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Original Article

Chemical composition and biological properties of *Ipomoea procumbens*

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Natural products have been the most valuable source of chemical compounds in the discovery of novel medicines. Secondary metabolites from terrestrial and marine organisms have found considerable use in the treatment of numerous diseases and have been considered lead molecules both in their natural form and as templates for medicinal chemistry. Brazil has an exceptionally rich biodiversity, and a valuable source of secondary metabolites that can be useful for the development of bioproducts. *Ipomoea* species, Convolvulaceae, are mostly found in tropical and sub-tropical regions, including South America and many are used for nutritional and medicinal purposes. *Ipomoea procumbens* Mart. & Choisy is endemic from South America, and this is the first study reported on the chemical composition and biological activities of this species. The present work reports the tentatively identification of natural products present in the extracts using a high performance liquid chromatography–high resolution mass spectrometry method. Additionally, the antioxidant and antifungal biological activities of the leaves, roots and steams extracts and fractions of this species were evaluated. While for the antioxidant activity the hydromethanol fractions (leaves, stem and roots) were more active, the methanol fractions of leaves and stem provided better results for the antifungal assay.

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Introduction

The *Ipomoea* genus belongs to the Convolvulaceae family, which contains between 500 and 600 species mostly found in tropical and sub-tropical regions, like South America. This genus has been used for nutritional, medicinal, ritual and agricultural purposes. Concerning the nutritional purpose, it is relevant to refer to *Ipomoea* as "batata" also known as sweet potato, which is commonly cultivated and consumed worldwide (Austin and Huamán, 1996).

Some *Ipomoea* species are used to treat diseases, and the most common use is as a purgative to treat constipation, using the roots (Pereda-Miranda and Bah, 2003). Additionally, they also present therapeutic effects or biological activities such as antimicrobial, analgesic, spasmolitic, spasmogenic, hypotensive, psychotomimetic and anticancer (Meira et al., 2012). The bioactive compounds found in the plants of this genus are ergoline alkaloids, indolizidine alkaloids, nortropane alkaloids, phenolic compounds, coumarins, norisoprenoids, diterpenes, isocoumarins, benzenoids flavonoids, anthocyanins, glycolipids, lignans and triterpenes (Meira et al., 2012). Several studies have shown that flavonoids and phenolic compounds contribute significantly to the antioxidant and anti-inflammatory activities of many plants used in traditional medicine (Zeraik et al., 2011; Fraige et al., 2017). The interest in polyphenols, particularly flavonoids, is due to their great abundance in our diet and their role in the prevention of various diseases associated with oxidative stress (Arwa et al., 2015).

An interesting class of compounds named glycolipids, such as batatins, batatinosides and orizabins can be found in several species

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of *Ipomoea* from Mexico (Hernandez-Carlos et al., 1999; Rosas-Ramírez and Pereda-Miranda, 2013). *Ipomoea batatas* is reported to have chlorogenic acids (Clifford et al., 2003), mono-caffeoylquinic acids and dicaffeoylquinic acids namely isochlorogenic acids A, B and C (Ishiguro et al., 2007). Isochlorogenic acids A, B and C were also isolated from *I. pes-caprae* (Teramachi et al., 2005). The leaves, stem and roots of *I. batatas* cultivated in China were analyzed by LC–MS in the search for chlorogenic acids (Zheng and Clifford, 2008). These compounds were not detected in the root, whereas caffeoylquinic acids were the main subgroup of chlorogenic acids detected in the stem and the only subgroup detected in the leaves. Three feruloylquinic acids, 3,5- and 4,5-dicaffeoylquinic acid, and small amounts of four caffeoyl-feruloylquinic acids were also detected in the stem. Five caffeoylquinic acids were isolated from *I. batatas* using AB-8 macroresin absorption and semipreparative HPLC–DAD and identified by ESI/MS and NMR as 5-caffeoylquinic acid, 6-*O*-caffeoyl-β-D-fructofuranosyl-(2-1)-α-D-glucopyranoside, *trans*-4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid. The scavenging activity of the compounds was evaluated by DPPH• assay and showed IC_{50} values ranging from 7.6 to 12.4 $\mu g\, mL^{-1}$ (Zhao et al., 2014).

Rehman et al. (2011) evaluated the antioxidant activity of different extracts of *I. hederacea* by four methods and reported that an ethyl acetate fraction showed an IC_{50} of 60.28 $\mu g\, mL^{-1}$ of DPPH• inhibition, as well as the highest content of total phenolic compounds. The aqueous extract of leaves of *I. fistulosa* exhibited high antioxidant activity (IC_{50} = 59.94 $\mu g\, mL^{-1}$) in DPPH assay, that was associated with the presence of higher amount of phenolic and flavonoid compounds in the extract (Phulera et al., 2014).

From *Ipomoea asarifolia* were isolated acylated anthocyanins and ergoline alkaloids (chanoclavine I, ergine, ergobalansinine and lysergic acid α-hydroxyethylamide) (Jenett-Siems et al., 1994). *I. asarifolia* is toxic to goats, sheep and cattle and causes depression, tremors of the head and hypermetria (Carvalho et al., 2014).

Ipomoea procumbens species was described by the authors Von Martius and Choisy, (Muséum National d'Histoire Naturelle, Paris), and it is found in South America (Flora do Brasil, 2017). Despite a wide research on scientific resources, no additional information such as chemical studies or biological activities about this plant could be found further than the taxonomy studies. The reported therapeutic effects are achieved by the use of the plant extract or herbal preparations. Therefore, the identification of the biologically active compounds using modern analytical techniques without time consuming isolation is an excellent strategy to understand the plant biological properties and detect compounds that could be further investigated for medicine properties. The main objective of the present work was to tentatively identify the chemical compounds present in the leaves, roots and stems of *I. procumbens* extracts by HPLC–HRMS method and to evaluate the antioxidant and antifungal of the extracts fractions.

Materials and methods

Plant material

Ipomoea procumbens Mart. & Choisy, Convolvulaceae, leaves, stem and roots were collected at Itirapina Ecological Station, Itirapina, SP, Brazil on March 2002. The species was identified by Dr. Inês Cordeiro from the Institute of Botany of São Paulo and a voucher specimen (MRSilva 402) was deposited in the State Herbarium 'Maria Eneyda P. Kaufmann Fidalgo (SP)', São Paulo, Brazil. The plant material was dried at 40 °C in a circulating air oven and pulverized by a mechanical grinder.

Extraction and solid phase extraction (SPE) microfractionation

Analysis of the extracts

The dried, powdered leaves (5.4 mg), stem (5.5 mg), and roots (5.2 mg) of *I. procumbens* were extracted by sonication with 1 mL MeOH/H_2O (9:1), for 30 min, at room temperature. The extracts were subjected to a clean-up procedure in SPE C_{18} cartridges (Chromabond C18 ec, 500 mg, 3 mL cartridge) initially activated with 5 mL MeOH and then conditioned with 5 mL MeOH/water (9:1). The cartridge was loaded with 1 mL sample extract (5 $mg\, mL^{-1}$) that was eluted with 3 mL MeOH/water (9:1). The solvents were removed by evaporation under reduced pressure at 40 °C using a Buchi Rotavapor® R-114 to yield dry extracts. These extracts were resolubilized, filtered and analyzed by HPLC–UV/PDA (SPD-M20A model, Shimadzu Lab Solutions, Kyoto, Japan) and evaluated for antioxidant and antifungal activity. The water user was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

Microfractionation

The microfractionation of the extracts of *I. procumbens* (50 mg each, in 1 mL H_2O/MeOH 8:2,) was performed with SPE C_{18} cartridges activated as previously described and conditioned with the initial solution (H_2O/MeOH 8:2, v/v), then the elution was performed using a mixture of H_2O/MeOH, 5 mL, in the following ratios: 8:2, 1:1, 0:1. The separation yielded 3 fractions of each different part of the plant, a total of 9 fractions (SPE1–9). These samples were analyzed by HPLC–UV/PDA under the same conditions used for the extract analysis and the dereplication using HPLC–HRMS was performed as described further. Each fraction was evaluated for the antioxidant and antifungal assay.

HPLC–UV/PDA and HPLC–HRMS analysis

Chromatographic analyses were carried out on a LC-10AD liquid chromatography system equipped with a binary pump, autosampler a photodiode array detector SPD-M20A model (Shimadzu Lab Solutions, Kyoto, Japan). The samples were filtered with a NORM-JECT® syringe and a Simplepure® NY (0.22 μm) filter, and then injected automatically (20 μL) with a flow rate of 1 $mL\, min^{-1}$. The separation was performed using a Phenomenex Luna® C_{18} column (250 mm × 4.6 mm i.d.; 5.0 μm, Torrance, CA, USA). The solvent system was a mixture of H_2O (A) and MeOH (B), both with 0.1% formic acid in a linear gradient mode from 5 to 100% B in 50 min. The chromatogram was monitored simultaneously at 254, 280 and 366 nm, and the UV spectra of individual peaks were recorded in the range of 200–400 nm.

HPLC–HRMS data were obtained in a MicrOTOF II (Bruker, Billerica, MA, USA) series system equipped with an electrospray interface (ESI), an auto sampler and a high-pressure mixing pump. The column and the chromatographic conditions were the same as those used for the HPLC–UV/PDA analysis. The ESI-MS conditions consisted of capillary voltage set at 3500 V, dry heater temperature 220 °C, and nitrogen as the sheath gas flow. The analyses were performed in positive and negative ion modes.

For data comparison and tentative identification of the compounds, a database containing 108 compounds reported for *Ipomoea* genus was created from the calculated the m/z $[M+H]^+$ and $[M-H]^-$ using Chem Draw Ultra 8.0 (CambridgeSoft, Cambridge, USA).

Antioxidant DPPH• scavenging assay

The DPPH• method reported by Brand-Williams et al. (1995), with some modifications (Zeraik et al., 2016) was used to evaluate the antioxidant capacity of the *I. procumbens* extracts and

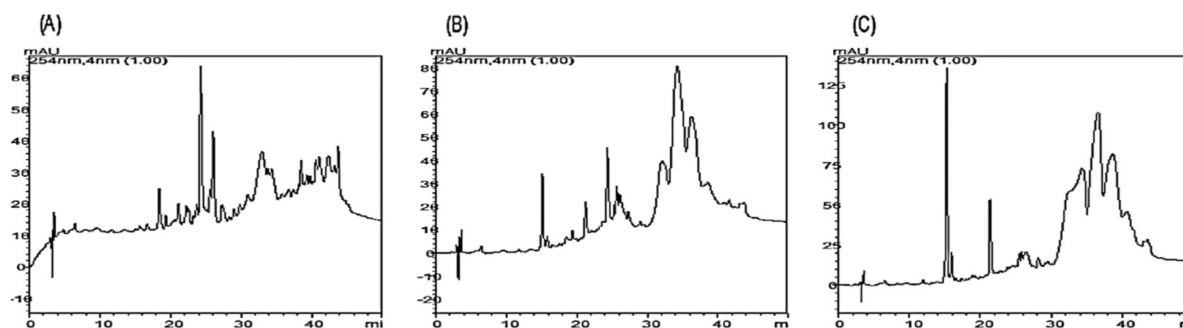

Fig. 1. Representative HPLC–UV analysis at 254 nm of the hydro-methanol extract of leaves (A), stem (B) and roots (C) of *Ipomoea procumbens*. Column: Phenomenex Luna® C_{18} column (250 mm × 4.6 mm i.d.; 5.0 μm). Mobile phase components: water (A) and methanol (B), both acidified with 0.5% formic acid and eluted in gradient mode, from 5% B to 100% B in 50 min. Flow-rate: 1.0 mL min^{-1}. Injection volume: 20 μL.

fractions. A range of concentrations (5–120 μg mL^{-1}) of the extracts and fractions, as well as the positive controls (rutin and gallic acid) were incubated for 30 min with 100 μmol l^{-1} of a DPPH• (2,2-diphenyl-1-picrylhydrazyl) solution in methanol in the dark. The scavenger activity was evaluated spectrophotometrically at 517 nm using a microplate reader (Synergy 2 Multi-Mode, BioTek, USA). The absorbance of the unreacted DPPH• radical was used as the control. The scavenging activity was calculated using the equation [(absorbance of control − absorbance of sample)/absorbance of control] × 100. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Chemical Co. The experiments were carried out in triplicate.

Antioxidant peroxyl radical scavenging assay

The peroxyl radical (ROO•)-scavenging capacity of the extracts was evaluated using the pyranine based procedure (Campos et al., 2004) with some modifications. The fluorescent compound pyranine (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt, 5 μmol l^{-1}) was incubated with 20 mmol l^{-1} AAPH (2,2′-azobis(2-methyl-propionamidine) hydrochloride)) in 10 mmol l^{-1} phosphate buffered saline (PBS, pH 7.4) at 37 °C in the absence (control) or presence of the tested extracts and fractions in the wells of a microplate. The final reaction volume was 300 μL and the extracts and fractions were tested in a range of concentrations (5–120 μg mL^{-1}). The fluorescence bleaching of the pyranine was measured at 485 nm (λ_{exc}) and 528 nm (λ_{em}) at 37 °C during 90 min in a Synergy2 Multi-Mode microplate reader (BioTek, EUA). Pyranine with AAPH (control 1) and without AAPH (control 2) were used as controls. Rutin and gallic acid were used as positive controls. The percentage of peroxyl radical scavenged was expressed by [(area sample − area control 2)/(area control 1 − area control 2)] × 100, using the values of the areas below the kinetic curves. 2,2′-azobis(2-methyl-propionamidine) hydrochloride (AAPH) (purity 97%), was purchased from Sigma-Aldrich Chemical Co. All reagents used for buffer preparation and mobile phases were of analytical grade. The experiments were carried out in triplicate.

Antifungal susceptibility test

Susceptibility tests of *Candida krusei*, *Cryptococcus neoformans*, *Candida parapsilosis*, *Cryptococcus gattii*, *Candida albicans* and *Candida tropicalis* to the *I. procumbens* fractions, amphotericin B (AmB) and fluconazole (FLZ) were performed according to the document M-27-A3 (Clinical and Laboratory Standards Institute, 2008). Inocula were prepared in Roswell Park Memorial Institute (RPMI-1640) medium purchased from Sigma-Aldrich, with L-glutamine, without sodium bicarbonate, supplemented with 2% glucose, and buffered to pH 7.0 using 0.165 M morpholinepropanesulfonic acid (MOPS; Sigma-Aldrich) to achieve a final concentration in microdilution plates of 2.5 × 10^3 colony-forming units (CFU)/mL. Work solutions of AmB and FLZ were tested in concentrations ranged from 64 to 0.0625 μg mL^{-1} and from 16 to 0.03 μg mL^{-1}, respectively. For the *I. procumbens* fractions, stock solution was prepared in appropriate quantities of dimethyl sulfoxide (DMSO) (purity 99.9%, Sigma-Aldrich Chemical Co.) and work solutions in RPMI medium. The amount of DMSO used was previously tested and did not affect the fungal viability (data not shown). The range of concentration tested was from 0.48 to 250 μg mL^{-1}. The plates were incubated at 37 °C under agitation of 150 rpm for up to 48 h. The readings were performed visually and confirmed using Alamar Blue® (Sigma-Aldrich). All the assays were performed in triplicate and in three independent experiments. All reagents used for buffer preparation and mobile phases were of analytical grade.

Table 1
Codes of the fractions obtained after the microfractionation of the extracts of leaves, stem and roots of *Ipomoea procumbens*.

Sample/H_2O:MeOH ratio	Fraction code
Leaves (8:2)	SPE1
Leaves (1:1)	SPE2
Leaves (0:1)	SPE3
Stem (8:2)	SPE4
Stem (1:1)	SPE5
Stem (0:1)	SPE6
Root (8:2)	SPE7
Root (1:1)	SPE8
Root (0:1)	SPE9

Results and discussion

The phytochemical screening of the leaves, stem and roots of hydro-methanol extracts of *I. procumbens* were performed by HPLC–UV and are shown in Fig. 1. The comparison of the chromatographic profiles obtained for each part of the plant showed that the leaves extract presents a richer composition in secondary metabolites that absorbs around 250 nm and 360 nm, probably belonging to the class of polyphenols. The extracts of stem and roots are very similar, with differences concerning the intensity of the peaks.

The extract of each part of the plant was microfractioned to yield three fractions each with different polarities (SPE1–9, Table 1), with the objective to make it easier to identify compounds present in the species by dereplication and to focus the antioxidant and antifungal activities. These fractions were analyzed by HPLC–UV under the same conditions used for the extracts and are shown in Fig. 2.

In an attempt to identify the compounds present in *I. procumbens*, we used the fast analytical technique dereplication, aimed at rapid on-line identification of known natural products contained in crude extracts or fractions. The database created for data comparison includes eighteen alkaloids, 34 phenolic compounds, 45

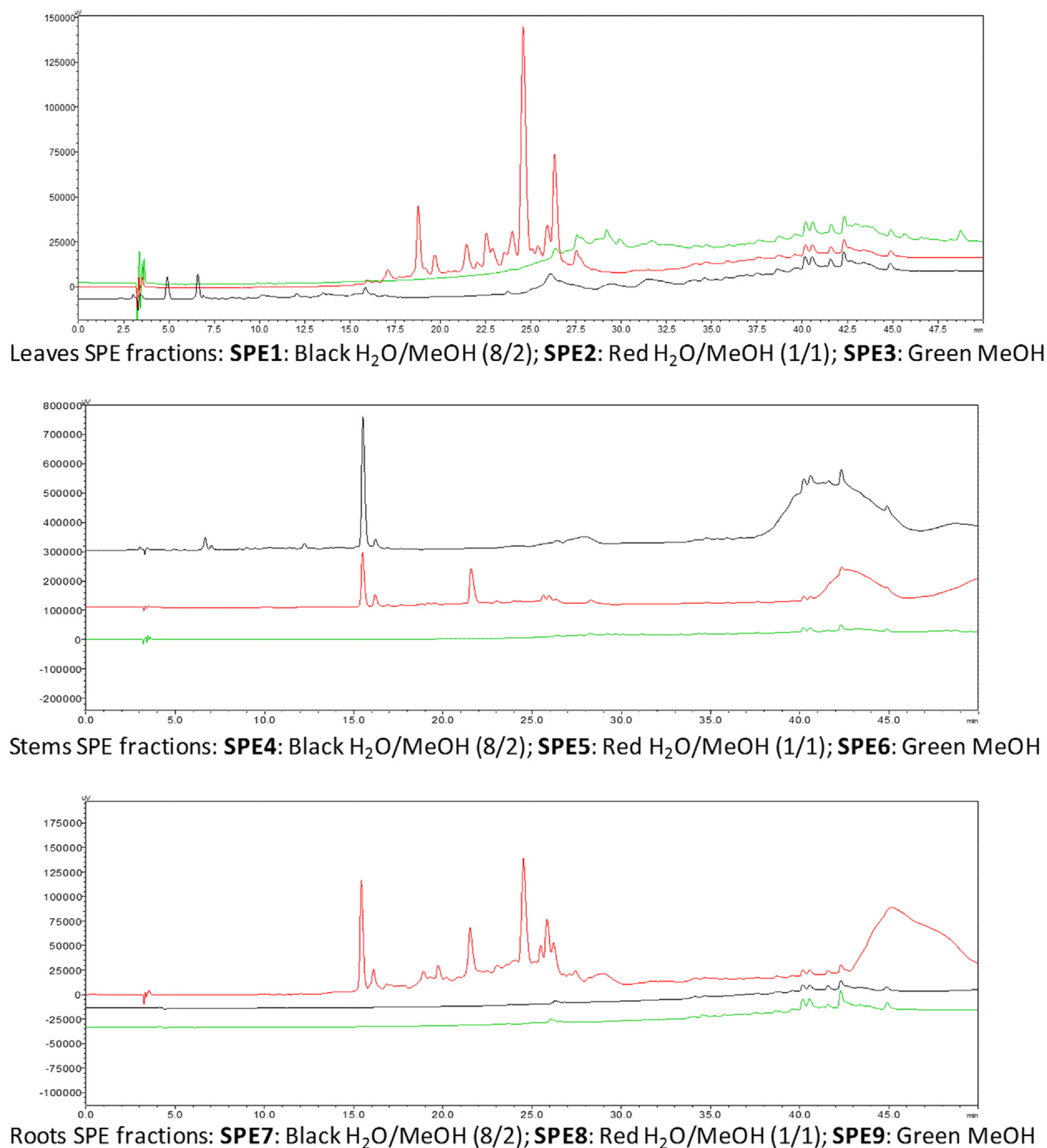

Fig. 2. HPLC–UV analysis at 254 nm of the SPE fractions of leaves (SPE1–SPE3), stems (SPE3–SPE6) and roots (SPE7–SPE9) of *Ipomoea procumbens*. Chromatographic conditions are the same as described in Fig. 1.

glycolipids, one lignan, seven triterpenes, among others. The application of LC coupled to MS in the analysis and characterization of natural products is a major breakthrough and it is frequently used for profiling extracts (Allard et al., 2017). Indeed, coupling LC with MS is extremely powerful in terms of time, detection, quantification and identification of a wide range of natural product. This method provides excellent sensitivity and selectivity, important structural information such as molecular weight (Wolfender et al., 2010). The observed *m/z* obtained for the major chromatographic peaks were compared with the *m/z* from the compounds in the database created (error less than $\pm$ 10 ppm). *I. procumbens* analysis allowed the detection of two isomers of dihydroxycinnamic acid, caffeoyl-quinic acid derivatives, the coumarins scopoletin and umbelliferone, and the alkaloids feruloyl tyramine and chanoclavine described in Tables 2 and 3 and Fig. 3.

Caffeoylquinic acids are phenolic compounds widely distributed in plants, and they are formed by esterification of quinic acid and caffeic acid, being chlorogenic acid the most known (Azuma et al., 2000). These compounds were also found in *I. batatas*, *I. aquatica*, *I. pes-caprae* and *I. fistulosa* (Meira et al., 2012; Zhao et al., 2014) and are associated with antioxidant and anti-inflammatory properties, and the dimers are reported to possess higher antioxidant activity than the monomer (Iwai et al., 2004), but poor bioavailability. Pale et al. (2003) reported the presence of two isomers of dihydroxycinnamic acids (caffeic acid and 3,5-dihydroxycinnamic acid) in the flowers of *I. asarifolia*. Scopoletin and umbelliferone were also found in *I. batatas*, *I. cairica* and *I. digitate* (Meira et al., 2012). Alkaloids such as chanoclavine, were also found in *I. asarifolia*, *I. hederacea*, *I. muelleri*, *I. corymbose*, *I. tricolor* and *I. violacea*.

Table 2
Compounds tentatively identified by dereplication studies in *Ipomoea procumbens* using HPLC–HRMS in the negative ion mode.

Retention time (min)	Observed *m/z* $[M-H]^-$	Calculated $[M-H]^-$	Error (ppm)	Compound	Fraction analyzed
14.3	179.0359	179.0350	5.02	Dihydroxycinnamic acid	SPE4
14.3	353.0888	353.0878	2.83	Caffeoyl-quinic acid	SPE4
20.2	529.1389	529.1351	7.18	Di-caffeoyl-quinic acid methyl ester	SPE8
22.4	677.153	677.1512	2.65	Tri-caffeoyl-quinic acid	SPE8

Table 3
Compounds tentatively identified by dereplication studies in *Ipomoea procumbens* using HPLC–HRMS in the positive mode.

Retention time (min)	Observed *m/z* $[M+H]^+$	Calculated $[M+H]^+$	Error (ppm)	Compound	Fraction analyzed
15.8/16.3	163.0397	163.039	4.29	Umbelliferone	SPE1/SPE4
18.2	193.0946	193.096	−7.25	Scopoletine	SPE2
23.7	314.1386	314.1387	−0.31	Feruloyl tyramine	SPE2
28.7/28.7	203.1055	203.1067	−5.90	Trimethyl-dihydro-hydroxy-naftalenone	SPE3/SPE9
14.3	179.0337	179.0339	−1.11	Esculetine/methylisocoumarins	SPE4
16.3	181.0498	181.0495	1.65	Dihydroxycinnamic acid	SPE4
12.1	257.1645	257.1648	−1.16	Chanoclavine	SPE8

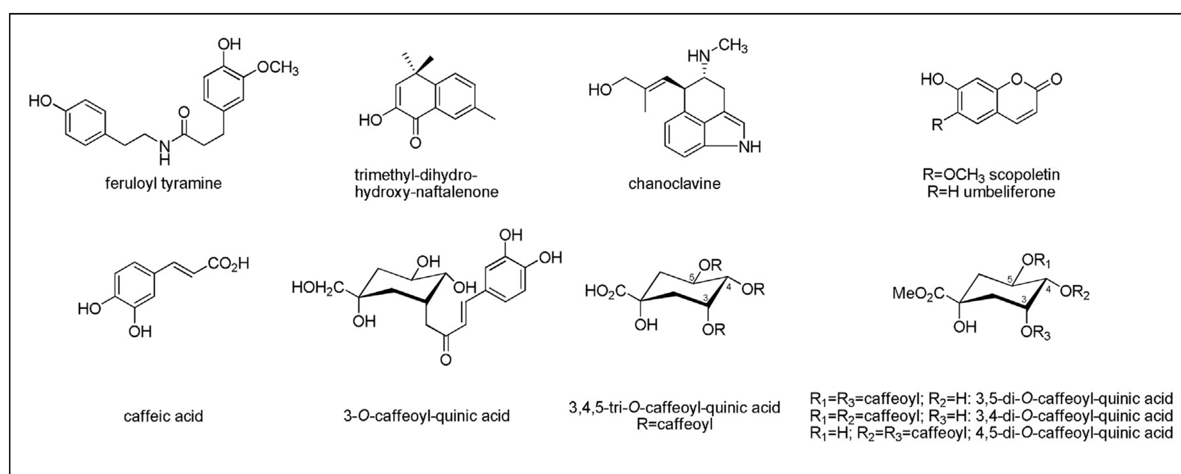

Fig. 3. Representative chemical structures of the tentatively identified compounds in *Ipomoea procumbens* (the positions for substitutions are merely representative and were not identified).

In Brazil there are a few studies that report the composition of the genus *Ipomoea*. To the best of our knowledge, none of the authors studying *Ipomoea* species from Brazil, including the present work, reported the glycolipid class of compounds found in *Ipomoea* species from Mexico. Apparently, there is a difference in the composition of *Ipomoea* genus found in these two countries and further studies could bring new information needed for confirmation. A difference in composition and biological activity was also reported when comparing the crude extract of *I. pes-caprae*, that reversibly inhibited the contractions induced by several spasmogens (Pongprayoon et al., 1989) and a similar study with the plant collected in Brazil (Emendorfer et al., 2005).

The antioxidant properties of the *I. procumbens* extracts were evaluated by two distinct methods, namely DPPH• (2,2-diphenyl-1-picrylhydrazyl) and peroxyl radical assays (ROO•), to reflect multifunctional properties in physiological processes (Muller et al., 2011). The DPPH• assay is based on the transfer of a hydrogen atom and an electron (ET), between the antioxidant compound and the DPPH radical, involving the change of color of the reaction medium from purple to yellow, wherein DPPH• itself reacts as a radical and a probe (Brand-Williams et al., 1995). This assay has been used widely to evaluate the radical scavenging activity of a variety of substances and plant extracts.

The peroxyl radical scavenging capacity procedure represents a typical hydrogen atom transfer based method, since it uses a competitive reaction scheme between antioxidants and a fluorescence probe (in this case pyranine), in which antioxidants and substrate compete for thermally generated peroxyl radicals generated by AAPH (2,2′-Azobis(2-methyl-propionamidine) hydrochloride) (Huang et al., 2005).

The results of the *in vitro* assays showed that the extracts of *I. procumbens* were able to scavenge DPPH• and ROO• in a dose dependent manner and presented antioxidant capacity comparable to that of the positive controls rutin and gallic acid, as well as most fractions as indicated in Table 4.

The leaves and steam fractions extracted with 1:1 H_2O:MeOH (SPE2 and SPE5) presented the higher antioxidant capacity, with EC_{50} values of 1.24 ± 0.08 and $1.29 \pm 0.10\,\mu g\,mL^{-1}$, respectively, while the most polar were the less active fractions. These results compared to the chromatograms presented in Fig. 2 suggest that the compounds related to the peaks observed can be the responsible for the activity. Considering the roots, the most polar fraction presented the higher antioxidant capacity, with EC_{50} of $1.48 \pm 0.10\,\mu g\,mL^{-1}$ (SPE7), followed by the fraction 1:1 H_2O:MeOH (SPE8).

Polyphenols are one of the most abundant groups of phytochemical compounds from plants, being phenolic compounds and flavonoids reported to present high antioxidant activity (Erkan et al., 2011; Kamiyama and Shibamoto, 2012). The content of total phenols, flavonoids, reducing power and antioxidant activity were compared for six *I. batatas* varieties commonly found in Malaysia. All varieties showed high radical scavenging activity by DPPH•

Table 4
Antioxidant capacity of *Ipomoea procumbens* extracts and fractions (SPE1–9) by DPPH• and ROO• scavenging methods.

Sample	Peroxyl radical scavenging EC_{50} ($\mu g\,mL^{-1}$)	DPPH• radical scavenging EC_{50} ($\mu g\,mL^{-1}$)
Leaves extract	4.14 ± 0.19	40.0 ± 0.50
Stem extract	5.32 ± 0.23	38.5 ± 0.41
Roots extract	3.34 ± 0.12	41.1 ± 0.41
SPE1	17.79 ± 0.27	>120.0
SPE2	1.24 ± 0.08	11.85 ± 0.35
SPE3	16.40 ± 0.25	83.10 ± 0.62
SPE4	17.27 ± 0.19	>120.0
SPE5	1.29 ± 0.10	13.8 ± 0.28
SPE6	4.70 ± 0.21	20.4 ± 0.32
SPE7	1.48 ± 0.10	16.0 ± 0.27
SPE8	3.13 ± 0.26	17.8 ± 0.33
SPE9	5.34 ± 0.29	19.1 ± 0.19
Rutin	1.36 ± 0.14	6.86 ± 0.13
Gallic acid	0.92 ± 0.07	2.01 ± 0.09

Table 5
Antifungal activity of SPE fractions of *Ipomoea procumbens*.

Fraction code	Minimum inhibitory concentration (MIC, $\mu g\,mL^{-1}$)					
	Candida krusei ATCC 6258	*Cryptococcus neoformans* 90012	*Candida parapsilosis* ATCC 22019	*Cryptococcus gattii* ATCC 56990	*Candida albicans* ATCC 90028	*Candida tropicalis* ATCC 750
SPE1	250	250	≥250	250	≥250	≥250
SPE2	125	125	≥250	250	≥250	≥250
SPE3	31.25	31.25	62.5	15.6	≥250	≥250
SPE4	≥250	250	≥250	125	≥250	≥250
SPE5	31.25	125	≥250	125	≥250	≥250
SPE6	15.6	15.6	31.25	62.5	≥250	≥250
SPE7	≥250	250	≥250	≥250	≥250	≥250
SPE8	250	125	≥250	125	≥250	≥250
SPE9	≥250	≥250	≥250	≥250	≥250	≥250
FLZ[a]	64	4	8	8	1	0.125–4
AmB[b]	0.25–2	0.25	1	0.125	1	0.5–2

[a] Fluconazole.
[b] Amphotericin B.

assay, ranging from 372.4 $\mu g\,mL^{-1}$ (IC_{50}) to 597.61 $\mu g\,mL^{-1}$ (IC_{50}) and these values were related to the high content of phenolics and flavonoids also reported in the varieties (Hue et al., 2012).

The evaluation of the antifungal properties of *I. procumbens* was important because of the medicinal plants use especially in South America, contributing significantly to primary health care. Many plants are used in Brazil in the form of crude extracts or herbal preparations to treat common infections without any scientific evidence of efficacy (Holetz et al., 2002). The phytochemistry of the *Ipomoea* genus has been studied since 1950 and some species of *Ipomoea* showed fungicide activity in the traditional use, particularly *I. batata* (Meira et al., 2012). The antifungal properties were evaluated in the present work according to the document M-27-A3 (Clinical and Laboratory Standards Institute, 2008) and the results are available in Table 5.

For this assay, it was considered that if the sample displayed a minimum inhibitory concentration (MIC) less than 100 $\mu g\,mL^{-1}$, the antimicrobial activity was good; from 100 to 500 $\mu g\,mL^{-1}$ the antimicrobial activity was moderate; from 500 to 1000 $\mu g\,mL^{-1}$ the antimicrobial activity was weak; over 1000 $\mu g\,mL^{-1}$ the sample was considered inactive (Holetz et al., 2002).

Candida parapsilopsis is sensitive to the methanol fractions of the leaf and the stem only (SPE3 and SPE6). The fractions SPE1 and SPE2 have a moderate activity on *C. krusei*, *C. neoformans* and *C. gattii*, whereas fraction SPE3 has a very good activity on all four *C. krusei*, *C. parapsilosis*, *C. neoformans* and *C. gattii*. The activity is particularly excellent on *C. gatti* with a MIC of 15.6 $\mu g\,mL^{-1}$. Concerning the stem, SPE4 fraction has a moderate activity on *C. neoformans* and *C. gatti* but has a better activity on *C. gatti*. The SPE5 fraction also has a moderate activity on *C. neoformans* and *C. gatti*, and the activity on *C. krusei* is very good. The methanol fraction (SPE6) has an excellent activity on *C. krusei* and *C. neoformans*, and very good activity on *C. parapsilosis* and *C. gattii*. The root fraction SPE7 has a moderate activity on *C. neoformans*, the SPE8 has a moderate action on *C. krusei*, but better on both *Cryptococcus*. There is no possibility to conclude on the methanol root fraction (SPE9) results. The methanol fractions of leaves and stem provided the best results for the antifungal activity. On *C. albicans* and *C. tropicalis*, all the results cannot be qualified; indeed, the MIC (>250 $\mu g\,mL^{-1}$) could not precisely be determined, so neither the activity.

While for the antioxidant activity the hydromethanol 1:1 fractions were more active, the methanol fractions (stems and leaves) provided better results for the antifungal assay.

Several species of *Ipomoea* (*I. batatas*, *I. muricata* and *I. aquatica*) have been explored extensively by various research groups for the search of compounds with good antifungal activity (Meira et al., 2012). In the present study, the methanol fractions of the leaf and the stem of *I. procumbems*, SPE3 and SPE6, showed a potent *in vitro* antifungal activity against *Candida* and *Cryptococcus* species and can be considered as promising antifungal compounds. In this scenario, more pharmacological studies will be necessary to evaluate these molecules as antifungal prototypes.

The findings herein reported are a preliminary study of chemical composition and biological activities and additional chemical studies are necessary to support biological studies.

Authors' contributions

SB (MSc student) contributed in running the laboratory work, extractions, acquisition and analysis of the data. MV supervised laboratory work, analyzed data and drafted the paper. MLZ and KF contributed to antioxidant biological studies and aided in the

drafting of the paper. GML contributed to chromatographic analysis and aided in the drafting of the paper. NSM and AMFA contributed to antifungal biological studies. MCMY contributed to plant collection, plant identification and herbarium confection. SM and VSB designed the study and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

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