

ORIGINAL ARTICLE

First phytochemical and biological study of the ethanolic extract from leaves of *Capirona decorticans* (Rubiaceae)

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ABSTRACT

Capirona decorticans (Rubiaceae) is popularly used to treat warts, wounds, mycoses and scabies, and is also a component of the Ayahuasca tea. Despite its popular use, the phytochemical and pharmacological research on this species is limited. Therefore, this work quantified phenolic compounds in the ethanolic extract (EE) and hydromethanolic fraction (FM) (406, 293 mgEAG g⁻¹, respectively) from leaves of *C. decorticans*. We identified flavonoids by LC-MS/MS-MMR-ESI (apigenin, rutin, luteolin, miricetin, quercetin, quercetin-3-β-D-glucoside, quercetrin), and evaluated oxidative stress and mutagenic/antimutagenic effect of EE and FM through an *in vivo* experiment using *Swiss* mice and cyclophosphamide (CP) as an inducer of DNA damage and oxidative stress. Mice were pretreated for 15 consecutive days with EE or FM (250 mg kg⁻¹) and then intraperitoneally injected with CP (25 mg kg⁻¹). Carbonylated proteins, ascorbic acid, catalase and thiobarbituric acid-reactive substances were measured in hepatic and renal tissues. The mutagenic/antimutagenic effect was evaluated through the Micronucleus Test. Protein carbonylation in the liver of animals exposed to CP was reduced by FM. There was no significant effect on other markers of oxidative stress. The groups treated with the extracts showed a significant percentage reduction (EE = 96% and FM = 71%) in the frequency of micronucleated polychromatic erythrocytes induced by CP. EE showed mutagenicity when used alone. The EE and FM of *C. decorticans* leaves showed antioxidant potential equivalent to that observed in other species, did not cause oxidative stress, nor toxicity, and had a protective and antimutagenic effect, although the EE showed signs of mutagenicity.

KEYWORDS: oxidative stress, cyclophosphamide, genotoxicity, phenolic compounds

Primeiro estudo fitoquímico e biológico do extrato etanólico de folhas de *Capirona decorticans* (Rubiaceae)

RESUMO

Capirona decorticans (Rubiaceae) é popularmente usada para tratar verrugas, feridas, micoses e sarna, e como um componente do chá de Ayahuasca. Apesar do uso popular, são limitadas as pesquisas fitoquímicas e farmacológicas sobre a espécie. Portanto, este estudo quantificou compostos fenólicos no extrato etanólico (EE) e na fração hidrometanólica (FM) (406 e 293 mgEAG g⁻¹, respectivamente) de folhas de *C. decorticans*. Identificamos flavonoides por LC-MS / MS-MMR-ESI (apigenina, rutina, luteolina, miricetina, quercetina, quercetina-3-β-D-glicosídeo, quercetrina), e avaliamos o estresse oxidativo e o efeito mutagênico/antimutagênico de EE e FM em um experimento *in vivo* utilizando camundongos *Swiss* e ciclofosfamida (CP) como um indutor de danos no DNA e estresse oxidativo. Os camundongos foram pré-tratados por 15 dias consecutivos com EE ou FM (250 mg kg⁻¹) e injetados intraperitonealmente com CP (25 mg kg⁻¹). Proteínas carboniladas, ácido ascórbico, catalase e substâncias reativas ao ácido tiobarbitúrico foram dosadas em tecidos hepáticos e renais. O efeito mutagênico/antimutagênico foi avaliado através do Teste de Micronúcleo. Houve carbonilação protéica no fígado de animais expostos à CP, que foi reduzida pela FM. Não houve efeito significativo sobre outros marcadores de estresse oxidativo. Os grupos tratados com os extratos apresentaram uma redução percentual significativa (EE = 96% e FM = 71%) na frequência de eritrócitos policromáticos micronucleados induzidos pela PC. O EE também apresentou mutagenicidade quando utilizado isoladamente. O EE e FM das folhas de *C. decorticans* apresentaram potencial antioxidante equivalente ao observado em outras espécies, não causaram estresse oxidativo, nem toxicidade, e tiveram efeito protetor e antimutagênico, embora a EE tenha apresentado mutagenicidade.

PALAVRAS-CHAVE: estresse oxidativo, ciclofosfamida, genotoxicidade, compostos fenólicos

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INTRODUCTION

Capirona decorticans Spruce (Rubiaceae) (*C. decorticans*) is popularly known in Brazil as *perna de moça* or *mulateiro*. This tree occurs in the Amazon forest (including Colombia, Venezuela, Bolivia, Peru, Ecuador, Guianas and Brazil) (Taylor *et al.* 2007). Plants of the Rubiaceae family are widely used in folk medicine, with numerous uses described (antimicrobial, anti-malarial, hepatoprotective, antioxidant, anticancer and psychoactive activities), being the source of several bioactive compounds including alkaloids, flavonoids, terpenes, anthraquinones and coumarins (Barrabé *et al.* 2014; Martins and Nunes 2015). Castillo *et al.* (2007) reported the popular use of *C. decorticans* for the treatment of warts, wounds, mycoses and scabies.

Since the 1980s, medicinal plants and their active ingredients have received increasing attention (Kren and Walterova 2005). Medicinal plants serve as therapeutic alternatives, being safer and effective treatment options, and an increasing number of these plants and their extracts have been shown to produce beneficial therapeutic effects, including antioxidant, anti-inflammatory, anticancer, antimicrobial and immune modulatory effects (Arafa 2009; Garcia-Nino and Pedraza-Chaverri 2014). The potential antioxidant activity of medicinal plants has been evaluated in many studies (Ramkissoon *et al.* 2013; Terpinic *et al.* 2016) and may be an important source of protection of the endogenous system from free radicals (Cândido *et al.* 2015). Natural products have been increasingly targeted as a source of antioxidants to counter the harmful effects of oxidative stress to body functions, specially polyphenols, that are present in many plant species (Coulibaly *et al.* 2014). In addition, phenolic compounds may have positive, inert or deleterious effects on mutagenesis, which makes them a frequent object of bioprospecting studies (Bourgaud *et al.* 2001). These phenolic compounds have the ability to suppress lipid peroxidation, prevent DNA oxidative damage, and scavenge free radicals, which cause the depletion of the immune system, changes in gene expression, and induction of abnormal proteins, resulting in degenerative diseases and aging (Cao and Cao 1999).

Cyclophosphamide (CP) is an alkylating agent widely used in the treatment of cancer and is classified as a human carcinogen based on the extensive evidence found not only in animal experimentation, but also in studies with humans (IARC 2012). The metabolites generated by CP induce oxidative stress and cause damage to DNA and toxicity to various target organs (Korkmaz *et al.* 2007).

Considering the above, the present work aimed to evaluate the phytochemical profile of the ethanolic extract of *C. decorticans* leaves and to determine the antioxidant action and mutagenic/antimutagenic effect of the ethanolic extract and the hydromethanolic fraction. We evaluated the effects

of *C. decorticans* extracts on the oxidative damage induced *in vivo* in mice exposed to cyclophosphamide.

MATERIAL AND METHODS

Collection and botanical identification

Leaves of *C. decorticans* were obtained in March 2015, during the rainy season, from an adult tree in a forest fragment in the city of Alta Floresta (Mato Grosso state, Brazil) (9°52'44.11"S, 56°6'7.00"W), at an altitude of 299 meters. The material was labeled and a sample was deposited in the Centro Norte Mato Grossense (CNMT) herbarium of Universidade Federal de Mato Grosso (UFMT) – Campus Sinop (deposit number 6557).

Preparation and fractionation of the extract

A sample (6800 g) of the leaves harvested was dried in an oven with forced air circulation at a mean temperature of 40 °C until completely dried. The dried material (1650 g) was ground and subjected to exhaustive extraction by maceration in ethanol at room temperature. The ethanolic extract (EE) was obtained by removing the ethanol with rotavaporation, with a pressure of -700 mmHg at 40 °C. Chlorophyll was removed from the crude extract by partitioning into methanol/water (1:1) (Morais *et al.* 2015), followed by filtration and evaporation under reduced pressure, resulting in 30 g EE.

The fractionation was carried out with 10 g of EE, which was dissolved in a methanol/water solution (9:1) and subjected to the liquid-liquid partitioning process by polarity gradient with hexane, dichloromethane and ethyl acetate. After this procedure, the hydromethanolic fraction (FM) (4 g) was obtained. Chemical and biological tests were conducted with EE and FM at the concentration of 250 mg kg⁻¹. The procedure followed Castillo *et al.* (2007), with adaptations.

Phenols, flavonoids and antiradical activity

To quantify the total phenols, the Folin-Ciocalteu method was used, as described by Costa *et al.* (2010). The phenol content was determined by linear regression, with a standard curve of gallic acid, with concentrations between 10 and 350 µg mL⁻¹. The analyses were performed in triplicate and the results were expressed in milligram gallic acid equivalent per gram of extract (mgEAG g⁻¹).

The total flavonoids were dosed in EE and FM by the colorimetric method with aluminum chloride (AlCl₃), according to Costa *et al.* (2010), with adaptations. The flavonoid contents were obtained by linear regression, with a calibration curve made with quercetin, with concentrations of 5, 10, 15, 20, 25 and 30 µg mL⁻¹. The results were expressed in milligram quercetin equivalent per gram of extract (mgEQ g⁻¹).

The anti-radical potential of the samples was determined by the free radical sequestration method, using 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to Costa *et al.* (2010). The CE₅₀ of DPPH consumption of the EE and FM samples was calculated using rutin and ascorbic acid as standards in the antiradical activity.

Flavonoid analysis by LC-MS/MS

The presence of flavonoids in EE and FM was confirmed by sequential mass spectrometry (LC-MS/MS), in the Multiple Reaction Monitoring (MRM) mode of acquisition and transition ions previously established by flavonoid pattern analysis, using a UHPLC 1290 Infinity equipped with 6460 Triple Quad LC/MS, both from Agilent Technologies. The procedure followed Ares *et al.* (2016) and Terpinic *et al.* (2016). The following parameters were used in the analysis: injected sample volume of 20 µL, mobile phase flow at 0.5 mL min⁻¹ with solvent A compound of water and formic acid 0.1%, solvent B acetonitrile and formic acid 0.1%, gradient 5% B 0-30 min, 100% B 30-32 min, 5% B 32-33 min; Column Zorbax Eclipse AAA, C-18, (4.6 x 150 mm, 3.5 µm), maintained at 25 °C; negative ionization mode by electrospray. In the mass spectrometer, the capillary voltage was adjusted to 3.5 kv, source at 300 °C and desolvation at 250 °C. Nitrogen was used for nebulization and as drying gas. The results were compared to the standards and to literature references.

In vivo experiment

Animals - Male Swiss mice aged 6-7 weeks (weighting 30-35 g), were obtained from the breeding colonies of the experimental animal facilities of Universidade Federal de Mato Grosso - UFMT, Campus Cuiabá, Mato Grosso, Brazil. The experimental procedures were authorized by the Ethics Committee for Animal Research of UFMT (CEUA/UFMT) (protocol number 23108.717375/2016-85). The animals were kept in plastic cages in an experimental room under controlled conditions of temperature (22 ± 2 °C), relative humidity (55 ± 10%) and light cycle (12 h light, 12 h dark), and were fed standardized commercial feed and filtered water *ad libitum*.

Experimental design - The animals were divided into 6 groups (treatments) containing 8 animals each. Treatments are defined in Table 1. The EE or FM dosage administered was established according to the Malone Hippocratic test (Malone 1983). The animals were treated orally by gavage (0.3 mL by day by animal of EE or FM) dissolved in filtered water, at a concentration of 250 mg kg⁻¹ for 15 consecutive days. Cyclophosphamide (CP) at a dosage of 25 mg kg⁻¹ was used as positive control, administered intraperitoneally (single dose) by injection on the 15th day of treatment (Delmanto *et al.* 2001). Twenty-four hours after intraperitoneal injection, the mice were anesthetized with Ketamine 50 mg kg⁻¹, Xylazine 20 mg kg⁻¹ and Acepromazine 20 mg kg⁻¹, followed by cervical dislocation and removal of femur, liver and kidneys. The liver and kidneys were washed with 0.9% NaCl and immediately frozen.

Table 1. Specification of the treatments used in the *in vivo* experiment to evaluate the mutagenic effect of the ethanolic extract (EE) and hydromethanolic fraction (FM) of *Capirona decorticans* leaves. CP = cyclophosphamide.

Group	Gavage	Intraperitoneal injection
Control (negative control)	H ₂ O	H ₂ O + NaCl 0.9%
CP (positive control)	H ₂ O	H ₂ O + CP 25 mg kg ⁻¹
EE	EE	H ₂ O + NaCl 0.9%
EE+CP	EE	H ₂ O + CP 25 mg kg ⁻¹
FM	FM	H ₂ O + NaCl 0.9%
FM+CP	FM	H ₂ O + CP 25 mg kg ⁻¹

Oxidative stress parameters

Carbonylated proteins - The oxidative damage to proteins in the liver and kidneys was assessed by determination of carbonyl groups, according to Yan *et al.* (1995). Each tissue was diluted in 10 mM Tris/HCl buffer (pH 7.4), in proportion 1:80 (w/v), in a blank and triplicate test. The amount of carbonylated proteins (PC) of samples was read at 370 nm in quartz cuvette and the results were expressed as nmol of carbonyl mg protein⁻¹.

Thiobarbituric acid reactive species (TBARS) - Oxidative damage to lipids in the liver was assessed by determining the levels of thiobarbituric acid reactive substances (TBARS) spectrophotometrically, according to Buege and Aust (1978) with some modifications. The liver was homogenized 1:8 (w/v) in 20 mM TFK pH 7.5 and centrifuged at 4000 rpm for 15 min. The reaction mixture (500 µL of the supernatant) were collected, 250 µL of 10% TCA and 1000 µL thiobarbituric acid (TBA) 0.67% was then incubated in a water bath at 100 °C for 30 minutes, and cooled and centrifuged at 3600 rpm for 10 minutes. The supernatant was read at 535 nm and compared to a calibration curve made with malonyldialdehyde (MDA). The amount of lipid peroxidation was expressed as nmol MDA mg⁻¹ protein⁻¹.

Ascorbic acid - The endogenous non-enzymatic antioxidant dosed for liver and kidneys was ascorbic acid, following Roe (1954). The tissue was diluted 1:15 (w/v) in 10 mM Tris/HCl, and centrifuged at 2000 rpm for 10 minutes at 4 °C. The solution was read at 520 nm and the results were compared to a standard calibration curve of ascorbic acid. The result was expressed in µmol ASA g⁻¹ of tissue.

Catalase - The enzymatic antioxidant activity of catalase (CAT) followed Nelson and Kiesow (1972). The principle is based on decomposition of H₂O₂ and measured spectrophotometrically at 240 nm. Tissues were homogenized in the following proportions: liver 1:30 and kidney 1:20 (w/v) in 20 mM potassium phosphate buffer (TFK) containing Triton X-100, NaCl, pH 7.4, then centrifuged at 10000 rpm for 15 minutes at 4 °C. The reaction mixture contained 25 µL of the supernatant, 1000 µL of 50 mM TFK pH 7.0 and 25 µL H₂O₂ added to a quartz cuvette and the absorbances were

read every 15 seconds for 1 minute. The result was expressed in $\mu\text{mol H}_2\text{O}_2$ consumed $\text{min}^{-1} \text{mg protein}^{-1}$.

Protein content of tissues – The protein content of the samples (except ASA) was determined with the Bradford method (1976) at 595 nm using bovine serum albumin as standard for calibration curve construction.

Micronucleus test – Micronuclei (MN) are small nuclei that arise from chromosome fragmentation and that are deposited in young erythrocytes of the bone marrow. The test allows the assessment of the clastogenic effects that damage the chromosome and aneugenic effects that induce aneuploidy or abnormal chromosome segregation. The test consists of assessing the frequency of micronucleated polychromatic erythrocytes (MNPCE) after exposure to the chemical agent under investigation (MacGregor *et al.* 1987). The procedures followed Ribeiro *et al.* (2003). The number of micronucleated cells was counted in 1000 polychromatic erythrocytes (PCE) per animal. The slides were analyzed in a blind test, using a light microscope with a 100x immersion objective for PCE.

The percentage reduction in the frequency of MNPCE was determined according to Manoharan and Banerjee (1985) and Waters *et al.* (1990), by the following formula:

$$\% \text{ reduction} = (\text{frequency MN in A} - \text{frequency MN in B}) \times 100 / (\text{frequency MN in A} - \text{frequency MN in C})$$

where: A is the group treated with CP (positive control); B is the group treated with extracts of *C. decorticans* plus CP; and C is the group treated with 0.9% NaCl (negative control).

Statistical analysis – Results are presented as mean \pm SD (standard deviation) and analyzed by one-way Anova followed by the post-hoc Tukey test. In all cases, a level of significance was accepted at $p < 0.05$. The frequency of micronucleated bone marrow cells was compared among treatments using a Chi-square test (Pereira 1991).

RESULTS

The results of chemical analysis showed high concentration of phenolic compounds, low concentration of total flavonoids and high antiradical potential (Table 2). Seven flavonoids were identified in the EE and FM samples: rutin, quercetin-3- β -D-glucoside, quercitrin, myricetin, quercetin, luteolin and apigenin (Table 3, Figure 1).

Regarding the *in vivo* experiment, there was a significant increase ($p < 0.05$) in the levels of carbonylated proteins in the liver tissue of the positive control group (that received only CP) when compared to the negative control group, and a significant decrease in the FM+CP group when compared to the positive control (Figure 2A). There was no significant difference among treatments for the levels of carbonylated

proteins in renal tissue (Figure 2B), nor for TBARS in hepatic tissue, or for ascorbic acid and catalase in liver and kidney.

Regarding CP-induced damage to DNA, the EE+CP and FM+CP groups showed a reduction of 96% and 71% ($p < 0.001$), respectively, in the frequency of MNPCE in the bone marrow when compared to the positive control group (Table 4). The FM group did not present any mutagenic potential, but the EE group was mutagenic when compared with the negative control group.

Table 2. Total phenol content, total flavonoid content, and antiradical activity (IC_{50}) of the ethanolic extract (EE) and hydromethanolic fraction (FM) of *Capirona decorticans* leaves.

Sample	Total phenols (mg EAG g^{-1})	Total flavonoids (EQ g^{-1})	IC_{50} ($\mu\text{g mL}^{-1}$)
EE	406	13	44
FM	293	8	50
Ascorbic acid	---	---	38
Rutin	---	---	43

Table 3. Characterization of the flavonoids identified in the ethanolic extract (EE) and hydromethanolic fraction (FM) of *Capirona decorticans* leaves, and the results of the LC-MS / MS analysis, MRM mode, with the main ions presented in the molecular breakdown of each compound.

Compound	Flavonoid identified	Molecular formula	Retention time	Molecular mass	[M-H] ⁻	MS^2 ions
1	Rutin	$\text{C}_{27}\text{H}_{30}\text{O}_{16}$	10	610.52	609.52	300.20
2	Quercetin-3- β -D-glucoside	$\text{C}_{26}\text{H}_{30}\text{O}_{15}$	10.5	464.38	463.38	300.00
3	Quercitrin	$\text{C}_{21}\text{H}_{20}\text{O}_{11}$	12	448.38	447.38	301.00
4	Miricetin	$\text{C}_{15}\text{H}_{10}\text{O}_8$	13	318.24	317.24	150.90
5	Quercetin	$\text{C}_{15}\text{H}_{10}\text{O}_7$	14.7	302.24	301.24	106.90
6	Apigenin	$\text{C}_{15}\text{H}_{10}\text{O}_5$	16.5	270.24	269.24	116.80
7	Luteolin	$\text{C}_{15}\text{H}_{10}\text{O}_6$	15.3	286.24	285.24	133.00

Table 4. Frequency of micronucleated polychromatic erythrocytes (MNPCE) in bone marrow of male Swiss mice after pre-treatment with ethanolic extract (EE) and hydromethanolic fraction (FM) of *Capirona decorticans* leaves and cyclophosphamide (CP). N analyzed cells = number of analyzed cells (1000 per individual); N = number of MNPCE; % = frequency of MNPCE.

Treatments	N analyzed cells	MNPCE	
		N	%
Control (negative control)	7000 ^a	87	1.24
CP (positive control)	7000 ^a	363	5.19
EE + CP	6000 ^b	97***	1.62
FM + CP	7000 ^a	168***	2.40
EE	7000 ^a	133*	1.90
FM	7000 ^a	89	1.27

^a One animal died; ^b Two animals died; * $p < 0.05$, in comparison with control group; *** $p < 0.001$, in comparison with positive control group according to Chi-square test.

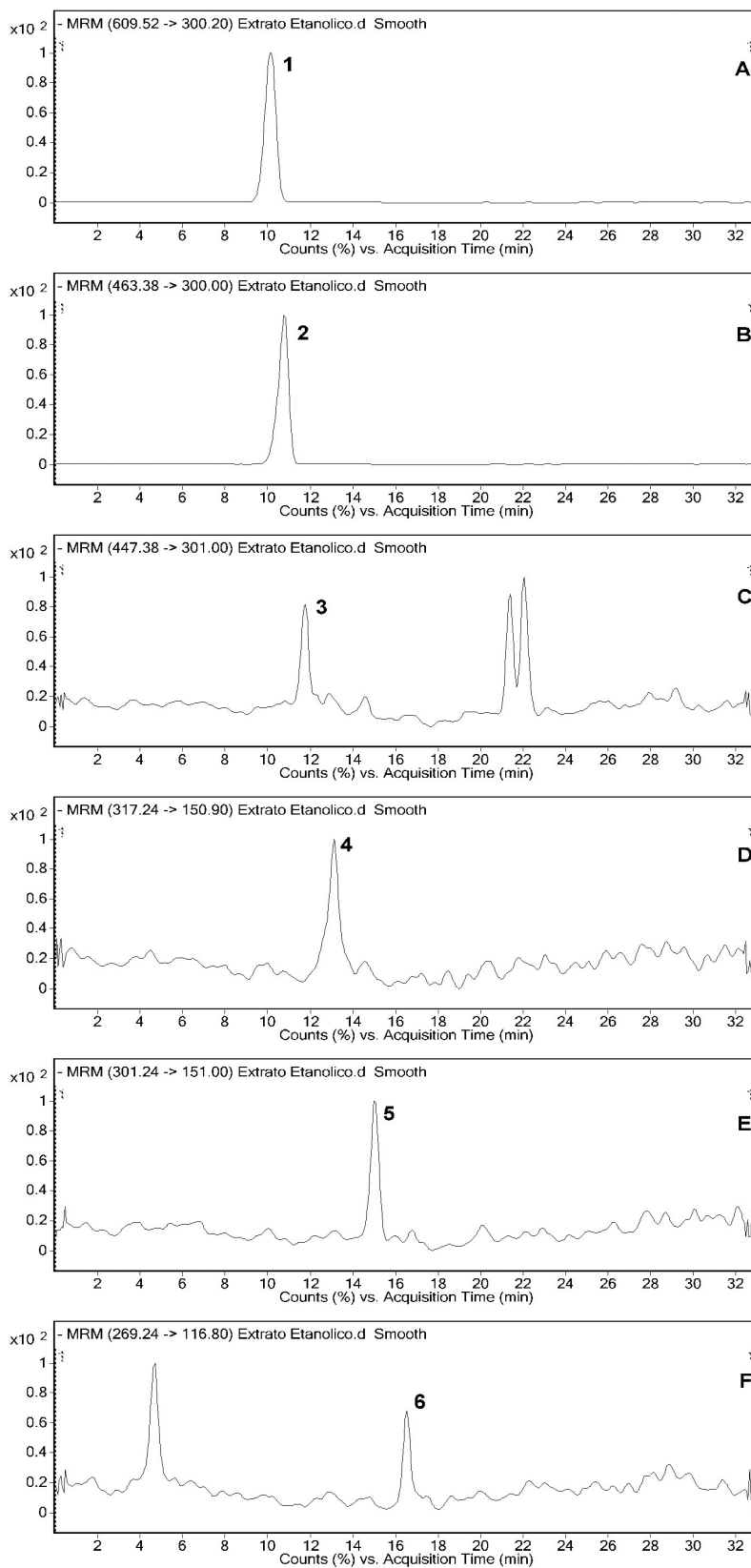


Figure 1. Flavonoid compounds 1 to 7 identified by LC-MS/MS in MRM mode in ethanolic extract (EE) samples of *Capirona decorticans*, showing the retention time, mass of molecular ions, and main fragment of the identified flavonoids.

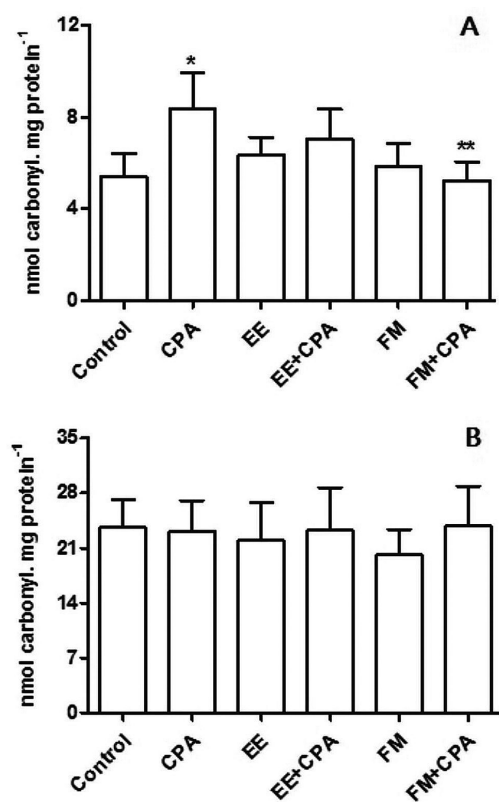


Figure 2. Levels of carbonylated proteins of the liver (A) and kidney (B) of Swiss mice treated with the ethanolic extract (EE) and hydrometanolic fraction (FM) of *Capirona decorticans* leaves and cyclophosphamide (CPA) (n = 8 per treatment). Treatment specifications are available in Table 1. Control = negative control; CPA = positive control; CPA dosage at 25 mg kg⁻¹; EE and FM dosage at 250 mg kg⁻¹. Asterisks indicate significant differences at p < 0.0001 (* relative to the control; ** relative to CPA).

DISCUSSION

The antiradical activity of *C. decorticans* detected in this study was comparable to that of the analyzed standards (ascorbic acid and rutin). Antiradical activity has been related to polyphenol action in the sequestration of free radicals and the prevention of mutagenic processes related to diverse human diseases (Yasir *et al.* 2016).

Compound **1** presented molecular ion at 609.52 [m/z-H] and fragment at 300.20 (m/z-309.32), which corresponds to the loss of two glycans, being similar to that obtained by Simirgiotis *et al.* (2016). Compound **2** presented molecular ion at 463.38 [m/z-H] and fragment at 300.00 [m/z-163.38] indicating the breakdown of the glycoside grouping of this aglycone. For Compound **3**, molecular ion was identified at 447.38 [m/z-H] and fragment at 301.00 [m/z-146.38], corresponding to the disruption of the glycone moiety (rhamnose), with a similar fragmentation pattern to that described by He *et al.* (2014). For compound **4** the molecular ion was identified at 317.24 [m/z-H] and fragment at 150.90 [m/z-166], that corresponds to the break in ring C, the same

pattern obtained by Sun *et al.* (2014). On the other hand, compound **5** had a molecular ion at 301.24 [m/z-H] and the fragment at 151.00 [m/z-150.24], a similar fragmentation to that found by Yasir *et al.* (2016) the yield of seeds extract was 20–41% (w/w and Sun *et al.* (2014), representing the C-ring rupture between carbon 2 and carbonyl. For compound **6** the molecular ion was at 269.24 [m/z-H] and fragment at 116.80 [m/z-155.44], which corresponds to the connection break between carbons 2 and 3 of ring C. The fragmentation profiles of compounds **3** and **7** were similar to those described by Dai *et al.* (2015). Compound **7** presented the molecular ion at 285.24 [m/z-H] and fragment at 133.00 [m/z-152.24], representing the C-ring breaking benzene diol; similar to the fragmentation in negative ionization mode described by Yasir *et al.* (2016) the yield of seeds extract was 20–41% (w/w).

The significantly lower level of carbonylated proteins in the liver of the FM+CP group relative to the CP control indicated that FM promoted a reduction of the damage caused by CP in this organ. No such effect was observed in the kidneys, possibly because the dose of CP was too low to cause renal damage. A dose of 27 mg kg⁻¹ CP every three weeks over 10 weeks promoted oxidative stress in renal tissue of rats (Kocahan *et al.* 2017). Protein carbonylation is characteristic of damage caused by oxidative stress and its occurrence can lead to changes in organ structure, decrease in enzymatic activity and destruction of proteins, being an important marker in toxicological studies (Qiu *et al.* 2016; Ramkissoon *et al.* 2013). Our differing results may be related to the CP dose and treatment duration (Horn *et al.* 2016; Qiu *et al.* 2016).

An increase in TBARS may result from oxidative processes that compromise antioxidant enzymes, leading to damage to cellular membranes and their functions (Borges *et al.* 2011). We observed no effect of CP and *C. decorticans* extract on TBARS in hepatic and renal tissues. TBARS results are dose-dependent (Horn *et al.* 2016). TBARS was significantly increased in mice liver exposed to 25 mg kg⁻¹ of CP for 10 days (Zarei and Shivanandappa 2013), also in mice 24 hours after having been administrated one dose of 200 mg kg⁻¹ CP (Valadares *et al.* 2010), and in the kidney of rats exposed to 27 mg kg⁻¹ CP administered four times in ten weeks (Kocahan *et al.* 2017). In a test of rutin as a potential inhibitor of the oxidative stress caused by CP, the levels of lipid peroxidation also remained unchanged (Nafees *et al.* 2015).

Ascorbic acid levels did not vary significantly among treatments, indicating that CP, EE and FM were inert in this test for oxidative stress. This is an important non-enzymatic antioxidant agent, since ascorbic acid acts in the defense of numerous cellular structures, and in low concentrations it prevents oxidation and consequent apoptosis (Head 1998). Similarly, the antioxidant activity of catalase did not change, indicating that our treatments caused no harmful effects involving a response of this enzyme. The catalase is

an enzymatic antioxidant that converts H_2O_2 (considered a reactive oxygen species) to water and oxygen, and is important in the inactivation processes of oxidative and/or xenobiotic agents. It is increased in response to liver damage and also by enzyme synthesis during oxidative stress (Bonfanti *et al.* 2016). Catalase activity can be increased or decreased in the presence of plant extracts (Olaleye *et al.* 2014), and also responds to the effects of CP, depending on dose and exposure time (Haque *et al.* 2001; Zarei and Shivanandappa 2013; Kocahan *et al.* 2017).

The known effects of CP, such as hepatotoxicity, nephrotoxicity, cardiotoxicity, oncogenic potential of secondary neoplasias, and generation of oxygen reactive species, are dependent on the administration dose and time of exposure (Mansour *et al.* 2015; Fahmy *et al.* 2016). In this study, CP had no significant effect on the studied parameters, which was also observed by Basu *et al.* (2015 *viz.*, oxovanadium(IV)). Probably the dose of 25 mg kg⁻¹ CP was inadequate in our experimental model and therefore the effects of the extracts on the evaluated parameters could not be clearly observed.

The reduction in the frequency of MNPCE in the groups treated with EE and FM indicated a potential for *C. decorticans* extracts to have a protective effect against *in vivo* DNA damage caused by CP, which acts indirectly as an alkylating agent (IARC 2012). These results suggest a possible concentration of antimutagenic substances in EE and FM or an adjuvant effect of the extracts, probably due to their content of phenolic compounds. In this context, the activity profile of the flavonoids identified in the extracts is indicative of antimutagenic potential. Rutin showed antioxidant and anti-inflammatory capacity against CP, preventing tissue and metabolic injuries (Nafees *et al.* 2015). Quercitrin also has antioxidant properties and acts in preventing liver damage (Hong *et al.* 2013). Luteolin is cited as having anticancer and antioxidant properties (Samy *et al.*, 2006). Apigenin has been suggested to have protective action against genotoxic effects (Siddique *et al.* 2010).

There was no indication of mutagenic potential for the FM extract, yet the EE extract showed mutagenic activity. Many substances with reported antimutagenic and anticancer activity may also show mutagenic and carcinogenic activity, mainly through their pro-oxidant effect (Zeiger 2003). Phenolic compounds such as flavonoids have genotoxic potential (León-González *et al.* 2015). For example, ascorbic acid can synthesize reactive oxygen species, which can promote mutagenic events when uncontrolled (Kramarenko *et al.* 2006; León-González *et al.* 2015). Rutin and quercetin have deleterious mutagenic effects when used in high and non-feasible dosages (Silva *et al.* 2002). Flavonoids may act as genotoxic substances depending on variation in pH, presence of antioxidants and metabolic factors, and their antioxidant or pro-oxidant activity is dose-

dependent, mainly in relation to myricetin (Hobbs *et al.* 2015). The antimutagenic and mutagenic effects observed in the treatment with *C. decorticans* extracts may be attributed to the presence of secondary metabolites (Gobbo-Neto and Lopes 2007; Sampaio *et al.* 2016).

CONCLUSIONS

Total phenol content, flavonoid compounds and antiradical activity determined in the ethanolic extract (EE) and hydromethanolic fraction (FM) of *Capirona decorticans* leaves supported the results obtained *in vivo*. EE and FM did not generate pathological alterations and prevented protein carbonylation caused by cyclophosphamide (CP) in hepatic tissue of mice. Seven flavonoids with biological activities were identified by LC-MS/MS. The reduction in MNPCE in mice treated with EE and FM plus CP indicated an antimutagenic effect, yet a mutagenic effect was also observed in mice treated only with EE, which may be related to the presence of mutagenic substances in the extract. The results suggest that *C. decorticans* leaf extract contains compounds with protective effect against pathological alterations in liver tissue, and that flavonoids may be involved in the observed antimutagenic action. The chemoprotective property of *C. decorticans* may be related to a possible anticarcinogenic effect.

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