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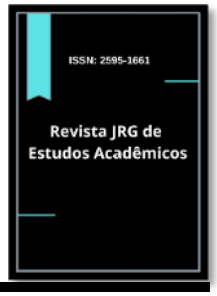

Antioxidant activity, total phenolics, total flavonoids and phytochemical screening of Solanum sanctae-catharinae Dunal (Solanaceae) aerial parts

Atividade antioxidante, fenólicos totais, flavonoides totais e screening fitoquímico das partes aéreas de *Solanum sanctae-catharinae* Dunal (Solanaceae)

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Abstract

Solanum sanctae-catharinae Dunal it's a native tree from Brazilian Atlantic Forest. The *Solanum* genus comprises numerous species that have been found to exhibit antidiabetic, antitumor, and antimalarial properties. The current study seeks to assess the antioxidant properties, of crude extracts and crude extracts fractions of *S. sanctae-catharinae* aerial parts. The Folin-Ciocalteu method and the aluminum-trichloride method were used to determine TPC and TFC in crude extracts and fractions of aerial parts. Two assays were conducted to evaluate antioxidant activity: the DPPH test and the phosphomolibdenium method. Differences between sample means and control groups were determined using ANOVA (one way) with Tukey's test coupled. Correlations were obtained using the Pearson correlation coefficient for bivariate correlations. Our findings shown that the remnant fraction of the ethanolic crude extract demonstrates the most relevant antioxidant activity, followed by the chloroform fraction of the ethanolic crude extract, the remnant fraction of the ethanolic-acetic crude extract. The presence of secondary metabolites such as phenols, flavonoids, coumarins, and alkaloids in *S. sanctae-catharinae* phytochemical screening suggest that this antioxidant activity can be attributed to the presence of this secondary metabolites. Furthermore, the results indicate a substantial quantity of polyphenols and flavonoids in both extracts.

Keywords: DPPH. TAC. Polyphenols. Alkaloids. Steroids

Resumo

Solanum sanctae-catharinae Dunal é uma árvore nativa da Mata Atlântica brasileira. O gênero Solanum abrange inúmeras espécies que apresentam propriedades antidiabéticas, antitumorais e antimaláricas. O presente estudo busca avaliar as propriedades antioxidantes, dos extratos brutos e frações dos extratos brutos das partes aéreas de S. sanctae-catharinae. O método Folin-Ciocalteu e o método do tricloreto de alumínio foram utilizados para determinar a quantidade total de compostos fenólicos e flavonoides em extratos brutos e frações das partes aéreas. Foram realizados dois ensaios para avaliar a atividade antioxidante: o teste DPPH e o método do fosfomolibdênio. As diferenças entre as médias das amostras e os grupos de controlo foram determinadas utilizando ANOVA (one way) com o teste de Tukey acoplado. As correlações foram obtidas utilizando o coeficiente de correlação de Pearson para correlações bivariadas. Os nossos resultados mostraram que a fração remanescente do extrato bruto etanólico demonstra a atividade antioxidante mais relevante, seguida da fração clorofórmica do extrato bruto etanólico e da fração remanescente do extrato bruto etanólico-acético. A presença de metabólitos secundários como fenóis, flavonóides, cumarinas e alcalóides no screening fitoquímico de S. sanctae-catharinae sugere que esta atividade antioxidante pode ser atribuída à presença destes metabólitos secundários. Além disso, os resultados indicam uma quantidade substancial de compostos fenólicos e flavonóides em ambos os extratos.

Palavras-chave: *DPPH. TAC. Polifenóis. Alcaloides. Esteroides*

1. Introduction

The interest in natural antioxidants, particularly those present in plants, has grown among both the consumer and scientific communities due to their potential to neutralize free radicals in biological systems (ZEHIROGLU & SARIKAYA, 2019).Cardiovascular and cerebrovascular diseases have been related as potential results of damage caused by free radicals to lipids, proteins, and nucleic acids in cellular tissues, to stabilize this effect, a safe way to combat these free radicals is to improve our body's defenses by using natural antioxidant substances in our diet (GIANAZZA & BRIOSCHI, 2021).

In the *Solanum* genus, there are many groups of secondary metabolites with potential therapeutic use, including steroidal saponins, pregnanes, lignans, sterols, phenols, coumarins, coumestanes (KAUNDA & ZHANG, 2019; MILNER *et al.*, 2011*)*. Many species of the *Solanum* genus are known to have antidiabetic, antitumor, and antimalarial properties and have been used by folk medicine to treat some diseases, as *Solanum americanum* Mill., which is used to treat ulcers, and *Solanum villosum* Mill., which is used to treat gonorrhea (SHAH, *et al.*, 2013). Previous studies by Fidrianny *et a*l., Gandhiappan & Rengasamy, Prakash *et al.*, and Loganayaki demonstrated the antioxidant capacities of some plants in the *Solanum* genus. Given the diversity of molecules from the secondary metabolism of *Solanum* genus that have antioxidant action, our study is justified.

S. sanctae-catharinae is a plant that belongs to *Solanum* genus, in Brazil, it's geographical distribution comprises the south and southeast regions of the Atlantic Forest (STEHMANN *et al.*, 2015). Although there has been a relative abundance of pharmacological research into the species included in the *Solanum* genus, this study focused on the *S. sanctae-catharinae* species, a plant that has not had any studies documenting its biological, pharmacological, or antioxidant properties. To date, this is the first study of the antioxidant activity of *Solanum sanctae-catharinae*.

2. Methodology

Plant Material

In March 2022, we gathered the aerial parts (stem and leaves) of *S. sanctae-catharinae* from an Atlantic Forest remnant area known as Capão CIFLOMA, situated within the Jardim Botânico campus of the Federal University of Paraná (UFPR) in Curitiba, Paraná, Brazil (S25°26' 49.4" WO 49° 14' 21.7"). The species identification was performed by M.Sc Alan Lessa the collected material as a *S. sanctae-catharinae* species by comparing it with the voucher n° 11836 registered at the Herbarium Escola de Florestas Curitiba of UFPR. Access to genetic heritage was registered and authorized by the Brazilian system SisGen (or the National System for the Management of Genetic Heritage and Associated Traditional Knowledge) registered under the code A11D443.

Phytochemical Screening

An extraction was carried out on 40 g dried aerial parts of *S. sanctae-catharinae* using a 200 mL of 70% hydro-ethanolic (v/v) solution, the plant material was extracted in hot water bath (70° C) for 90 minutes. Then we use the hydro-ethanolic extract to obtain the fractions by liquid/liquid partitioning with solvents of different polarities in the following order: hexane, chloroform, and ethyl acetate. The qualitative reactions were then conducted using the fractions of hydro-ethanolic extract to perform a

phytochemical screening of the secondary metabolites present in *S. sanctae-catharinae* aerial parts. The objective of these reactions was to observe color changes and/or the formation of precipitates, which are characteristic of each class of phytochemicals. Based on this, a qualitative phytochemical assay was carried out to investigate the presence of the following secondary metabolites: alkaloids, flavonoids, coumarins, and steroids (MOREIRA, 1979; HOMEM, SZABO & MIGUEL, 2015; MIGUEL, 2003).

Preparation of the extracts and fractions

The *S. sanctae-catharinae* ethanolic crude extract was obtained from 2 kg of dried aerial parts in ethanol using a Soxhlet apparatus for 11 hours. After this stage, the remaining plant material from the ethanolic extraction was extracted again with an ethanolic-acetic solution (95:5) in Soxhlet apparatus for 8 hours.

The crude extracts were concentrated in a rotary evaporator (70° C) on a low-pressure system. The crude extracts were then used to obtain the fractions by liquid/liquid partitioning with solvents of different polarities in the following order: hexane, chloroform, and ethyl acetate. The partiotioning of the crude extract was performed using a modified Soxhlet as described by Carvalho in 2009. After partitioning, they were placed in a hot water bath (70° C) so that as much of the solvent as possible was evaporated.

According to the following formula, the extraction yield has been calculated as a percentage of the weight of the plant powder used:

$$Y\ (\%) = (EW/WP) \times 100$$

Y: yield of extract in %; EW: Extract weight obtained; WP: weight of the plant material obtained.

Determination of total phenolic content

The total phenolic content was determined using the Folin–Ciocalteu method, with Woiski & Salatino (1998) methodology modified by Surek (2022). In a 96-well microplate, 100 µL of the aqueous Folin – Ciocalteu reagent (2N), 20 µL of the crude extract or fractions samples (200 µg/mL), then were added 80 µL of sodium carbonate solution (7.5 g/100 mL). The plate was left to rest in the dark for 60 min, and absorbance was measured using a Multiskan FC microplate reader (Thermo Scientific, Waltham, MA, USA) at 690 nm. The concentration of total phenols in the extracts and fractions was determined using an external calibration curve with gallic acid (25, 50, 75, 100, 125 and 150 µg/mL) and expressed in mg gallic acid equivalents (mg GAE/g) of plant extract or fraction (GAE = gallic acid equivalents). The concentration of the total phenolics was determined as mg of gallic acid equivalent using the equation of the line obtained from the gallic acid standard curve. The estimation of phenolic compounds in the fractions was performed in triplicate, and the results were averaged.

Determination of total flavonoid content

Total flavonoid contents of plant extracts and fraction were determined by using the aluminium chloride colorimetric method described by Devequi-Nunes (2018), with minor modifications (Dowd method). In a 96-well microplate, 100 µL of the crude

extracts or fractions samples (200 µg/mL), then were added 100 µL of aluminum chloride in methanol (2 g/100 mL). The plate was left to rest in the dark for 60 min. The absorbance was measured using a Multiskan FC microplate reader (Thermo Scientific, Waltham, MA, USA) at 414 nm against the blank, which consisted of the plant extract or fraction and methanol (100 µL each). The concentration of total flavonoids was compared with the standard curve of quercetin in methanol (5, 10, 15, 20, 25 and 30 µg/mL) and expressed in mg QE/g of plant extract or fraction (QE = quercetin equivalents). The concentration of the total flavonoids was determined as mg of quercetin equivalent using the equation of the line obtained from the gallic acid standard curve. The estimation of total flavonoids in the extracts was carried out in triplicate and the results were averaged.

Evaluation of total antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of plant extracts and fractions was evaluated using the phosphomolibdenium method described by Prieto, Pineda, and Aguilar (1999), with minor modifications. An aliquot of 300 µL of the crude extract or fraction partitioned samples (200 µg/mL), except the remnant fraction of ethanolic crude extract, due to the absorbance at this concentration was <1000 we adjust the ascorbic acid sample concentration at 20 µg/mL. Then we combined with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and 1.5 mL of purified water. The tubes were slightly capped and incubated in a boiling water bath at 95 °C for 90 min. After the samples had cooled to room temperature, 200 µL of each tube was added to the cavity of a 96-well microplate, and the absorbance of the aqueous solution was measured using a Multiskan FC microplate reader (Thermo Scientific, Waltham, MA, USA) at 690 nm against the blank. A typical blank solution containing 1 mL of reagent solution, 300 µL of the same solvent used for the sample, and 1.5 mL of purified water was incubated under the same conditions as the rest of the samples. For the crude extract samples and fraction partitioned samples, the water-soluble antioxidant capacity was expressed as the percentage equivalent of ascorbic acid as 100% (% EQAA).

Radical scavenging activity using DPPH method

The radical scavenging activity of the extracts and fractions was determined as described by Salgueiro (2016). To conduct the test, 29 µL DPPH solution diluted in methanol (0.3 mMol/L) were added to 96-well microplates using a micropipette, then were added 71 µL of the extract or fraction sample (200 µg/mL). For each sample we performed a negative group, prepared the same way descripted above but we substitute DPPH reagent for methanol, in order to discount the natural color from our extract or fraction sample. A typical blank solution was prepared the same way described above but instead a plant extract or fraction samples we used methanol. After 30 min of the reaction at rest in the dark, the absorbances were measured at 540 nm using a Multiskan FC microplate reader (Thermo Scientific, Waltham, MA, USA). A positive control was prepared with ascorbic acid at the concentration of 200 µg/mL in methanol for comparison with the *S. sanctae-catharinae* extracts and fractions.

Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

AAR % = (AE − AN / AB) × 100.

AAR %: % radical scavenging activity AN: uptake of the negative control; AE: uptake for the extracts; and AB: uptake for the blank solution

The results obtained were compared with the standard curve of Trolox in methanol (2, 2, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 µg/mL) and expressed in trolox EQ µg /mL and the percentual value compared plant extract or fraction (TroloxEQ = trolox equivalents). The estimation of results of the antioxidant activity in the extracts were carried out in triplicate and the results were averaged.

Statistical analysis

Experimental results were mean ± S.D. of three parallel measurements and analyzed with the statistical program Past® 4.03. Differences between sample means and control groups were determined using ANOVA (one way) with Tukey's test coupled. Correlations were obtained using the Pearson correlation coefficient for bivariate correlations. P values bellow 0.05 were considered significant.

3. Results and Discussion

The yields in of plant drug extracted from the crude ethanolic extract and its fractions and the yields of the crude ethanolic-acetic extract and its fractions of *S. sanctae-catharinae* aerial parts were presented in Table 1.

Table 1. Yields extracted and fractionated from aerial parts of *S. sanctae-catharinae*.

Sample (g)	Yield	Yield %
CEE [a]	155	1.754
HFCEE [b]	55.032	0.119
CHCEE [c]	11.962	27.52
EAFCEE [d]	2.841	8.5
RFCEE [e]	66.386	5.98
CEAE [f]	20.3	12.9
HFCEAE [g]	1.565	1.42
CFCEAE [h]	2.385	0.35
EAFCEAE [i]	0.648	33.19
RFCEAE [j]	11.064	76.2

a CEE) Crude Ethanolic Extract, b HFCEE) Hexan Fraction of Crude Ethanolic Extract, c CFCEE) Chloroform Fraction of Crude Ethanolic Extract, d EAFCEE) Ethyl Acetate Fraction of Crude Ethanolic Extract, e RFCEE) Remnant Fraction of Crude Ethanolic Extract, f CEAE) Crude Ethanolic Acetic Extract, g HFCEAE) Hexan Fraction of Crude Ethanolic Acetic Extract, h CFCEAE) Chloroform Fraction of Crude Ethanolic Acetic Extract, i EAFCEAE) Ethyl Acetate Fraction of Crude Ethanolic Extract and j RFCEAE) Remnant Fraction of Crude Ethanolic Acetic Extract.

Secondary Metabolites	Fractions			
	Hexan	Ethyl Acetate	Chloroform	Remnant
Alkaloids	- [b]	+ [a]	- [b]	+ [a]
Coumarins	- [b]	- [b]	+ [a]	+ [a]
Flavonoids	- [b]	- [b]	- [b]	+ [a]
Steroids	+ [a]	+ [a]	+ [a]	- [b]

The phytochemical screening involved qualitative characterization processes to identify the various secondary metabolites present in the hydroethanolic extract of S. sanctae-catharinae aerial parts. These reactions resulted in either precipitation or coloration, and the results of this phytochemical screening are presented in Table 2.

Table 2. Secondary metabolites present in *S. sanctae-catharinae* extracts and fractions.

a +) positive reaction, b -) negative reaction.

Using the Folin-Ciocalteu reagent, the polyphenol content of the various extracts and fractions were quantified. The regression equation obtained from the different concentrations of gallic acid, y = 0.0324 + 0.0373, R2 = 0.9982, demonstrated that the concentration of polyphenols in the remaining fraction of ethanolic crude extract present the most prominent activity (20009.722 mg GAE/g), followed by the chloroform fraction from the ethanolic crude extract (4079.167 mg GAE/g), ethyl acetate fraction from the ethanolic crude extract (3412.5 mg GAE/g), ethyl acetate fraction of the ethanolic-acetic crude extract (3148.611 mg GAE/g), hexane fraction of the ethanolic crude extract (2940.278 mg GAE/g), remnant fraction of the ethanolic-acetic crude extract (2912.5 mg GAE/g), hexane fraction of the ethanolic-acetic crude extract (2481.944 mg GAE/g), ethanolic-acetic crude extract (1398.611 mg GAE/g), and ethanolic crude extract (1176.389 mg GAE/g). Remnant fraction from the crude ethanolic extract was 390% higher than the chloroform fraction of the crude ethanolic extract, indicating that this fraction contains the most polyphenols per g of sample. The results of the total polyphenol analysis are shown in Table 3, values with * were

statistically different between all samples. The difference between the samples and positive and negative controls was statistically significant ($p < 0.05$).

Table 3. Results of total phenolics of *S. sanctae-catharinae* extracts and fractions.

Sample	mg EQ/g	± S.D. [k]
CEE [a]	1176.389	2.32
HFCEE [b]	2940.278	3.101
CHCEE [c]	4079.167*	2.357
EAFCEE [d]	3412.5	1.543
RFCEE [e]	20009.722*	2.537
CEAE [f]	1398.611	0.786
HFCEAE [g]	2481.944	2.312
CFCEAE [h]	1815.278	1.572
EAFCEAE [i]	3148.611	0.789
RFCEAE [j]	2912.5	1.819

a CEE) Crude Ethanolic Extract, b HFCEE) Hexan Fraction of Crude Ethanolic Extract, c CFCEE) Chloroform Fraction of Crude Ethanolic Extract, d EAFCEE) Ethyl Acetate Fraction of Crude Ethanolic Extract, e RFCEE) Remnant Fraction of Crude Ethanolic Extract, f CEAE) Crude Ethanolic Acetic Extract, g HFCEAE) Hexan Fraction of Crude Ethanolic Acetic Extract, h CFCEAE) Chloroform Fraction of Crude Ethanolic Acetic Extract, i EAFCEAE) Ethyl Acetate Fraction of Crude Ethanolic Extract and j RFCEAE) Remnant Fraction of Crude Ethanolic Acetic Extract, k ± S.D.) Standard Deviation, *) $p<0.05$.

The total phenolic values determined using this technique are not absolute measures of the quantities of phenolic compounds but are in fact based on their relative chemical reduction capacity, in this case to gallic acid (HOSSAIN *et al.*, 2011). The antioxidant phenol index is a combined measure of the quality and quantity of antioxidants in vegetables (ELLIOTT, 1999). Phenolic compounds are considered the mainly responsible for the antioxidant activity of plant drugs (HEIM, TAGLIAFERRO & BOBILYA, 2002). Several studies have revealed that most of the antioxidant activity may come from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechins, and other phenolics (MHATRE & DEVASAGAYAM, 2009; ISABELLE *et al.*, 2010; DANINO *et al.*, 2009).

Other species of the Solanum genus in total phenolics test, such as *Solanum elangnifolium* Cav. presented a value of 2.54 mg GAE/g in its ethanolic extract of the leaves17, *Solanum virginianum* L. showed a value of 0.69 mg EqAG/mL in its methanolic extract made from the whole plant18 (MORANKAR, 2019), and *Solanum fastigatum* Var. showed a value of 141.1 mg GAE/g in its methanolic extract made from the whole plant19.

The leaves of the species *Solanum guaraniticum* A. St-Hil were extracted with ethanol, chloroform, and ethyl acetate, and its total phenolics assay showed values of 259.95, 195.9, and 546.47 mg GAE/g, respectively20. Another species of the genus, *Solanum nigrum* L., had its aerial parts extracted with methanol and then partitioned

with n-hexane, dichloromethane, and ethyl acetate, and its total phenolics assay showed the respective values 128, 228, 416, and 426 mg GAE/g 21. In addition, Saddiqe's research in 2013 mentioned that the *Solanum nigrum* L. species, according to the values obtained in the total phenolics test, is a plant rich in phenolic substances. The values obtained in the total phenolics test with the extracts and fractions *of S. sanctae-catharinae* were not similar to any of the results previously presented for species of its genus cited in this research. Despite this, the value obtained in the total phenolics test with the remaining fraction from the ethanolic extract was a significant result, with a value of 740.79 mg GAE/g, which is higher than all the others mentioned in this research. Indicating that *S. sanctae-catharinae* is a plant rich in phenolic substances.

Using aluminum trichloride, the flavonoid content in various extracts and fractions was quantified. A regression equation was derived from the different concentrations of quercetin, with the equation y = -0,01013 + 0.7368 and R2 = 0.9908, we calculated that the flavonoid concentration in the remnant fraction of ethanolic-acetic crude extract present the most prominent activity (69.312 mg QE/g). The results of the total flavonoids analysis are shown in Table 4. It was observed that the samples from ethanolic crude extract (excluding the ethyl acetate fraction) had lower flavonoid contents in quercetin when compared to the fractionated samples of the ethanolic-acetic crude extract. The difference in total antioxidant activity between the samples and positive and negative controls was statistically significant ($p < 0.05$).

Table 4. Results of total flavonoids of S. sanctae-catharinae extracts and fractions.

Sample	mg EQ/g	± S.D.
CEE [a]	66.186	0.099
HFCEE [b]	49.454	0.148
CHCEE [c]	55.015	0.352
EAFCEE [d]	66.449	0.148
RFCEE [e]	64.163	0.050
CEAE [f]	65.298	0.197
HFCEAE [g]	64.064	0.248
CFCEAE [h]	68.440	0.198
EAFCEAE [i]	54.061	0.247
RFCEAE [j]	69.312	0.643

a CEE) Crude Ethanolic Extract, b HFCEE) Hexan Fraction of Crude Ethanolic Extract, c CFCEE) Chloroform Fraction of Crude Ethanolic Extract, d EAFCEE) Ethyl Acetate Fraction of Crude Ethanolic Extract, e RFCEE) Remnant Fraction of Crude Ethanolic Extract, f CEAE) Crude Ethanolic Acetic Extract, g HFCEAE) Hexan Fraction of Crude Ethanolic Acetic Extract, h CFCEAE) Chloroform Fraction of Crude Ethanolic Acetic Extract, i EAFCEAE) Ethyl Acetate Fraction of Crude Ethanolic Extract and j

RFCEAE) Remnant Fraction of Crude Ethanolic Acetic Extract. , k ± S.D.) Standard Deviation.

The ethanolic extract of the leaves from *Solanum elangnifolium* Cav. showed a content of 0.067 mg EQ/mL in quercetin, while the methanolic extract from the whole plant of *Solanum virginianum* L. had a result of 1.37 mg EQ/mL in quercetin (BOUSLAMTI *et al.*, 2022; MORANKAR & JAIN, 2019). Additionally, the methanolic extract from the whole plant of *Solanum fastigatum* Var. showed a value of 224 mg EQ/mL in quercetin (SABIR & ROCHA, 2008). Saddiqe's research in 2013 found that *Solanum nigrum* L. had total phenolics values of 128, 44, 140 and 188 mg EQ/mL in quercetin in the n-hexane, dichloromethane, and ethyl acetate partitions, respectively. However, the total flavonoids test values obtained for *S. sanctae-catharinae* were not similar to those previously reported for other species in *Solanum* genus.

The majority of the fractions from the ethanolic-acetic crude extract showed higher values in the total flavonoids test compared to those from the ethanolic extract, suggesting that the ethanol-acetic extraction removed a significant amount of flavonoids compared to the ethanolic extraction. It's important to take account that the first extraction presented the highest yields.

Using, phosphomolibdenium method the water-soluble total antioxidant capacity of *S. sanctae-catharinae* extracts and fractions were quantified. *S. sanctae-catharinae* extracts and fractions results of total antioxidant activity are presented in table 5. We noted in our study that the remnant fraction of ethanolic extract was the most outstanding sample and presented 67.85% activity compared with the ascorbic acid (100 %). The results of the total DPPH assay are shown in Table 5, values with * were statistically different between all samples. The difference in total antioxidant activity between the extracts/fractions and positive and negative controls was statistically significant ($p < 0.05$).

Table 5. Results of the water-soluble antioxidant capacity of *S. sanctae-catharinae* extracts and fractions.

Sample	**% Ascorbic acid**	**± S.D.** [k]
CEE [a]	7.2	0.46
HFCEE [b]	11.63	0.84
CHCEE [c]	19.31*	1.05
EAFCEE [d]	13.88	0.53
RFCEE [e]	69.84*	1.79

CEAE [f]	8.98	0.31
HFCEAE [g]	12.05	0.95
CFCEAE [h]	13.22	1.23
EAFCEAE [i]	18.47*	0.14
RFCEAE [j]	11.69	0.10

a CEE) Crude Ethanolic Extract, b HFCEE) Hexan Fraction of Crude Ethanolic Extract, c CFCEE) Chloroform Fraction of Crude Ethanolic Extract, d EAFCEE) Ethyl Acetate Fraction of Crude Ethanolic Extract, e RFCEE) Remnant Fraction of Crude Ethanolic Extract, f CEAE) Crude Ethanolic Acetic Extract, g HFCEAE) Hexan Fraction of Crude Ethanolic Acetic Extract, h CFCEAE) Chloroform Fraction of Crude Ethanolic Acetic Extract, i EAFCEAE) Ethyl Acetate Fraction of Crude Ethanolic Extract and j RFCEAE) Remnant Fraction of Crude Ethanolic Acetic Extract, k ± S.D.) Standard Deviation, *) $p<0.05$.

Using DPPH method we performed the radical scavenging activity of *S. solanum-catharinae* extracts and fractions. The regression equation obtained from the different concentrations of Trolox, y = -0.0115 + 0.1956, R2 = 0.9893 and the formula used to calculate the % of free radicals scavenging, indicate that the most effective sample was the chloroform fraction from the ethanolic crude extract present the most effective activity (9539.057 TroloxEQ µg /mL and 110.71%). The results of the total DPPH assay are shown in Table 6, values with * were statistically different between all samples. The difference in total antioxidant activity between the samples and positive and negative controls was statistically significant ($p <0.05$).

Table 6. Results of the radical scavenging using DPPH method of *S. sanctae-catharinae* extracts and fractions.

Sample	**EqTrolox ug/mL**	**AAR %**
CEE [a]	389635.3	89.81
HFCEE [b]	390367.6	90.60
CHCEE [c]	476952.9*	110.71
EAFCEE [d]	367242.9	85.80
RFCEE [e]	414708.7	95.97
CEAE [f]	80915.81	93.25
HFCEAE [g]	78909.86	90.94
CFCEAE [h]	81995.69	94.49

EAFCEAE [i]	78562.98	90.54
RFCEAE [j]	85055.35*	98.01
Ascorbic acid	-	95.68

a CEE) Crude Ethanolic Extract, b HFCEE) Hexan Fraction of Crude Ethanolic Extract, c CFCEE) Chloroform Fraction of Crude Ethanolic Extract, d EAFCEE) Ethyl Acetate Fraction of Crude Ethanolic Extract, e RFCEE) Remnant Fraction of Crude Ethanolic Extract, f CEAE) Crude Ethanolic Acetic Extract, g HFCEAE) Hexan Fraction of Crude Ethanolic Acetic Extract, h CFCEAE) Chloroform Fraction of Crude Ethanolic Acetic Extract, i EAFCEAE) Ethyl Acetate Fraction of Crude Ethanolic Extract and j RFCEAE) Remnant Fraction of Crude Ethanolic Acetic Extract, k ± S.D.) Standard Deviation, *) $p<0.05$.

Pearson's correlation test demonstrated the that only correlation was between total phenolics and total water-soluble antioxidant capacity (phosphomolibdenium method) $p = 0.995573$. The polyphenol content in the *S. sanctae-catharinae* aerial parts was statistically related to the TAC test's antioxidant capacity.

4. Conclusions

This study investigated the amount of total phenolic and flavonoid content, potential antioxidant activity and we also performed a phytochemical screening of extracts and fractions from aerial parts of *S. sanctae-catharinae*. The results indicate that the remnant fraction of the ethanolic crude extract exhibit the most effective antioxidant activity, followed by chloroform fraction of the ethanolic crude extract, remnant fraction of the ethanolic-acetic crude extract and the ethyl acetate fraction of ethanolic-acetic crude extract. These findings also reveal that a relevant amount of polyphenols and flavonoids in both extracts and a positive correlation between the phenolics and the TAC test. These antioxidant activities can be attributed to the presence of phenols, flavonoids, coumarins and alkaloids in *S. sanctae-catharinae* secondary metabolism. These outcomes motivate further investigation into the plant's other potential biological activities. Future studies could involve advanced techniques such as HPLC or GC/MS to identify the main molecules of *S. sanctae-catharinae*, as well as in silico tests, docking tests and structural elucidations for the secondary metabolites of this plant.

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