Antioxidant Activity and Phenolics Analysis by HPLC-DAD of *Solanum thomasiifolium* Sendtner (Solanaceae)

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ABSTRACT

Introduction: The genus *Solanum* is the most representative of the Solanaceae families and is considered one of the largest and most diverse among the Angiosperms. Chemotaxonomic studies have recognised the genus *Solanum* as a source of phenolic compounds, which present a wide variety of biological actions, including antioxidant activity. **Methods:** The hexane, ethyl acetate, ethanol and aqueous extracts of aerial parts of the *Solanum thomasiifolium* Sendtner were studied for antioxidant activity by using different assay *in vitro* such as inhibition of DPPH, superoxide anions and lipid peroxidation. The total phenolic content in the extracts was analysed by the Folin-Ciocalteu method. In addition, the active extracts were fractionated by open column chromatography containing Sephadex LH-20 and the phenolic compounds presents in the fractions were analysed by High Liquid Chromatography coupled with diode array detector (HPLC-DAD). **Results:** The better results were ethanolic and aqueous extracts which exhibited good scavenging activity of DPPH (IC_{50} = 23.9±0.7 and 28.3±3.1 μg/mL), ABTS (IC_{50} = 10.5±0.4 and 13.3±0.5 μg/mL), β-carotene t = 60min (83.3±0.1% and 91.3±1.5 %), and phenolic content (93.0±6.9 and 82.5±1.4 mgEAG/g). The analysis of UV profiles obtained by HPLC-DAD showed the presence of flavones and flavonols in the active fractions. **Conclusion:** This work shows that polar extracts of *S. thomasiifolium* were good antioxidant activity.

Keywords: *Solanum thomasiifolium;* Antioxidant activity; Total phenolic content; High performance liquid chromatography coupled with diode-array detector; Flavonoid.

INTRODUCTION

Phenolic compounds are a class of secondary plant metabolite with relevant antioxidant action. These compounds were presents in medicinal plants and human diet which development a protection against free radical preserving a good health.[1]

Solanaceae A. L. Jussieu is one of the largest families of Angiosperms with 96 genera and approximately 2,300 species. This family has a wide distribution, but South America is considered one of the main centres of diversity with approximately 50 endemic genera.[2,3] The species of this family have great economic importance, because many plants are sources of chemical compounds of relevance in modern medicine and pharmacology, as well as

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important sources of human food.[4] The species that are widely cultivated as food include are the potato (*Solanum tuberosum* L.),[5] the tomato (*S. lycopersicon* L.),[6] and cayenne (*Capsicum frutescens* L.).[7] Plants of this family used in medicine include *Solanum paniculatum* (*jurubeba* true) to regulate intestinal functions,[8] and *lobeira* (*S. lycocarpum*) for hypertension, diabetes and high cholesterol.[9] For pharmacologically active drugs, species that produce alkaloids have been the most commonly used for therapeutic purposes, such as *Atropa belladonna* (atropine), *Hyoscyamus niger* L. (hyoscyamine) and *Datura* spp. (hyoscine).[10] The Solanaceae family also includes ornamental plants, such as *petúnias* (*Petunia* spp.) and jasmine (*S. jasminoides*), and tobacco (*Nicotiana tabacum*). Worldwide, these plants are economically significant along with other toxic and medicinal plants.[11]

The genus *Solanum* is the most representative of the Solanaceae family and is considered one of the largest and most diverse among the Angiosperms. This genus consists of approximately 1,500 species and is well represented in Brazil, where it is widely distributed across all ecosystems. This genus is represented by 80 species in northeastern Brazil, including 32 species that are endemic in Brazil, of which 20 species are endemic in the Northeast. The genus *Solanum* has been extensively studied, mainly due to the many biological activities presented by its representative species.[12] In Brazil, almost 90 different uses in traditional medicine have been reported for 61 species of *Solanum*. Most of the biological activities reported were related to medicinal plants used in treating skin (fungus, warts, acne, erysipelas), gastrointestinal (liver, laxative, purgative) and respiratory problems (asthma, bronchitis, influenza, tuberculosis), as well as reproductive system diseases (syphilis, gonorrhoea, leucorrhoea).[13] Many species of the genus *Solanum* have great economic importance because they produce a variety of steroidal saponins[14] beyond alkaloids.[10] Flavonoids are also commonly found in species of this genus.[15]

Chemotaxonomic studies have recognised the genus *Solanum* as a source of phenolic compounds, which present a wide variety of biological actions, including antioxidant activity.[15] A seed dispersal study of *S. thomasiifolium* has been reported;[16] however, no study on *S. thomasiifolium* chemical composition and biological activity has been published. The aim of this work is to evaluate the antioxidant properties and total phenolic content of different extracts from *Solanum thomasiifolium* Sendtner and to characterise the flavonoids present in the active extracts by using HPLC-DAD.

MATERIALS AND METHODS

Plant material

Aerial parts of *S. thomasiifolium* were collected in the city of Morro do Chapéu, Bahia State, Brazil in September 2008. A voucher specimen is deposited in the Herbarium of State University of Feira de Santana (HUEFS 146443).

Extract preparation

Dried and powdered aerial parts of *S. thomasiifolium* (370 g) were extracted at room temperature with hexane, ethyl acetate, ethanol and water successively for 48 hours each. The solvents were evaporated under reduced pressure to obtain the hexane (7.3 g), ethyl acetate (4.4 g), ethanolic (22.4 g) and aqueous (173.4 g) extracts.

Chemicals and instruments

The Folin-Ciocalteu reagent, Trolox, potassium persulphate, β-carotene, linoleic acid, and Tween-40 were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Gallic acid and ABTS were purchased from Fluka Chemie AG (Buchs, Switzerland) and DPPH was purchased from Alfa Aesar. The standards used in HPLC-DAD analysis were quercetin, hesperidin, rutin, naringin, chlorogenic acid, caffeic acid and ferulic acid (Sigma-Aldrich). The solvents employed for extraction and HPLC analysis were of analytical and HPLC grade and purchased from Quimex and Sigma-Aldrich, respectively. All standard solutions were diluted in MeOH (1 mg/mL) and filtered through a 0,45 μm Millipore filter (Supelco, USA). CC was performed using Sephadex LH-20® (Amersham Biosciences, Sweden). TLC was performed on aluminium sheets of silica gel 60 (Merck) and TLC detection was provided by UV absorption at 254 and 360 nm. The absorbance measurements were recorded using a Vankel 50 ultraviolet-visible spectrophotometer (Varian, Australia). HPLC analysis was carried out using analytical reversed-phase liquid chromatography in a Hitachi model LaChrom Elite with an auto-sampler and quaternary pump coupled to a diode array detector (DAD). For the chromatographic separation, a LiCospher® 100 RP-18 column (150 mm x 4 mm i.d., 5 μm, Merck) was used. Samples and mobile phases were filtered through a 0,45 μm Millipore filter (Supelco, USA) prior to HPLC injection.

Determination of DPPH free radical scavenging activity

The DPPH radical scavenging activity of the extracts of *Solanum thomasiifolium* was measured according to a

procedure previously described in the literature.[17] Stock solutions (1 mg/mL) of extracts and DPPH were prepared in ethanol. Ethanol solutions of the samples, at different concentrations, were mixed with 4.0 mL of DPPH (23,2 μg/mL) yielding final concentrations of extracts that ranged from 6,2 to 272,7 μg/mL. Absorbance was measured in a spectrophotometer at 517 nm after 30 minutes of stirring in an ultrasonic bath. Ascorbic acid was used as the positive control and DPPH in ethanol was used as the negative control. The DPPH free radical scavenging activity (%) was calculated using the following equation:

$$\% \text{ inhibition} = 100 \times (Abs_{control} - Abs_{sample})/Abs_{control}$$

where $Abs_{control}$ is the absorbance of DPPH and ethanol and Abs_{sample} is the absorbance of the DPPH radical and sample extract or standard.

ABTS scavenging activity

The scavenging activity of plant extracts against the ABTS radical was determined according to methodology described in the literature.[18] Stock solutions of 7 mM ABTS and 2,45 mM potassium persulphate were mixed and allowed to stand in the dark for 12 hours at room temperature. The resultant $ABTS^{\bullet+}$ solution was diluted in ethanol (1:90 v/v) to obtain an absorbance of 0.70 at 734 nm. Stock solutions (1 mg/mL) of extracts were prepared in ethanol, diluted to the appropriate concentrations and mixed with 3.0 mL of $ABTS^{\bullet+}$ solution yielding final concentrations of extracts that ranged from 10 to 150 μg/mL. Solutions were shaken for 10 minutes, and the absorbance values of the samples and controls were measured in a spectrophotometer at 734 nm. Trolox was used as the positive control, $ABTS^{\bullet+}$ in ethanol was used as the negative control and solutions of the extracts in ethanol were used as blanks. The ABTS scavenging activity (%) was calculated using the following equation:

$$\% \text{ inhibition} = 100 \times (Abs_{control} - Abs_{sample})/Abs_{control}$$

where: $Abs_{control}$ is the absorbance of $ABTS^{\bullet+}$ in ethanol and Abs_{sample} is the absorbance of the ABTS radical and sample extract or standard.

β-carotene/linoleic acid assay

The β-carotene/linoleic acid assay was originally described by Marco[19] and subsequently modified by Miller.[20] A solution of β-carotene/linoleic acid was prepared by adding 50 μL of β-carotene solution (20 mg/mL in chloroform), 80 μL of linoleic acid and 660 μL of Tween 20 to an Erlenmeyer flask (250 mL). Next, 90 mL of distilled water saturated with oxygen (for 30 minutes) was added with vigorous shaking. Absorbance of this reaction mixture was adjusted to 0.70 at 470 nm. Aliquots of the extracts (100 μg/mL) were tested, and the results were compared to the negative (no antioxidant) and positive (Trolox 16 μg/mL) controls. Absorbance was measured immediately after the addition of samples and standard and monitored every 20 minutes up to 120 minutes. The reaction solutions were kept in a water bath at 40 °C during this time. The antioxidant capacity of the extracts was expressed as the percentage inhibition of oxidation. The decrease in the absorbance reading of the samples was compared with the system and used to establishes the percentage oxidation.

$$\% \text{ inhibition} = 100 - (Abs_{sample}/Abs_{control} \times 100)$$

where: $Abs_{control}$ is the absorbance of negative control and Abs_{sample} is the absorbance of the extracts.

Determination of the total phenolics

The total phenolic content from crude extracts was determined by the Folin-Ciocalteau method[20] with some modifications. Aliquots of 0.5 mL of each solution of crude extracts (1 mg/mL) were mixed with 0.1 mL Folin-Ciocalteau reagent and 3 mL of distilled water. After shaking for 1 minute, 0.3 mL of 15% Na_2CO_3 was added, and the solution was diluted to 5 mL with water in a volumetric flask. After 2 hours incubation at room temperature, the absorbance was measured at 760 nm on a spectrophotometer using distilled water as the blank. Standard gallic acid solutions (0.5 to 10.0 μg/mL) were used for the calibration curve. Total phenolics contents was calculated as mg of gallic acid equivalent per gram (mg GAE/g) of extract using the following equation based on the calibration curve $Y = 0.1032x - 0.0323$, $r^2 = 0.9993$, where x is the absorbance and Y is the gallic acid equivalent in mg/g.

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as the mean value ± standard deviation (SD). IC_{50} ± SD is the concentration of sample necessary to decrease the absorbance of DPPH and ABTS by 50 %. Linear regression analysis was performed, quoting the correlation coefficient r^2, using GraphPad Prism 4.0 software (San Diego, CA, EUA). Significant levels were tested at $p < 0.05$. The extracts and compounds were considered actives when $IC_{50} < 500$ μg/mL.[21]

Fractionation of extracts

Ethyl acetate (3.40 g) and ethanolic (21.35 g) extracts were submitted to chromatographic purification in Sephadex LH-20® using $CHCl_3$:MeOH (1:1) as the elution system resulting in 22 and 25 fractions, respectively. The collected fractions were analysed by TLC, and the visualisation of the spots was achieved by spraying 1% NP solution in methanol and observing under UV light at 366 nm. Fractions that showed similar chromatographic profiles were gathered to yield 8 fractions from the ethyl acetate extract and 6 fractions from the ethanolic extract.

HPLC-DAD analysis

The mobile phase was composed of solvent (A) H_2O/H_3PO_4 0.1% and solvent (B) MeOH. The solvent gradient was composed of A (75–0%) and B (25–100%) for 20 minutos, solvent B (100%) for four minutes and retuning to initial conditions (75% A; 25% B) for 10 minutes. A flow rate of 1.0 mL/min was used and 20 µL of each sample or standard (1 mg/mL in MeOH) was injected. Spectra data were recorded from 200 to 400 nm during the entire run and the oven temperature was at 30 °C.

RESULTS AND DISCUSSION

Determination of DPPH free radical scavenging activity

Different extracts of *S. thomasiifolium* exhibited considerable free radical scavenging activity as indicated by their IC_{50} values (Figure 1). The lower the IC_{50} value is, the greater the consumption of DPPH• by the sample and hence the greater its antioxidant capacity. In comparison to ascorbic acid (IC_{50} = 6.9±0.0 µg/mL), the ethanolic and aqueous extracts were shown to have good antioxidant activities (IC_{50} = 23.9±0.7 and 28.3±3.1 µg/mL, respectively). The hexane and ethyl acetate extracts had higher IC_{50}values (272.3±1.8 and 109.1±3.0 µg/mL, respectively), indicating weak antioxidant activities by the DPPH free radical assay.

Compounds with antioxidant activity were found to concentrated in the more polar extracts (ethanolic and aqueous extracts). Except for tocopherols, phenolic compounds are known to be polar due to their degree of hydroxylation and have higher affinities to more polar solvents. This finding suggested that phenolic compounds were present in higher concentrations in the aqueous and ethanolic extracts. These extracts showed similar IC_{50} values compared to ascorbic acid (IC_{50} = 6.9±0.0 µg/mL).

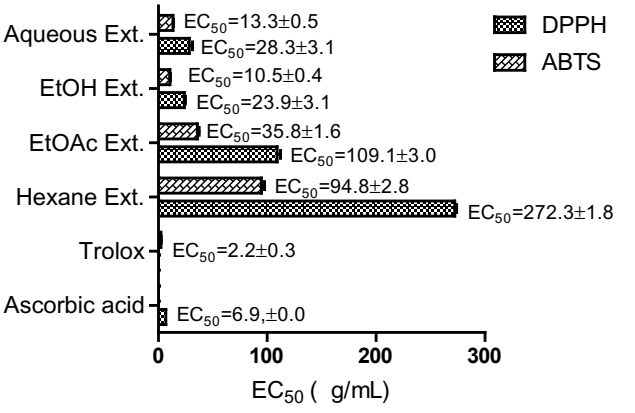

Figure 1. DPPH and ABTS free radical scavenging activities of hexane, ethyl acetate, ethanolic and aqueous extracts from *S. thomasiifolium*.

This finding may be due to the existence of similar phenolic compounds with antioxidant activities in both extracts. Several studies have demonstrated the antioxidant activity of extracts of *Solanum* species, specially their polar extracts.[15] Antioxidant activities have been demonstrated for the ethyl acetate extract of the leaves of *S. guaraniticum* (IC_{50} = 9.11±0.75 µg/mL)[22] and the methanol extract of berries of *Solanum aculeastrum* (IC_{50} = 65.5±0.49 µg/mL).[23]

ABTS scavenging activity

The results of ABTS scavenging activity in extracts of *S. thomasiifolium* were expressed as IC_{50} values and are shown in Figure 1. The results showed that all extracts exhibited antioxidant activity, but the ethanolic extract showed the best ABTS free radical scavenging activity (IC_{50} = 10.5±0.4 µg/mL), followed by the aqueous (IC_{50} = 13.3±0.5 µg/mL), ethyl acetate (IC_{50} = 35.8±1.6 µg/ml) and hexane extracts (IC_{50} = 94.8±2.8 µg/ml). These results were in agreement with data obtained for the DPPH free radical scavenging activity. However, the IC_{50} values presented were distinct, possibly due to different in the phenolic compositions in the tested extracts.

β-carotene/linoleic acid assay

Percentages of inhibition of oxidation of linoleic acid obtained with the hexane, ethyl acetate, ethanolic and aqueous extracts in comparison to the standard (Trolox) are shown in Figure 2. Additionally, the ability of the extracts to inhibit the oxidation of β-carotene was lower than the standard. At the reaction time of 60 minutes the ethyl acetate and aqueous extracts showed higher activities (89.2±0.2% and 91.3±1.5%, respectively), although

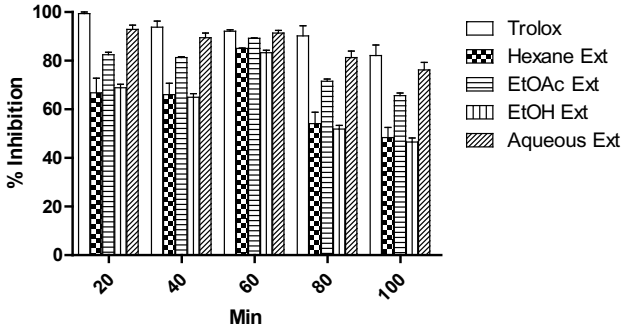

Figure 2. Inhibition of linoleic acid oxidation by extracts of *S. thomasiifolium* measured in β-carotene/linoleic acid assays.

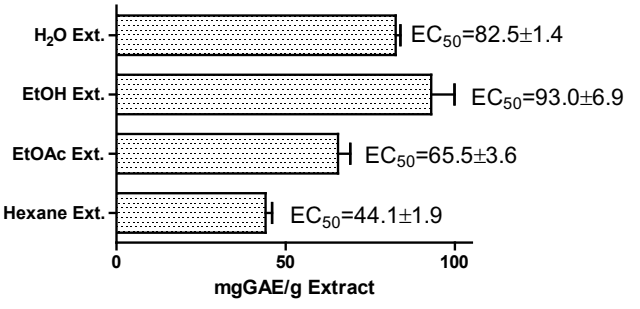

Figure 3. Total phenolic contents of crude extracts of *S.thomasiifolium.*

were as effective as Trolox (92.0±0.1%). The hexane (85.0±0.1%) and ethanolic (83.3±0.1%) extracts had the least inhibition of oxidation. In this test, a different behavior was observed: the ethanol extract had the lowest ability to inhibit the oxidation process compared to the other extracts.

Determination of the total phenolics

Figure 3 shows the values for the phenolic content in extracts of *S. thomasiifolium* expressed as gallic acid equivalents (mg gallic acid/g of crude extract). The ethanolic extract had the greatest total phenolic content (93.0±6.9 mgEAG/g), followed by the aqueous (82.5±1.4 mgEAG/g), ethyl acetate (65.5±3.6 mgEAG/g) and hexane extract (44.1±1.9 mgEAG/g).

Several studies showed considerable levels of phenolic compounds in the extracts of the *Solanum* species tested using the Folin-Ciocalteu method. For example, the methanol extracts of the roots of *S. granulosoleprosum*, *S. torvum*, *S. paniculatum* and *S. mauritianum* showed phenolic contents of 27.46±0.91, 38.37±0.47, 21.02±0.16 and 10.01±0.21 mgEAG/g, respectively. Furthermore, the chloroform extracts of the leaves of *S. nigrum* and fruits of *S. torvum* showed phenolic contents of 5.01 and

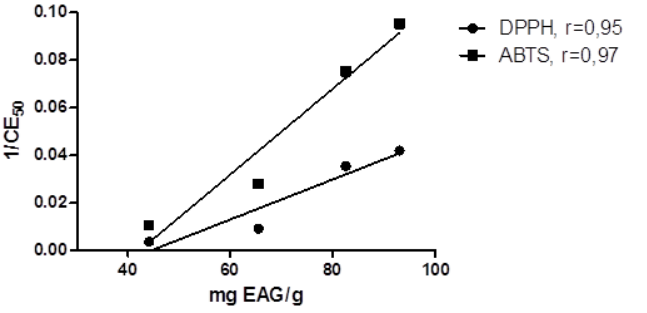

Figure 4. Correlation between total phenolic content and the $1/IC_{50}$ values of DPPH and ABTS scavenging activity.

8.50±0.1 mgEAG/g, respectively.[24] In the present study, a high phenolic content was observed in the polar extracts of *S. thomasiifolium* compared to its other extracts.

Several authors[25,26] have described a positive correlation between phenolic content and antioxidant activity using similar assay systems. The linear regression curve between the total phenolic content and the $1/IC_{50}$ values of the DPPH and ABTS scavenging activities is shown in Figure 4. The Pearson correlation coefficients (r) obtained from the extracts were 0.95 and 0.97, suggesting 95% and 97 % DPPH and ABTS radical scavenging ability, respectively, of *S. thomasiifolium* extracts because of the content of total phenolics. In this study, ethanolic and aqueous extracts had higher phenolic contents and were the most active extracts in term of antioxidant activity (DPPH and ABTS scavenging activity).

HPLC-DAD analysis

Ethyl acetate and ethanolic extracts were selected for fractionation on Sephadex LH-20 and the fractions (see experimental) obtained were analysed by HPLC-DAD for the presence of phenolic compounds. Using the UV spectra of the different compounds contained in the extracts, it was possible to characterise individual components through comparison with data in the literature.[27]

The ultraviolet spectrum for flavonoids in methanol typically consists of two absorptions in the region of 240–400 nm: band I, absorbing between 300–400 nm (ring B, cinnamoyl group), and band II, absorbing between 240–285 nm (ring A, benzoyl group).[27] Another parameter used for characterization of flavonoids by HPLC-DAD is injection of comparative standards.[28] For this study, the following flavonoid standards were analysed and compared with *Solanum thomasiifolium* extract: rutin, quercetin, hesperidin, naringin and 5-hydroxy-3,7,3',4'-tetramethoxy flavone.

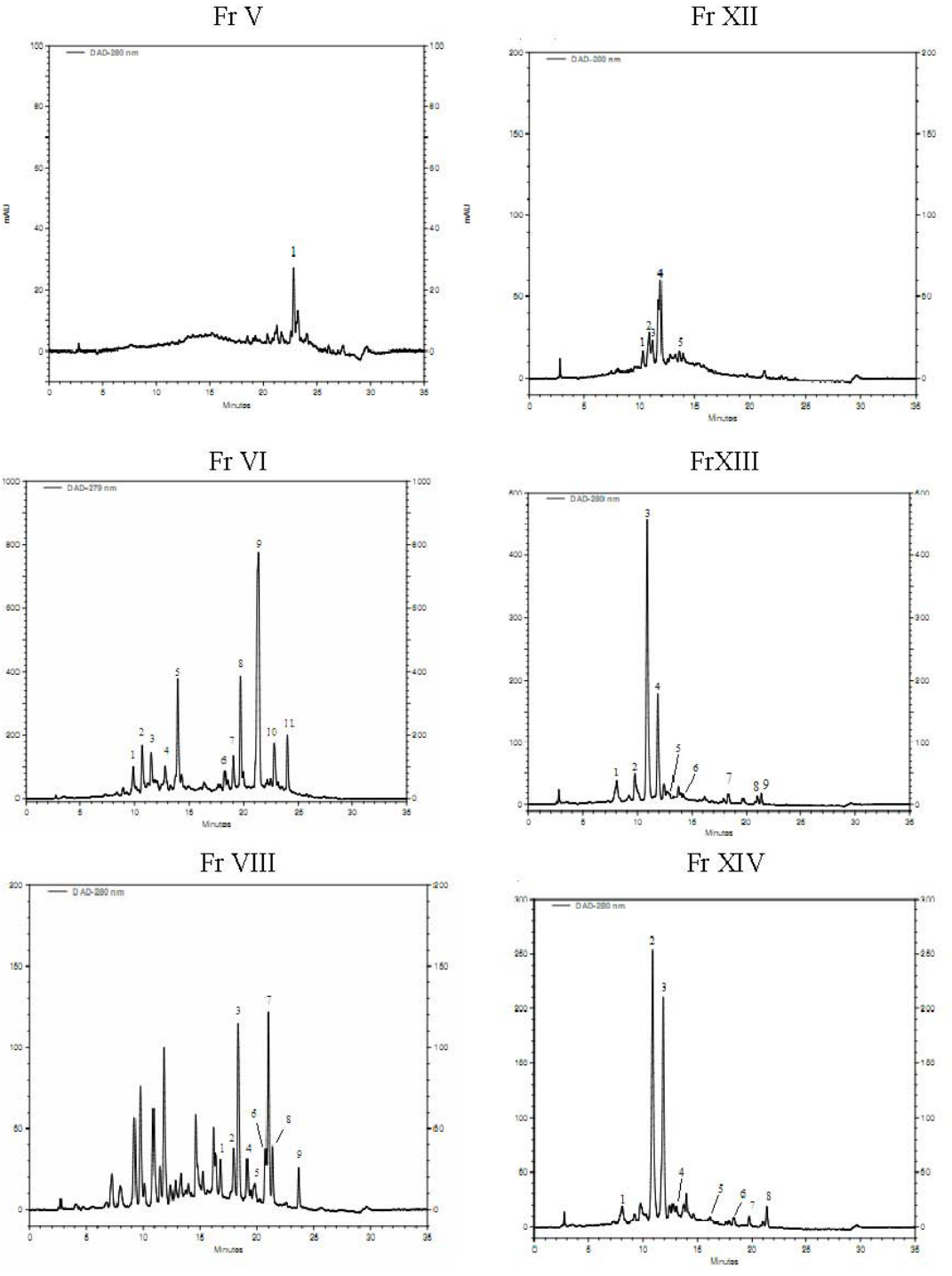

Figure 5. HPLC-DAD Chromatogram of fractions obtained from ethyl acetate (Fr V, VI and VIII, left) and ethanol extracts (Fr XII, XIII and XIV, right).

All analysis of standards and fractions of *S. thomasiifolium* were performed under the same chromatographic conditions. Elution bands were separated according to retention time (R_t) of the standards: glycosides flavonoids eluted with lower retention time (R_t = 10–13 min), flavonols with free hydroxyls showed intermediate retention time (R_t approximately 15 min) and polymethoxylated flavonoids presented a retention time of 20 min. Figure 5 shows the chromatograms of fractions obtained from active extracts of *S. thomasiifolium* obtained by HPLC-DAD. The observed peaks are attributed to the presence of flavonoids based on their UV spectra and retention time (Tables 1 and 2). Analysis of the ultraviolet absorption spectra of the fractions indicated the types of flavonoids

Table 1 Characterisation of the phenolic compounds classes by UV spectra of peaks present in chromatograms of the fractions obtained from the ethyl acetate extract of *S. thomasiifolium*

Fraction	Peak	Rt (mim)	UV	Class compounds
V	1	22,83	254, 268, 346	Flavone
VI	1	9,88	220, 274	Phenolic acid
	2	10,72	230, 280, 312	Cinnamic acid derivative
	3	11,56	224, 276, 308	Flavanone
	4	12,76	276, 304	Phenolic acid
	5	13,93	222, 294, 318	Cinnamic acid derivative
	6	18,27	252, 265, 360	Flavonol
	7	19,01	258, 272, 342	Flavone/flavonol
	8	19,69	252, 264, 300, 359	Flavonol
	9	21,37	258, 302, 354	Flavonol
	10	22,83	252, 268, 350	Flavone or flavonol
	11	24,07	268, 350	Flavone or flavonol
VIII	1	16,81	255, 267, 298, 358	Flavonol
	2	17,94	248, 268, 300, 355	Flavonol
	3	18,35	267, 298, 352	Flavonol
	4	19,15	275, 300, 375	Flavonol
	5	19,82	256, 268, 360	Flavonol
	6	20,78	254, 268, 370	Flavonol
	7	20,98	255, 268, 368	Flavonol
	8	21,35	268, 301, 356	Flavonol
	9	2370	260, 320, 366	Flavonol

Table 2 Characterisation of the phenolic compounds classes by UV spectra of peaks present in chromatograms of the fractions obtained from the ethanolic extract of *S. thomasiifolium*

Fraction	Peak	R_t (mim)	UV	Class compounds
XII	1	10,31	244, 270, 340	Flavone or Flavonol
	2	10,90	254, 268, 353	Flavonol
	3	11,20	241, 268, 353	Flavonol
	4	11,89	265, 298, 348	Flavone or Flavonol
	5	13,60	247, 262, 323	Flavone
XIII	1	8,06	214, 244, 304, 325	Cinnamic acid derivative
	2	9,72	219, 260, 293	–
	3	10,90	255, 264, 300, 354	Flavonol
	4	11,89	264, 291, 350	Flavone or Flavonol
	5	12,96	254, 265, 352	Flavonol
	6	14,17	254, 352	Flavonol
	7	18,37	258, 264, 350	Flavone or Flavonol
	8	20,99	252, 370	Flavonol
	9	21,35	268, 350	Flavone or Flavonol
XIV	1	8,06	220, 251, 300, 330	–
	2	10,90	259, 264, 298, 356	Flavonol
	3	11,89	265, 298, 346	Flavone or Flavonol
	4	13,03	254, 263, 357	Flavonol
	5	15,99	252, 370	Flavonol
	6	18,37	267, 252, 353	Flavonol
	7	19,70	262, 252, 362	Flavonol
	8	21,36	268, 300, 349	Flavone or Flavonol

present and their patterns of oxygenation, particularly from examining band I. Specifically, band I in flavones occurs in the range of λ_{max} 310–350 nm, whereas the flavonols exhibit band I between 350 and 385 nm. Furthermore, flavanones, isoflavones and dihydroflavonols have low absorption intensity in band I, usually appearing as a shoulder of band II.

Most of the flavonoids contained in the ethyl acetate and ethanol extracts fractions displayed two absorption bands, one with λ_{max} between 250–280 nm (band II) and the other in the range 310–380 nm (band I). Based on these results, flavonoids present in these fractions were predominantly flavones and flavonols, in agreement with the literature data. A review on the occurrence of flavones, flavonols and their heteroglycosides in *Solanum* species also highlighted their ability to produce 3-*O*-glycosylflavonols. The authors also reported 86 flavonoids, flavones and flavonols already isolated or identified from *Solanum* species that constitute a group of compounds that are common in the genus.[29]

An analysis of fractions (Fr) VI and VIII of the ethyl acetate extract by HPLC-DAD indicated the presence of cinnamic acid derivatives and flavonols. Peaks 9 and 11 of fraction VI corresponded to the flavonols hesperidin (λ_{max} = 354 and 259 nm) and kumatakenin (λ_{max} = 350 and 267 nm), respectively, which have been previously isolated from *Solanum* species.[29] In fraction VIII, peak 1 was characterised as 3-*O*-methylquercetin (λ_{max} = 358 and 256 nm) and peaks 3, 6 and 7 corresponded to other derivatives of quercetin.[24] Fraction V contained one flavone that was likely a polymethoxylated derivative because of its retention time of 22.83 min and a UV spectrum similar to the standard 5-hydroxy-3,7,3',4'-tetra-methoxy-flavone. Chromatographic analysis revealed a higher content of phenolic compounds in the ethanol extract than the ethyl acetate extract, confirming the results obtained through the Folin-Ciocalteu test and assay of antioxidant activity. Flavonols dominated the ethanol extract, especially quercetin derivatives, which were present in all fractions and correlated to peaks 2 (Fr XII), 5/6 (Fr XIII) and 7 (Fr XIV). In addition, peak 4 from fraction Fr XII of the ethanol extract likely indicated the presence of 3-*O*-[β-glucopyranosyl (1→6) α [rhamnopyranosyl]-7-*O*-α rhamnosilkaempferol] (λ_{max} = 348 and 265 nm), isolated from other species of the *Solanum* genus.[29,30]

CONCLUSIONS

This work reports the first study of antioxidant activity of *Solanum thomasiifolium* extracts. The data obtained for antioxidant activity (DPPH, ABTS and β-carotene/acid linoleic co-oxidation assays) indicated that secondary metabolites with antioxidant activity were concentrated in the polar extracts of *S. thomasiifolium* (aqueous and ethanolic extracts). The antioxidant activity was shown to be directly related to the presence of phenolic compounds through HPLC-DAD analyses and the Folin-Ciocalteu test. The UV spectra by HPLC-DAD showed the presence of phenolic compounds in fractions of the ethanolic and ethyl acetate extracts. The ethanolic extract was distinguished by a greater number of flavonoids, predominantly flavones and flavonols. These results showed that extracts of *S. thomasiifolium* are an important source of natural antioxidants, which may be of interest in treating diseases related to oxidative stress.

Abbreviations: ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; HPLC-DAD, High Liquid Chromatography coupled with diode array detector; CC, Column chromatography; TLC, Thin-layer chromatography; NP, diphenylboryloxyethylamine.

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