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# Antioxidant Activity and Phytochemical Composition of *Solanum corymbiflorum* Fractions (Leaves and Fruits)

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**Abstract** The antioxidant activity and phenolic compounds of the chloroform ($CHCl_3$), ethyl acetate (AcOEt) and *n*-butanol (*n*-BuOH) fractions from *Solanum corymbiflorum* leaves and fruits were evaluated. The AcOEt fraction of the leaves presented the highest content of total polyphenols (114.00 mg GAE/g) and the best antioxidant capacity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) test ($IC_{50}$ = 31.90 µg/mL). For the fruits, the same fraction exhibited the highest content of phenolics (99.77 mg GAE/g) and best results in the DPPH test ($IC_{50}$ = 141.47 µg/mL). In relation to 2′,7′-dichlorofluorescein diacetate (DCFH-DA) and thiobarbituric acid reactive substances (TBARS) assays, the $CHCl_3$ fraction of leaves and fruits showed better results than the other samples analyzed. Besides of the phenolic compounds, the alkaloids contributed in the activity. Rutin, chlorogenic and caffeic acids quantified by HPLC are some of phenolic compounds responsible by this activity *S. corymbiflorum* can be a promising source of natural antioxidants. However, more in vivo studies are required to stimulate the consumption and its other potentialities.

***Keywords:*** *Solanum corymbiflorum, antioxidant activity, HPLC, chlorogenic acid, Cyphomandra corymbiflora*

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## 1. Introduction

Several crude extracts and pure natural compounds from plants are reported for having radical scavenging capacity. Intensive research has been performed to characterize the antioxidant properties of extracts or isolates, and identify the compounds that have this activity leading to the development of natural antioxidant formulations in areas as food, medicine and cosmetics [1]. This is possible due the ability of these substances to reduce oxidative stress by neutralizing of the reactive species by hydrogen donation, before they attacking cells and other biological components [2].

For this reason, the commercial value of fruits is increasing in domestic and international markets due also to the recognition of its nutritional and therapeutic properties, so it have been subject to several studies conducted around the world, reporting their nutritional values, especially in relation to the evaluation of the antioxidant activity [3]. Concern about improving health involving natural products with benefits, has enhanced research on antioxidants, because many degenerative human diseases including cancer, cardiovascular and brain diseases have been recognized as being a possible consequence of free radical damage to lipids, proteins and nucleic acids [4].

This way, epidemiological studies suggest the consumption of natural antioxidant such as polyphenol rich foods, teas, fresh fruits or vegetables that have protective effects against diseases [5].

*Solanum corymbiflorum* (syn. *Cyphomandra corymbiflora*) is popularly known as "baga-de-veado". Occurs in the southern states of Brazil [6], and in Argentina, where is known as "ka'a Kururu" (Herb of frog), its leaves are popularly applied on swollen or inflamed legs caused by an infection, in scabies, tick bite, boils, mastitis, low back pain and otitis. Piana et al. [7] confirmed anti-edematogenic and anti-inflammatory activities of this species. In addition to these properties, the fruits of this species are consumed in many Guarani communities and border areas of Brazil [8], second Kinupp [9], when mature with green pulp possess very sweet flavor and pleasant aroma, in the form of juices have mild foaming and potential for making syrup, jams, liqueurs and other desserts.

Considering the several popular uses of the leaves and fruits and absence of research related to this species. The aim of this study was quantify for the first time total polyphenols, flavonoids, condensed tannins and alkaloids in the chloroform ($CHCl_3$), ethyl acetate (AcOEt) and *n*-butanol (*n*-BuOH) fractions from *S. corymbiflorum* leaves

and fruits, also evaluate the antioxidant activity by thiobarbituric acid reactive substances (TBARS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the 2′,7′-dichlorofluorescein diacetate (DCFH-DA) oxidation methods. Taking into account phytochemical analysis, polyphenols were quantified by high performance liquid chromatography (HPLC).

# 2. Methodology

## 2.1. Chemicals

All chemicals used in the tests were of analytical grade. Solvents for the extractions (ethanol, methanol, chloroform, ethyl acetate, and *n*-butanol), Folin-Ciocalteau reagent and iron sulfate ($FeSO_4$) were acquired from Merck (Darmstadt, Germany). Rutin, gallic acid, chlorogenic acid, caffeic acid, DPPH, tris-HCl, thiobarbituric acid and DCFH-DA were acquired from Sigma Chemical Co. (St. Louis, MO, USA).

## 2.2. Plant Collection and Extractions

Fruits and leaves of *S. corymbiflorum* were collected in Gaurama (Rio Grande do Sul State of Brazil) in October (2012). A dried voucher specimen is deposited in the herbarium of the Department of Biology at Federal University of Santa Maria (SMBD 13159). The fruits were used in natura and the leaves were dried in stove for 36 h (temperature of 40°C) and powdered in a knife mill, both were separately macerated with 70% ethanol for a week with daily shake-up. After filtration, the hydroalcoholic extracts were evaporated under reduced pressure to remove the alcoholic solvent. The remaining aqueous was partitioned with solvents of increasing polarity (chloroform, ethyl acetate and *n*-butanol), and were dried (temperature below 40°C) to give each corresponding fraction.

## 2.3. Phytochemical Composition

### 2.3.1. Polyphenols Content

The polyphenol content was evaluated by the colorimetric method described by Chandra and Mejia [10], using the Folin-Ciocalteau reagent. Samples were prepared at a concentration of 0.15 mg/mL. The absorbance were measured at 730 nm. Gallic acid (10 – 100 µg/mL) were used in the calibration curve and the results were expressed in mg of gallic acid equivalents per g of fraction (mg GAE/g).

### 2.3.2. Flavonoids Content

The flavonoids were quantified by method described by Woisky and Salatino [11] which used aluminum chloride (AlCl3 2%) as reagent. The absorbances were measured at 420 nm. Samples were prepared at a concentration of 1 mg/mL. The data were evaluated based on the calibration curve (10 – 100 µg/mL) of rutin and expressed in mg of rutin equivalents per g of fraction (mg RE/g).

### 2.3.3. Determination of Condensed Tannins

Condensed tannins were quantified by vanillin method described by Morrison et al. [12]. Solutions of 25 mg/mL of the fractions were used, and absorbance were measured at 500 nm. The data were expressed in mg of catechin equivalent per g of fraction (mg CaE/g), based on the calibration curve (5 – 30 µg/mL) of catechin.

### 2.3.4. Determination of Total Alkaloids

Total alkaloids were determined by reaction of precipitation with Dragendorff's reagent, described by Sreevidya and Mehrotra [13]. The extracts were prepared at concentration of 80 mg/mL and the absorbance used was 435 nm. Total alkaloid content was quantified by a calibration curve of bismuth nitrate (5 – 30 µg/mL), the values were expressed in mg of total alkaloids per g of fraction (mg/g).

### 2.3.5. HPLC analysis Phenolic Compounds

HPLC analysis was assessed on a Shimadzu HPLC system (Kyoto, Japan), Prominence Auto-Sampler (SIL-20A) with Shimadzu LC-20 AT reciprocating pumps connected to a DGU 20A5 degasser, CBM 20A integrator, UV–VIS detector DAD SPD-M20A and LC Solution 1.22 SP1 software. The chromatographic analyses were carried out under gradient conditions using a C-18 column (250 mm × 4.6 mm) packed with 5 µm diameter particles. Solvent 1 (water containing 2% acetic acid) and Solvent 2 (methanol) composed the mobile phase used, according to slightly modified method of Piana et al. [14]. The samples were prepared at concentrations of 10 mg/mL were filtered through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA). The flow rate was 0.6 mL/min and the injection volume was 40 µL. Identification of phenolics was performed by comparing retention times and the Diode-Array-UV spectra with those of standards. For quantification, was used integration of the peaks using the external standard method and calibration curve (5–40 µg/mL). Chlorogenic acid, caffeic acid, gallic acid and rutin.

## 2.4. Antioxidant Activity

### 2.4.1. DPPH Test (Radical scavenging Capacity)

The fractions of the fruits and leaves were assessed in the presence of DPPH stable radical, according to method of Choi et al. [15]. The samples were tested at 7.81, 15.62, 31.25, 62.50, 125 and 250 µg/mL. Spectrophotometric analysis were used to measure the antioxidant capacity and to determine the inhibitory concentration required to inhibit 50% of the DPPH in the assay, expressed as IC50 (µg/mL).

Each sample was mixed with 1.0 mL of DPPH 0.3 mM in ethanol solution for 30 min. The absorption was measured at 518 nm. A solution of DPPH in ethanol was used as negative control and gallic acid as positive control. The tests were performed in triplicate and the calculation of the antioxidant capacity followed the equation:
Where: Abssample is absorbance of each fraction; Absblank is absorbance of the samples without adding the DPPH; Abscontrol is absorbance the solution of ethanol in DPPH.

$$\%\ \text{inhibition} = 100 - \frac{(\text{ABS}_{\text{sample}} - \text{ABS}_{\text{blank}}) \times 100}{\text{Abs}_{\text{control}}}. \quad (1)$$

### 2.4.2. DCFH-DA Oxidation Assay

Male Wistar rats with 3.0–3.5 months of age and weighing 270–320 g were kept in 3–4 animals per cage. They had continuous access to water and food in the place

with controlled temperature (22±3°C). The animals were used in accordance to the guidelines of the Brazilian Association for Laboratory Animal Science (COBEA). The rats were killed and the brain tissue was quickly dissected, weighed and solubilized in Tris-HCl 10 mM, pH 7.5 (1/10, w/v). The homogenate was centrifuged for 10 min at 4,000 rpm and the supernatant was used in the analyses. The experimental protocols were approved by the Ethics Animal Committee of Universidade Federal de Santa Maria (CEUA UFSM; Protocol 23081.005770/2009-38).

The substrate DCFH-DA was used to evaluate intracellular formation of reactive species, according to Myrhe et al. [16]. The supernatant of the homogenate was incubating at 37°C with different concentrations of the fractions (7.81, 15.62, 31.25, 62.50, 125 and 250 µg/mL) for 60 min. Aliquots were removed and placed to a assay tube with DCFH-DA for 1 hour in the dark. The fluorescence was measured using 488 nm for excitation and 520 nm for emission. The results were expressed as µmol of oxidized DCF per mg protein and calculated by interpolation in a standard curve of oxidized DCF (constructed in parallel), corrected by the content of protein. Ethanol was used as negative control, gallic acid as positive control, and the concentration of the fraction required to reduce intracellular formation of reactive species was expressed as $IC_{50}$ (µg/mL).

#### 2.4.3. TBARS Assay - Inhibition of Lipid Peroxidation

The same homogenate of the previous assay was used in the TBARS assay. An aliquot of 100 µL was incubated for 1 h at 37 °C with recently prepared $FeSO_4$ (10 µM), in the presence of the different concentrations of samples (7.81, 15.62, 31.25, 62.50, 125 and 250 µg/mL). The TBARS production was determined as described by Ohkawa et al. [17]. Gallic acid was used as positive control and the concentration of the fraction required to reduce the lipid peroxidation in 50% was expressed as $IC_{50}$ (µg/mL).

### 2.5. Statistical Analysis

All assays were performed in triplicate. For phytochemical composition were used calibration curves, the values found were expressed as mean±S.D. and statistically analyzed by analysis of variance (one-way ANOVA) followed by Tukey test, and $p < 0.05$ were considered significant. For antioxidante activity assays, the $IC_{50}$ values were statistically analyzed by same way and expressed mean±S.E.M.

## 3. Results and Discussion

### 3.1. Phytochemical Composition

The contents of total polyphenols, flavonoids and condensed tannins were higher in the fractions of the leaves than in the fruit fractions, as shown in the Table 1.

For the leaves, the AcOEt fraction presented the highest content of total polyphenols (114.00 mg GAE/g), followed by *n*-BuOH and $CHCl_3$ fractions. The quantity of flavonoids showed the same trend of the polyphenols, which were higher in the AcOEt fraction (54.66 mg RE/g). Piana et al. [7] also found these compounds in the leaves crude extract of the same species, agreeing with the results of this study.

The AcOEt fraction of the fruits exhibited the highest content of phenolics compounds (99.77 mg GAE/g) followed by *n*-BuOH fraction (49.90 mg GAE/g). For the determination of flavonoids contents, all samples exhibited modest values, while the AcOEt and *n*-BuOH fractions did not show significant differences of values ($P > 0.05$). For the leaves and fruits, the condensed tannins are present only in the AcOEt and *n*-BuOH fractions and the total alkaloids were found in all the fractions, but the highest content are in the $CHCl_3$ fractions, as expected.

**Table 1. Total polyphenols (TP), flavonoids (TF), condensed tannins (CT) and total alkaloids (TA) in the fractions of *S. corymbiflorum* leaves and fruits**

| | TP (mg GAE/g) | TF (mg RE/g) | CT (mg CaE/g) | TA (mg/g) |
|---|---|---|---|---|
| **Leaves** | | | | |
| $CHCl_3$ | 54.11 ± 1.21$^c$ | 23.33 ± 1.53$^c$ | - | 17.73 ± 1.54$^a$ |
| AcOEt | 114.00 ± 1.62$^a$ | 54.66 ± 0.65$^a$ | 19.82 ± 1.55$^a$ | 14.58 ± 1.62$^b$ |
| *n*-BuOH | 63.00 ± 1.55$^b$ | 28.9 ± 0.81$^b$ | 16.22 ± 0.92$^b$ | 10.05 ± 1.87$^c$ |
| **Fruits** | | | | |
| $CHCl_3$ | 44.57 ± 0.74$^c$ | 3.30 ± 0.76$^c$ | - | 27.82 ± 0.06$^a$ |
| AcOEt | 99.77 ± 0.33$^a$ | 5.32 ± 0.14$^a$ | 7.11 ± 0.15$^b$ | 19.13 ± 0.04$^b$ |
| *n*-BuOH | 49.90 ± 0.11$^b$ | 5.40 ± 0.23$^a$ | 11.64 ± 1.34$^a$ | 10.27 ± 0.05$^c$ |

Values are expressed as mean ± standard deviation. GAE: gallic acid equivalents, RE: rutin equivalents, CaE: catechin equivalents, $^{a-c}$Means with the different letters in each column for leaves and fruits are significantly different ($p < 0.05$), by analysis of variance (One-way ANOVA) (n = 3).

Studies by Hari et al. [18] found alkaloids, flavonoids, tannins in *Solanum nigrum* extracts. Another study in *Solanum guaraniticum* leaves, Zadra et al. *[19]* found the same trend for the amount of polyphenols (AcOEt fraction > *n*-BuOH fraction > $CHCl_3$ fraction), and showed presence of alkaloids and flavonoids in similar quantity in the AcOEt fraction when compared with same fraction of the *S. corymbiflorum* leaves.

In all fractions of the leaves analyzed by HPLC were possible find antioxidant compounds (Table 2 and Figure 1). The AcOEt and *n*-BuOH fractions of the leaves showed a large amount of rutin (53.55 and 24.79 mg/g, respectively), chlorogenic (49.11 and 47.38 mg/g, respectively) and caffeic acids. Gallic acid was found in AcOEt fraction in a lesser amount, as well as the chlorogenic and caffeic acid in the $CHCl_3$ fraction. Research performed by Piana et al. [7] showed which the chlorogenic acid and rutin were some the compounds responsible by anti-inflammatory activity of this species.

The AcOEt fraction of the fruits showed large amount of chlorogenic (87.37 mg/g) and caffeic (11.59 mg/g) acids, and modest values these compounds in CHCl3 fraction. Gallic and chlorogenic acid were found in the n-BuOH fraction (Table 2 and Figure 2). Chlorogenic acid and caffeic acid both have vicinal hydroxyl groups, they have antimutagenic, carcinogenic and antioxidant activities [20].

**Table 2. Amount of phenolic compounds analyzed by HPLC/DAD.**

| | $CHCl_3$ (mg/g) | AcOEt (mg/g) | *n*-BuOH (mg/g) |
|---|---|---|---|
| **Leaves** | | | |
| Gallic acid | - | 3.10 ± 0.22[a] | - |
| Chlorogenic acid | 2.04 ± 0.37[a] | 49.11 ± 0.23[d] | 47.38 ± 0.38[d] |
| Caffeic acid | 3.04 ± 0.11[b] | 28.73 ± 1.99[c] | 12.14 ± 0.49[a] |
| Rosmarinic acid | | 19.14 ± 0.45[b] | 15.32 ± 0.89[b] |
| Rutin | - | 53.55 ± 0.13[e] | 24.79 ± 1.31[c] |
| **Fruits** | | | |
| Gallic acid | - | - | 2.05 ± 0.08[b] |
| Chlorogenic acid | 2.04 ± 0.17[a] | 87.37 ± 0.21[a] | 47.38 ± 0.17[a] |
| Caffeic acid | 1.67 ± 0.03[b] | 11.59 ± 0.33[b] | - |

Mean ± standard deviation; - phenolic compounds not found in the fraction. [a–e] Means with the different letters in each column for leaves and fruits are significantly different ($p < 0.05$), by analysis of variance (n = 3).

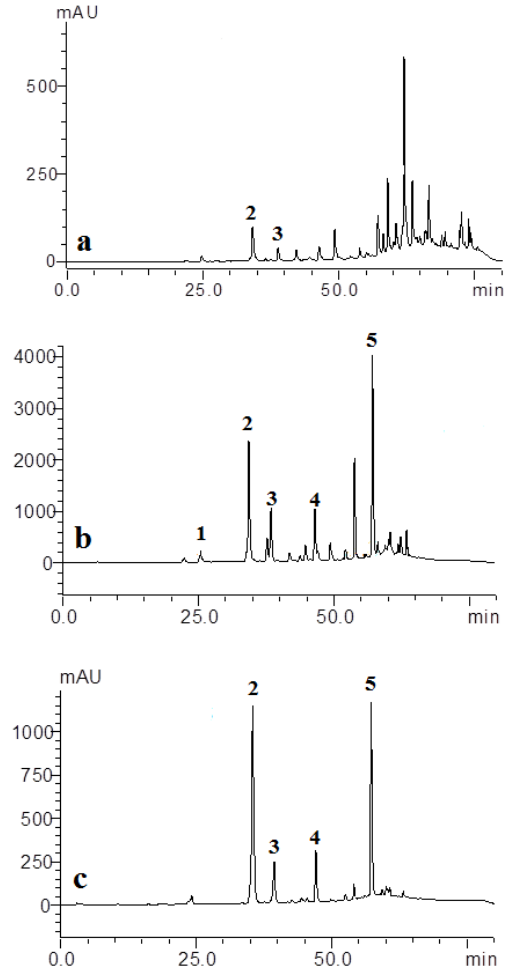


**Figure 1.** Chromatographic profile of the fractions leaves (a) $CHCl_3$, (b) AcOEt (c) *n*-BuOH of *S. Corymbiflorum* leaves at 326 nm. 1 correspond to gallic acid peak (RT: 14.06 min), 2 chlorogenic acid (RT: 34.73 min), 3 caffeic acid (RT: 38.12), 4 rosmarinic acid (RT: 44.72) 5 rutin (53.48 min); RT: retention time

## 3.2. DPPH Test

Taking into consideration the $IC_{50}$ values, the fractions of the leaves showed better antioxidant activity than fractions of the fruits.

The AcOEt fraction of the leaves showed the best antioxidant capacity ($IC_{50}$ = 31.90 µg/mL), similar value of the gallic acid standard ($IC_{50}$ = 29.12 µg/mL) (Table 3), followed by the *n*-BuOH fraction ($IC_{50}$ = 63.97 µg/mL), and finally, the higher $IC_{50}$ value was of the $CHCl_3$ fraction. The results found in the DPPH test followed the same trend of the amount of polyphenols and flavonoids found in each fraction. Boligon et al. [21]. showed similar results in fractions of *Tabernaemontana catharinensis* leaves, who found lower $IC_{50}$ value in the AcOEt followed by *n*-BuOH fractions. In accordance with the authors, this fact could be explained based on the similarity between compounds with high antioxidant capacity, which were extracted by these organic solvents. In study of Piana et al. [7], the crude extract of the leaves showed $IC_{50}$ = 23.94 µg/mL, agreeing to our results.

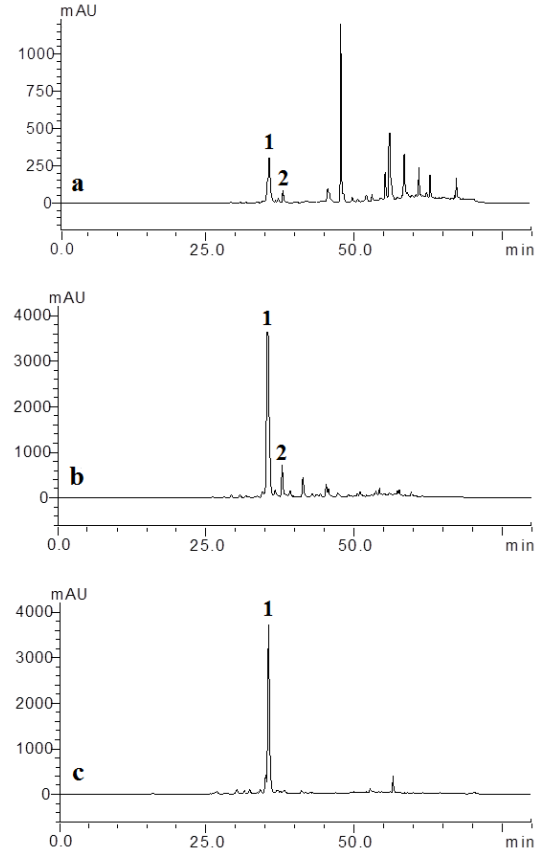


**Figure 2.** Chromatographic profile of the fractions of the fruis. (a) $CHCl_3$, (b) AcOEt (c) *n*-BuOH of *S. Corymbiflorum* leaves at 326 nm. 1 correspond to chlorogenic acid (RT: 34.73 min), 2 caffeic acid (RT: 38.12) RT: retention time

**Table 3. $IC_{50}$ values for antioxidant activity assays.**

| | DPPH (µg/mL) | DCFH-DA (µg/mL) | TBARS (µg/mL) |
|---|---|---|---|
| **Fruits** | | | |
| $CHCl_3$ | - | 136.17 ± 0.54 | 105.59 ±10.62[b] |
| AcOEt | 141.47 ± 0.25[b] | - | - |
| *n*-BuOH | 165.69 ± 0.27[c] | - | - |
| **Leaves** | | | |
| $CHCl_3$ | 126.55 ± 0.74[d] | 57.55 ± 0,63 | 100.73 ± 2.37[b] |
| AcOEt | 31.90 ± 1.63[b] | - | 162.29 ± 10.48[c] |
| *n*-BuOH | 63.97 ± 0,21[c] | - | - |
| Gallic acid | 29.12 ± 0.32[a] | - | 80.07 ± 2.89 |

Values are expressed as mean ± S.E.M.[a–d] Means with the different letters in each column for leaves and fruits are significantly different ($p < 0.05$), by analysis of variance (One-way ANOVA) (n = 3).

Just the AcOEt and *n*-BuOH fractions of the fruits showed good DPPH radical scavenging capacity, with $IC_{50}$ values of 141.47 and 165.69 µg/mL, respectively (Table 2), higher slightly values compared to the gallic acid standard ($IC_{50}$ 29.12 µg/mL). It is probable that

mainly the phenolic compounds, flavonoids and condensed tannins found in higher concentrations in these fractions, have greatly contributed in this activity. Studies of Piana et al. [14], showed the best antioxidant capacity for AcOEt fraction by DPPH test in *Tabernaemontana catharinensis* fruits. Furthermore, Sudha et al. [22] found antioxidant properties in AcOEt extract of *Solanum muricatum* fruit associated to phenolic and flavonoids contents.

So, the phenolic compounds found in plants demonstrated potent antioxidant activity mainly due their redox properties, which allow them to act as reducing agents, singlet oxygen quenchers, hydrogen donators and chelating agents of metal ions [20].

### 3.3. DCFH-DA Oxidation Assay

Only the $CHCl_3$ fractions of the leaves and fruits were able to reduce the oxidation of DCFH-DA in 50% compared to the basal group ($IC_{50}$ = 57.55 and 136.17 µg/mL, respectively), demonstrating excellent antioxidant activity (Table 2).

These results are agreeing with Zadra et al. [19] where the $CHCl_3$ fraction from *Solanum guaraniticum* showed activity in this assay. Besides the phenolic compounds and flavonoids, other substances as alkaloids may have contributed in this activity and acted synergistically, mainly, in this fraction. Research conducted by Jung et al. [23] found inhibitory effects of two isolated alkaloids from rhizoma of *Coptis chinensis* (groenlandicine and coptisine) by DCFH-DA oxidation assay.

Furthermore, Koduru *et al*. [24] isolated two steroid alkaloids (tomatidine and solasodine) from *Solanum aculeastrum* and found strong antioxidant activity and synergistic effect in DPPH test of the isolated compounds. This fact can explain, at least in part, the activity of the $CHCl_3$ fractions that has considerable amount of alkaloids.

### 3.4. TBARS Assay

The antioxidant activity evaluated by the TBARS assay is based on the formation of malondialdehyde (MDA), a subproduct of lipid peroxidation. The level of MDA in brain tissue was affected mainly by the treatment with the $CHCl_3$ fraction of the leaves, that showed the best result ($IC_{50}$ = 100.73 µg/mL), followed by AcOEt fraction. The $CHCl_3$ fraction of the fruits presented similar activity ($IC_{50}$ = 105.59 µg/mL) compared with same fraction of the leaves (Table 3). The gallic acid showed $IC_{50}$ = 80.07 µg/mL.

Lipids are components of cell membranes and its have as function maintain the control and cell structure. They are the first target of the attack of reactive species. The lipid peroxidation is associated with various human diseases such as atherosclerosis, cancer, diabetes, lung and neurodegenerative disorders. The TBARS assay is the most commonly used to evaluate this condition [25].

To protect the cells, humans developed an antioxidant protection system, which works interactively and synergistically with antioxidant compounds of the plants, neutralizing free radicals before they start attacking the cells [26].

In $CHCl_3$ fractions of the leaves and fruits besides polyphenols, larger quantity of alkaloids were found, these compounds have shown antioxidant properties [27]. A possible hypothesis for the mechanism of action is the presence of aromatic hydroxyl group, that may be responsible for its antioxidant efficiency, similarly to phenolic antioxidants, which have chain-breaking mechanism by donation of phenolic hydrogen. Moreover, antiperoxidative activity of alkaloids has been also reported [28].

## 4. Conclusion

The results clearly prove by the first time that the fractions leaves and fruits of *S. corymbiflorum* possess notable antioxidant activity and contributed to reveal some phytochemical characteristics of this species. All the fractions showed considerable presence of phenolics and alkaloids. For leaves and fruits, the AcOEt fraction showed the best antioxidant capacity in the DPPH test, which can be attributed to its high content of total polyphenols, mainly the rutin and chlorogenic acid. Otherwise, the $CHCl_3$ fraction showed the best result in the DCFH-DA oxidation and TBARS assays. Besides of the phenolic compounds, the alkaloids also contributed in this activity. Caffeic acid and a small quantity of gallic acid were found by HPLC confirms the free radical scavenging activity. *S corymbiflorum* have antioxidant potential and can be a promising source of natural antioxidants. However, more in vivo studies are required to stimulate the consumption and its other potentialities.

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