academicJournals

Vol. 9(7), pp. 207- 215, 17 February, 2015
DOI: 10.5897/JMPR2014.5555
Article Number: 044362E51208
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Identification of flavonol glycosides and *in vitro* photoprotective and antioxidant activities of *Triplaris gardneriana* Wedd

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Received 10 September, 2014; Accepted 16 February, 2015

Triplaris gardneriana **belongs to the family Polygonaceae, known for producing a number of biologically important molecules. The present study was aimed at identifying and quantify its total flavonoid content and determining the antioxidant and photoprotective potential of the plant's leaves. The flavonoids present in the extract and fractions were analyzed using Liquid chromatography–mass spectrometry (LC-MS). The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and autoxidation of β-carotene. The absorbance of the extracts was measured at different concentrations between 260 to 400 nm wavelengths. Calculation of sun protection factor (SPF) was determined using the formula developed by Mansur. The total flavonoid content was determined by the method developed by Dewanto. In the study, the following four flavonols were identified: quercetin-hexoside (2a), quercetin-pentoside (2b), quercetin-ramnobioside (2c) and myricetin-hexoside (2d). The crude ethanol extract, ethyl acetate, and methanol fractions showed higher flavonoid content and also exhibited excellent antioxidant and photoprotective activity. The SPF values were best observed for the crude ethanol extract and for the chloroform, ethyl acetate and methanol fractions. The good antioxidant and photoprotective potential can be attributed to the presence of flavonols identified for the first time in this species.**

Key words: *Triplaris gardneriana*, Polygonaceae, antioxidant activity, photoprotective activity, glycosylated flavonols, LC-MS.

INTRODUCTION

The species *Triplaris gardneriana* Wedd (Polygonaceae) is popularly known in the northeast as "Pajeu". It is used

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in popular medicine to treat bleeding hemorrhoids, coughing and bronchitis; decoction of the bark or root is used to treat gonorrhea, leucorrhea and inflammation of internal organs, as well as to stimulate the uterus of rats and as a molluscicide (Cartaxo et al., 2010). Recent studies of seed extracts showed antibacterial, antioxidant, and anticholinesterase activities (Farias et al., 2013). The plants of the family Polygonaceae are known to produce a number of important secondary metabolites such as flavonoids. We emphasized the class of flavonols occurring in 25% of the genera of the family, including *Triplaris* (Oliveira et al., 2008).

Recently, plants that have antioxidant activity have been of great interest, given the distinct roles of free radical presence in the body (Alves, 2010). Phenolic compounds such as flavonoids exhibit intense absorption in the UV region and have antioxidant action (Zuanazzi and Montanha, 2012). Such intrinsic features of these secondary metabolites allow the possibility of plant extracts that contain them in their composition to be used for their photoprotective action. This is owing to their confirmed capacity to absorb solar radiation; as an antioxidant, they may neutralize free radicals produced in the skin after exposure to sunlight (Souza et al., 2013).

In recent studies, hyphenated techniques such as Liquid chromatography–mass spectrometry (LC-MS) have been applied in the metabolomic analysis of plants, as the combination of these two techniques provides a powerful tool in the analysis of flavonoids in crude extracts by detecting ions produced by electrospray ionization (ESI) (Boulekbache-Makhlouf et al., 2012). Considering that in Brazil *T. gardneriana* presents no chemical and biological studies *in vitro*, the present study aimed, through LC-MS, to identify and quantify the total flavonoid content and determine the antioxidant and photoprotective potential of the extracts and fractions of the species' leaves.

MATERIALS AND METHODS

Plant

The leaves were collected in the city of Santa Maria of Boa Vista in the state of Pernambuco, Brazil in July, 2013, located at 349 m elevation (08° 47' 59, 00 S, 039° 50' 42, 40 W). Specialist Diogo de Oliveira Gallo confirmed the botanical identity of the plant. A voucher specimen of the plant was deposited in the Herbarium of the Federal University of San Francisco Valley (HVASF) under registration number 21221.

Obtaining and fractionation of the crude ethanol extract

The dried and powdered leaves (5,000 g) were continuously extracted three times during a 72-h period with 95% ethanol at room temperature. The ethanol solution obtained was filtered, and then the solvent was evaporated with the aid of a rotaevaporator at reduced pressure with an average temperature of 50°C, yielding 724 g of crude ethanol extract after the distillation of the solvent. This was mixed with silica gel 60 (0.04 to 0.063 mm, MACHEREY-NAGEL), subjected to a vacuum-liquid chromatography (VLC), and fractionated with hexane, chloroform, ethyl acetate and methanol (Sigma-Aldrich, USA) to yield the following four fractions: hexane (9.30 g), chloroform (42.17 g), ethyl acetate (19.29 g) and methanol (193.48 g).

Determination of total flavonoid content

The determination of total flavonoid content was determined using the methodology previously described by Dewanto et al. (2002), with adaptations. The absorbance was measured against the blank at 510 nm using a spectrophotometer (QUIMIS, Brazil) in comparison with the standard prepared similarly with known concentrations. The results were expressed as mg of catechin equivalent to a gram of extract/fractions (mgCE/g) using the calibration curve of catechin (R^2 = 0.9943). The range of the calibration curve was 50 to 1000 mg^{-1}.

LC-MS analysis

Analyses were performed by the Analytical Center of the Institute of Chemistry, University of São Paulo, using the apparatus of high-efficiency liquid chromatography coupled to ion trap mass spectrometer model Esquire 3000 plus Bruker Daltonics, equipped with electrospray ionization-ESI. The LC system consisted of two LC10AD solvent pumps, a SLC 10A system controller, a CTO-10AS column oven (Shimadzu, Japan), a 7125 Rheodyne injector with a 20 ml loop, and an UV detector (SPD 10A, Shimadzu, Japan). The samples were diluted in methanol (EM Science, USA) in 1 mg ml^{-1} concentrations, injected into the apparatus, and subjected to LC-18 Shimadzu Shim-Pack® column (250 × 4.6 mm, Japan). For elution of the column, solvent A (water: acetic acid 99:1) and solvent B (acetonitrile: acetic acid 99:1) were used with the following program: 10% B (0 to 5 min), 15% B (50 to 55 min), 20% B (65 to 75 min), 100% B (80 to 85 min), and finally 10% B (up to 90 min). The flow rate was maintained at 1.0 ml min^{-1}, and the injection volume was 10 µl. In the electrospray ionisation mass spectrometry (ESI-MS) analyses, the general conditions were: source temperature of 40°C and capillary voltage of 4.0 Kv in positive mode, data acquisition in MS. The compounds were identified according to the interpretation of their fragmentation spectra and comparison with literature data.

Determination of antioxidant activity

DPPH free radical scavenging assay

The free radical scavenging activity was measured according to the method developed by Mensor et al. (2001) with adaptations, using 2,2-diphenyl-1-picryl-hydrazyl (DPPH•). Stock solutions of 1.0 mg ml^{-1} of the extract and fractions were diluted in ethanol to final concentrations of 243, 81, 27, 9, 3 and 1 µg ml^{-1}. One ml of a 50 µg ml^{-1} DPPH ethanol solution was added to 2.5 ml of diluted sample and allowed to react at room temperature. After 30 min, the absorbance values were measured at 518 nm and converted into percentage of antioxidant activity (AA) using the following formula:

$$\%AA = [(A_C - A_A) / A_C] * 100$$

Where A_C is equivalent to absorbance of the control and A_A absorbance of the sample. Ethanol 1.0 ml with extract solution 2.5 ml were used as a blank. DPPH solution 1.0 ml with ethanol 2.5 ml was used as negative controls. The positive controls were the ascorbic acid, butylhydroxyanisole and butylhydroxytoluene. The effective concentration (EC_{50}) values were calculated by linear regression using the GraphPad Prism® 5.0 program.

Inhibition of auto oxidation of β-carotene

The ability of the extracts to prevent the oxidation of β-carotene was evaluated according to the methodology described by Wannes et al. (2010). The β-carotene 2 mg was dissolved in 10 ml of chloroform, linoleic acid 40 mg and Tween 40 (400 mg) were added to 2 ml of this solution. The chloroform was evaporated under vacuum at 40°C and 100 ml of distilled water was added; afterwards, the emulsion was vigorously shaken for two minutes. The standard compounds (ascorbic acid, butylhydroxyanisole and butylhydroxytoluene) and extracts were diluted in ethanol. The 3.0 ml emulsion was added to a tube containing 0.12 ml of the standard solutions and 1.0 mg ml^{-1} of the extracts. Absorbance was measured immediately at 470 nm and the samples were incubated in a water bath at 50°C for 120 min, when absorbance was measured again. In the negative control, extracts were replaced with an equal volume of ethanol. The antioxidant activity (%AA) was evaluated in terms of bleaching of β-carotene using the following formula:

$$\%AA = [1 - (A_0 - A_t) / (A_0^0 - A_t^0)] * 100$$

Where A_0 is the initial absorbance and A_t is the measured absorbance for the final sample. A_0^0. is the initial absorption and $A_t^0)$ is the final absorbance measured for the negative contro. Results are expressed as percentage of antioxidant activity (%AA).

Determination of *in vitro* sun protection factor (SPF) and the maximum absorption wavelength

Determining the wavelength of maximum absorption of the extract and fractions was performed by diluting these in absolute ethanol according to the method described by Violante et al. (2009) to yield concentrations of 5, 25, 50 and 100 mg L^{-1}. Subsequently, the reading was performed in a UV-VIS spectrophotometer (QUIMIS, Brazil) at wavelengths between 260 and 400 nm, with intervals of 5 nm. Readings were taken using a quartz cell of 1.0 cm optical path and ethanol was used as a blank. Calculation of SPF was determined using the equation developed by Mansour et al. (1986):

$$SPF = CF. \sum_{290}^{320} . EE(\lambda). I(\lambda). abs(\lambda)$$

Where EE (λ) equals erythematogenic effect of radiation of wavelength; I (λ) Intensity of solar radiation at a wavelength; Abs (λ) Spectrophotometric determination of the absorbance of the solution in wavelength; CF Correction factor (= 10). The values of EE (λ). I(λ) are constants.

Statistical analysis

Data obtained in triplicate experiments were analyzed statistically using the GraphPad Prism® version 5.0 and expressed as mean ± SD. Differences were considered significant when $P < 0.05$.

RESULTS

LC-MS analysis

The LC-MS-coupled technique used in this study allowed for the identification of the presence of flavonols in the crude ethanol extract and fractions of *T. gardneriana*. This technique was used to characterize the presence of compounds with the antioxidant and photoprotective potential presented in this study. The electrospray ionisation mass spectrometry (ESI-MS) analyses were performed in positive mode, and adducts formed were predominantly as $[M+Na]^+$. Analysis through ESI-MS showed that the information of the mass spectra of some constituents present in the crude ethanol extract and fractions coincided with the mass identified as the flavonol derivatives quercetin and myricetin, based on the presence of the signals at m/z 303 and m/z 319 of aglycones, respectively. The following compounds were identified: (2a) quercetin-hexoside, (2b) quercetin-pentoside, (2c) quercetin-ramnobioside and (2d) myricetin-hexoside (Figure 2, Table 1). The chromatogram obtained from the conditions of the HPLC analysis of the crude ethanol extract is shown in Figure 1. The peak corresponding to the compound 1 (Figure 2a) was detected in the crude ethanol extract, ethyl acetate fraction and chloroform in 17.6 and 17.5 min in the methanol fraction. With the compound of m/z 465 $[M+H]^+$ and m/z 487 $[M+Na]^+$, thereby eliminating the values of the atomic mass of the protonated adduct, it was possible to get the molecular formula $C_{21}H_{20}O_{12}$ referring to 464 amu. The spectrum was presented as quercetin aglycone, with protonated fragment m/z 303 (Y^+) and sodiated m/z 325. The formation of the fragment of m/z 185 sodiated and 163 not sodiated is consistent with a hexose. The substance 2 was detected in the chromatograms of the crude ethanol extract and of the ethyl acetate and methanol fractions with retention times equal to 18.4, 18.2, and 18.3 min, respectively. The compound of mass m/z 435 $[M+H]^+$ and m/z 457 $[M+Na]^+$ showed peaks for quercetin m/z 303 (Y^+) and m/z 325 quercetin sodiated after loss of one molecule of pentose 132 amu. The fragments sodiated and not sodiated m/z 155 and 133 confirm that a pentose is linked in the aglycone. The peaks allowed us to suggest the flavonoid quercetin- pentoside (Figure 2b) mass 434 amu and molecular formula $C_{20}H_{18}O_{11}$. The peak corresponding to the compound 3 was only detected in the crude ethanol extract and in the chloroform fraction at 21.6 and 21.5 min, respectively. The substance exhibited molecular ion peak equal to m/z 617, which refers to the atomic mass of the substance added to a sodium adduct $[M+Na]^+$ and m/z 595 consistent with

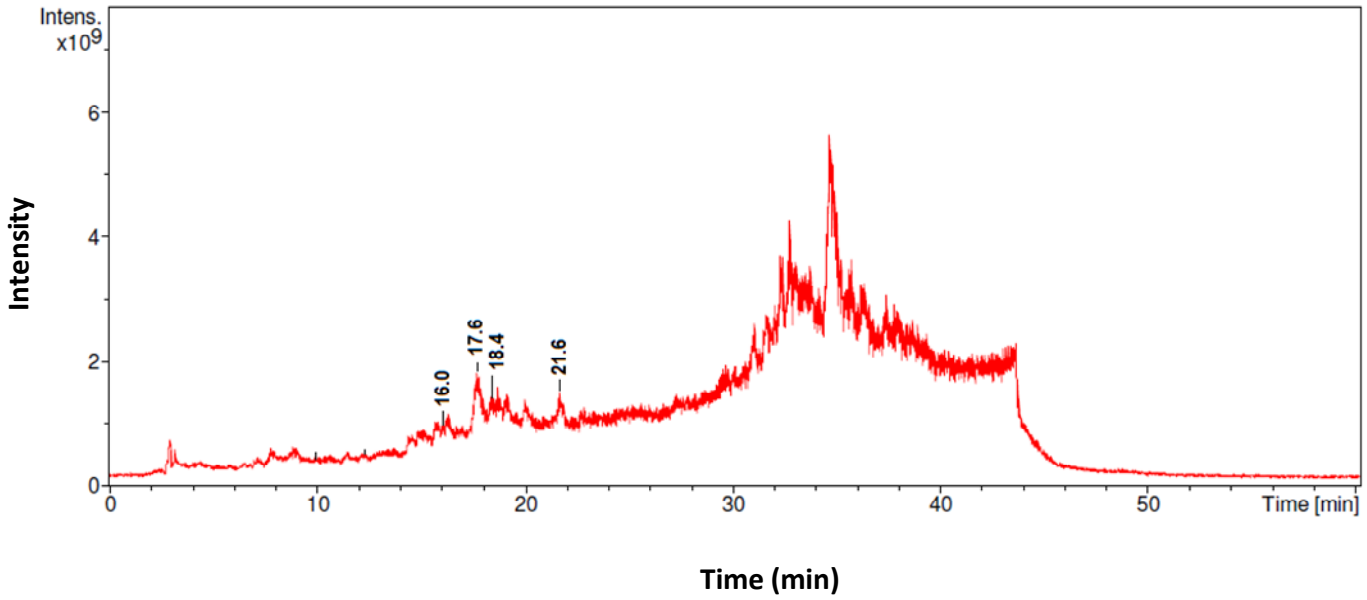

Figure 1. Chromatogram HPLC/UV-ESI-MS of the crude ethanol extract of *T. gardneriana*.

the hydrogen adduct $[M+H]^+$, coinciding with quercetin-ramnobioside (Figure 2c) with molecular formula $C_{27}H_{30}O_{15}$ and 594 amu. The fragment m/z 303 (Y^+) is consistent with quercetin aglycone with a neutral loss of 292 amu, which corresponds to two rhamnoses linked. This information has been confirmed with the fragmentation of the ions sodiated m/z 617, a small fragment of m/z 315, consistent with the disaccharide Rha-Rha, which is connected to quercetin. The fragment m/z 449 and 471 sodiated is compatible with quercetin except for a rhamnose unit. The peak m/z 169 rhamnose sodiated and 147 correspond to one rhamnose unit. Compound 4 was identified only in the crude ethanol extract and methanol fraction in 16.0 and 15.9 min, respectively. The compound showed a molecular ion peak equal to m/z 503 that refers to the atomic mass of the substance added to a sodium $[M+Na]^+$ and m/z 481 consistent adding a hydrogen $[M+H]^+$, indicating molecular formula $C_{21}H_{21}O_{13}$ and 480 amu. The spectrum showed a fragmentation of ion m/z 319 (Y^+), being compatible with myricetin, with neutral loss of 162 amu indicating the loss of a hexose unit, identified as myricetin-hexoside (Figure 2d). Figure 3 shows the mass spectra of the flavonols. All substances identified are reported for the first time in the species *T. gardneriana*.

Determination of total flavonoid content

The crude ethanol extract and the ethyl acetate and methanol fractions showed the highest content of total flavonoids 281.35 ± 6.23, 287.14 ± 2.23 and 271.35 ± 1.32, respectively (Table 2).

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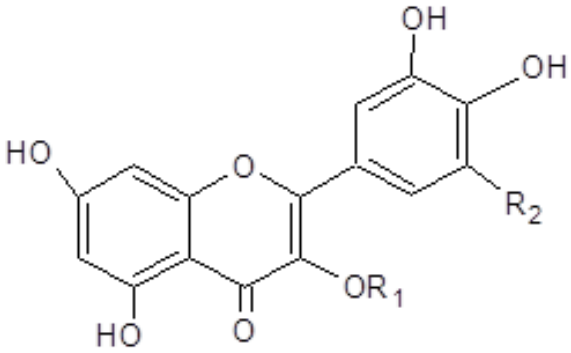
Determination of antioxidant activity

The results of the evaluation of the antioxidant activity are shown in Table 2. Data showed that the crude ethanol extract, ethyl acetate and methanol fractions exhibited excellent activity in the scavenging of DDPH with lower EC_{50} values (2.27 ± 0.19, 5.42 ± 0.84 and 3.35 ± 0.15 µg ml^{-1}), respectively. The hexane and chloroform fractions showed low antioxidant activity, with higher EC_{50} values. In the β-carotene method, the crude ethanol extract also showed better activity, 67.62 ± 2.48%, followed by chloroform fraction, 54.74 ± 1.28% (Table 2).

Photoprotective activity

The crude ethanol extract and the fractions chloroform, ethyl acetate and methanol showed spectrophotometric absorption profile within the range of UVC region (100 to 290 nm) and UVB (290 to 320 nm). However, only the chloroform and ethyl acetate fractions absorbed in the UVA region (320 to 400 nm) had maximum wavelength in this region, emphasizing the chloroform fraction, which was more efficient to range UVA as well as UVB followed by the ethyl acetate fraction. The maximum wavelength



	R_1	R_2
(a) Quercetin- hexoside	Glc or Gal	H
(b) Quercetin- pentoside	Ara or Xyl	H
(c) Quercetin- ramnobioside	Rha-Rha	H
(d) Myricetin- hexoside	Glc or Gal	OH

Figure 2. Glycosylated flavonols identified in the crude ethanol extract and fractions of *T. gardneriana*.

exhibited by the crude ethanol extract and fractions is shown in Table 3. All samples exhibited a higher SPF of 6.0 in a concentration in 100 mg L^{-1}, except for the hexane fraction. The SPF values at this concentration were 7.410 ± 0.132, 12.794 ± 0.163, 11.280 ± 0.127 and 11.891 ± 0.291 for crude ethanol extract, the chloroform fraction, ethyl acetate fraction and methanol fraction, respectively. The results of *in vitro* SPF of crude ethanol extract and fractions using the methodology proposed by Mansur (1986) are shown in Table 4.

DISCUSSION

LC-MS analysis

Structural analysis of the individual ions in the mass spectra was performed by comparison with the literature data (Souza et al., 2008). In compound 1, the removal of ring B resulted in the fragmented peak m/z 181 allowing profiling of the sugar substituent in ring C. Generally, sugar substituents linked to a hydroxyl group of the aglycone are located at position 3 or 7; however, substituent located at the 3-position is more easily lost than in position 7 (Cuyckens and Claeys, 2005; Rijke et al., 2006). The hexose identified in compound 1 and 4 possibly refers to glycosides as galactose or glucose, as it has been common in flavonol glycosides in the family Polygonaceae. With this, the compound 1 was identified as quercetin-hexoside (Figure 2a), consistent with the structure of isoquercitrin or hyperoside, both already reported in the family; the compound 4 refers to myricetin-hexoside (Figure 2d). In compound 2 (Figure 2b), the sugar residue may consist of an arabinose or xylose, both already reported in the family Polygonaceae. However, the pentose arabinose appears in most cases in the structures of flavonols in the family, what leads to the supposition that it is the sugar linked in the aglycone. The peaks m/z 169 and 147 that may correspond to a rhamnose unit in compound 3 (Figure 2c) may also be a coumaroyl unit, but there is no report of this acyl group in flavonols isolated in the family. It therefore suggests that it is a quercetin-ramnobioside (Oliveira, 2008).

Determination of total flavonoid content

As expected, the compounds of phenolic nature were mainly present in the samples that have moderate to high polarity as the crude ethanol extract and the ethyl acetate and methanol fractions (Table 2).

Determination of antioxidant activity

Values of antioxidant activity of the crude ethanol extract,

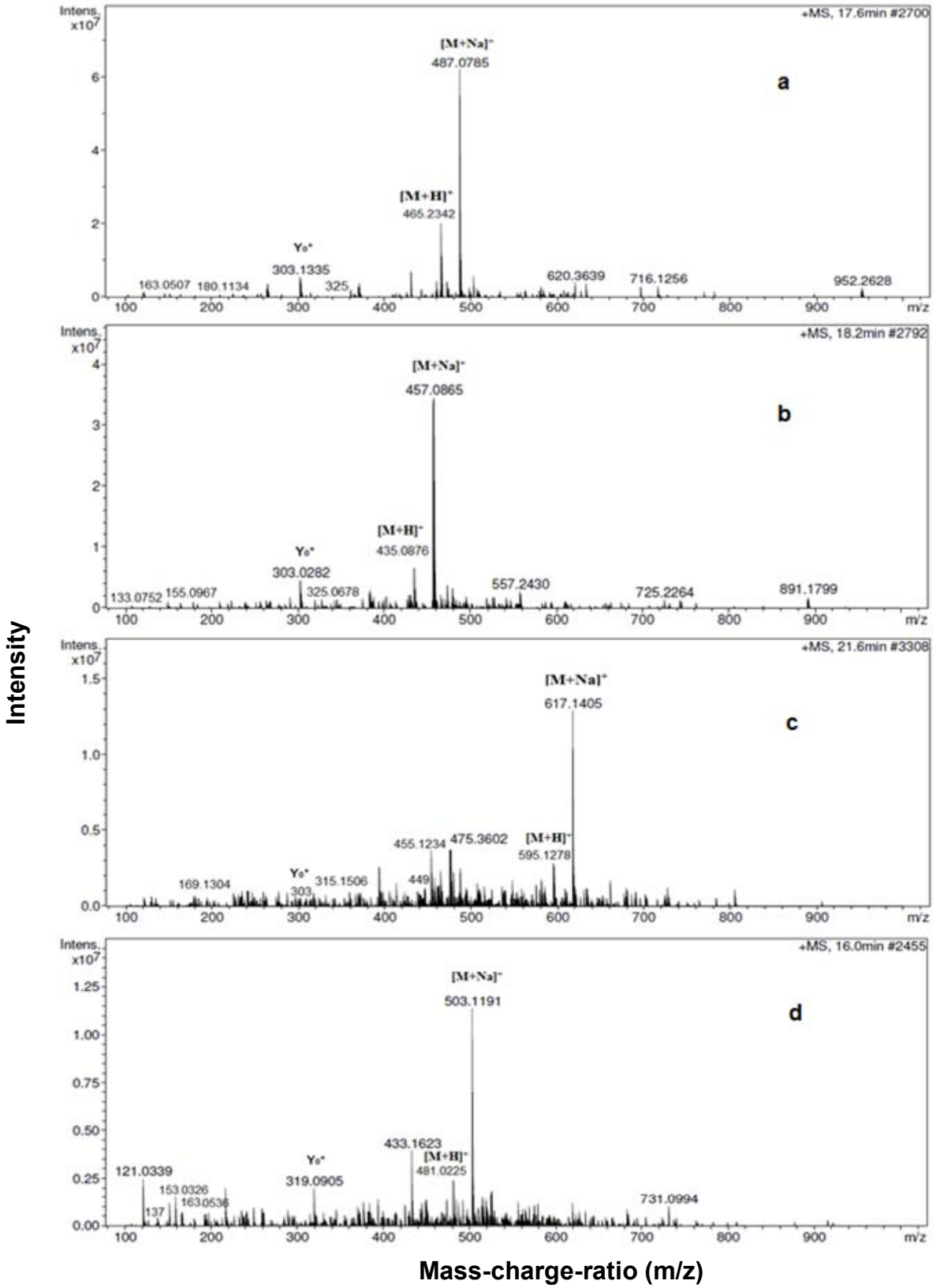


Figure 3. ESI-MS mass spectrum registered in positive mode: (a) quercetin-hexoside, (b) quercetin-pentoside, (c) quercetin-ramnobioside and (d) myricetin-hexoside.

Table 1. Retention time and fragmentation in positive mode of the identified compounds of T. gardneriana.

Compound	T_R EEC	T_R $CHCl_3$	T_R AcOEt	T_R MeOH	$[M+H]^+$ (m/z)	$[M+Na]^+$ (m/z)	Fragmentation in positive mode (MS+)
1	17.6	17.6	17.6	17.5	465	487	303, 181, 185, 163
2	18.4	-	18.2	18.3	435	457	303, 325, 133, 155
3	21.6	21.5	-	-	595	617	303, 471, 455, 449, 315, 147, 169
4	16.0	-	-	15.9	481	503	319, 163,153

*EEC= Crude ethanol extract. $CHCl_3$=Chloroform fraction. AcOEt= Ethyl acetate fraction. MeOH= Methanol fraction. T_R= Retention time.

Table 2. Total flavonoids and antioxidant activity of *T. gardneriana*.

Extract/Fractions/Standards	Flavonoids (mg de ECAT/g)	DPPH (EC_{50}, µg ml^{-1})	β-Carotene (%AA)
Crude ethanol extract	281.35± 6.23	2.27± 0.19	67.62± 2.48
Hexane fraction	n.d	32.91± 0.76	48.90± 1.12
Chloroform fraction	61.88± 3.50	50.35± 10.27	54.74± 1.28
Ethyl acetate fraction	287.14± 2.23	5.42± 0.84	45.04± 1.12
Methanol fraction	271.35± 1.32	3.35± 0.15	47.64± 2.59
Ascorbic acid	-	3.21± 0.30	-7.83± 2.58
Butylhydroxyanisole	-	3.05± 0.40	70.68± 0.55
Butylhydroxytoluene	-	5.38± 0.11	69.96± 3.07

*Values are presented as mean ± SD (n = 3). ECAT= Catechin equivalents. EC50 is defined as sufficient for 50% maximal effect concentration. (%AA)= percentage of antioxidant activity. n.d.= not determined.

Table 3. Maximum wavelength and absorption type of crude ethanol extract and fractions of T. gardneriana.

Extract / Fractions	λ maximum (nm)	Absorption region
Crude ethanol extract	280	UVC/UVB
Hexane fraction	-	-
Chloroform fraction	280/330/395	UVA/UVB/UVC
Ethyl acetate fraction	280/370/400	UVA/UVB/UVC
Methanolic fraction	280	UVC/UVB

*UVA (320 to 400 nm), UVB (280 to 320 nm) and UVC (100 to 280 nm). Concentrations of the analysis were 5, 25, 50 and 100 mg L^{-1}.

ethyl acetate and methanol fractions are not statistically different ($P < 0.05$) from that found for standards. The crude ethanol extract showed better antioxidant activity than did the standards used. In the β- carotene method, the values of the hexane, ethyl acetate and methanol fractions were not significantly different (Table 2). According to the results shown in Table 2, all samples exhibited antioxidant activity. These results can be justified by the presence of higher levels of flavonoids in the crude ethanol extract, methanol and ethyl acetate fractions. Phenolic compounds, particularly flavonoids, possess ideal structure for the scavenging of radicals, since they are very reactive as a hydrogen and electron donor (Barreiros et al., 2006). The antioxidant activity presented by the leaf extract and fractions of *T. gardneriana* corroborates studies with seed extract (Farias et al., 2013).

Photoprotective activity

According to RDC Resolution No. 30 (National Health

Table 4. Sun Protection Factor (SPF) of crude ethanol extract and fractions of *T. gardneriana*.

Concentration (mg L^{-1})	Crude ethanol extract	Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction
5	0.700±0.127	0.343±0.031	0.669±0.022	0.775±0.052	0.685±0.069
25	2.042±0.050	0.731±0.020	3.169±0.032	2.937±0.123	2.769±0.049
50	3.734±0.051	1.267±0.053	6.324±0.069	5.608±0.084	5.379±0.145
100	7.410±0.132	2.393±0.084	12.794±0.163	11.280±0.127	11.891±0.291

* Values are presented as mean ± SD (n = 3).

Surveillance Agency of Brazil (ANVISA), 2012), a product suitable for use in cosmetics such as sunscreen products should have SPF values of at least 6.0. Therefore, all samples, except for the hexane fraction, showed photoprotective effect. Such a high level of photoprotection can be explained by the content of flavonoids present in the samples (Table 2), since, as already mentioned, the presence of these metabolites indicates a potential in the absorption of UV radiation. Therefore, assays like this are important, since they guide the selection of plant species with potential sun protection factor in a simple and inexpensive manner. According to the literature, the genus has been little explored from the phytochemical point of view, previously reporting only the isolation of five flavonol glycosides and gallic acid from the ethyl acetate fraction of the leaves of *T. cumingiana* (Hussein et al., 2005) and phenylpropanoid, one flavonol glycoside and gallic acid on leaves, stems and fruit of *T. americana* (Oliveira et al., 2008). The presence of glycosylated flavonols and gallic acid in both plant species may serve as useful chemo-taxonomic markers for the genus. Thus, glycosylated flavonols identified in this study are in agreement with the data described in studies of genus and confirm further these chemical markers.

Conclusion

In this study, the coupled LC-MS technique has proved to be a powerful tool for the identification of flavonols, which allowed for the identification of four flavonol glycosides which are described for the first time in the species. The results obtained from this study show that the excellent antioxidant and photoprotective activities can be attributed to its chemical composition rich in flavonols. It also suggests that this species, which is already widely used in folk medicine, may become a great alternative for use in pharmaceuticals.

Conflict of interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENTS

This work was supported by grants from Brazilian agencies CNPq and FACEPE. We also would like to thank Teacher Abilio Borghi for the grammar review of the manuscript.

REFERENCES

Agência de Vigilância Sanitária- ANVISA (2012). RDC Resolution No. 30, approved the Technical Regulation on sunscreens in cosmetics. ANVISA: Brasília. Available at: http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2012/rdc0030_01_06_2012.html Accessed April 2014.

Alves CQ, David JM, David JP, Bahia MV, Aguiar RM (2010). Methods for determination of in vitro antioxidant activity in organic substrates. Quim. Nova 33(10):2202-2210.

Barreiros ALBS, David JM, David JP (2006). Oxidative stress: relationship between generation of reactive species and organism defense. Quim. Nova 29(1):113-123.

Boulekbache-Makhlouf L, Meudec E, Mazauric JP, Madani K, Cheynier V (2012). Qualitative and semi-quantitative analysis of phenolics in *Eucalyptus globulus* leaves by High- performance Liquid Chromatography coupled with Diode Array Detection and Electrospray Ionisation Mass Spectrometry. Phytochem. Anal. 24:162-170.

Cartaxo SL Souza MMA, Albuquerque UP (2010). Medicinal plants with bioprospecting potential used in semi-arid Northeastern Brazil. J. Ethnopharmacol. 131:326-342.

Cuyckens F, Claeys M (2005). Determination of the glycosylation site in flavonoid mono-O- glycosides by collision-induced dissociation of electrospray-generated deprotonated and sodiated molecules. J. Mass Spectrometry 40:364-372.

Dewanto V, Wu X, Adom KK, Liu RH (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50(10):3010-3014.

Farias DF, Souza TM, Viana MP, Soares BM, Cunha AP, Vasconcelos IM, Ricardo NMPS, Ferreira PMP, Melo VMM, Carvalho AFU (2013). Antibacterial, antioxidant, and anticholinesterase activities of plant seed extracts from Brazilian semiarid region. Biomed. Res. Int. 510736:9.

Hussein AA, Barberena I, Correa M, Coley PD, Solis PN, Gupta MP (2005). Cytotoxic flavonol glycosides from *Triplaris cumingiana*. J. Nat. Prod. 68:231-233.

Mansur JS, Breder MNR, Mansur MCA, Azulary RD (1986). Determination of the sun protection factor by spectrophotometry. Anal. Bras. Dermatol. 61:121-124.

Mensor LL, Menezes F, Leitão GG, Reis AS, Santos TC, Coube TS, Leitão SG (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15:127-130.

Oliveira PES, Conserva LM, Lemos RPL (2008). Chemical constituents from *Triplaris americana* L. (Polygonaceae). Biochem. Syst. Ecol. 36:134-137.

Rijke E, Out P, Niessen WMA, Ariese F, Gooijer C, Brinkman UAT (2006). Analytical separation and detection methods for flavonoids. J. Chromatogr. A. 1112:31-63.

Souza FP, Campos GR, Packer FJ (2013). Determination of the photoprotective and antioxidant activity in emulsions containing extract *Malpighia glabra* L. – Acerola. Rev. Cienc. Farm. Basica Appl. 34(1):69-77.

Souza LM, Cipriani TR, Serrato RV, Costa DE, Iacomini M, Gorin FAJ, Sassaki GL (2008). Analysis of flavonol glycoside isomers from leaves of *Maytenus ilicifolia* by offline and online high performance liquid chromatography–electrospray mass spectrometry. J. Chromatogr. A. 1207:101-109.

Violante IMP, Souza IM, Venturini CL, Ramalho AFS, Santos RAN, Ferrari M (2009). In vitro evaluation of the photoprotective activity of plant extracts from the Cerrado of Mato Grosso. Rev. Bras. Farmacogn. 19(2A):452-57.

Wannes WA, Mhamdi B, Sriti J, Jemia MB, Ouchikh O, Hamdaoui G, Kchouk ME, Marzouk B (2010). Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. Food Chem. Toxicol. 48(5):1362-1370.

Zuanazzi JAS, Montanha JA (2012). Flavonoids. In: Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR (eds.), Pharmacognosy of the plant to the medicine. Federal University of Rio Grande do Sul: Porto Alegre. pp. 577-614.