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Mutagenic activity and chemical composition of phenolic-rich extracts of leaves from two species of *Ficus* medicinal plants

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ABSTRACT

Plant species from the *Ficus* genus are widely used as food, and in folk medicine as anti-inflammatory, antioxidant and anticancer agents, although some of these species are known to produce adverse effects. The aim of this study was to determine and compare the chemical composition as well as *in vitro* antioxidant and mutagenic activity of the aqueous extracts of leaves from *F. adhatodifolia and F. obtusiuscula*. Phytochemical screening using thin-layer chromatography identified 6 classes of secondary metabolites in the extracts. Total phenolic content was estimated by the Folin-Ciocalteau method and the phenolic profile was determined by UPLC-DAD-ESI/MS/MS. Antioxidant activities were evaluated by the DPPH radical assay and by the β-carotene/linoleic acid system. Mutagenic activity was measured by the *Salmonella typhimurium* reverse mutation test with 4 strains, in both the presence and absence of metabolic activation. Flavonoids, coumarins, and tannins were detected in both extracts, and 6 major derivatives were identified as flavone compounds. Antioxidant activities were demonstrated for both extracts, while *F. obtusiuscula* contained higher concentrations of phenolic compounds. Mutagenic activity of the TA97 strain without metabolic activation was observed for both tested extracts, as well as the TA102 strain with metabolic activation. In addition, the extract of *F. adhatodifolia* was shown to be mutagenic to the TA102 strain without metabolic activation. Evidence indicates that the use of teas obtained from these two plant extracts in folk medicine may raise concerns and needs further investigation as a result of potential pro-oxidant mutagenic effects in the absence or presence of metabolic activation.

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Introduction

Since ancient times, the fruits, leaves, latex and bark from plants of the genus *Ficus* have been used as either food or medicine (Barolo, Mostacero, and López 2014; Solomon et al. 2006; Wang et al. 2008). Commonly known as fig trees, *Ficus* (Moraceae) is a genus of angiosperms containing approximately 800 species (Loutfy et al. 2005; Ronsted et al. 2008). Some of *Ficus* species are used in traditional medicine predominantly for treatment of worms (Breitbach et al. 2013; Hansson, Zelada, and Noriega 2005; Ricardo et al. 2017). However, pharmacological and chemical studies noted distinct biological activities including antioxidant (Manian et al. 2008; Verma et al. 2010), anti-inflammatory (Lansky et al. 2008) and anticancer activities (Abdel-Hameed 2009; Yap et al. 2015). These biological activities are mainly attributed to the secondary metabolites present in extracts of *Ficus*.

Flavonoids, coumarins and alkaloids were isolated from the leaves (Chang et al. 2005), roots and bark of *Ficus* species (Lansky et al. 2008). Despite the widespread use of these plants in popular medicine, adverse effects have also been attributed to some *Ficus* species (Bafor and Igbinuwen 2009; Lansky et al. 2008; Petersen 2011). It is well-known that furanocoumarins, a class of phototoxins found in *Ficus* were linked to genotoxic and carcinogenic activities (Oliveira et al. 2012; Singh, Singh, and Goel 2011; Sproll et al. 2008). Studies in rats and mice revealed that chronic oral exposure to coumarin may lead to hepatic adenomas and carcinomas (Carlton, Aubrun, and Simon 1996; Lake 1999). Further, phenolic compounds, frequently

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found in *Ficus*, are related to antioxidant activities (Carmona et al. 2017; Q-N. et al. 2017; Trindade et al. 2016), and are widely investigated for the prevention of human diseases, including cancer, atherosclerosis and other inflammatory diseases (Arunachalam and Parimelazhagan 2013; Shi et al. 2011). Conversely, antioxidants may also exhibit pro-oxidant activities that, depending on the target cell, degrade DNA by generating reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) (Choueiri et al. 2012; Labieniec, Gabryelak, and Falcioni 2003; Lambert and Elias 2010). In some cases, antioxidants may lower ROS to levels that compromise cell signaling, which might enhance the risk of diseases such as lung cancer (Albanes et al. 1995).

In Brazilian folk medicine, teas prepared from leaves of *Ficus adhatodifolia* Schott ex Spreng. and *Ficus obtusiuscula* (Miq.) Miq., both belonging to the subgenus *Pharmacosycea*, are used as anthelmintics and antianemics (Breitbach et al. 2013; Duke and Vasquez 1994). However, there are no apparent studies regarding the chemical composition, pharmacology, and toxicology of these two species. Investigations on the use of these two medicinal plants are needed because certain common secondary metabolites found in the *Ficus* genus exert cytotoxic and genotoxic effects (Abraham et al., 2010; Labieniec, Gabryelak, and Falcioni 2003). Thus, the aim of this study was to determine the chemical composition as well as *in vitro* antioxidant and mutagenic activity of aqueous extracts of leaves, as teas used in folk medicine originate from *F. adhatodifolia* and *F. obtusiuscula* species.

Materials and methods

Plant material

Leaves of *F. adhatodifolia* (W42°52′13.7”; S20°45′ 39.51”) and *F. obtusiuscula* (W42°58′13”; S20°40′ 47”) were collected in the city of Viçosa, Minas Gerais, Brazil. The leaves were identified by Sérgio Romaniuc Neto (Botanical Institute of São Paulo, São Paulo, Brazil); and herbarium voucher specimens were herborized and deposited in the Herbarium VIC at the Universidade Federal de Viçosa under the register numbers VIC 31644 (*F. adhatodifolia*) and VIC 31713 (*F. obtusiuscula*).

Extract preparation

A portion of the collected leaves was dried in a kiln at 39°C for 48 hr, and dried leaves then crushed. Aqueous leaves extracts were prepared by infusion, using the same methods as used in folk medicine. Briefly, 50 g of each plant material at a 1:20 ratio (leaves:distilled water, w/v) was boiled in water for 15 min. The aqueous extracts were filtered through a Whatman filter paper using a vacuum pump followed by lyophilization. Totals of 4.7 and 3.8 g of *F. adhatodifolia* and *F. obtusiuscula* extract were obtained, respectively. The solvent-free extracts were those used in the present study.

Phytochemical screening

The following classes of secondary metabolites were investigated in the extracts using thin-layer chromatography (TLC) as described by Wagner and Bladt (1996) on a sheet containing silica-gel F254 (Merck®, Darmstadt, Germany): coumarins, alkaloids, triterpenes/steroids, flavonoids, tannins, and saponins. For some experiments, light at wavelengths of 254 nm and 365 nm was employed to examine the chromatograms. As a control, reference patterns of each of the metabolites were placed together on the sheets.

Determination of total phenolic compounds

The concentration of total phenolics was determined by colorimetry using the Folin-Ciocalteau method as described by (Coelho et al. 2012). For each extract, 8.5 ml of 32 µg/ml extract was added along with 1 ml sodium carbonate (150 mg/ml) and 0.5 mol Folin-Ciocalteau (Dinâmica®, Diadema, Brazil) reagent. A third test tube was prepared by substituting the plant extract with distilled water to calibrate the spectrophotometer. The test tubes were shaken and incubated in the dark for 30 min. All reactions were performed in triplicate; and measurements taken at 760 nm in a spectrophotometer (UV-1600 Pro-Análise, Porto Alegre, Brazil). For quantification of polyphenols, a standard curve was generated with a tannic acid solution (CRQ, São Paulo, Brazil) at different concentrations (10, 20, 30, 40, 50, and 60 µg/ml). The

results were expressed in tannic acid equivalent per gram of dry extract (TAE/g).

Analysis of ultra performance liquid chromatography (UPLC) with diode-array detection (DAD) coupled to electrospray ionization (ESI) mass spectrometry/mass spectrometry (MS/MS)

UPLC-DAD-ESI/MS/MS analyses were carried out using an ACQUITY Ultra Performance LC™ system (Waters, Milford, MA, USA) linked simultaneously to both a photodiode array (PDA) 2996detector (Waters, Milford, MA, USA) and a ACQUITY TQ Detector (Waters MS Technologies, Manchester, UK), equipped with a Z-spray electrospray ionization (ESI) source operating in positive and negative modes. The MassLynx™ software (version 4.1, Waters, Milford, MA, USA) was used to control instruments for data acquisition and processing. Sample solutions (3 µl; 0.5 mg/ml) were injected into a reversed phase column (Acquity UPLC BEH C18, 1.7 µm, 1 mm × 50 mm, Waters, Milford, MA), which was maintained at 40°C. The mobile phase consisted of a linear gradient system of 0.1% formic acid in water (*v*/*v*) (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.3 ml/min. Elution was achieved with the following stepwise gradient: 0 min, 5% B; 10 min, 95% B; 11 min, 5% B; 13 min, 5% B. The effluent was introduced into a PDA detector (scanning range 210–400 nm, resolution 1.2 nm) and subsequently into an electrospray source (source block temperature 120°C, desolvation temperature 400°C, capillary voltage 3.5 kV, cone voltage 30 V). Nitrogen was used as the desolvation gas (500 L/hr).

Antioxidant activity measures

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay was performed as described by Shirwaikar and co-workers (2006) with some modifications. For each of the 2 ml extract samples diluted with methanol (Vetec, São Paulo, Brazil) at different concentrations (25, 75, 125, 175 or 225 µg/ml), 2 ml 0.1 mM DPPH (Sigma-Aldrich, São Paulo, Brazil) solution were added. A control solution containing 2 ml methanol and 2 ml DPPH solution was employed. The absorbance was measured at 517 nm after 30 min of initiating the reaction. The reactions were performed in triplicate, and % DPPH free radical capture was calculated utilizing the following equation:

$$\%\ \text{of DPPH capture} = \left(1 - A_{sample}/A_{control}\right) \times 100$$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample. The extract concentration required to inhibit 50% of DPPH free radicals (IC_{50}) was estimated using the regression equation. Further, to compare the results, the antioxidant activity of the synthetic compound butylated hydroxytoluene (BHT) was evaluated at concentrations of 1, 5, 10, 20, and 30 µg/ml.

β-carotene/linoleic acid system

To evaluate the total antioxidant activity of the extracts, the β-carotene/linoleic acid system was employed as described by Hua-Hua et al. (2006) with modifications. A β-carotene/linoleic acid emulsion was prepared by dissolving 2 mg β-carotene (Fluka, São Paulo, Brazil) in 10 ml chloroform (Vetec, São Paulo, Brazil), followed by adding 250 µl linoleic acid (Vetec, São Paulo, Brazil) and 1.835 ml Tween 20 (Vetec, São Paulo, Brazil). The chloroform was evaporated at 50°C for 2 min. Next, 500 ml distilled water, pre-aerated for 30 min, was added to the semi-solid residue and subsequently agitated to generate the emulsion. An aliquot 5 ml emulsion was added to test tubes containing 1 ml of each extract at a concentration 100 µg/ml. The absorbance was initially measured at 470 nm using a spectrophotometer (UV-1600 Pro-Análise, Porto Alegre, Brazil) and then at 20 min intervals for 120 min. The control solution contained 5 ml of emulsion and 1 ml of distilled water. In addition, the total antioxidant activity for BHT (75 µg/ml) was measured for comparison. To determine the total antioxidant activity as % decrease in absorbance compared to control was calculated using the following equation:

$$AA = (1 - A_0 - A_t/A0_o - A0_t) \times 100$$

where AA is the antioxidant activity, A_0 is the initial absorbance of the sample, At is the absorbance of the sample after 120 min, $A0_0$ is the initial absorbance of the control and $A0_t$ is the absorbance of control after 120 min.

Mutagenicity assay

Cytotoxicity assay

The maximal extract concentration for cytotoxicity experiments was determined by the solubility of the extract in water (15 mg/plate). To determine the cytotoxicity of *F. adhatodifolia* and *F. obtusiuscula* extracts using the *Salmonella typhimurium* assay, the TA100 strain without metabolic activation was used in accordance with Stankevicins and co-workers (2008) with some modifications. The bacterial culture was diluted to 0.4 x 10^2 bacteria/ml. In the test tube, a 100 µl aliquot of each extract at 150 mg/ml was added to 500 µl 0.2 M phosphate buffer (pH 7.4) and 100 µl bacterial culture. The test tubes were incubated at 37°C for 30 min. After incubation 2 ml top agar was added containing agar and sodium chloride. The contents of each tube were poured onto plates containing a minimum amount of glycosylated agar and Vogel Bonner E 50X enriched with histidine and biotin (0.5 mM). The plates were incubated at 37°C for 24 hr before the number of colonies was counted. The experiment was performed in triplicate (Maron and Ames 1983).

Salmonella mutagenicity assay

The mutagenicity of leaf extracts from the two *Ficus* species was evaluated using the Ames test (Maron and Ames 1983). Different strains (TA97, TA98, TA100, and TA102) of *Salmonella typhimurium* were pre-incubated with 5 different amounts of each extract diluted in distilled and autoclaved water. Because some chemicals become mutagenic after being metabolized by cytochrome P450 enzymes, pre-incubation of each sample was also performed using the S9 Mix (Moltox, USA), which mimics hepatic metabolism. For pre-incubation, 5 different concentrations (15, 11.25, 7.5, 3.75 or 1.875 mg/plate) of each extract were mixed with 0.5 ml 0.2 M phosphate buffer (pH 7.4) and 0.1 ml bacterial culture (0.2 x 10^8 bacteria/ml), and then incubated at 37°C for 30 min. The S9 mix was prepared by mixing 19.75 ml distilled water, 25 ml 0.2 M phosphate buffer (pH 7.4), 2 ml 0.1 M NADP, 0.25 ml 1 M glucose-6-phosphate, 1 ml saline solution (1.65 M KCl + 0.4 M $MgCl_2 \cdot 6H_2O$) and 2 mlS9 fraction diluted in autoclaved and distilled water. After pre-incubation, 2 ml top agar containing L-histidine/D-biotin (0.5 mM) were added. The contents of each tube were poured onto plates containing a minimum amount of glycosylated agar. After the top agar solidified, the plates were incubated at 37°C for 48 hr. Subsequently, the number of revertant colonies per plate was counted. All assays were performed in triplicate, and negative control was generated by replacing the plant extract with water.

Statistical analysis

The significance of the differences between antioxidant activities of each extract and BHT was assessed by analysis of variance (ANOVA) followed by Tukey's test. The significance of the differences between total antioxidant activities of each extract and BHT was assessed by ANOVA followed by Tukey's test. The average number of colonies grown on the plates treated with the extracts was compared to a negative control (no extract) by ANOVA followed by a Tukey's test. The concentrations of the extract that inhibited colony growth by greater than 30% when compared to the negative control were considered cytotoxic. The confidence limit was set at $p < 0.05$. ANOVA and Tukey's test were used to analyze differences between mean number of revertant colonies at different concentrations determined and negative control followed by linear regression. The Mutagenicity Index (MI) was calculated for each concentration using the mean number of revertants per plate divided by the average number of spontaneous revertants per plate of the negative control. The extracts were considered positive for mutagenic activity when the MI was greater or equal to 2 in at least two

of the concentrations analyzed. An extract was considered weakly mutagenic if the concentration exhibited an MI greater than 1.5 and less than 2. However, the extract was considered negative for mutagenicity if the number of revertants was not sufficient to produce an MI greater than 1.5 for all of the concentrations analyzed (Nunes et al. 2012).

Results

Chemical characterization of the extracts

The TLC phytochemical screening carried out on extracts of the *F. adhatodifolia* and *F. obtusiuscula* species revealed the presence of flavonoids, coumarins, and tannins in both extracts. The *F. obtusiuscula* extract presented a significantly higher concentration of phenolic compounds (127.26 mg TAE/g) than the extract of *F. adhatodifolia* (82.36 mg TAE/g).

The chromatographic profiles obtained by UPLC-DAD from the extracts of the two targeted *Ficus* species were similar when analyzed in the 254 nm region (Figure 1), with both chromatograms containing peaks characteristic of flavonoids in the ultraviolet spectrum (Mabry, Markham, and Thomas 1970). Six derivatives were identified as flavone compounds, and were common to both targeted species. Although both species displayed similar chromatographic profiles, differences in intensity of the major peaks between the extracts were observed. The peak for *F. adhatodifolia* was found at R_t 2.79 min, whereas the peak with R_t 2.69 min was most intense for *F. obtusiuscula*.

Data from UPLC-ESI/MS/MS were used to identify the 6 flavonoid compounds detected in extracts of both targeted species. The retention times (R_t), the UV λ_{max} values, and molecular and fragmentation ions are presented in Table 1.

Antioxidant activity

The antioxidant activity measured by the capture of DPPH free radicals revealed IC_{50} concentrations

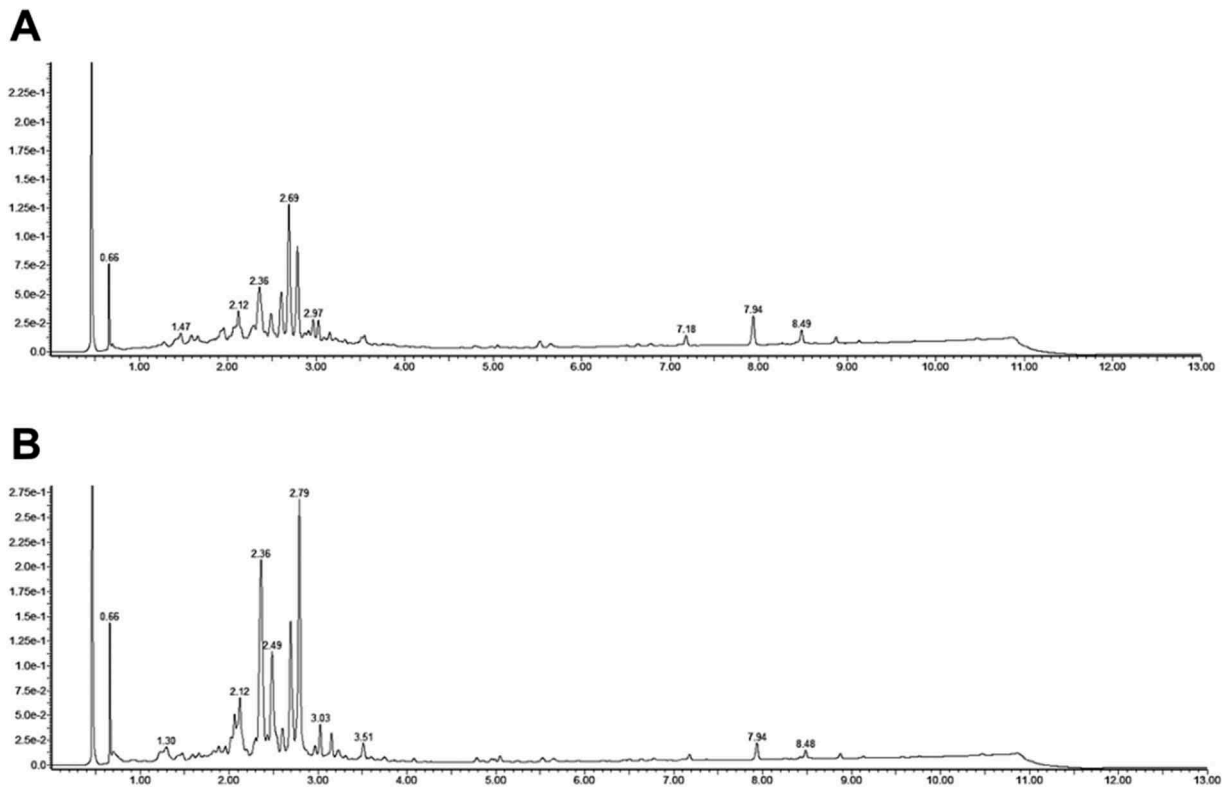

Figure 1. UPLC-DAD profile of leaf aqueous extract from Ficus obtusiuscula (A) and Ficus adhatodifolia (B). Conditions: CHS130 100 RP-18 column (1.7 m, 50 × 3 mm i.d.). Elution was carried out with a linear gradient of water 0.1% formic acid (A) and acetonitrile 0.1% formic acid (B) (from 5% to 95% of B in 11 min) and the UPLC fingerprints were registered on a ACQUITY Waters apparatus with a UV-DAD detector in 254 nm.

Table 1. UPLC-DAD-ESI/MS/MS analyses of polyphenols present in Ficus adhatodifolia and Ficus obtusiuscula.

R_t (min)	Compound	$\lambda_{máx.}$ (nm)	$[M + H]^+$ (*m/z*)	MS (*m/z*)	$[M-H]^-$ (*m/z*)	MS (*m/z*)	*Ficus adhatodifolia*	*Ficus obtusiuscula*
2.12	Vicenin II or isomers of Vicenin II	221.13 272.13 323.13	595.17	-	593.28	503.19; 473.20	+	+
2.36	Vicenin I or isomers of Vicenin I	219.13 271.13 333.13	565.37	-	563.42	503.25; 473.13; 443.33	+	+
2.49	Orientin or isoorientin	219.13 271.13 324.13	449.32	-	447.17	357.38; 327.16	+	+
2.60	Vitexin or isovitexin	221.13 268.13 333.13	433.19	-	431.18	341.46; 311.16	+	+
2.69	Vitexin-7-*O*-rhamnoside or isovitexin-7-*O*-rhamnoside	216.13 269.13 334.13	579.30	433.38	577.47	457.32; 311.22	+	+
2.79	Vitexin or isovitexin	216.13 270.13 333.13	433.32	415.05; 313.23	431.37	341.20; 311.34	+	+

of 124.68 and 111.48 μg/ml for *F. adhatodifolia* and *F. obtusiuscula* extracts, respectively, which were not significantly different. The synthetic compound BHT exhibited a significantly different IC_{50} of 3.21 μg/ml, from those of the extracts. In the β-carotene/linoleic acid system, the *F. adhatodifolia* and *F. obtusiuscula* extracts inhibited 36% and 39.9% of β-carotene oxidation, respectively, after 120 min. The inhibition % (total antioxidant activity) of these two extracts were not markedly significantly different, whereas BHT inhibited 80% of β-carotene oxidation and was significantly different from those of the extracts.

Mutagenic assessment

Cytotoxicity testing did not reveal a marked difference between mean number of colony-forming units (cfu) for the TA100 strain (S9-) treated with 15 mg/plate of both *Ficus* extracts or with negative control. Data demonstrated that both *Ficus* extracts were not cytotoxic. The *F. adhatodifolia* extract exhibited mutagenic activity to strain TA97 without metabolic activation (S9-), with MI values greater than 2 for the 4 concentrations tested, in addition to a concentration-response correlation (R^2 = 0.83) (Table 2). Mutagenic activity was also found using *F. adhatodifolia* extract on the TA102 strain with metabolic activation (S9+) at the two highest concentrations tested. Weak indications of mutagenicity were observed for *F. adhatodifolia* extracts on strains TA98 (S9+) and TA102 (S9-). Despite a lack of observed mutagenic activity of the plant extract of *F. adhatodifolia* on the strain TA100 (S9-), a concentration-response correlation was observed (R^2 = 0.9), which may be an indication of mutagenicity. The *F. obtusiuscula* extract was considered mutagenic to the TA97 strain (S9-) (Table 2). However, MI > 2 was not noted for any of the tested concentrations after metabolic activation. This extract was also mutagenic to the TA102 strain (S9+) exhibiting an MI greater than 2 for 3 of 4 concentrations tested with a significant concentration-response correlation (R^2 = 0.99). The mutagenic analysis of this extract with other bacterial strains, TA98 and TA100, did not induce an MI greater than 2 or a concentration–response relationship in the presence or absence of metabolic activation.

Discussion

All of the compounds detected in the *Ficus* extracts by UPLC-DAD exhibited UV absorption maxima with two bands at 220–272 nm and 324–334 nm, which is characteristic of flavonoids with an absence of hydroxylation at *C*-3 (flavones) (Mabry, Markham, and Thomas 1970) (Figure 2). The identification of these detected compounds was based upon a search of the main molecular ions, in addition to some useful observed fragmentations. Flavonoid aglycones were characterized by

Table 2. Mutagenic activity expressed by the mean number of revertents, standard deviation and MI (in parentheses) of strains TA97, TA98, TA100 and TA102 of *S. typhimurium* exposed to different doses of leaf extracts of two *Ficus* species without (S9-) or with (S9+) metabolic activation.

		TA97		TA98		TA100		TA102	
Sample	mg/plate	S9-	S9+	S9-	S9+	S9-	S9+	S9-	S9+
F. adhatodifolia	1.87	858.7 ± 157.0 (2.8)	1256.0 ± 157.0 (1.3)	19.0 ± 3.0 (1.0)	21.0 ± 6.0 (0.8)	151.3 ± 19.0 (0.9)	130.0 ± 21.0 (0.8)	318.0 ± 13.0 (1.3)	81.0 ± 16.0 (1.9)
	3.75	1040.0 ± 49.0* (3.5)	1220.0 ± 277.0 (1.2)	19.7 ± 4.0 (1.0)	30.7 ± 6.0 (1.2)	175.3 ± 11.0 (1.0)	180.3 ± 15.0 (1.1)	358.7 ± 36.0 (1.5)	72.0 ± 8.0 (1.7)
	7.50	1098.7 ± 170.0* (3.6)	996.0 ± 293.0 (1.0)	33.3 ± 17.0 (1.8)	23.3 ± 2.0 (0.9)	182.5 ± 30.0 (1.0)	159.0 ± 24.0 (0.9)	397.3 ± 49.0 (1.6)	76.3 ± 11.0 (1.8)
	11.25	1166.7 ± 307.0* (3.9)	1113.3 ± 129.0 (1.1)	36.7 ± 11.0 (2.0)	35.3 ± 3.0* (1.4)	191.0 ± 38.0 (1.1)	171.5 ± 19.0 (1.0)	442.7 ± 63.0* (1.8)	101.7 ± 8.0 (2.4)
	15.0	568.0 ± 34.0 (1.9)	1325.3 ± 96.0 (1.3)	34.3 ± 12.0 (1.8)	43.0 ± 1.0* (1.7)	229.0 ± 9.0* (1.3)	200.5 ± 19.0* (1.2)	449.3 ± 39.0* (1.9)	118.3 ± 15.0* (2.8)
F. obtusiuscula	1.87	722.7 ± 265.0 (2.4)	994.7 ± 154.0 (1.0)	20.7 ± 6.0 (1.1)	29.0 ± 10.0 (1.1)	192.3 ± 5.0 (1.1)	168.3 ± 25.0 (1.0)	305.3 ± 52.0 (1.3)	64.3 ± 12.0* (1.5)
	3.75	633.3 ± 191.0 (2.1)	1382.7 ± 173.0 (1.4)	30.3 ± 7.0 (1.6)	19.3 ± 2.0 (0.8)	174.7 ± 20.0 (0.9)	157.3 ± 12.0 (0.9)	344.0 ± 53.0 (1.4)	83.3 ± 22.0 (2.0)
	7.50	417.3 ± 36.0 (1.4)	1573.3 ± 70.0* (1.6)	29.7 ± 10.0 (1.6)	26.0 ± 6.0 (1.0)	164.7 ± 39.0 (0.9)	162.0 ± 11.0 (0.9)	368.0 ± 93.0 (1.5)	118.3 ± 10.0 (2.8)
	11.25	608.0 ± 147.0 (2.0)	1340.0 ± 152.0 (1.3)	33.7 ± 3.0 (1.8)	39.0 ± 9.0 (1.5)	209.0 ± 6.0 (1.2)	166.0 ± 15.0 (1.0)	370.7 ± 68.0 (1.5)	139.3 ± 7.0 (3.3)
	15.0	710.7 ± 25.0 (2.4)	1397.3 ± 416.0 (1.4)	35.0 ± 4.0 (1.9)	29.7 ± 8.0 (1.2)	188.0 ± 18.0 (1.1)	162.7 ± 32.0 (1.0)	274.0 ± 18.0 (1.1)	178.0 ± 18.0* (4.2)
Control +		1188.0 ± 70.0* (3.9)	1709.3 ± 106.0* (1.7)	165.0 ± 24.0* (8.84)	174.0 ± 57.0* (6.9)	1629.3 ± 237.0* (9.3)	538.7 ± 24.0* (3.2)	1584.0 ± 52.0* (6.6)	83.0 ± 11.0 (2.0)
Control −		301.0 ± 10.0	994.7 ± 66.0	18.7 ± 4.0	25.3 ± 6.0	175.0 ± 9.0	169.7 ± 242.0	240.3 ± 25.0	42.3 ± 2.0

* $P < 0.05$ (ANOVA). Positive Control: 5 µg/plate of 4-nitro-*o*-phenylenediamine (TA97 S9- and TA98 S9-); 5 µg/plate of sodium azide (TA100 S9-); 0.5 µg/plate of mitomycin C (TA102 S9-); 5 µg/plate of 2-aminoanthracene (TA97 S9+; TA98 S9+; TA100 S9+ and TA102 S9+). Negative Control: 100 µL of water.

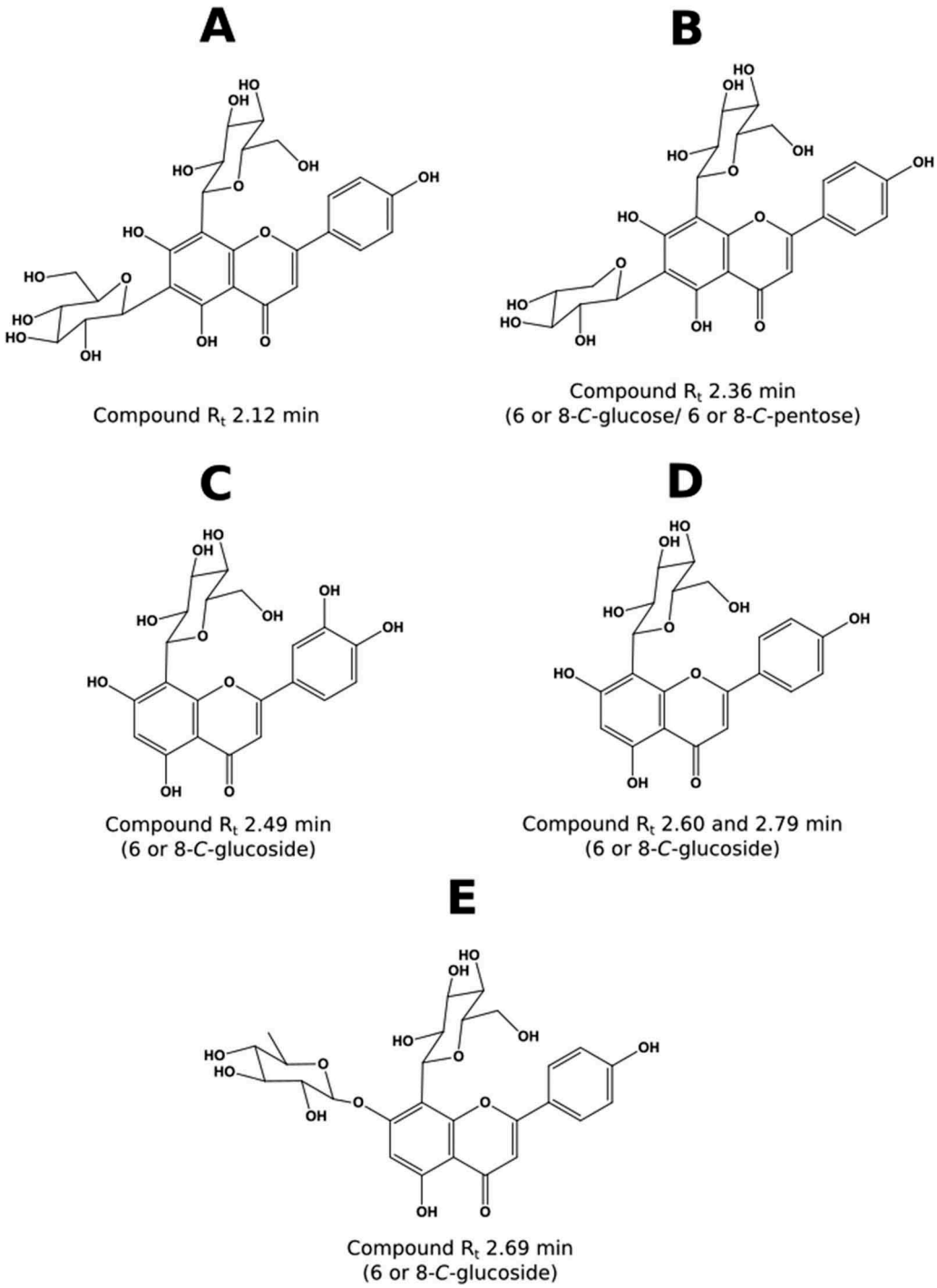

Figure 2. Phenolics compounds identified of leaf aqueous extracts from Ficus obtusiuscula and Ficus adhatodifolia. (A) Compounds Rt 2.12 min: Isomers Vicenin II; (B) Compounds Rt 2.36 min: Isomers Vicenin I; (C) Compound Rt 2.49 min: Orietin or isoorietin; (D) Compounds Rt 2.60 and 2.79 min: Vitexin or isovitexin; and (E) Compound Rt 2.69 min: vitexin-7-O-rhamnoside.

ESI-MS/MS, present in the major diagnostic fragments, which are those involving the cleavage of *C*-*C* bonds, with the loss of 60 Da [(M–H) – 60 Da]$^-$ (*C*-pentoside), 90 Da [(M–H) – 90 Da]$^-$, and 120 Da [(M–H) – 120 Da]$^-$ mass units from the deprotonated species [M–H]$^-$ of MS-MS spectra. These losses are typical of *C*-glucoside linkages (Colombo, Yariwake, and McCullagh 2008). In addition, fragmentation of the two *C*-*C* bonds of the *C*-ring provided information on the number and type of substituents in the A- and B-rings (Oliveira et al. 2012). Thus, 6 *C*-glycosyl

derivatives of flavones were identified for both extracts of *F. adhatodifolia* and *F. obtusiuscula*.

All 6 flavones identified in this investigation (Figure 2) were described for the first time in the *F. adhatodifolia* and *F. obtusiuscula* species, although these flavonoid glycosides were previously identified for other *Ficus* species. In general, plants of the *Ficus* genus are rich in flavonoids (Fongang et al. 2015). The phytochemical composition of 14 *Ficus* species was investigated (Greenham et al. 2007) and 39 phenolic compounds identified, including 14 flavonol *O*-glycosides, 6 flavone *O*-glycosides and 15 flavone *C*-glycosides. Among these were the luteolin 7-*O*-rutinoside, vitexin, isovitexin, apigenin *C*-hexoside-*C*-pentoside and other flavonoid-like compounds with C-, and/or *O*-hexosides, and/or pentosides.

Phenolic compounds such as flavonoids, including those found in Ficus extracts, may act as potent antioxidants, which are of important biological interest (Acésio et al. 2017; White, Howard, and Prior 2010). Antioxidants are believed to reduce the accumulation of ROS such as hydroxyl radicals, hydrogen peroxide, superoxide, and singlet oxygen and RNS such as nitric oxide and peroxynitrite that are generated through normal physiological processes in the mammalian organism. Conversely, antioxidants may deplete ROS to levels that compromise cell signaling enhancing the risk of diseases (Finley et al. 2011). Some secondary metabolites, including tannins (Labieniec, Gabryelak, and Falcioni 2003), flavonoids (Procházková, Boušová, and Wilhelmová 2011) and coumarins (Abraham et al., 2010; Sproll et al. 2008), may exhibit pro-oxidant and genotoxic effects on specific cellular targets. Previous investigators reported the presence of antioxidant activity in some Ficus species, correlating this activity with the content of phenolic compounds present in these plants (Abdel-Hameed 2009; Al-Fatimi et al. 2007; Daniel et al. 1998; Manian et al. 2008; Shukla et al. 2004). Therefore, Ficus species examined here were found to be potential sources of antioxidants, which may be attributed to the polyphenol content. Although there was a lack of significant difference between antioxidant potential of the two studied species, the extract of *F. obtusiuscula* showed greater antioxidant activity and polyphenolic content than *F. adhatodifolia*.

Phenolics constitute one of the major groups of compounds acting as primary antioxidants in both of the *Ficus* extracts. This effect may be attributed to its redox properties, which enables them to act differently as reducing agents, hydrogen donators or function as metal chelating compounds (Abdel-Hameed 2009).

The results of the test with *Salmonella typhimurium* indicate that both *Ficus* extracts exhibited mutagenic activity to TA97 (S9-) and TA102 (S9+) strains. The TA97 strain contains a hisD6610 mutation and detects substances that induce reading frame errors. In addition, the TA97 strain contains a second mutation site in GC base pairs and is sensitive to compounds that might also mutate the TA98 and TA1538 strains (Levin, Yamasaki, and Ames 1982). The TA102 strain detects oxidative mutagens, including X-rays, ultraviolet light, mitomycin C, hydrogen peroxide, aldehydes, and quinones, which act by base pair substitutions and target the adenine-thymine bond. In particular, the *F. adhatodifolia* extract was considered mutagenic to the TA97 strain (S9-) and exhibited weak mutagenicity to the TA102 (S9-) and TA98 (S9+) strains. Metabolic activation of the *F. adhatodifolia* extract by enzymes from the S9 mix decreased the observed MI for the TA97 and TA100 strains. This mutagenic effect was also found to be reduced when *F. obtusiuscula* extract was tested on the strain TA97. Conversely, metabolic activation increased the mutagenicity of both extracts on the TA102 strain. These data indicate that the secondary metabolites contained in extracts of both *Ficus* species may exert pro-oxidant effects against specific cellular targets and produce enhanced toxicity to DNA after metabolism by hepatic enzymes.

Structure-mutagenicity studies demonstrated increased rate of mutagenicity of flavonoids in the Ames test; however, this was attributed to the presence of a 3-hydroxyl group in the C-ring (Elliger, Henika, and MacGregor 1984; Resende et al. 2012; Rietjens et al. 2005). Several other flavone compounds (absence of 3-OH in C-ring) were also reported to be exhibit mutagenic activity using the same test (Beudot et al. 1998; Elliger, Henika, and MacGregor 1984). Our findings support the postulation that the absence of the catechol system in the flavones was not sufficient to inhibit the mutagenic activity of the extracts of the two targeted species of *Ficus*.

The mutagenic effect detected by extracts of both *F. adhatodifolia* and *F. obtusiuscula* may be associated with their polyphenolic composition. Reversions in the strain TA102 might be attributed to the presence of phenolic compounds with pro-oxidative activity, which might induce DNA mutations despite the *in vitro* antioxidant activity. In addition to the 6 different flavones identified in this study, other secondary metabolites such as coumarins, detected by TLC in both extracts, and compounds containing an aromatic ring fused to a condensed lactone ring, were previously identified in *Ficus* species, and related to mutagenic effects of other plant extracts (Varanda et al. 2002).

Conclusions

Chromatographic analysis enabled identification of the main components present in the aqueous extracts of leaves from *F. adhatodifolia* and *F. obtusiuscula*. The 6 flavone glycosides were identified as constituents of these species for the first time. Data demonstrated antioxidant activity and mutagenic effects of the extracts of both *Ficus* species. Our findings suggest that the use of these plant extracts may induce DNA damage, regardless of metabolic activation in the organism, inducing both read frame errors and base pair substitutions. The widespread use of these extracts in folk medicine and/or as food, in addition to their potential for carcinogenesis predicted by the Ames test suggests the need for caution when utilizing aqueous extracts of leaves from *F. adhatodifolia* and *F. obtusiuscula*.

Conflict of interest

The authors declare no conflict of interest.

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