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Article

Determination of phenolic and triterpenic compounds in *Jatropha gossypiifolia* L by Ultra-high performance liquid chromatography-tandem mass spectrometric (UHPLC-MS/MS)

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This work describes the chemical characterization of extracts of *Jatropha gossypiifolia* (from Amazonia region) concerning the presence of phenolic and triterpenic compounds using Ultra High Perfomance Liquid chromatography coupled to Mass Spectrometry in Tandem (UHPLC-MS/MS) methods. The studied compounds belong to the most frequently found classes in medicinal plants (triterpenes, flavonoids, flavones, hydroxycinnamic acids, coumarins, catechins and stilbenes), which have been analyzed as chemical and bioactive markers in hydroethanolic and aqueous extracts. Several polyphenolic compounds identified herein are unprecedented in the scientific literature for this species. The chemical markers identified and quantified in the studied extracts of *J. gossypiifolia* were gallic acid, chlorogenic acid, catechin, caffeic acid, vanillic acid, *p*-coumaric acid, ferullic acid, rutin, quercitrin, 3-acetylcoumarin, *trans*-cinnamic acid, quercetin, luteolin, apigenin, kaempferol, chrysin. α-amyrin, β-amyrin and lupeol. As the majority compounds, (+)-catechin, *p*-coumaric acid, ferulic acid, luteolin, α-amyrin and β-amyrin were found to be present at mg kg^{-1} levels. *J. gossypiifolia* extracts presented a high *in vitro* activity against different reactive oxygen species (hydroxyl, peroxyl, and superoxide anion radicals). Several polyphenolic compound data presented herein are unprecedented in the scientific literature for this plant species. As a result, this plant can be a new source of bioactive molecules for therapeutic purposes.

Keywords: *Jatropha gossypiifolia* L. UHPLC-MS/MS. Phenolic compounds. Triterpenic compounds.

INTRODUCTION

Traditional medicine (TM) is an important and often underestimated part of health services. In some countries, including Brazil, traditional medicine or non-conventional medicine may be termed complementary medicine. TM has a long history of use in health maintenance and in disease prevention and treatment, particularly for chronic diseases. According to the World Health Organization (WHO) Traditional Medicine Strategy 2014-2023, policies and regulations to promote the safe and effective use of Traditional and complementary medicine (T&CM) products have increased all over the world (WHO, 2016).

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Herbs are used throughout the world and their use is on the rise, with available data suggesting that the T&CM product market is substantial. However, the diversity of regulatory categories for T&CM products makes it difficult to assess the size of the market with any degree of accuracy. For example, the output of Chinese *materia medica* was estimated to be about US$83.1 billion in 2012, annual expenditure on TM in the Republic of Korea was US$7.4 billion in 2009 and out-of-pocket spending for natural products in the United States reached US$14.8 billion in 2008 (WHO, 2016).

The genus Jatropha belongs to the Euphorbiaceae and comprises about 200 species which are distributed mainly in the tropical and subtropical regions of Americas and Africa (Zhang *et al.*, 2009). Species from this genus are popularly used to cure stomachache, toothache,

swelling, inflammation, leprosy, dysentery, dyscrasia, vertigo, anemia, diabetes, as well as to treat HIV and tumors, ophthalmia, ringworm, ulcers, malaria, skin diseases, bronchitis, and asthma and as an aphrodisiac (Sabandar *et al.*, 2013).

Jatropha gossypiifolia Linneus is popularly known worldwide as "bellyache bush" or "black physicnut". In Brazil, it is known by various popular names, the most common of which are "pinhão-roxo", "pião-roxo", "peão-roxo", "batata-de-teu", "erva-purgante", "jalapão", "mamoninha", "raiz-de-teiú", "peão-curador", "peão-pajé", "pião-caboclo" and "pião-preto". *J. gossypiifolia* is a small shrub with dark green or more frequently purplish-red dark leaves, with a length of 16–19 cm and a width of 10–12.9 cm; they are alternate, palmate and pubescent, with an acuminate apex, cordate base and serrated margin. The flowers are unisexual, purple and in cymose summits, with the calyx having five petals; in male flowers, these may form a petaloid tube. The fruit is capsular, with three furrows, containing a dark seed with black spots (Félix-Silva *et al.*, 2014). Regarding its phytochemical constitution, alkaloid, steroids, saponin, lignan, tannin, phenolic compounds, flavonoid, curcin, triterpenes, diterpene, jatrophone, jatropholones A and B, jatrophatrione, apigenin, and cyclogossine A have been reported in different extracts from different parts of this plant (Apu *et al.*, 2013; Félix-Silva *et al.*, 2014; Khyade, Vaikos, 2011; Saini *et al.*, 2015).

J. gossypiifolia is applied for the treatment of various types of disorders in T&CM. Several uses are described for different parts (leaves, stems, roots, seeds, and latex) and preparations (infusion, decoction, and maceration, among others) based on this plant, via different routes (oral or topical) (Félix-Silva *et al.*, 2014). The leaves of the plant are traditionally topically applied to boils, carbuncles, eczema, itches and venereal diseases. Orally, the most frequent reports concern its use as an antihypertensive, anti-inflammatory, analgesic, antipyretic, antimicrobial, emetic, purgative, anti-anemic, antidiabetic and anti-hemorrhagic (Sabandar *et al.*, 2013). To establish its traditional uses, *J. gossypifolia* has been investigated for its antihypertensive, anti-allergic, molluscicidal, larvicidal, antimicrobial, anti-inflammatory, antioxidant, anti-neoplasic, coagulating and anti-coagulating activities (including various types of extracts from different parts of the plant) (Apu *et al.*, 2013; Félix-Silva *et al.*, 2014; Khyade, Vaikos, 2011).

J. gossypiifolia is included in the National List of Medicinal Plants of Interest to the Brazilian Public Health System (Relação Nacional de Plantas Medicinais de Interesse ao Sistema Único de Saúde – RENISUS). In February 2009, the Brazilian Health Ministry published the relation that contains 71 medicinal plants with the potential to generate pharmaceutical products of interest in Brazilian public health (Brazil, 2016). Therefore, this study was carried out aiming to define the activity of *J. gossypiifolia* extracts against different oxygen free radicals and quantify 24 phenolic compounds and 12 triterpenic compounds by Ultra High Perfomance Liquid chromatography coupled to Mass Spectrometry in Tandem (UHPLC-MS/MS) methods. The studied compounds belong to the most frequently found classes in medicinal plants (terpenics, flavonoids, flavones, hydroxycinnamic acids, coumarins, catechins and stilbenes), which have been analyzed as chemical and bioactive markers in extracts.

MATERIAL AND METHODS

Instrumentation, reagents and solutions

An Agilent 1260 Infinity UHPLC-MS chromatograph (Santa Clara, CA, United States) with automatic injection and an Agilent 6430 triple quadrupole mass spectrometer were used. High purity nitrogen (99.999%) obtained from Linde (Munich, Germany) was used as the gas to induce a collision at the ESI source and as the drying gas. A Zorbax SB-C18 Rapid Resolution HD column (2.1 × 50 mm, 1.8 μm, Agilent) was used at a temperature of 40 °C. Ultrapure water was obtained from a Milli-Q Synergy UV (Merck Millipore, Darmstadt, Germany) system. Acetonitrile and methanol were of Chromasolv LC-MS grade and supplied by Sigma-Aldrich (St. Louis, MO, United States). Acetic acid was obtained from Sigma-Aldrich (St. Louis, USA).

The standards (+)-catechin, 3-acetyl coumarin, 3,6-dihydroxyflavone, 4-hydroxycoumarin, 6-hydroxycoumarin, apigenin, chlorogenic acid, chrysin, fisetin, galangin, gallic acid, kaempferol, luteolin, myricetin, *p*-coumaric acid, quercetin, quercitrin, *trans*-resveratrol, rosmarinic acid, rutin, *trans*-cinnamic acid and vanillic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Caffeic and ferulic acids were obtained from Fluka Analytical (Buchs, Switzerland). All standards were of analytical grade with a minimum of 95% purity and were used as received. Stock solutions of the phenolic standards (1000 mg L^{-1}) were prepared by dissolution of the appropriate amounts of substances in LC-MS grade methanol.

The triterpenoids: arjunic acid, maslinic acid, betulinic acid, ursolic acid, erythrodiol, lupeol, β-amyrin, α-amyrin, friedelin, sitosterol, stigmasterol

and campesterol were purchased from Fluka and Sigma-Aldrich (St. Louis, MO, United States). The standard solutions of each triterpenic compound as well as the mixtures were prepared in acetonitrile as follows: 1000 mg L^{-1} for maslinic acid, arjunic acid, β-amyrin, α-amyrin, and erythrodiol; 985 mg L^{-1} for ursolic acid; 125 mg L^{-1} for campesterol; 196 mg L^{-1} for betulinic acid; 378 mg L^{-1} for sitosterol; 365 mg L^{-1} for lupeol; 481 mg L^{-1} for stigmasterol; and 500 mg L^{-1} for friedelin.

All solutions were stored in amber glass vessels at -30 °C until use. Working solutions of the studied phenolic and terpenic compounds were prepared by dilution of the stock solutions in the respective solvents.

For the evaluation of antioxidant activity, carbon dioxide (99.9% purity) was purchased from White and Martins®. Sodium hydroxide, xanthine oxidase (XOD) 25UN, hypoxanthine (HPX), nitro tetrazolium blue chloride (NBT) were obtained from Sigma-Aldrich. DMSO (dimethyl sulfoxide) was obtained from Isofar® (Rio de Janeiro, Brazil). Sodium carbonate was obtained from Merck. Ethylenediamine tetraacetic acid (EDTA) was obtained from Nuclear® (Brazil).

Sample collection

Jatropha gossypiifolia L. (deposit n° IAN 192905) was obtained from the herbal collection of the Brazilian agricultural research corporation Embrapa Amazônia Oriental, Belém/PA, Brazil. The species was sourced from the Amazonian region and cultivated in the Horto of medicinal and aromatic plants. The geographical location of collection was 1°27'21''S latitude and 48°30'14''W longitude. The fresh plant species were manually cleaned for macroscopic particles, dried at 40 °C for 12 hours, ground into a fine powder in a laboratory mill and finally supplied as a dry, powdered material. Three harvests were received: (1) August 2013, (2) January 2015 and (3) September 2015. The dried sample materials were stored in desiccators under vacuum at room temperature until their sample preparation for use in HPLC analysis.

Sample preparation

Dry extract of *Jatropha gossypiifolia* L. was obtained by maceration at room temperature (25 °C). Then, 450 g of the dried plant material was maintained in 70% (v/v) ethanol for 7 days with occasional agitation of the maceration bath. The solvent was renewed and the procedure was repeated three times, over a 4-week extraction process. After completing the maceration process, part of the extract was filtered and evaporated under pressure in a rotary evaporator to obtain the crude hydroalcoholic extract. In this way, the remaining ethanolic extract was subjected to successive liquid-liquid extractions with chloroform, ethyl acetate and n-butanol. The resulting ethyl acetate and butanolic liquid extracts were evaporated under pressure in a rotary evaporator to obtain dry extracts. These dry extracts were stored at 4°C until further use.

For the analysis of phenolic compounds, approximately 0.06 g of each of the dried extract was resuspended in 3 mL of methanol, sonicated until complete dissolution, diluted with 12 mL of ultrapure water and then acetic acid was added to a final proportion of 0.1% (v/v). Then, the extracts were filtered through hydrophilic PTFE membranes with a 0.2 μm pore size. The final extracts obtained were submitted to a cleanup step using solid phase extraction (SPE). The Strata C18-E cartridges (Phenomenex, Torrance, USA), 500 mg, 3 mL, were conditioned with 6 mL of MeOH:0.2% CH_3COOH (1:1, v/v) and equilibrated with 6 mL of 0.1% CH_3COOH (v/v) in water. A fixed volume of 2 mL of the obtained extracts with a final MeOH:H_2O:CH_3COOH composition of 20:80:0.1 (v/v) was percolated with a 2 mL min^{-1} flow rate, followed by washing with 2 mL of 0.1% CH_3COOH. Lastly, the cartridge was eluted with 2 mL of MeOH. Just before the chromatographic analysis, the eluate obtained from the SPE procedure was diluted in a MeOH:0.2% CH_3COOH (1:1, v/v) solution.

For the analysis of triterpenic compounds, approximately 50 mg of the dried extract was resuspended in 5 mL of 50% acetonitrile in H_2O (v/v) and vortexed for 30 seconds followed by 10 minutes of sonication. This 1% extract was diluted 20 times in acetonitrile, filtered through a 0.2 μm PTFE filter and injected into the chromatographic system.

UHPLC-MS/MS conditions

Chromatographic analysis of phenolic compounds

The phenolic compounds were determined by the method previously developed by Faccin *et al.* (2016). This method used a gradient elution composed of 0.1% acetic acid in water (A) and acetonitrile (B) as the mobile phase at a constant flow rate (0.8 mL min^{-1}) according to the following elution program: 8.0% B (0.00-0.10 min); 8.0-25.8% B (0.10-3.45 min); 25.8-54.0% B (3.45-6.90 min); 54.0-100.0% B (6.90-7.00 min); and 100.0% B (7.00-9.00 min). The injection volume was 5 μL, and the injected aliquots were acidified to a final concentration of 0.1% acetic acid (v/v). An electrospray ionization source (ESI) was used to ionize

the chromatographic effluent generated up until 7 minutes. The parameters for ESI were optimized to give the best response for the analytes, especially for intensity and signal stability. The source and detector final parameters conditions are shown in Table I. The mass spectrometer operates in the multiple reaction monitoring (MRM) mode with a resolution of 0.7 *m/z* (FWHM). The quantification transitions were divided into three temporal segments of acquisition and the dwell time for each transition was optimized to 20 ms. The collision energies for each analyte are shown in Table II, along with the transitions monitored by the mass spectrometer and the retention times as well.

After finishing the gradient program, a post-run time of 4 minutes was adopted for re-equilibrating the system. The acquired data were analyzed by using the MassHunter Workstation Software Qualitative Analysis (version B.07.00, Build 7.0.7024.0, Agilent Technologies, Inc. 2014). The standard addition method was used to quantify the samples. The calibration curves were built with seven equally spaced concentration levels, in addition to a blank extract. The data points of the calibration curve were determined in triplicate.

Chromatographic analysis of triterpenic compounds

The triterpenic compounds were determined by the method previously developed by Gobo *et al.* (2016). This method used an Atmospheric Pressure Photoionization (APPI) ion source and toluene as a dopant. The source and detector conditions are shown in Table I. The collision energies for each triterpenic compound are also shown in Table II, which describes the transitions monitored by the mass spectrometer and the retention times as well. The determination of triterpenes was performed using a gradient elution that consisted initially of 70% acetonitrile/water from 0 to 3.5 minutes followed by a ramp gradient, which achieved the end condition of 100% acetonitrile after 4 minutes. This condition was maintained until the end of the run (8 minutes). The flow rate was 0.6 mL min^{-1} during the first 4.5 minutes and was increased up to 0.8 mL min^{-1} at 5 minutes run. The flow rate was maintained at 0.8 mL min^{-1} until the end of the chromatographic run. The standard addition method was used to quantify the samples.

Radical scavenging activity

Radical scavenging activity against peroxyl radicals was evaluated through ROS determination in samples treated or not with a peroxyl radical generator (Amado *et al.*, 2009). Briefly, a white 96-well microplate was divided into two regions, where region 1 (background) corresponds lines A, B, C and D, and region 2 corresponds to the lines E, F, G and H. The first 3 wells in both regions were reserved for the solvent; here, 10 µL of solvent used for the samples was added. In the rest of the microplate, 10 µL of sample was added in triplicate (three wells) in region 1 and region 2. The reaction buffer (127.5 µL) containing 30 mM HEPES (pH 7.2), 200 mM KCl and 1 mM $MgCl_2$ was added to all plate pools. Then, 7.5 µL of ultrapure water was added in region 1 and 7.5 µL of 2,2′-azobis 2 methylpropionamidine dihydrochloride (ABAP; 4 mM; Aldrich) in region 2 of the microplate. After this step, the microplate was put into a fluorescence microplate reader (Victor 2, Perkin Elmer), programmed to maintain the temperature at 37°C. At this temperature, peroxyl radicals were produced by the thermal decomposition of ABAP. Immediately before the microplate reading, 10 µL of the fluorescent probe 2′,7′ dichlorofluorescein diacetate (H2DCF-DA) at a final concentration of 40 µM was added to all wells. Thereafter, the non-fluorescent compound H_2DCF was oxidized by ROS to the fluorescent compound DCF, which is detected at wavelengths of 488 and 525 nm, for excitation and emission, respectively. The thermal decomposition of ABAP and ROS formation was monitored for 30 minutes, with readings every 5 minutes.

Total fluorescence production was calculated by integrating the fluorescence units (FU) along the time

TABLE I - Parameters and conditions of the UHPLC-MS/MS analysis of phenolic and triterpenic compounds

Parameter	*Phenolic compounds*	*Triterpenic compounds*
Vaporizer temperature (°C)	–	300
Gas temperature (°C)	250	350
Nebulizer (psi)	30	45
Gas flow (L min^{-1})	11	10
Capillary Voltage (V)	2400	5500
Dopant flow (µL min^{-1})	–	55

TABLE II - Retention time windows (RTW) and MS/MS parameters of the phenolic and triterpenic compounds

Compound	RTW (min)	Fragmentor (V)	Source polarity	Quantification transition*	Confirmation transition*
Phenolic compounds					
Gallic acid	0.24-0.43	106	–	169.0 > 125.1 (10)	-
Chlorogenic acid	0.61-0.94	104	–	353.1 > 191.1 (5)	-
(+)-catechin	0.66-0.88	134	–	289.1 > 245.1 (10)	289.1 > 203.2 (15)
Vanillic acid	0.87-1.13	92	–	167.0 > 152.2 (10)	167.0 > 123.1 (8)
Caffeic acid	0.89-1.10	106	–	179.0 > 135.1 (10)	-
6-hydroxycoumarin	1.48-1.77	124	–	161.0 > 133.2 (17)	161.0 > 105.2 (17)
p-coumaric acid	1.70-2.01	96	–	163.0 > 119.1 (9)	-
Ferulic acid	2.40-2.66	88	–	193.1 > 134.1 (9)	193.1 > 178.1 (7)
Rutin	2.87-3.43	210	–	609.1 > 300.1 (31)	-
4-Hydroxycoumarin	3.26-3.54	130	–	161.0 > 117.2 (15)	-
Rosmarinic acid	3.50-3.77	138	–	359.1 > 161.0 (7)	359.1 > 197.1 (7)
Quercitrin	3.48-3.82	164	–	447.1 > 301.1 (17)	-
Myricetin	3.63-3.91	128	–	317.0 > 150.9 (21)	317.0 > 178.8 (21)
Fisetin	3.71-4.07	120	–	285.0 > 135.0 (15)	285.0 > 163.0 (15)
trans-Resveratrol	3.82-4.10	124	–	227.1 > 185.2 (13)	227.1 > 143.0 (13)
3-acetylcoumarin	4.02-4.31	80	+	211.0 > 211.0 (0)	-
trans-cinnamic acid	4.26-4.54	90	–	147.0 > 103.2 (5)	147.0 > 77.1 (15)
Quercetin	4.37-4.96	126	–	301.0 > 151.1 (17)	301.0 > 179.0 (15)
Luteolin	4.37-4.97	162	–	285.0 > 133.2 (27)	285.0 > 151.1 (27)
Apigenin	5.19-5.53	136	–	269.0 > 117.1 (29)	269.0 > 149.0 (20)
Kaempferol	5.25-5.49	164	–	285.0 > 239.0 (29)	285.0 > 117.2 (31)
3,6-dihydroxyflavone	6.16-6.51	126	–	253.1 > 197.0 (20)	253.1 > 208.0 (21)
Chrysin	6.26-6.84	130	–	253.1 > 143.1 (25)	253.1 > 106.9 (25)
Galangin	6.60-6.87	156	–	269.0 > 169.1 (30)	269.0 > 211.2 (29)
2-naphthol (IS)	5.15-5.40	134	–	143.0 > 115.1 (24)	
Triterpenic compounds					
Arjunic acid	0.440	120	+	489.3 > 408.9 (10)	489.3 > 472.7 (10)
Maslinic acid	0.847	75	+	473.3 > 411.0 (6)	473.3 > 149.0 (6)
Betulinic acid	1.966	90	+	439.4 > 393.5 (15)	439.4 > 287.0 (15)
Ursolic acid	2.136	120	+	439.4 > 203.3 (20)	439.4 > 191.0 (20)
Erythrodiol	3.271	124	+	425.4 > 217.3 (20)	425.4 > 177.0 (20)
Lupeol	6.590	120	+	409.1 > 109.1 (32)	409.1 > 137.2 (32)
β-amyrin	6.980	120	+	409.1 > 109.1 (32)	409.1 > 149.1 (32)
α-amyrin	7.150	135	+	409.1 > 109.1 (32)	409.1 > 134.9 (32)
Friedelin	7.335	110	+	409.1 > 109.1 (32)	409.1 > 121.1 (32)
Sitosterol	7.779	130	+	396.9 > 109.1 (32)	396.9 > 146.9 (32)
Stigmasterol	7.390	80	+	394.7 > 159.0 (33)	394.7 > 118.9 (33)
Campestrol	7.324	135	+	382.8 > 160.8 (25)	382.8 > 133.0 (25)

*Collision energy (V) is given in brackets.

of the measurement, after adjusting FU data to a second order polynomial function. The results were expressed as area difference of FU × min in the same sample with and without ABAP addition and standardized to the ROS area without ABAP (background area). The relative difference between ROS area with and without ABAP was considered as a measure of antioxidant capacity, with high area differences meaning low antioxidant capacity, since high fluorescence levels were obtained after adding ABAP, meaning a low ability to neutralize peroxyl radicals.

Radical scavenging activity against hydroxyl radical was determined according to the deoxyribose method with some modifications (Zhao *et al.*, 2006). The solution of $FeCl_3.6H_2O$ and ascorbic acid was prepared exclusively with deionized water. Thus, the following reagents were added to a test tube containing the plant extract: 100 µL of 1 mM EDTA, 100 µL of 1 mM $FeCl_3.6H_2O$, 100 µL of 36 mM deoxyribose, 100 µL of 10 mM H_2O_2, 100 µL of 1 mM ascorbic acid and 100 µL of the extract. The volume was made up to 1 mL using phosphate buffer (25 mM, pH 7.4). The mixture was incubated for one hour at 37 °C and the reaction stopped by the addition of 1 mL 10% trichloroacetic acid (w/v) and 1 mL of 1% thiobarbituric acid (m/v) in buffer phosphate (pH 7.4). The tube was heated in a water bath for 15 minutes at 37 °C, removed and cooled. Water was added (final volume = 5 mL) and the absorbance measured at 532 nm.

Radical scavenging activity against superoxide radical was measured using the hypoxanthine/xanthine oxidase system (HPX/XOD) following the procedure described by Zhao *et al.* (2006) with short modifications. First, 100 µL of EDTA (30 $mmol.L^{-1}$), 100 µL of HPX (3 $mmol.L^{-1}$) and 200 µL of NBT (1.42 $mmol.L^{-1}$) were mixed with 100 µL of plant extract. After 3 minutes, the xanthine oxidase enzyme (0.75 unit mL^{-1}) was added to initiate the reaction. The reaction mixture was left in the dark for 40 minutes and quenched with buffer solution (0.05 $mol.L^{-1}$, pH 7.4) until the final volume = 3 mL. Then, the absorbance was measured at 560 nm, in an UV-Vis 8453 Hewlett Packard spectrophotometer (Agilent Technologies, Santa Clara, EUA). After, a blank sample was assessed, which was prepared in the same manner, but without the presence of NBT and was run with a control test containing all reagents with the solvent employed in the sample, both as a blank control.

RESULTS AND DISCUSSION

In Brazil, many plants are used for domestic use as medicaments. Several of these plants are still unexplored and need to be scientifically investigated for their use as new drugs. Medicinal plants represent the greatest sources of therapeutic agents due to the large structural diversity of metabolites produced (Fabricant, Farnsworth, 2001). However, there are phytochemical compounds found in just one specie or genus, or in taxonomically related groups (Mazid, Khan, Mohammad, 2011). *J. gossypiifolia* presents an important potential for the generation of pharmacological and/or biotechnological products, based on popular uses and biological studies. However, their major bioactive compounds are not fully exploited up to date (Félix-Silva *et al.*, 2014).

The concentration of secondary metabolites depends on factors such as cultivation techniques, cultivar, growing conditions, ripening process, processing and storage. Some factors may increase their presence, such as stress, UV radiation, infection by pathogens and parasites, injuries, air pollution and exposure to extreme temperatures (Barcia *et al.*, 2014; Rockenbach *et al.*, 2011). There are few studies applying water as an extraction solvent of constituents from *J. gossypiifolia*, but the people use this extractor when use this plant for medicinal purposes. Thus, there are few polar compounds identified as tannins and flavonoids. Normally, nonpolar solvents are used, which, due to their features, contribute to the characterization of non-polar compounds, such as terpenes. Mono-, di- and triterpenes are widely present in the genus Jatropha (Félix-Silva *et al.*, 2014). This work led a phytochemical study considering also the infusion as an extraction method, solvent with greater popular use. The chemical composition of infusion was characterized and compared with the ethanolic extract, which is the most frequently applied solvent in scientific works. This contributes to the identification and quantification of polar components in this species.

As plant extracts constitute a mixture of molecules with entirely different physicochemical properties, the use of analytical tools to allow the identification of the main bioactive compounds is crucial to asses any information of those properties as well as to establish quality control parameters (Borges *et al.*, 2007). Liquid chromatography coupled to mass spectrometry (LC-MS) has excelled as an analytical tool because it is able to provide the high selectivity required for analyzing complex matrices, such as vegetable matrices. Moreover, the use of ultra-high-performance LC (UHPLC) allows an increase in the separation efficiency, while the analysis times can be considerably reduced. Additionally, tandem mass spectrometry using triple quadrupole analyzers is a powerful quantification tool (Faccin *et al.*, 2016).

Analysis of phenolic compounds

Phenolic compounds have great distribution in living beings, both in plants and microorganisms and are among the classes of compounds that have a wide range of structures. The structural element that characterizes them is a benzene nucleus with at least one hydrogen substituted by a hydroxyl, found in the free form or as part of another function such as, for example, ether, ester, glycosides. Therefore, these exhibit solubility in water and polar organic solvents. The classes of phenolic compounds addressed in this study were some derivatives of benzoic acid and hydroxycinnamic acid, flavonols, flavanols, flavone, coumarin and stilbene.

The chromatographic method applied in this study was developed by Faccin *et al.* (2016), aimed at the determination and quantification of some of the phenolic constituents of *J. gossypiifolia*. The traditional use of this plant is through teas; thus, infusions were made to elucidate some of the potentially bioactive compounds to be extracted in this way. However, in the hypothesis that more phenolic compounds could be extracted, ethanolic maceration was also made, as described in section 3.3. Since the plant extracts were not free of phenolic compounds (non-availability blank samples) and considerable effects of ion suppression were seen by UHPLC-ESI-MS/MS, the quantifications were performed by standard addition methods.

Figure 1A presents the chromatograms obtained for the analysis of the hydroalcoholic extract of *J. gossypiifolia* (first harvest) by UHPLC-ESI-MS/MS in MRM mode. Sixteen of the twenty-four phenolic compounds analyzed were identified: gallic acid, chlorogenic acid, catechin, caffeic acid, vanillic acid, *p*-coumaric acid, ferullic acid, rutin, quercitrin, 3-acetylcoumarin, *trans*-cinnamic acid, quercetin, luteolin, apigenin, kaempferol and chrysin. Concentrations of phenolic compounds in *Jatropha gossypiifolia* L. extracts determined by UHPLC-MS/MS are given in Table III.

Apigenin has already been found in ethanolic extracts of stems and leaves of *Jatropha gossypiifolia L* (Mariz *et al.*, 2010; Sabandar *et al.*, 2013). Ferulic acid was found in its roots, after extraction with ethyl acetate (Mariz *et al.*, 2010). For other phenolic compounds, no data were found in the presence of the studied species. To the best of our knowledge, the data published here appear to be unprecedented in the literature.

Analysis of triterpenic compounds

The main compounds isolated from Jatropha genus are the terpenoids. In fact, many of them were isolated

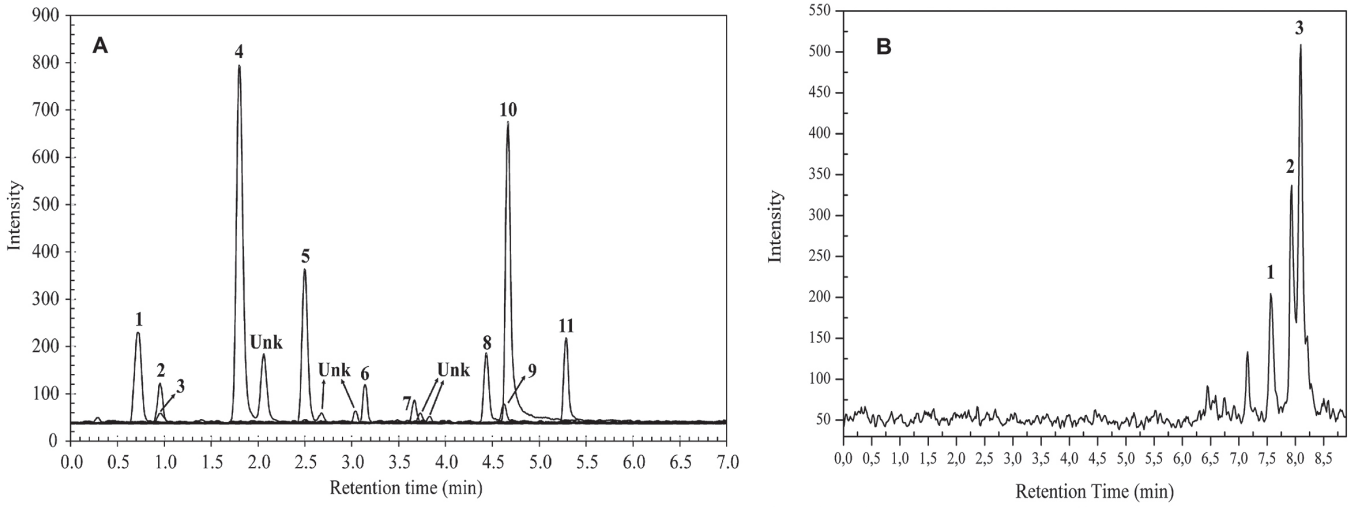

FIGURE 1 - (A) Chromatograms obtained for the analysis of the hydroalcoholic extract of Jatropha gossypiifolia L. (first harvest) by UHPLC-ESI-MS/MS in MRM mode. Column: Zorbax SB C18 2.1x50 mm (1.8 µm). Gradient elution with 0.1% acetic acid in water (A) and acetonitrile (B) as the mobile phase at a constant flow rate (0.8 mL min^{-1}) according to the following elution program: 8.0% B (0.00–0.10 min); 8.0–25.8% B (0.10–3.45 min); 25.8–54.0% B (3.45–6.90 min); 54.0–100.0% B (6.90–7.00 min); and 100.0% B (7.00–9.00 min). Phenolic compounds identified: (1) Catechin, (2) Caffeic acid, (3) Vanillic acid, (4) *p*-coumaric acid, (5) Ferullic acid, (6) Rutin, (7) Quercitrin, (8) trans-cinnamic acid, (9) Quercetin, (10) Luteolin, (11) Apigenin. (B) Chromatograms obtained from UHPLC-APPI-MS/MS analysis of the hydroalcoholic extract of *Jatropha gossypiifolia* L. (first harvest) in MRM mode. Column: Zorbax SB C18 2.1x50 mm (1.8 µm). Mobile phase gradient: 70% acetonitrile/water from zero to 3.5 min and 100% acetonitrile after 4.0 min (8 min run). Flow rate gradient: 0.6 mL min-1 from zero to 4 min and 0.8 mL min-1 after 5 min run (8 min run). Triterpenic compounds identified: (1) lupeol, (2) β-amyrin, (3) α-amyrin.

TABLE III - Concentrations of phenolic and triterpenic compounds in *Jatropha gossypiifolia* L. extracts determined by UHPLC-MS/MS. Values are given in $\mu g.g^{-1}$ of plant followed by standard deviation of concentration (n=3)

Compound	Infusion		Hydroethanolic crude extract			Fruit
	1st harvest	2nd harvest	1st harvest	2nd harvest	3rd harvest	
Gallic acid	0.4 ± 0.2	n.d.	n.d.	n.d.	n.d.	11.2 ± 0.8
Chlorogenic acid	n.d.	n.d.	n.d.	n.d.	n.d.	1.3 ± 0.6
(+)-catechin	402.6 ± 22.9	27.5 ± 1.1	78.1 ± 2.7	21.9 ± 0.6	360.9 ± 3.2	45.8 ± 2
Vanillic acid	6.4 ± 1.2	15.9 ± 2.0	2.4 ± 0.3	1.2 ± 0.2	6.4 ± 0.9	22.2 ± 0.4
Caffeic acid	5.2 ± 0.5	-	3.4 ± 0.1	1.6 ± 0.04	6.6 ± 0.3	3.4 ± 0.4
p-coumaric acid	117.8 ± 7.3	28.6 ± 0.7	92.5 ± 4.0	26.3 ± 0.3	89.6 ± 0.9	3.6 ± 0.3
Ferulic acid	50.5 ± 1.3	3.2 ± 0.3	27.2 ± 0.4	6.5 ± 0.1	39.5 ± 0.2	9.1 ± 0.1
Rutin	8.9 ± 0.3	1.1 ± 0.2	4.6 ± 0.1	0.2 ± 0.1	1.4 ± 0.2	3.3 ± 0.2
Quercitrin	1.0 ± 0.1	0.4 ± 0.1	0.7 ± 0.02	0.2 ± 0.01	1.3 ± 0.02	0.3 ± 0.01
3-acetylcoumarin	n.d.	n.d.	n.d.	n.d.	28.1 ± 1.6	n.d.
trans-cinnamic acid	15.7 ± 0.5	n.d.	6.8 ± 0.2	1.9 ± 0.1	4.5 ± 0.1	1.8 ± 0.3
Quercetin	n.d.	n.d.	1.6 ± 0.2	1.0 ± 0.1	16.4 ± 0.7	0.7 ± 0.2
Luteolin	9.7 ± 0.3	14.9 ± 0.4	80.9 ± 3.7	13.4 ± 0.4	81.1 ± 1.8	5.7 ± 0.1
Apigenin	2.2 ± 0.2	5.6 ± 0.2	7.0 ± 0.2	6.9 ± 0.2	32.3 ± 1.0	0.6 ± 0.1
Kaempferol	n.d.	n.d.	n.d.	1.2 ± 0.2	10.7 ± 1.3	n.d.
Chrysin	n.d.	n.d.	n.d.	0.1 ± 0.05	n.d.	n.d.
α-amyrin	n.a.	n.a.	470.9 ±2.3	1267.8 ±3.6	n.a.	n.a.
β-amyrin	n.a.	n.a.	209.5 ±4.9	196.9 ±5.4	n.a.	n.a.
Lupeol	n.a.	n.a.	5.0 ±4.8	46.6 ±3.4	n.a.	n.a.

n.d.=not detected; n.a.= not analyzed.

from different parts of *J. gossypiifolia*. These compounds belong to a class of secondary metabolites in plants and are widely present in the plant kingdom, especially in plants that possess abundant chlorophyll (Gobo *et al.*, 2016).

The hydroalcoholic extracts were analyzed for screening of the studied triterpenes. Figure 1B presents the chromatograms obtained from UHPLC-APPI-MS/MS analysis of the hydroalcoholic extract of *Jatropha gossypiifolia L* (first harvest) by UHPLC-ESI-MS/MS in MRM mode. The results reported here indicate the presence of three triterpenic compounds, α-amyrin, β-amyrin and lupeol, which can be seen in Table III. These species are compounds that are widely found in medicinal plants according to the literature (Hernández-Vázquez *et al.*, 2012). α-amyrin has already been found in leaves of *J. gaumeri* (Sabandar *et al.*, 2013). β-amyrin was found in seeds of *J. gossypiifolia*, *J. gaumeri* leaves, and in *J. curcas* stems (Sabandar *et al.*, 2013; Mariz *et al.*, 2010). β-Sitosterol has already been found in the seeds of *J. gossypiifolia* stems (Mariz *et al.*, 2010).

Seasonal variation of chemical markers analyzed

Jatropha gossypiifolia L. is commonly found vegetating in several Brazilian states; in semi-arid areas, this is of great importance due to its easy cultivation, adaptation to low fertility soils, degraded soils and drought tolerance (Albuquerque, Andrade, 2002). The samples analyzed in this work came from the Brazilian Amazon region, which is characterized by climate with high annual temperatures and pluviometric regime marked by the occurrence of two seasons: rainy summer and dry winter. Herein, the harvest that refers to the dry season showed less quantity of all phenolic substances analyzed. However, the triterpenes α-amyrin and lupeol were significantly higher in that harvest, as can be seen in Table III. Finally, these results confirm that seasonal variation has a direct and significant influence on the amount of the compounds in this plant species.

Radical scavenging activity

According to the results shown in Table IV, *J. gossypiifolia* L. extracts presented *in vitro* activity against different reactive oxygen species in three concentration levels. The radical scavenging activity observed was not equal for all concentrations and reactive oxygen species, since these radicals have a half-life time

and different potential reductions between them, and different concentrations of the extracts leads to changes in the concentration of antioxidant species shown in the plant. Moreover, the quantitative determination of phenolic compounds by UHPLC-MS/MS has shown flavonoids and phenolic compounds to be the main constituents of the plant. Infusion and ethanolic extracts of *J. gossypiifolia L.* showed the presence of flavonoids and phenolic acids such as gallic, ferulic, p-coumaric acid, catechin, quercitrin, luteolin and others (Table III), which could be associated with the results of *J. gossypiifolia L.* scavenging activity against superoxide, hydroxyl and peroxyl radicals. The higher antioxidant activity was found for the peroxyl radical, probably because of its greater stability when compared to other radicals.

The infusion presented antioxidant activity against all oxygen species studied, while acetate, butanolic and ethanolic fractions showed pro-oxidant activity against superoxide radicals. It could be explained by the propensity of a flavonoid to inhibit free radical-mediated events being governed by its chemical structure. Multiple hydroxyl groups confer upon the molecule substantial antioxidant, chelating and pro-oxidant activities (Heim, Tagliaferro, Bobilya, 2002). In this context, the observed pro-oxidant activity against superoxide radical for the fractions in higher concentration (1.0 and 2.0% w/v) is thought to be directly proportional to the number of hydroxyl groups of the flavonoids found in these extracts. Moreover, in higher concentrations, flavonoids and phenolic compounds could present *in vitro* pro-oxidant activity (Eghbaliferiz *et al.*, 2016). In this way, a toxicity study of these extracts in different concentrations has been suggested for better understanding of the effects of *J. gossypiifolia L.* extracts *in vivo* and *in vitro*.

Pharmacological potential of *Jatropha gossypiifolia L.*

Considering the diverse uses of the genus Jatropha, knowledge about the chemical compounds present in the species *J. gossypiifolia*, in different parts like leaves and fruits, is of common interest. This is particularly in relation to the consumption of this plant by traditional communities and for being potential in the production, for example, herbal medicines. Table V presents the compounds analyzed, the compounds identified and quantified in the species, their respective chemical classes and pharmacological activities.

Catechin, *p*-coumaric acid and luteolin were phenolic compounds analyzed with higher levels. From these findings, the plant would have the potential to present the following pharmacological activities: anticoagulant, anti-mutagenic, antioxidant, beta-adrenergic receptor blocker, cardiotonic, allelopathic, anti-hepatotoxic, antispasmodic, cytotoxic, antibacterial and anti-inflammatory.

It can be highlighted that the ethanolic extract of *J. gossypiifolia* L. induced oral hypotension in conscious normotensive rats and vasorelaxant activity (Abreu *et al.*, 2003). This activity may occur due to the presence of

TABLE IV - Antioxidant activity of different extracts of *Jatropha gossypiifolia* against superoxide, hydroxyl and peroxyl radicals

Extract	Concentration (%)	Antioxidant Activity (%)		
		Free radical		
		Superoxide	Hydroxyl	Peroxyl
Infusion	0.1	8.4 ±3.6	19.9 ±6.2	84.8 ±5.8
	1.0	66.8 ±0.2	56.8 ±0.7	99.7 ±13.9
	2.0	22.2 ±0.4	11.3 ±4.4	38.0 ±4.8
Hydroethanolic Extract	0.1	90.4 ±1.9	96.6 ±3.0	51.3 ±5.2
	1.0	POA	87.3 ±4.7	74.7 ±6.3
	2.0	POA	63.1 ±7.3	83.8 ±3.3
Acetate Fraction	0.1	74.2 ±0.1	72.3 ±5.1	47.4 ±10.3
	1.0	POA	84.8 ±8.5	69.2 ±13.6
	2.0	POA	86.5 ±3.1	95.0 ±4.4
Butanolic Fraction	0.1	89.7 ±1.3	53.2 ±5.3	78.4 ±4.5
	1.0	POA	29.6 ±6.6	72.4 ±4.3
	2.0	POA	11.8 ±1.5	92.0 ±3.8

POA = prooxidant activity

TABLE V - Chemical class and pharmacological activity of compounds isolated from *Jatropha gossypifolia*

Chemical class	Compound	Pharmacological activity
Hydroxybenzoic acids	Vanillic acid	Anthelmintic, antibacterial, anti-inflammatory and laxative.[1]
	Gallic acid	Antiadenovirus, antiallergenic, antianaphylactic, antiasthmatic, antibronchitic, antiflu, antihepatotoxic, antiinflammatory, antimutagenic, antiseptic, astringent, bacteristat, bronchodilator, hepatoprotective, immunostimulant and myorelaxant.[1]
Hydroxycinnamic acids	Caffeic acid	Antiadenoviral, antiflu, antispasmodic, antihypercholesterolemic and sunscreen.[1]
	Ferulic acid	Allelopathic, analgesic, antiaggregant, antiallergic, antibacterial, antiestrogenic, antihepatotoxic, antiinflammatory, antimutagenic, antiviral, candidicide, cholagogue, choleretic, fungicide, hepatoprotective, herbicide, insectifuge, sunscreen.[1]
	p-coumaric acid	Allelopathic, antibacterial, antioxidant, antihepatotoxic, antispasmodic, and cytotoxic.[1]
	trans-cinnamic acid	Antimicrobial, antifungal and antitumor activity.[3]
Coumarins	3-acetylcoumarin	Anti-oxidant and anti-inflammatory activity.[4]
Flavones	Apigenin	Antiallergic, antibacterial, antidermatitic, antimutagenic, cytotoxic, diuretic, estrogenic and hypotensive.[1]
	Luteolin	Antibacterial and anti-inflammatory.[1]
Flavanols	(+)-catechin	Anticoagulant, antimutagenic, antioxidant, beta-adrenergic receptor blocker and cardiotonic.[1]
Flavonols	Kaempferol	Antihistaminic, antimutagenic, antispasmodic and choleretic.[1]
	Quercetin	Antianaphylactic, antibacterial, antimutagenic, antioxidant, antiviral, bradycardiac and cytotoxic.[1]
	Quercitrin	Antibacterial, antiflu, antihemorrhagic, antiherpetic, anti-inflammatory, antimutagenic, antiulcer, choleretic, diuretic and hypoglycemic.[1]
	Rutin	Antibacterial, anticonvulsant, antidermatitic, antidiabetic, antihepatotoxic, antiherpetic, antihypertensive, antimalarial, hypocholesterolemic, hypotensive, insecticide, mutagenic and myorelaxant.[1]
Triterpenes	α-amyrin	Antinociceptive and anti-inflammatory properties.[5]
	β- amyrin	Antinociceptive and anti-inflammatory properties.[5]
	Lupeol	Anti-arthritis, anti-inflammatory, antimalarial, antitumor, chemopreventive agent, hepatoprotective.[2]

[1]Duke, 1992; [2]Gallo, Sarachine, 2009; [3]Gravina *et al.,* 2011; [4]Lakshmi *et al.,* 2012; [5]Silva *et al.,* 2011.

catechin in large quantities, which exerts blocking activity of beta-adrenergic receptors and cardiotonic. Apigenin also has a hypotensive effect. The presence of α-amyrin compounds, β-amyrin and lupeol that could infer that this species can present anti-inflammatory potential.

The use of *J. gossypiifolia* as a medicinal plant demonstrates that it has great potential as a source of bioactive molecules with pharmacological relevance. However, it is necessary to know the really important compounds in the pharmacological actions, aspiring to the chemical refinement of the products to eliminate the eventual toxic effects, or which could reduce the medicinal value of the species. In this way, it is possible to use them in the production of drugs or natural bioactive products for their application in T&CM (Félix-Silva *et al.*, 2014).

Finally, other analytes not covered by this study were found to be present based on the interpretation of the fingerprints chromatograms obtained by LC-MS/MS in full scan mode from *J. gossypiifolia* extracts. Some of these compounds were even more intense when paralleled to the studied analytes and may be associated with a higher concentration and consequent greater pharmacological relevance.

CONCLUSION

Jatropha gossypiifolia has a therapeutic potential as a phytomedicine and therefore the establishment of innovative analytical methods to characterize their active components is crucial to the future development of a product with chemical markers of its quality. In the last few years, plant secondary metabolites with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. In this work, *J. gossypiifolia* extracts showed a large number of chemical constituents, which may be responsible for various pharmacological activities. Several polyphenolic compounds identified herein are unprecedented in the scientific literature for this plant species. Thus, this plant can be a new source of bioactive molecules for therapeutic purposes. The major chemical markers identified and quantified in the studied extracts of *J. gossypiifolia* were (+)-catechin, *p*-coumaric acid, ferulic acid, luteolin, α-amyrin and β-amyrin at mg kg^{-1} levels.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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