of morbidity and mortality for thousands of people of different ages, posing a threat to public health. Some studies have reported three endemic African species, *C. angolense;*

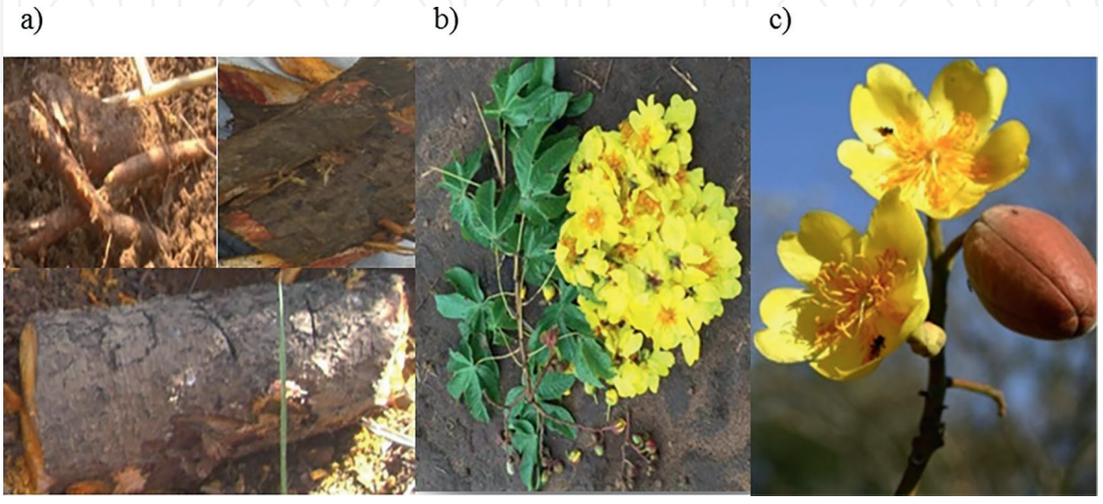

Figure 1.
Parts of the borututu plant. (a) roots, (b) leaves and flowers, (c) flowers and fruit.

C. planchonii; and C. tinctorium, for their hepatoprotective properties and their use in the treatment of malaria [12]. Antiplasmodial activity has been demonstrated through both in vivo and in vitro tests, mainly in essential oils from extracts of roots and leaves of *C. planchonii* and *C. tinctorium* [7]. However, most of the studies carried out were based on obtaining extracts through conventional methods, mainly with boiling water, while the use of efficient new extraction techniques and green solvents to obtain functional ingredients rich in bioactive compounds remains underexplored [6], carried out the fractionation of a borututu extract obtained with methanol, using several solvents of different polarity, with the aim of determining its antioxidant capacity and trying to relate it to its chemical composition, using the extraction technique with pressurized liquids, reported for the first time, whose objective differs from that presented in this study. In recent decades, there has been growing interest in the use of plant matrices through the application of a systematic approach, based on scientific evidence, to obtain bioactive plant extracts, with application in the food and pharmaceutical industries. The pressurized liquid technique (PLE) has been successfully applied as a green extraction technology to extract bioactive compounds from different plant materials [17–19]. The basic principle is based on the use of solvents at temperatures above their boiling point. For this, a certain pressure is applied that can keep the solvent in a liquid state (at subcritical conditions) during the extraction process [20, 21].

The PLE technique was recently investigated for the production of bioactive extracts from borututu roots and flowers using different temperatures (50–200°C) and different solvents: water, ethanol, and ethyl acetate [8].

2. Materials and methods

2.1 Obtaining natural extracts of borututu (*Cochlospermum angolense welw*): Collection and selection of the vegetable matrix, roots, and flowers

Borututu roots and flowers were harvested in the northern region of Angola and dried at room temperature to approximate the conditions used traditionally. They were then packaged in plastic bags and transported to the laboratory for analysis. Subsequently, the samples were ground, and particles smaller than 250μm were obtained using a laboratory sieves column.

2.2 Obtaining natural borututu extracts (*Cochlospermum angolense welw*) by PLE (pressurized liquids extraction)

The natural extracts of borututu roots and flowers were obtained using the PLE technique (using accelerated solvent extraction equipment) (ASE35 0 Dionex Corporation, Sunnyvale, CA, USA), equipped with a solvent control unit [21, 22]. This method allowed the evaluation of three ecological solvents (water, ethanol, and ethyl acetate) at different temperatures (50, 100, 150, and 200°C) with a static extraction mode for 10 minutes, applying a fixed pressure of 10 MPa to keep the solvent in a liquid state above its boiling point. The system was automatically washed with fresh solvent and purged with nitrogen to promote the recovery of the extract, which was collected in a vial. **Figure 2** shows the simplified extraction scheme for PLE [8].

For instance, water, ethanol, and ethyl acetate were chosen as solvents due to their varying polarities, allowing the extraction of a broad range of bioactive compounds.

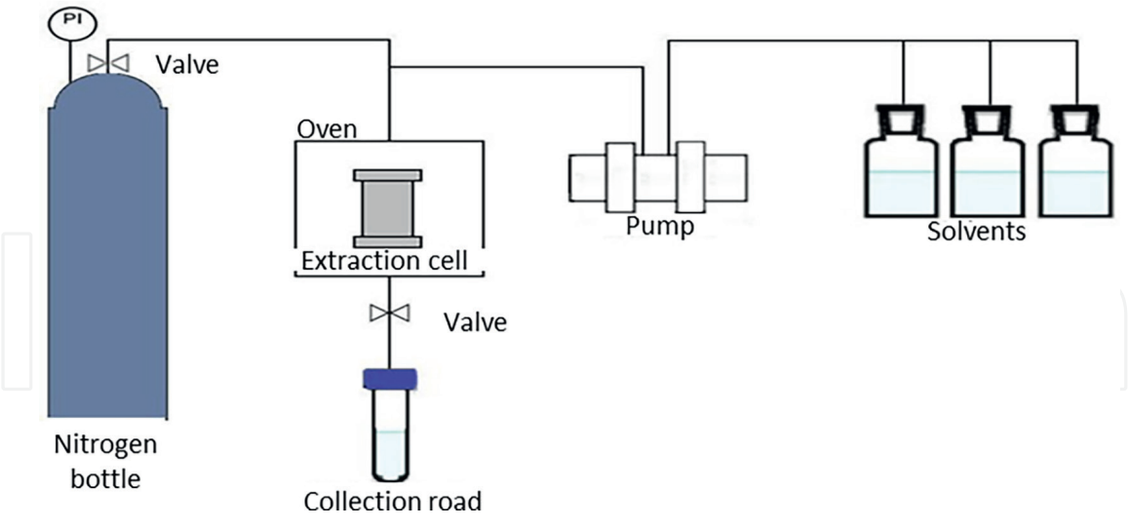

Figure 2.
Simplified extraction diagram using the method PLE. Valve; nitrogen bottle; oven; extraction cell; collection road; pump and solvents.

The temperature range of 50, 100, 150, and 200°C was selected to optimize the extraction of different compounds, as higher temperatures generally improve the solubility of bioactive molecules.

PLE is a highly efficient extraction method that uses high pressure and temperature to increase the solubility of compounds, allowing for faster and more complete extractions compared to traditional methods.

2.3 Obtaining extracts by infusion

For the traditional infusion extraction process, the extracts were obtained using a solvent/sample ratio of 200mL/g, using boiling water and allowed to rest for 5 minutes. All samples obtained with organic solvents were evaporated using a rotavapor (R-210 Buchi) and turbo Vap to eliminate the solvent. Aqueous samples were lyophilized in a Telstar LyoBeta freeze dryer.

2.4 Extraction yields

The extraction yields (extract mass/plant mass) obtained are shown in **Table 1**, indicating their dependence on the variables under analysis. As shown in **Table 1**, the extraction yield increased with the temperature for all solvents and for the two matrices (root and flowers).

3. Results and discussion

The highest extraction yields were obtained with the aqueous solvent, i.e., pure water. From both the roots (57.32%) and flowers (47.33%), both extracted at a maximum temperature of 200°C. It can be deduced that the extracts in general contain a large amount of water-soluble compound. Especially the root extracts, which could explain why they are used as an infusion in African countries and are currently expanding to Europe.

Extraction yields (%)		Temperature (°C)				
		Solvent	**50**	**100**	**150**	**200**
PLE	Root	water	17.94 ± 2.60	19.25 ± 2.89	43.63 ± 0.10	57.32 ± 0.83
		Ethanol	4.58 ± 0.27	7.17 ± 0.76	10.24 ± 0.54	18.89 ± 1.10
		Ethyl acetate	3.80 ± 0.18	3.73 ± 0.16	4.42 ± 0.22	6.43 ± 0.25
		Root infusion		28.39 ± 2.1		
PLE	Flowers	water	17.05 ± 2.4	24.79 ± 6.58	39.44 ± 2.36	47.33 ± 5.69
		Ethanol	6.71 ± 0.90	13.21 ± 1.65	17.09 ± 2.30	18.80 ± 6.28
		Ethyl acetate	5.62 ± 1.85	8.03 ± 0.60	12.42 ± 0.75	15.44 ± 1.41
		Flower infusion		18.64 ± 2.3		

Table 1.
Extraction yields by PLE of the root and flowers (g of extract/g plant).

It was also observed that the yields obtained by infusion of root extracts (28.39%) were higher than those obtained at a temperature of 100°C (19.25%) with pure water, while for flower infusion extracts (18.64%), they presented a lower yield than that obtained by PLE at 100°C (24.79%). We observed a yield greater than 1.5 times the yield obtained with flowers, demonstrating the same behavior with extracts obtained by PLE at 100°C, with a greater presence of more water-soluble compounds in root extracts. On the other hand, as the polarity of the solvents is reduced, the extraction yields by PLE decreased for both vegetable matrices, presenting lower values obtained with ethyl acetate for root extracts varying (3.80–6.43%) while flower extracts will present higher values than the previous ones (5.62–15.44%). If we compared both parts, in flower extracts obtained with this solvent, the yield was between 1.5 and 2.8 times higher than that obtained from the root, suggesting the presence of a greater quantity of non-polar compounds in this raw material. The use of high temperatures allowed the obtaining of extracts from borututu roots and flowers with high extraction yields, as expected when producing an increase in the solubility of solutes and also, to a lesser extent, a decrease in viscosity and surface tension of the solvent with increasing temperature, which favors better penetration of the solvent into the vegetable matrix [21, 23]. The extraction yield also increases with the polarity of the solvent. Therefore, the greatest yields were obtained with water at 200°C (57.32% in the case of roots and 47.33% in the case of flowers), as we mentioned previously, which shows the greatest number of polar compounds containing this material, which allows high yields to be obtained with this technique, just like what has been described from other plants [18, 24, 25]. This is possibly the reason why infusion with water has been used as a common practice by communities for the traditional therapeutic use of borututu [2]. These results in **Table 1** were achieved within just 10 minutes of extraction time. The increase in extraction yield produced with the increase in temperature for each of the solvents, in the case of the root (**Table 1**), the largest increases are produced when high temperatures are used, which is why the PLE technique was especially efficient to obtain extracts with high extraction yields. The extraction yield at 200°C using water increased 3.2 times compared to that obtained at 50°C with the same solvent. From 50 to 100°C, the increase was only 1.1, but this increased considerably before increasing from 100 to 150°C (increase of 2.3), leading to the production of an extraction yield that was 3 times greater when raising the temperature from 100°C (maximum that, theoretically, can be reached with water

in a conventional process where pressure is not applied) to 200°C. Something similar occurred with the remaining solvents, especially from 150°C. In the case of ethanol, the extraction yield increased linearly between 50 and 150°C. In this temperature range, the yield increased around 1.4 times for each 50°C increase in extraction temperature. However, when rising from 150 to 200°C, the yield almost doubled. Therefore, the PLE technique allowed for obtaining high extraction yields, which could not have been achieved by conventional extraction methods carried out at room temperature, where the maximum extraction temperature applied is limited by the boiling temperature of the solvent.

3.1 Concentration of phenolic compounds

In the case of the root (**Table 2**), the highest concentration of TPC was obtained at temperatures of 50 and 100°C (≈21.4%), after which it slightly decreased and remained stable at 150°C (17.30%).

However, the lowest concentrations were generally obtained with ethyl acetate, especially at 50°C (5.27%). Therefore, the presence of water facilitated the extraction of higher TPC values from the root (21–17%), which suggests a higher presence of water-soluble phenolic compounds or relatively high polarity in this material. In contrast, with ethanol and ethyl acetate, there was a progressive increase in concentration as the extraction temperature rose, with the highest results being obtained at 200°C for both the root and flower matrices. For the root extracts, the TPC was 15.28 and 16.30% for ethanol and ethyl acetate, respectively. The root was thus suggested as the best raw material to obtain extracts with a high concentration of TCP, based on the solvents and conditions applied. Regarding the flowers, a different pattern was observed (**Table 2**). The highest concentration was obtained with ethanol at 200°C (16.27%), which was considerably lower than the highest concentrations obtained from the root extracts.

The lower concentrations of phenolic compounds obtained with water from the flowers, compared to the root, suggest that flowers contain fewer water-soluble phenolic compounds. No major differences were observed between the flowers and the root in the maximum concentration of phenolic compounds obtained in the ethanol extracts, which were 16.27 and 15.28% at 200°C.

TPC (%)			**Temperature (°C)**			
		Solvent	**50**	**100**	**150**	**200**
PLE	Root	Water	21.39 ± 0.87	21.37 ± 0.83	17.30 ± 0.28	17.16 ± 0.59
		Ethanol	7.24 ± 0.28	11.07 ± 0.22	13.09 ± 0.58	15.28 ± 0.10
		Ethyl acetate	5.27 ± 0.04	7.84 ± 0.06	8.77 ± 0.17	16.30 ± 0.29
		Root infusion		17.52 ± 0.13		
PLE	Flowers	Water	9.27 ± 0.04	11.77 ± 0.07	9.01 ± 0.05	7.06 ± 0. 16
		Ethanol	5.47 ± 0.28	7.25 ± 0.38	9.08 ± 0.16	16.27 ± 0.13
		Ethyl acetate	4.77 + 0.07	6.96 ± 0.20	7.18 ± 0.06	11.17 ± 0.16
		Flower infusion		28.79 ± 0.11		

Table 2.
Concentration of total phenolic compounds (TPC) of borututu root and flower extracts, expressed as % by weight (mg GAE/100mg extract).

For the flower extracts, the maximum concentration obtained with ethyl acetate at 200°C was 11.17%, which was significantly lower than the 16.30% obtained from the root with the same solvent at the same temperature.

In the extracts obtained by infusion, the concentration of phenolic compounds from the root was 17.52%, similar to that obtained in the PLE extracts with water at 100°C (21.37%) (**Table 2**).

These large differences could be due to the higher solvent/feed ratio used in the case of infusion.

3.2 Identification of the phenolic profile in the root and flower extracts of borututu (*Cochlospermum angolense welw*)

Chromatographic analyses were performed using an HPLC-DAD-ESI (Ion Trap) -MSn system, as described by Herrero et al. [20]. The exact mass determination was carried out using UPLC-ESI-QTOF-MS2 with an Agilent 1290 Infinity LC system coupled to a 6550 Accurate-Mass QTOF (Agilent Technologies, Waldbronn, Germany) with an electrospray interface."

The analyses were performed according to the method described by Garcia-Mendoza et al. [26]. The compounds identified in the two matrices (**Figure 3**) are as follows: roots (A) and flowers (B).

In the identification of phenolic compounds, the different root extracts showed very similar profiles at 250 nm (**Figure 3A**), regardless of the solvent used, and the same occurred with those of the flowers at 340 nm (**Figure 3B**). It was possible to confirm the presence of the same compounds in the extracts obtained from each of the matrices.

Therefore, the solvent did not seem to exert a considerable action on the type of compound extracted.

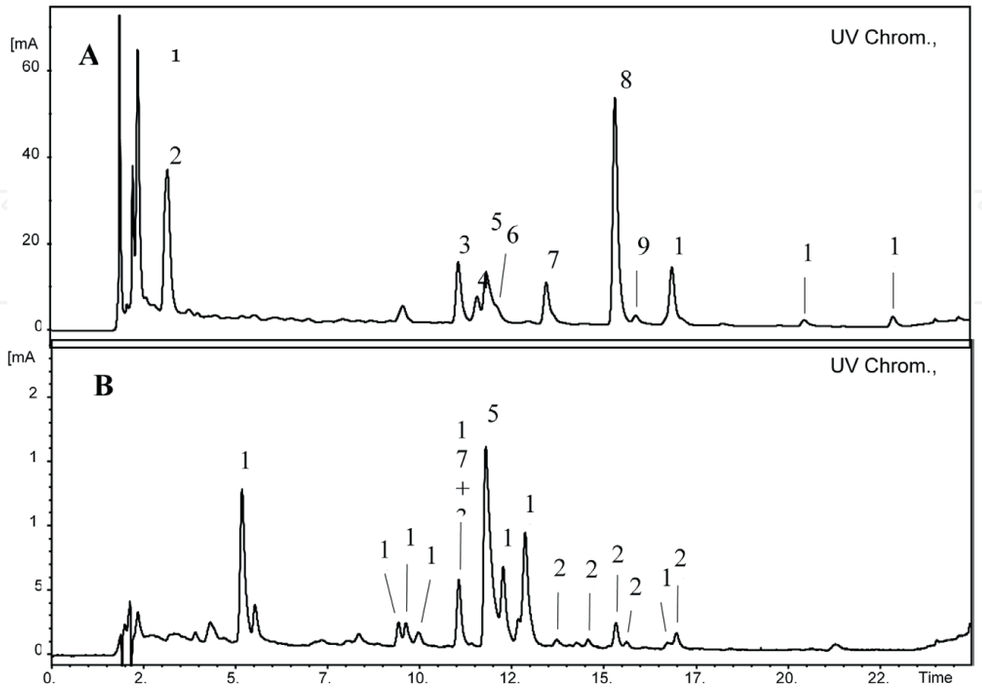

Figure 3.
*UV-Visible chromatograms of borututu extracts obtained by PLE with water, at 100°C, root extracts (250 nm) (***Figure 3A***) and flower extracts (340 nm) (***Figure 3B***).*

The compounds shown in **Figure 3A** correspond to the root extract from the UV-Visible chromatogram at 250 nm, with the main compounds (peaks 3–12) derived from ellagic acid.

These peaks presented UV spectra characteristic of these compounds (~248, 300 sh, 350 sh, 356 nm). In the case of compound 1 (2.3 minutes retention time; UV: 270 nm; MS: [M-H]−: 169.0148 [C7H5O5]−, MS2 [M-H]−: 125.0242 [C5H5O3, (M-H) − CO2]−), this was identified as possibly gallic acid, indicating they could be the pentoside (3) and rhamnoside (6) of ellagic acid (**Figure 3A**).

Compound 5 presented a deprotonated molecular ion m/z 300.9979, with the formula C14H6O8, corresponding to ellagic acid, whose retention time and fragmentation profile coincided with the pure pattern of this compound (**Figure 3A**). Compounds 3 and 6, with deprotonated molecular ions 132 and 146 atomic mass units (amu) higher than compound 5, respectively, presented in their mass fragments a base peak with an m/z ratio of 300.9970, corresponding to the deprotonated ion of ellagic acid, so they could be the pentoside (3) and rhamnoside (6) of ellagic acid (**Figure 3A**). The remaining compounds (4 and 7–12) showed in their spectra the presence of an ion with an m/z ratio of 315.0145, which could correspond to deprotonated methyl ellagic acid (molecular formula [C5H7O8]). Still in the same **Figure 3A**, in 4 and 7–10, it is the base peak, and it is produced by the loss of a 162 amu fragment (hexosyl radical) in compound 4, of a 146 amu fragment (rhamnosyl radical) in compound 10, and of a 132 amu fragment (pentosyl radical) in compounds 7–9. Therefore, these compounds could be the hexoside (4) and rhamnoside (10) of methyl ellagic acid, as well as isomers of the pentoside of methyl ellagic acid (7–9).

Compounds 11 and 12 (**Figure 3A**) exhibit the same deprotonated molecular ion with m/z 599.0690 (molecular formula C27H20O16).

Both present, in addition to the deprotonated methyl ellagic acid ion, an ion (base peak) with m/z 447.0567 ([C20H15O12]), as a consequence of the loss of the 152 amu fragment ([C7H4O4], galloyl radical). Fragmentation of this ion (MS2 [599 → 447]) gives rise to the deprotonated methyl ellagic acid ion. All this led to indicate that these compounds were galloyl isomers of methyl ellagic acid pentosides (**Figure 3A**).

Table 3 lists the phenolic compounds identified in the root extracts.

In this case, ellagic acid, methyl ellagic acid, and their respective glycosides (mainly hexosides and pentosides) are the most representative compounds commonly found in borututu root, depending on the origin of the plant and the extraction technique.

In the case of flowers, the identified compounds were flavonoids, which can be divided into two main categories: flavonoid-C-glycosides (**Table 4**)—mainly luteolin glycosides and apigenin—and flavonoid-O-glycosides (**Table 5**), primarily luteolin glycosides, quercetin, and myricetin. These compounds are common constituents in flowers.

Flavonoids-C-glucosides (compounds 14-15-17-18-21-24), as shown in **Figure 3B** and **Table 4**, compounds 14 and 15 presented UV spectra of luteolin derivatives and their deprotonated molecular ions (m/z447.0940), with molecular formula C21H20O11, indicated to be, possibly, luteolin-hexoside isomers. Based on their fragment masses, ions with m/z of 357 and 327 ([(MH) - 90/−120]-), characteristic of C-hexosylation, were observed, which in mono-C-glucosyl flavones coincide with [aglycone +71/+41]- ions, which confirmed luteolin (286 + 71/+41) as the aglycone. Moreover, the higher abundance of the aglycone+71 ion in the 6-C-glucosyl derivatives compared to the 8-C-isomer, as well as the presence of the ([(M-H) − 18]−) ion, indicated the C-glucosylation position [10].

Compound	Identification*	Rt	Molecular formula	[M-H]$^-$ *(m/z)*	MS^2 *(m/z)*
1	Gallic acid	2.3	C5H5O3	169.0148	125.0242
2	Protocatechuic acid	3.1	C5H5O2	153.0193	109.0293
3	EA pentoside	11.0	C19H14O12	433.0402	300.9966
4	MEA hexoside	11.6	C21H18O13	477.0682	315.0140
5	EA	11.8	C14H6O8	300.9979	
6	EA rhamnoside	12.1	C20H16O12	447.0569	300.9970
7	MEA pentosid	13.4	C20H16O12	447.0570	315.0145
8	MEA pentosid	15.3	C20H16O12	447.0574	315.0142
9	MEA pentosid	16.0	C20H16O12	447.0569	315.0147
10	MEA rhamnoside	16.8	C21H18O12	461.0732	315.0147
11	MEA galloyl pentoside	20.4	C27H20O16	599.0689	447.0567 315.0146
12	MEA galloyl pentoside	22.8	C27H20O16	599.0691	447.0569 315.0144

**EA: ellagic acid; MEA: methylellagic acid.*

Table 3.
Retention time (Rt), molecular formula, and main ions generated in the analysis of phenolic compounds from root extracts of borututu.

Compound	Identification	Rt	Molecular formula	[M-H]$^-$ *(m/z)*	MS^2 *(m/z)*
14	luteolin –8-*C*- hexoside	9.4	C21H20O11	447.0939	357.0617
15	luteolin –6-*C*- hexoside	9.6	C21H20O11	447.0941	357.0615
17	Apigenin-8-6-*C*- hexoside	11.1	C21H20O10	431.0992	341.0663
18	Apigenin-6-*C*- hexoside	12.3	C21H20O10	431.0989	341.0665
21	Apigenin-8-*C*-(6- Acetyl) hexoside	14.5	C22H22O11	473.1095	341.0660
24	Apigenin-6-*C*-(6- Acetyl) hexoside	16.9	C22H22O11	473.1091	341.0662

Table 4.
Retention time (Rt), molecular formula, and main ions generated in the analysis of derivados Flavonides-C-glucosidos from borututu flower extracts.

The phenolic compound profiles were markedly different between the root and flower extracts. Only three compounds were common to both matrices: ellagic acid, its pentoside, and the rhamnoside of methyl ellagic acid."

Similarly, isomers 17 and 18 differed from the previous ones in their deprotonated molecular ions, as well as in the lower fragment ions 16 amu. On the other hand, the aglycone+71/+41 ions (341/311), characteristic of mono-C-glucosyl apigenin derivatives, allowed them to be formulated as apigenin-8-C-hexoside and apigenin-6-C-hexoside, which could be vitexin (apigenin-8-C-glucoside) (17) and isovitexin (apigenin-6-C-glucoside) (18) (**Table 4**). For compounds 21 and 24, the characteristic mono-C-glucosyl-apigenin ions (341/311) were also detected, as in 17 and 18, but instead of the [(M-H)-90/−120]-ions, losses of-132(90 + 42)/−162(120 + 42)-from

Compound	Identification	Rt	Molecular formula	$[M-H]^-$ *(m/z)*	MS^2 *(m/z)*
16	Myricetin-3-O-glucuronide	9.9	C21H18O14	493.0634	317.0307
19	Quercetin-3-O-glucuronide	12.8	21H18O13	477.0669	301.0355
20	Quercetin-3-O-pentoside	13.7	C20H18O1	433.0776	
22	Isorhamnentin-3-O-pentoside	15.3	C21H20O11	447.0945	
23	Luteolin-7-O-glucuronide	16.6	C21H18O12	461.0729	285.0395

Source: Ref. [8].

Table 5.
Retention time (Rt), molecular formula, and main ions generated in the analysis of phenolic compounds from borututu flowers extracts.

the [M-H]-, which indicated the presence of an acetyl radical (42 amu) on the C-glucosylation sugar, were observed. Moreover, this acetylation was at the 6″-position of the hexose, and the [(M-H) -(120 + 42)]-ion implies an overall loss of the acetyl radical plus the remaining sugar moiety from C-glycosylation, which contains carbons 6″-3″. Therefore, these compounds could be named as apigenin-8-C-(6-acetyl) hexoside and apigenin-6-C-(6-acetyl) hexoside, so they could be 6″-acetyl-vitexin (apigenin-8-C-(6-acetyl) glycoside) (21) and 6″-acetyl-isovitexin (apigenin-6-C-(6-acetyl) glycoside) (24).

b) *Flavonoids-O-glucosides* The rest of the compounds (16, 19, 20, 22, and 23) also show the peaks identified in **Figure 3** and **Table 5**, which present deprotonated molecular ions of flavonoid monoglucosides and fragmentations corresponding to O-glucosides, in which the losses of the glucuronyl (−176, in compounds 16, 19, and 23) and pentosyl (−132, in compounds 20 and 22) radicals are observed to give rise to deprotonated ions of their respective aglycones, specifically, myricetin (16), quercetin (19 and 20), isorhamnetin (22), and luteolin (23).

Their spectra indicated that 16, 19, 20, and 22 are substituted at position 3, so they could be myricetin-3-O-glucuronide (16), quercetin-3-O-glucuronide (19), quercetin-3-O-pentoside (20), isorhamnetin-3-O-pentoside (22), and, possibly, luteolin-7-glucuronide (23). The phenolic compound profile was very different from that determined in the root extracts. Only three compounds were found in common in both plant matrices (roots and flowers): ellagic acid, its pentoside, and the rhamnoside of methyl ellagic acid.

3.3 Quantification of phenolic compounds in root and flower extracts

The quantification of phenolic compounds identified in the root (**Table 6**) and flower (**Table 7**) extracts, obtained by pressurized liquid extraction (PLE) with water at 100°C, is shown in **Table 6**.

Differences were observed in the abundance of these compounds in each of the extracts. For the analyses, the samples were injected at the same concentration, so that a greater area of one of the identified compounds meant a greater abundance of that compound in the extract with respect to the others. In general, the concentration of most compounds decreased as the polarity of the solvent decreased. Thus, the higher amount, in turn, shows a higher concentration of the identified compounds, which was obtained with water, both for root and flower extracts. The type of extraction (infusion and PLE with water at 100°C) did not seem to exert any effect, obtaining practically identical abundances for each of the

Peak	Compound	(mg/g extract)	CV (%)
3	Ellagic acid pentoside	0.37 ± 0.01	2.18
4	Methyl ellagic acid hexoside	*<LOQ	—
5 + 6	Ellagic acid and rhamnoside of ellagic acid	0.66 ± 0.00	0.68
7	Methyl ellagic acid pentoside	0.19 ± 0.01	5.26
8	Methyl ellagic acid pentoside	1.51 ± 0.04	2.50
9	Methyl ellagic acid pentoside	*<LOQ	—
10	Methyl ellagic acid rhamnoside	0.39 ± 0.01	1.34
11	Galloyl pentoside of methyl ellagic acid	*<LOQ	—
12	Galloyl pentoside of methyl ellagic acid	*<LOQ	—
Total		3.12	

Source: Ref. [8].

Table 6.
Concentration (mg/g) of phenolic compounds identified in root extracts obtained by PLE at 100°C, with water.

Peak	*Compound	(mg/g extract)	CV (%)
14	Luteolin-8-C-hexoside	0.29 ± 0.02	7.21
15	Luteolin-6-C-hexoside	0.49 ± 0.06	12.98
16	Myricetin-3-O-glucuronide	0.13 ± 0.03	22.08
5	Ellagic Acid	5.28 ± 0.01	0.10
18	Apigenin-6-C-hexoside	4.27 ± 0.17	4.02
19	Quercetin-3-O-glucuronide	5.22 ± 0.08	1.47
20	Quercetin-3-O-pentoside	*<LOQ	—
21	Apigenin-6-C-(6-acetyl) hexoside	0.06 ± 0.03	
22 + 23	Isohamentin-3-O-pentoside and Luteolin-7-O-glucuronide	0.99 ± 0.07	7.56
10 + 24	Rhamnoside-methyl ellagic acidand Apigenin-6-C-(6-acetyl) hexoside	0.30 ± 0.06	19.78
Total		17.03	

**<LOQ: Below limit of quantification. Source: Ref. [8].*

Table 7.
Concentration (mg/g) of phenolic compounds identified in flower extracts obtained by PLE at 100°C, with water.

compounds. In contrast, the lowest abundances were obtained with the solvent of lower polarity, namely ethyl acetate.

Among the phenolic compounds identified, a higher content of phenolic compounds was determined in the flower extracts, with a total of 17.03 mg/g, being more than 5 times higher than in the root extracts, which presented a total of 3.12 mg/g, as shown in **Table 6** for the root extracts and for the flower extracts (**Table 7**).

The coefficient of variation of each of the compounds identified from the root (**Table 6**) was less than 6%, showing a good repeatability of the results. In this case, the highest variability (5.26%) was shown by the methyl ellagic acid pentoside (7),

and the lowest (0.68%) corresponded to the sum of the ellagic acids and their rhamnoside structure (5 + 6). As for their concentration in the extract, the methyl ellagic acid pentosides (7–9) are the compounds present in the highest amount (1.7 mg/g), followed by the sum of ellagic acid and its rhamnoside (0.66 mg/g). In contrast, the galloylated forms of methyl ellagic acid (11 and 12) were found in concentrations below the limit of quantification. Although ellagic acid and its derivatives were the main phenolic compounds identified in root extracts (3.12 mg/g), the amount of ellagic acid (5.28 mg/g) in flower extracts (**Table 7**) was 1.7 times higher (**Table 7**).

In the case of flowers, along with ellagic acid, the other two compounds (18 and 19) that occurred in high concentration were quercetin-3-O-glucuronide (5.22 mg/g), followed by apigenin-6-C-hexoside (4.27 mg/g). Based on the low coefficients of variation (less than 4.1%), the reproducibility obtained in relation to the concentration of these three major compounds in the flower extracts was high. For the rest of the compounds quantified, the concentrations were less than 1 mg/g.

Flavonoids, including the flavonols quercetin and myricetin and the flavones luteolin and apigenin, which were identified in the PLE extracts of flowers (**Figure 3B** and **Table 7**), have also been described as potent antioxidants [27–30]. These compounds could also be responsible, together with ellagic acid, for the antioxidant capacity of these extracts. Pereira et al. [12], observed a positive correlation between the content of phenolic compounds and the antioxidant capacity, determined by DPPH of an extract obtained by water infusion of borututu root. Moreover, although their antioxidant action in Cochlospermum extracts is discrete, especially compared to that provided by phenolic compounds, the presence of specific apocarotenoids of this genus, such as cocloxanthin and dihydrococloxanthin, would help to increase the antioxidant capacity of root and flower extracts [6, 8, 31]. In a general way, phenolic compounds are bringing much interest due to scientific evidence suggesting potential antioxidant effects related to cardiovascular activities, anticancer, reduction of inflammatory damage, and also as a modulator in immune response among other diseases [32]. Other authors consider that phenolic compounds, such as tannins and flavonoids, are responsible for the antioxidant defense of the plant when it suffers environmental stress or when it undergoes different morphological stages of development that cause an increase in ROS, helping the chemical adaptation of the plant to these conditions [33]. This adaptation occurs through genes that activate the biosynthesis of antioxidant mechanisms, in particular, secondary metabolism genes that help in plant development [33]. It should be noted that the levels of polyphenols and bioactive compounds present in plant extracts can be affected by many factors such as plant growth conditions (soil, climate) [34].

3.4 Evaluation of the biological activity of borututu root and flower extracts

The antioxidant activity of the extracts obtained by PLE and infusion by in vitro methods was determined by two methods: by neutralization of the DPPH radical (**Table 8**), SET method, mediated by one-electron transfer reactions (described by Brand-Williams et al. [35]), and by ABTS (**Table 4**), the method mediated by one-hydrogen atom transfer reactions described by Re et al. [36]. In **Table 8**, the results for the case of root extracts obtained with pressurized water at 50°C and by conventional infusion were quite similar (1208 and 1179 μmol/g, respectively). At 100°C it was slightly higher (1286 μmol/g). However, the antioxidant capacity was lower when using pressurized water at 150°C and reached the maximum antioxidant activity (1413 μmol/g).

AO/DPPH		Solvent	Temperature (°C)			
PLE			50	100	150	200
	Root	Water	1208 ± 1	1286 ± 1	1413 ± 2	1276 ± 1
		Ethanol	1108 ± 3	1156 ± 1	1300 ± 2	1386 ± 4
		Ethyl acetate	770 ± 4	919 ± 1	948 ± 1	1051 ± 4
	Flowers	Water	1295 ± 4	1587 ± 2	2029 ± 2	1820 ± 6
		Ethanol	205 ± 21	210 ± 23	671 ± 23	735 ± 9
		Ethyl acetate	99 ± 63	98 ± 23	241 ± 23	171 ± 6
Infusion		Flowers		1225 ± 2		
		Root		1179 ± 2		

Source: Ref. [8].

Table 8.
Antioxidant activity of borututu root and flower extracts determined by the ABTS method and expressed as Trolox equivalents (μmol Trolox/g extract).

A progressive decrease in antioxidant capacity can be observed as solvent polarity decreases, with the lowest antioxidant capacity values being obtained with ethyl acetate (770 μmol/g, at 50°C). Regarding the behavior at different temperatures by PLE, in general, the antioxidant capacity was relatively similar, even increasing with increasing temperature for the different solvents. In the case of water, this is true up to a temperature of 150°C, after which the TEAC value of the extracts decreases slightly. However, in the case of ethanol and ethyl acetate, the highest antioxidant capacity using these solvents was obtained at 200°C. At that temperature, there was a 25% increase in the antioxidant capacity of the extract with respect to that obtained at 50°C using ethanol. In the case of ethyl acetate, this increase was 36%. As for the results obtained from flowers (**Table 8**), the use of ethanol and ethyl acetate generated extracts with considerably lower TEAC values than those obtained with the same solvents from the root. Considering the flower extracts with the highest antioxidant capacity obtained with each of these two solvents, the highest TEAC values using ethanol and ethyl acetate were 735 and 171 μmol/g, respectively, while the values obtained from the root were 1386 μmol/g with ethanol and 1051 μmol/g with ethyl acetate, which means antioxidant capacities are 1.9 and 6.1 times higher than in the flower extracts using the same solvents. Based on these results, the root gave rise to extracts with considerably higher TEAC values when organic solvents were used. However, the highest TEAC values were obtained with the extracts obtained with pressurized water from the flowers.

In this case, at 150°C it was possible to obtain an extract with a TEAC value of 2029 μmol/g, which was 36% higher than the extract with the highest antioxidant capacity from the root (**Table 8**). As for the effect of temperature on the TEAC values of the flower extracts, their behavior was similar to that observed with the root, with an increase in antioxidant capacity as the extraction temperature increased. In the case of water and ethyl acetate, the highest TEAC values were obtained at 150°C and decreased somewhat as the temperature was increased to 200°C. On the contrary, using ethanol, the highest value was obtained at the maximum temperature of 200°C. When comparing PLE extraction with water at 100°C with respect to traditional infusion, it was observed that the results obtained with pressurized water were 23% higher than those obtained by infusion (1587 and 1225 μmol/g, respectively). As in the case of the root, the temperature that allows PLE extraction led to obtaining extracts

from flowers with higher TEAC values (2029 μmol/g with water at 150°C) than those obtained by infusion (1225 μmol/g), which meant a 66% increase in antioxidant capacity. As with the results obtained in the DPPH method, the antioxidant capacity determined by the ABTS method (**Table 9**) showed that the increase in temperature in the extractions from the root generated an increase in the antioxidant capacity when pure water was used with higher TEAC values, obtained at 150°C, producing a decrease at the maximum extraction temperature, while for ethanol and ethyl acetate, the maximum values were reached at 200°C. In any case, the increase in temperature above the boiling point of the solvent that allows extraction by PLE to be achieved generated substantial increases (**Table 9**).

The TEAC value of the extract obtained by root infusion (1698 μmol/g) was considerably lower than that obtained with pressurized water at 100°C (3475 μmol/g) and those obtained with pressurized water at the four temperatures (2812–3591 μmol/g). In the case of the extracts obtained from borututu flowers (**Table 9**), a behavior similar to that determined for the root extracts was observed. The decrease in solvent polarity led to a reduction in the TEAC value of the extracts so that the extracts with the lowest antioxidant capacity were obtained with ethyl acetate, followed by ethanol (**Table 9**).

The extract with the highest antioxidant capacity was obtained with water at 150°C (4457 μmol/g), which coincides with the behavior observed on the basis of the results obtained with the DPPH method (**Table 8**). Also, as for the results obtained in the DPPH method, the TEAC values obtained with water in the ABTS from flowers (**Table 9**) were considerably higher than those obtained with organic solvents. In the case of flowers, there were large differences in the values obtained between solvents and the antioxidant capacity increased with solvent polarity in both test methods, with the highest values obtained with water at 150°C (2029 μmol/g, determined by DPPH and 4457 μmol/g by ABTS) (**Table 9**).

Therefore, the highest values obtained, both of root and flowers, were reached at temperatures above the boiling point of the solvent (in subcritical conditions) in short periods of time, obtaining higher extraction yields, resulting in a lower use of solvent volume and minimal environmental impact. This demonstrates that PLE was an efficient technique for obtaining extracts with high antioxidant capacity. This can also be verified by comparing the results obtained with an infusion with respect to those of PLE with water. When the temperature was raised to 150°C, the increase in antioxidant

AO/ABTS	**Solvent**		**Temperature (°C)**			
PLE			50	100	150	200
	Root	Water	2812 ± 49	3475 ± 22	3645 ± 52	3590 ± 9
		Ethanol	639 ± 27	1005 ± 25	1366 ± 42	1693 ± 59
		Ethyl acetate	301 ± 1	553 ± 7	445 ± 9	1322 ± 22
	Flowers	Water	2597 ± 18	2952 ± 21	4457 ± 36	3919 ± 56
		Ethanol	483 ± 6	594 ± 7	728 ± 3	1156 ± 5
		Ethyl acetate	275 ± 0	281 ± 2	263 ± 3	809 ± 9
Infusion		Flowers		3097 ± 13		
		Root		1698 ± 12		

Table 9.
Antioxidant activity of borututu one and flower extracts determined by the ABTS method and expressed as Trolox equivalents.

Variable								
	Y (%) Flower	Y (%) Root	TPC Flowers	TPC Root	ABTS Flower	ABTS Root	DPPH Flower	DPPH Root
p-valor*	0.000[1]	0.000[1]	0.000[1]	0.000[1]	0.000[1]	0.000[1]	0.000[1]	0.000[1]
Treatments	Averages (homogeneous groups)**							
Water 50°C	17.047[bcd]	17.917[d]	9.287[d]	21.393[i]	2596.670[g]	2812.000[e]	1295.057[g]	1208.360[g]
Water 100°C	24.795[e]	19.251[d]	11.773[f]	21.270[i]	2951.330[h]	3474.330[f]	1587.103[h]	1285.727[h]
Water 150°C	39.438[f]	43.120[f]	9.010[d]	17.297[h]	4456.330[j]	3645.000[fg]	2028.870[j]	1412.920[k]
Water 200°C	47.315[g]	57.325[h]	7.060[c]	17.157[h]	3918.670[i]	3589.330[f]	1823.117[i]	1276.040[h]
Ethanol 50°C	6.807[a]	4.583[ab]	5.470[b]	7.540[b]	481.670[b]	638.670[ab]	205.150[c]	1107.827[e]
Et. 100°C	13.237[bcd]	7.173[b]	7.253[c]	11.067[d]	598.000[c]	1005.000[bc]	209.777[c]	1155.653[f]
Et. 150°C	17.090[bcd]	10.237[c]	9.083[d]	13.087[e]	728.000[d]	1366.000[cd]	671.437[e]	1299.860[h]
Et. 200°C	18.803[de]	18.888[d]	16.267[g]	15.280[f]	1153.330[f]	1692.670[d]	735.283[f]	1386.360[j]
EA 50°C	5.613[a]	3.870[a]	4.727[a]	5.270[a]	275.330[a]	301.330[a]	99.093[a]	770.380[a]
EA 100°C	8.023[a]	3.730[a]	6.932[c]	7.840[b]	281.330[a]	553.670[ab]	97.737[a]	919.123[b]
EA 150°C	12.417[b]	4.420[a]	7.182[c]	8.770[c]	263.670[a]	444.670[ab]	241.247[d]	947.973[c]
EA 200°C	15.352[bc]	6.433[ab]	11.173[e]	16.300[g]	807.330[e]	1321.330[cd]	171.353[b]	1051.087[d]

Et. -Ethanol; EA. -Ethyl acetate.

Table 10.
Analysis of variance of the results obtained for each of the extraction conditions (different letters indicate significant differences between means at level) (P < 0.05).

capacity of the extract obtained by PLE was 20% higher (DPPH assay) (**Table 8**) and even 2 times higher (ABTS assay) (**Table 9**) than that of the extract obtained by infusion from the root. In the case of flowers, this increase with respect to the extract obtained by infusion was also considerable (66%, determined by DPPH, and 44% by ABTS). In the case of the antioxidant capacity determined by ABTS from the flowers, water at 150°C allowed the highest values to be obtained; likewise, for the root, the most appropriate treatment to achieve the TEAC value with water was at 150°C.

Likewise, for the results determined in the DPPH assay from the flower extracts, as for the ABTS, the best treatment corresponded to water at 150°C, which generated the extract with the highest antioxidant capacity. **Table 10** shows a summary of the main results obtained and their statistical treatment with Duncan's post hoc and Games-Howell tests as appropriate, which made it possible to determine r ($p < 0.05$). Both for the yield obtained from flowers and from the root, the highest significant values were obtained with water at 200°C obtained when water was used as a solvent and water at 100°C. (**Table 10**).

4. Conclusions

- Extraction yields increased with solvent polarity and extraction temperature, reaching a maximum value at 200°C of 57.32% in water extracts from the root and 47.33% in those from flowers.

- The main phenolic compounds identified in the root extracts were gallic, protocatechuic, ellagic, methyl ellagic acids, and the glycosides of the latter two. The glycosides were mainly hexosides and pentosides; one also found rhamnoside forms and a recently described galloyl pentoside derivative.

- The main phenolic compounds identified in the flower extracts were ellagic acid, ellagic acid and methyl ellagic acid glycosides, and flavonoids, further divided into C-glycosides of luteolin and apigenin and O-glycosides of luteolin, quercetin, and myricetin.

- The extracts obtained from each part of the plant presented the same profile of phenolic compounds, independent of the solvent used, varying only the relative abundance of each one of them. This abundance decreased as the solvent polarity decreased.

- The highest antioxidant capacity presented the extract obtained by PLE (1413 μmol/g in DPPH and 3645 μmol/g in ABTS) for roots and (2029 μmol/g in DPPH and 4457 μmol/g in ABTS) for flowers, both obtained at a temperature of 150°C, using water as a solvent. Both matrices present a similar profile and a great potential for obtaining antioxidant ingredients. These values were higher than those obtained by infusion, which for root extracts were (1179 μmol/g for DPPH and ABTS 1698 μmol/g) and (1225 μmol/g for DPPH and 3097 μmol/g for ABTS) for flowers, respectively.

- The PLE extraction technique allowed for obtaining extracts with high antioxidant capacity, both from the root and the flowers, as well as a considerable

concentration and yield of phenolic compounds, using water in only 10 minutes of extraction. These extracts were obtained at a temperature of 150°C, which is above the boiling point of the solvent. The use of water is postulated as an efficient and clean alternative for obtaining extracts with antioxidant capacity from the borututu plant (roots and flowers).

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Acronyms and abbreviations

ABTS	2,2′-azino-bis (3-ethybenzothiazoline-6-sulfonic acid)
ASE	accelerated solvent extraction
AMU	atomic mass unit
AO	antioxidant activity
C. angolense	cochlospermum angolense
DPPH	2,2-diphenyl-1-picryl hydrazyl
EA	ellagic acid
MEA	methyl ellagic acid
GAE	gallic acid equivalent
PLE	pressurized liquid extraction
TEAC	trolox antioxidant capacity
TPC	total phenolic compounds
Trolox	6-hydroxy-2,5,2,8-tetramethylchroman-2-carboxylic acid with 97% of purity
Rt	retention time

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