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Pyrrolizidine alkaloids in medicinal tea of *Ageratum conyzoides*

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Abstract: It is now widely-recognized that the view that herbal remedies have no adverse effects and/or toxicity is incorrect; some traditionally-used plants can present toxicity. The well-established popular use of *Ageratum conyzoides* has led to its inclusion in a category of medicinal crude drugs created by the Brazilian Health Surveillance Agency. *Ageratum* belongs to the Eupatorieae tribe, Asteraceae, and is described as containing toxic pyrrolizidine alkaloids. Aqueous extracts of *Ageratum conyzoides* L. harvested in Brazil (commercial, flowering and non-flowering samples) were prepared according to the prescribed method and analyzed by HPLC-HRMS. The pyrrolizidine alkaloids lycopsamine, dihydrolycopsamine, and acetyl-lycopsamine and their *N*-oxides, were detected in the analyzed extracts, lycopsamine and its *N*-oxide being known hepatotoxins and tumorigens. Together with the pyrrolizidine alkaloids identified by HPLC-HRMS, thirteen phenolic compounds were identified, notably, methoxylated flavonoids and chromenes. Toxicological studies on *A. conyzoides* are necessary, as is monitoring of its clinical use. To date, there are no established safety guidelines on pyrrolizidine alkaloids-containing plants, and their use in Brazil.

Article

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Introduction

The *Ageratum* genus, Asteraceae, comprises *ca* thirty species that are not yet well-investigated. The widespread neotropical species *Ageratum conyzoides* L. is an annual aromatic plant that is considered an invasive and cosmopolite weed that grows in tropical areas, and is very common in Brazil. Its peculiar odour has been likened to the smell of goats, giving rise to the popular name of *goatweed*. In Brazil it has several names, such as mentrasto, maria-preta, picão-branco; picão-roxo, erva-de-são-joão, erva-de-são-josé, erva-de-santa-lúcia. *Ageratum conyzoides* has a long history of medicinal use in several tropical countries around the world, and has a wide range of indications, from skin diseases to mental disorders and infectious diseases (Okunade, 2002).

In Brazil, its aerial parts are widely used (internally and externally, fresh or dried, in tinctures or infusions) for their supposed analgesic and anti-inflammatory properties, and are commonly used to treat menstrual cramps, arthritis, rheumatism, and diarrhea (Okunade, 2002; Lorenzi & Matos, 2008).

The well-established popular use of this plant has led to its inclusion in the list of notified herbal drugs, a category of medicinal crude drugs created by the Brazilian Health Surveillance Agency, Anvisa (RDC No. 10, March 9th, 2010). This means the crude drug (aerial parts, crushed or powdered) is now authorized for marketing without medical prescription, for use in the preparation of infusions.

The majority of publications on the biological activity of *A. conyzoides* focus on its essential oil, and not on substances isolated from its extracts, such as pyrrolizidine alkaloids (PA), which were isolated from *A. conyzoides* collected in Kenya (Wiedenfeld & Roder, 1991). These alkaloids are potentially hepatotoxic, and acute intoxications caused by PA are characterized by haemorrhagic liver necrosis in animals, being rare in humans. The main problem associated with PA is that long-term exposure can cause hepatic megalocytosis and veno-occlusive disease (to a lesser extent in the lungs), fatty liver degeneration and cirrhosis, and proliferation of the bile duct epithelium. Moreover, many PA are genotoxic and carcinogenic in rodents (Chen & Huo, 2010).

Since there is no published paper that confirms the presence of PA in the Brazilian *Ageratum conyzoides*, this work investigated the presence of PA in tea (aqueous extract) obtained by infusion of harvested plants (with and without flowers) and from a commercial sample (prepared according to the Anvisa procedure). It also attempted to isolate PA and further constituents from this medicinal plant.

Material and Methods

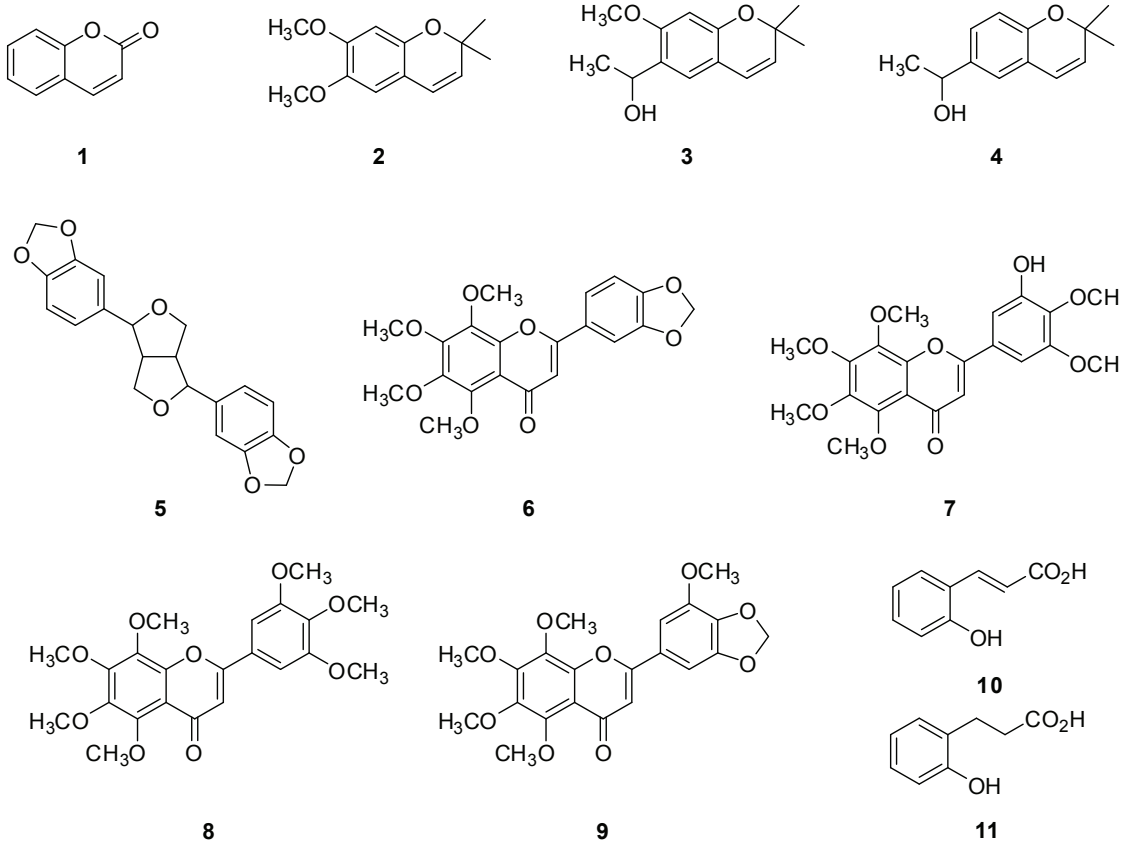
Plant material

The whole plant (*Ageratum conyzoides* L., Asteraceae) was harvested from the Garden of Medicinal Plants of the University Hospital of the Universidade Federal de Santa Catarina and from vacant lots near Praia Brava Beach, Florianópolis, SC. A voucher was identified by the botanist Renato Záchia and is deposited in the Herbarium of the Universidade Federal de Santa Maria (No. SMDB 13.138). The commercial sample was purchased from a Pharmacy in Ouro Preto, MG.

Plant processing and extraction

The aerial parts of flowering and non-flowering species were separated and air-dried at 50 ºC (six days), then crushed, and stored in a refrigerator (4 ºC). The aqueous extract (infusion) was prepared by placing 3 g of crude drug in 150 mL of boiling water (according to RDC 10/10, Anvisa). The resulting aqueous extract was lyophilized and then analysed by NMR and HPLC-HRMS. The following samples were prepared: Flowering aerial parts (FS), non-flowering aerial parts (NFS), commercial sample (CS), purchased from a Pharmacy in Ouro Preto (MG).

In order to isolate the main compounds, selective extraction of the crude drug with flowers (1 kg) was performed with solvents of increasing polarity, using *n*-hexane (28 g of *n*-hexane extract), dichloromethane (34 g of dichloromethane extract), ethyl acetate (3 g of ethyl acetate extract) and ethanol (11 g of ethanol extract). From *n*-hexane extract, the following known compounds were obtained by classic silica gel CC, using several proportions of hexane-acetone as eluents (spectral data in accordance with the literature): β-sitosterol and stigmasterol (Kongduang et al., 2008), coumarin (**1**) (Kupriyanova, 1997), precocene II (**2**) (Adebayo et al., 2010), encecalol and demethoxyencecalol (**3** and **4**) (González et al., 1991), sesamin (**5**) (Moazzami et al., 2007), linderoflavone B (**6**) (Saxena; Shrivastava, 1994), 3'-hydroxy-5,6,7,8,4',5'-hexamethoxyflavone (**7**) (Herz & Kulanthaivel, 1982), 5'-



methoxynobiletin (**8**) (Le-Van & Van Cuong Pham, 1979), and eupalestin (**9**) (Herz et al., 1980). From the ethanol extract, the following were obtained: 2-hydroxycinnamic and 2-hydroxydihydrocinnamic acids (**10** and **11**) (Gerothanassis et al., 1998).

Pyrrolizidine alkaloids extraction procedures

In an attempt to isolate the PA, the method described previously for *A. conyzoides* by Wiedenfeld & Roder was reproduced, but without success. Additionally, traditional and improved methods for PA extraction were used, but no alkaloids were isolated (Frahn et al., 1980, Mroczek et al., 2006).

Aqueous extract fractionation

In order to screen the chemical composition of the medicinal aqueous extract of *A. conyzoides*, CC fractionation was performed with Amberlite resin (Rohm and Hass Co) XAD-4 (230 g) and XAD-7 (250 g), using the hot aqueous extract (10 L), prepared according to the official Brazilian method (M&M) (Figure 1). The non-retained extract in the resin XAD-7 (*i.e.* the aqueous extract that eluted from the resin, 21 g) was solubilized in water and partitioned with *n*-butanol. The resulting fraction (5 g) was chromatographed in silica gel with an *n*-hexane-ethyl acetate-methanol gradient, leading to the identification of two compounds (1.7 g of 2-hydroxycinnamic acid, **16** and 2-hydroxydihydrocinnamic acid, **17**). The aqueous extract retained in XAD-4 (eluted with methanol) yielded 3.6 g, which was partitioned with dichloromethane (68.8 mg) and chromatographed in silica gel with an *n*-hexane-ethyl acetate-methanol gradient, furnishing 5.3 mg of compounds **16** and **17** and 8.5 mg of precocene II (**8**).

All the other fractions obtained, shown in Figure 1, were lyophilized and analyzed by UHPLC-HRMS: XAD-4 aqueous fraction, XAD-4 aqueous fraction after partition with dichloromethane, XAD-7 aqueous fraction, XAD-7 aqueous fraction after partition with ethyl acetate, and aqueous extract not retained in XAD.

Equipment

NMR data for the isolated compounds were recorded on Bruker AVANCE 400 and AVANCE III 400 NMR spectrometers, both operating at 9.4 T. ^{1}H and ^{13}C nuclei were observed at 400.13 and 100.61 MHz, respectively. The spectrometers were equipped with either a 5 mm multinuclear direct detection probe with *z*-gradient or a 5 mm multinuclear inverse detection probe with *z*-gradient. One-bond and long-range ^{1}H-^{13}C correlations from HSQC and HMBC NMR experiments were performed with average coupling constants $^{1}J_{(H,C)}$ and $^{LR}J_{(H,C)}$ optimized for 140 and 8 Hz, respectively. The NMR chemical shifts are given in ppm related to the TMS signal at 0.00 ppm as internal reference. The UHPLC-ESI-(+)-HRMS and UHPLC-ESI(+)-HRMS/MS analyses were performed using the Accela system (San Jose, CA, USA) equipped with a binary pump, autosampler, online vacuum degasser, and temperature-controlled column compartment. High resolution mass spectrometry was performed on a hybrid LTQ-Orbitrap XL Discovery mass spectrometer equipped with an ESI probe (Thermo Scientific, San Jose, CA, USA).

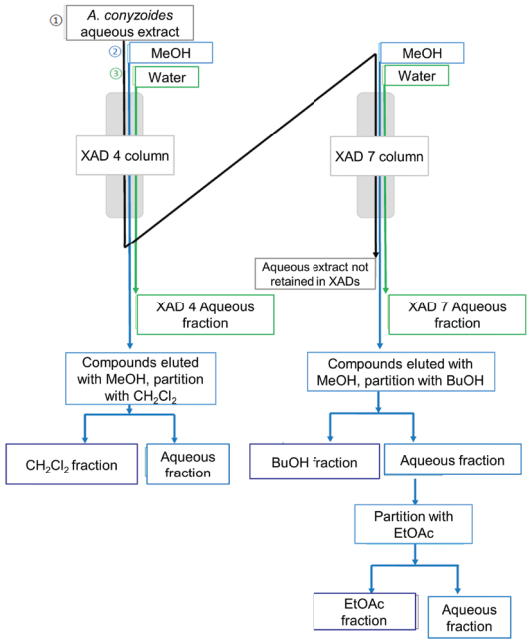


Figure 1. Flow chart of the *Ageratum conyzoides* aqueous extract fractionation using Amberlite XAD-4 and XAD-7 resins.

UHPLC-ESI(+)-HRMS & HRMS/MS analysis of extracts/ isolated compounds

The solvents used in this study were of LC-MS grade, purchased from Fluka/Riedel-de Haën (St. Gallen, Switzerland). The freeze-dried extracts were stored in the dark at 4 °C prior to preparation for LC-MS, being diluted at the moment of analysis in MeOH at a final concentration of 100 µg/mL. An Express Gold C18 100 x 2.1, 3 µm reversed phase column (Thermo Scientific, Brehmen, Germany) was used at a flow rate of 300 µL/min for the chromatographic separation. The mobile phase consisted of solvents A: water, 0.1% formic acid and B: ACN 0.1% formic acid. A gradient method (total run time of 20 min) was used for the profiling of the samples as follows: 0 to 5 min: 5% B, 5 to 7 min: 25% B, 7 to 14 min: 95% B, 14 to 16 min: 95% B, 16 to 17 min: 5% B and 17 to 20 min: 5% B. The injection volume was 2 µL. The hybrid high resolution mass spectrometer (LTQ-Orbitrap Discovery XL) was operated in positive ion mode under

the following optimized conditions: capillary temperature, 270 °C; capillary voltage, 20 V; tube lens, 110 V; source voltage, 3.5 kV; sheath gas flow, 40 arb. units; aux gas flow, 20 arb. units. Analysis was performed in full scan mode, with resolution of 30,000 at *m/z* 400 and scan range set to *m/z* 100 -1000.

For the HRMS/MS analysis, a "data-dependent" method was used, enabling the fragmentation of the three highest peaks detected for each scan. Two main scan events were performed successively, using only Orbitrap analyzers, the first consisting of a full scan (3 microscans, 50000 injection time and 50-1000 *m/z* scan range) and the second consisting of three HRMS/MS acquisitions of the three most intense peaks (2 microscans, 10000 injection time, 45V CID / Q:0.25).

Results and Discussion

The use of herbal plants as natural remedies, functional foods, and dietary supplements for health care has been increasing worldwide. Market estimates suggest that the rate of growth in sales of traditional medicinal products in recent years has been between 5 and 18% per annum (Kohler & Baghdadi-Sabeti, 2011). It is now well-recognized that the concept of no adverse effects and/or toxicity of natural products is an incorrect one (Li et al., 2011). It is highly possible that PA-containing plants are the most common poisonous plants affecting livestock, wildlife, and humans, and are probably also the leading hepatotoxic and tumorigenic phytochemicals associated with human and animal diseases.

Consequently, it is appropriate and imperative to identify plants and herbal products that contain toxic PA, and to assess the risk posed by exposure to these substances present in herbal products (Molyneux et al., 2011). It has been reported that 3% of all flowering plants contain PA, but they are primarily found in members of three plant families: Asteraceae, Boraginaceae and Fabaceae (Fu et al., 2002).

Ageratum belongs to the Eupatorieae tribe of the Asteraceae family, the Eupatorieae and Senecioneae tribes, the two described as containing the toxic PA compounds: 1,2-dehydropyrrolizidine ester alkaloids (dehydroPA, as in lycopsamine (**12**) and their *N*-oxides) (Hartmann & Conner, 2009). Worldwide, human hepatotoxicity due to 1,2-dehydropyrrolizidine alkaloids can be associated with the consumption of herbal remedies, the main problem being related to contamination of food or feed (Edgar et al., 2002).

In mammals, ingested dehydroPA are oxidized in the liver by mixed-function oxidases (cytochrome P450) to pyrrole derivatives. The pyrrole ring system renders the C-7 and/or C-9 position highly electrophilic, and capable of reacting with tissue nucleophiles, concomitant with cleavage of the ester substituents, thereby binding to proteins and/or nucleic acids. This, in turn, can alter cell function, leading to cell damage or cell death (Molyneux et al., 2011).

The presence of dehydroPA and its *N*-oxides has been reported in *A. conyzoides* harvested in Kenya (Wiedenfeld & Roder, 1991), Ethiopia (Wiedenfeld, 2011) and Hawaii (Molyneux et al., 2011). PA and other toxins occur in low concentrations, and as predicted by the shifting defense hypothesis, toxin concentrations are significantly higher in invasive weed species than in native species (Doorduin & Vrieling, 2011). *Ageratum* is native to tropical America, and is highly invasive, especially under the drought conditions, which are alien to its natural environment. The shifting defense hypothesis could help explain why, in this work, it was not possible to isolate the alkaloids using the specific method described for these dehydroPA or using the same method as that previously used for the same species (M&M).

Aqueous extracts of *A. conyzoides* harvested in South Brazil (samples FS, NFS, CS), and its XAD fractions, were analyzed by HPLC-HRMS (Table 1) in full scan mode, to investigate the presence of the previously isolated dehydroPA isomers lycopsamine and echinatine, and other biogenetically related PA, such as acetyl-lycopsamine, dihydrolycopsamine and their *N*-oxide forms.

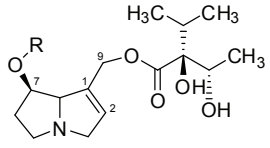
In the full scan HPLC-HRMS analysis, there were only a few peaks representing the relatively higher content of components in the TIC (Figure 2, 1st line); however, in the extracted ion chromatograms (XIC, 2nd to 7th lines in Figure 2), six PA were detected. To verify their identities, HRMS/MS, generated by a newly developed data compound-dependent method for PA, were recorded. The resulting retention times, exact mass of protonated molecular ions, and characteristic fragment ions are summarized in Tables 1 and 3. In the full scan (TIC), six peaks with protonated molecular ions ($[M+H]^+$) were observed at *m/z* 300.1805 (3.05 min, lycopsamine; **12**), 316.1755 (3.36 min, lycopsamine *N*-oxide, **13**), 302.1962 (3.38 min, dihydrolycopsamine, **14**), 318.1911 (3.75 min, dihydrolycopsamine *N*-oxide, **15**), 342.1911 (4.62 min, acetyl-lycopsamine, **16**), and 358.1860 (4.83 min, acetyl-lycopsamine *N*-oxide, **17**). In the HRMS/MS experiment, these six peaks showed the characteristic retronecine-type specific diagnostic fragment ion at 138 *m/z* (Table 3). Of these six alkaloids identified, only two peaks were related to the dihydroPA form of lycopsamine and its *N*-oxide, which were not characterized as carcinogenic or hepatotoxic. It is important to note that together with the above mentioned PA, several mass isomers, mainly of dihydroPA, were also present (Figure 2, Table 1).

Few peaks were observed in the total ion chromatogram (TIC), confirming that the content of PA in the samples is low (Figure 2). Lycopsamine and its *N*-oxide were the two major PA (known hepatotoxins and tumorigens), while the other PA identified were only

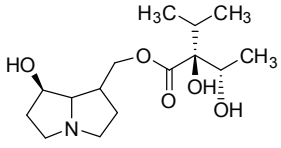
Table 1. LC-HRMS data of compounds identified in aqueous extract samples of *Ageratum conyzoides.*

Compounds	LC-HRMS analysis						Identification	Samples		
	[M+H]$^+$									
	t_R (min)	Theoretical *m/z*	Experimental *m/z*	Delta (ppm)	RDB	Molecular Formula		CS[1]	FS[2]	NFS[3]
1	3.05	300.1805	300.1811	2.00	3.5	$C_{15}H_{25}O_5N$	lycopsamine (**12**)	++	+++	+
2	2.90	302.1962	302.1966	1.32	2.5	$C_{15}H_{27}O_5N$	dihydro-lycopsamine (**14**)	+	+	+
	3.38		302.1967	1.65			possible isomer I	+	+	+
	4.03		302.1966	1.32			possible isomer II			
3	4.65	342.1911	342.1917	1.75	4.5	$C_{17}H_{27}O_6N$	acetyl-lycopsamine (**16**)	+	+++	++
4	3.36	316.1755	316.1760	1.58	3.5	$C_{15}H_{25}O_6N$	lycopsamine *N*-oxide (**13**)	+++	++	+
5	3.21	318.1911	318.1915	1.26	2.5	$C_{15}H_{27}O_6N$	dihydro-lycopsamine *N*-oxide (**15**)	+	+	+
	3.75		318.1915	1.26			possible isomer I	++	+	+++
	3.94		318.1915	1.26			possible isomer II	++	+	+++
6	4.83	358.1860	358.1866	1.68	4.5	$C_{17}H_{27}O_7N$	acetyl-lycopsamine *N*-oxide (**17**)	++	+	+++
	11.99		358.1869	2.51			possible isomer I	+	++	+++
7	12.50	417.1180	417.1174	-1.44	11.5	$C_{21}H_{20}O_9$	eupalestin (**9**)	+	+	+
8	11.87	433.1493	433.1486	-1.62	10.5	$C_{22}H_{24}O_9$	5'-methoxynobiletin (**8**)	+	+	+
9	4.83	205.1223	205.1219	-1.95	5.5	$C_{13}H_{16}O_2$	demethoxyencecalol (**4**)	+	+	+
10	12.70	235.1329	235.1327	-0.85	5.5	$C_{14}H_{18}O_3$	encecalol (**3**)	+	+	+
12	12.66	167.0703	167.0700	-1.80	4.5	$C_9H_{10}O_3$	2-hydroxydihydrocinnamic acid (**11**)	+	+	+
13	10.95	163.1117	163.1113	-2.45	4.5	$C_{11}H_{14}O$	2,2-dimethylchromane	+	-	-
14	5.96	223.1329	223.1324	-2.24	4.5	$C_{13}H_{18}O_3$	3,4-dihydroprecocene II	-	-	-
15	10.66	419.1337	419.1336	-0.24	10.5	$C_{21}H_{22}O_9$	3'-hydroxy-5,6,7,8,4',5'-hexamethoxyflavone (**7**)	+	+	+
16	11.68	387.1074	387.1065	-2.32	11.5	$C_{20}H_{18}O_8$	linderoflavone B (**6**)	+	+	+
17	7.49	147.0441	147.0437	-2.72	6.5	$C_9H_6O_2$	coumarin (**1**)	+	+	+
18	12.14	221.1172	221.1169	-1.36	5.5	$C_{13}H_{16}O_3$	precocene II (**2**)	-	+	+
19	11.41	403.1387	403.1392	1.24	10.5	$C_{21}H_{22}O_8$	5,6,7,3',4',5'-hexamethoxyflavone	+	+	+
20	10.26	389.1231	389.1239	2.06	10.5	$C_{20}H_{20}O_8$	ageconyflavone C	+	-	-

[1]Commercial sample; [2]Flowering sample; [3]Non-flowering sample.



12 R=H
13 R=H; N➔O
16 R=Ac
17 R=Ac; N➔O



14
15 N➔O

minor ingredients in the plant. Analysing the data shown in Table 1, it can be clearly seen that in the non-flowering sample, the dihydro and *N*-oxide forms were predominant, but toxic dehydroPA were also present, contrary to the expectation that the plant would only produce these alkaloids when in flower, or only in the flowers, sequestered by butterflies (Hartmann & Ober, 2000). Moreover, due to the rhizomatous propagation of *A. conyzoides*, it is very difficult to separate the non-flowering from the flowering specimens. Despite the recommendation of Brazilian Health Surveillance Agency regarding the marketing of the non-flowering plant, only samples containing flowers were commercially available. In this work, we used only non-rhizomatous specimens for the non-flowering sample. Indeed, semi-quantitative comparison with the flowering and commercial samples showed that their alkaloid contents were very similar.

Together with the PA identified by HPLC-HRMS, thirteen phenolic known compounds were detected in the aqueous extract, particularly methoxylated flavonoids and chromenes, together with coumarin and simple phenolics. Table 2 shows the PA distributed in various fractions, similar to the flavonoids after XAD fractionation, which was performed as shown in Figure 1. The possible role of these abundant phenolics in the medicinal qualities of this plant should also be explored, to determine both its beneficial and its adverse effects.

Conclusions

In the present study, hepatotoxic and tumorigenic PA and *N*-oxide PA were identified in *A. conyzoides*. The

Table 2. LC-HRMS data of compounds identified in XAD fractions of *Ageratum conyzoides*.

Identification	Samples				Not retained[e]
	XAD4A[a]	XAD4AG[b]	XAD7A[c]	XAD7AG[d]	
lycopsamine (**12**)	+	+	+	+	+
dihydrolycopsamine (**14**)	+	+	+	+	-
acetyl-lycopsamine (**16**)	-	-	-	+	-
lycopsamine *N*-oxide (**13**)	+	+	+	+	+
dihydrolycopsamine *N*-oxide (**15**)	-	-	-	+	-
acetyl-lycopsamine *N*-oxide (**17**)	-	+	+	+	-
eupalestin (**9**)	+	+	-	+	+
5'-methoxynobiletin (**8**)	+	+	+	+	+
demethoxyencecalol (**4**)	-	-	-	-	-
encecalol (**3**)	-	-	-	-	-
2-hydroxydihydrocinnamic acid (**11**)	+	+	-	-	-
2,2-dimethylchromane	-	-	-	-	-
3,4-dihydroprecocene II	-	-	-	+	-
3'-hydroxy-5,6,7,8,4',5'-hexamethoxyflavone (**7**)	-	+	+	-	-
linderoflavone B (**6**)	+	+	+	+	+
coumarin (**1**)	-	+	+	+	-
precocene II (**2**)	-	-		-	-
5,6,7,3',4',5'-hexamethoxyflavone	+	+	+	+	+
ageconyflavone C	-	+	-	-	-

[a]XAD-4 aqueous fraction; [b]XAD-4 aqueous fraction after partition with dichloromethane; [c]XAD-7 aqueous fraction; [d]XAD-7 aqueous fraction after partition with ethyl acetate; [e]Aqueous extract not retained in XAD.

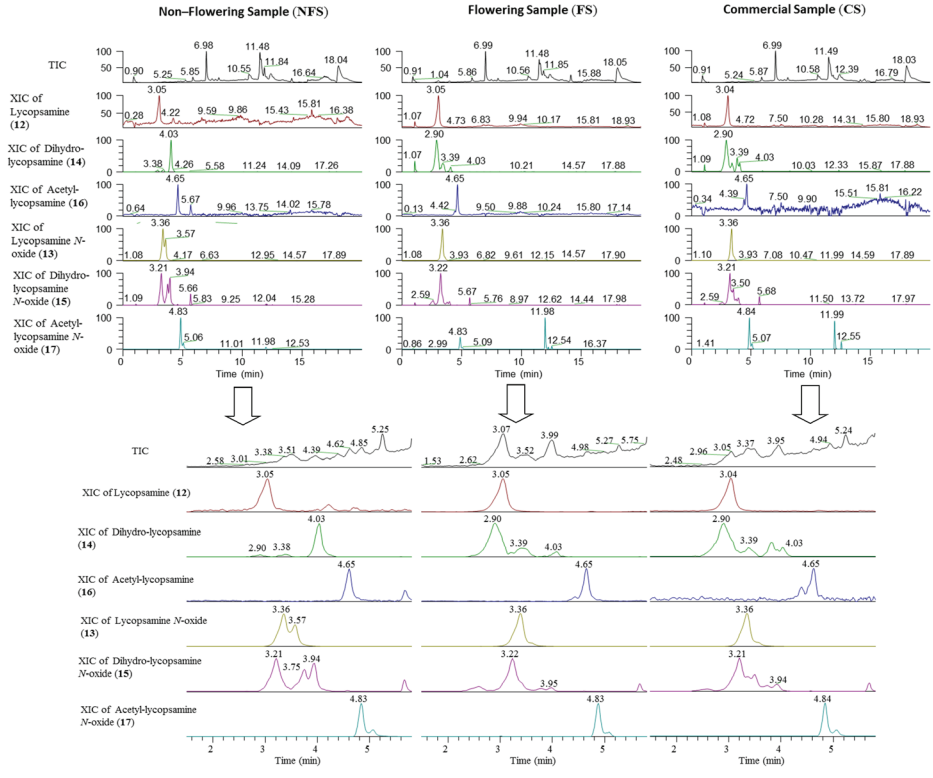


Figure 2. Total ion chromatogram and extracted peaks obtained for the three analysed samples of *Ageratum conyzoides* aqueous extract, together with the respective extracted ion chromatograms (XIC), with several PA detected.

Table 3. HRMS/MS data of major PA found in *Ageratum conyzoides* aqueous extract.

Compounds	LC-HRMS analysis						Identification	Main product ions *m/z* (RDB)					
		[M+H]$^+$											
	t_R (min)	Theoretical *m/z*	Experimental *m/z*	Delta (ppm)	RDB	Formula							
1	3.05	300.1805	300.1811	2.00	3.5	$C_{15}H_{25}O_5N$	lycopsamine (**12**)	282.1704 (3.5)	156.1019 (2.5)	138.0912 (3.5)			
2	2.90 3.38 3.82	302.1962	302.1966	1.32	2.5	$C_{15}H_{27}O_5N$	dihydro-lycopsamine (**14**)	138.0911 (3.5)					
3	4.65	342.1911	342.19171	1.78	4.5	$C_{17}H_{27}O_6N$	acetyl-lycopsamine (**16**)	282.1700 (4.5)	138.0912 (3.5)				
4	3.36	316.1755	316.1760	1.58	3.5	$C_{15}H_{25}O_6N$	lycopsamine *N*-oxide (**13**)	298.1647 (4.5)	272.1495 (3.5)	226.1439 (3.5)	172.0967 (2.5)	155.0939 (3.0)	138.0912 (3.5)
5	3.21 3.75 3.94	318.1911	318.1915	1.26	2.5	$C_{15}H_{27}O_6N$	dihydro-lycopsamine *N*-oxide (**15**)	300.1802 (3.5)	274.1647 (2.5)	174.1121 (1.5)	138.0909 (3.5)		
6	4.83 11.99	358.1860	358.18658	1.62	4.5	$C_{17}H_{27}O_7N$	acetyl-lycopsamine *N*-oxide (**17**)	177.0907 (5.5)					

use of extracts of this plant for medicinal preparations could potentially be harmful to human health, despite the low content of these substances in plants harvested in Brazil; chronic exposure to these toxigenic PA can present a risk of liver damage. In some countries, its clinical use is only authorized within certain limits. In Germany, for example, it is recommended that daily exposure to PA be no more than 0.1 mg for less than six weeks per year; and in Belgium, the limit for PA in plants is 1 ppm (1 mg per gram of plant) (Chen; Huo, 2010). Systematic toxicological studies on *A. conyzoides* with accurate quantification of toxic PA in plants are necessary, as is monitoring the clinical use of this drug. To date, there are no established safety guidelines on PA-containing medicinal plants and their use in Brazil.

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Author contributions

CFB and DWR harvested, extracted and isolated compounds. RG worked with XAD resins. MH, MWB and NL performed MS experiments. AS contributed to the manuscript. MWB supervised the work and wrote the manuscript.

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