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Preliminary analysis of Brazilian Amazonian plant extracts using bench-top assays in a through-put basis aiming the identification of potential antioxidant natural product

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ABSTRACT

Plants are a source of compounds that are used for the treatment of human and veterinary diseases. Brazil is one of the richest countries in the world in terms of biodiversity. The present study evaluated extracts using thin-layer chromatography to identify antioxidant activity and determine the presence of groups of compounds, in a large-scale basis. A total of 1,260 aqueous and organic extracts were obtained from plants that were collected in the Amazon and Atlantic rain forests. Thin-layer chromatography was performed to evaluate the presence of alkaloids, anthraquinones, cardioactive glicosides, terpenes, and phenolics and determine antioxidant and radical scavenging activity using the following reagents: Dragendorff's reagent, KOH, Kedde's reagent, sulphuric acid, NP reagent, β-carotene, and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Of the 1,260 extracts, 837 (66.43%) presented a β-carotene/bleaching response, and 1,205 (95.63%) presented a radical scavenging response. Alkaloids were found in 203 extracts (16.11%). Anthraquinones were found in 14 extracts (1.11%), cardenolides were found in eight extracts (0.63%). The present findings shows the importance of the Brazilian Amazon plants as sources of antioxidant and radical scavenging active compounds.

Keywords: Plants Extracts. Biodiversity. Annonaceae. Alkaloids. Antioxidants.

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INTRODUCTION

Plants are widely used in traditional medicine for the treatment of several disorders including cancer, heart disease, infectious disease, gastritis, among many others. Nonetheless, more studies have yet to be done focusing in the potential of Amazon and Atlantic rain forest plant extracts as a source of new natural products that are active against tumor cell lines (Ozi *et al*., 2011; Suffredini *et al*., 2007a; Suffredini *et al*., 2007b; Suffredini *et al*., 2006a; Suffredini *et al*., 2006b), microorganisms (Barnabé *et al*., 2014; Camargo & Suffredini, 2014; Castilho *et al*., 2014; Silva *et al*., 2014; Barrella *et al*., 2012; Silva *et al*., 2012; Matheus de Assis *et al*., 2009; Suffredini *et al*., 2004), parasites that have importance in human and veterinary health conditions (Cunha *et al*., 2014;) and about their toxicological profile (Estork *et al*., in press; Estork *et al*., 2014; Gusmão *et al*., 2013a; Gusmão *et al*., 2013b; Ribeiro-de-Assis *et al*., 2006).

Research in natural products mainly focused on traditional plants; despite of this group represents less than 10% of the flora. For that reason, a large amount of plants must be prospected for their potentialities. High-throughput assays are strategically the fast way of assessing chemical and biological information, once they are reliable, retional, not expensive and easy-to-implement techniques.

The use of thin-layer chromatography (TLC; Brazilian Ministry of Health, 2014; World Health Organization, 2011; Wagner & Bladt, 1996) fits the requirements to be used in high-throughput assays, as it is a widespread technique. TLC was adopted in the present study as to create a support for further spectroscopical analyses (Jakimska *et al*., 2014; Singh *et al*., 2014). In the present work, 1,260 plant extracts were chemically screened in TLC using traditional reagents to identify their antioxidant and radical scavenging potential, as to track for chemical compounds as alkaloids, phenolic compounds, anthraquinones, terpenes and cardenolides.

MATERIALS AND METHODS

Plant collection

Plant materials were collected under Brazilian Government plant collect license no. MMA/ICMBio/SISBIO#14895. The license to access genetic material was IBAMA/MMA/CGen#012A-2008. Plants were collected in two major localities in the Amazon rain forest, specifically in the Anavilhanas National Park (2° 23' 41" S 60° 55' 14" O) and surroudings, both in *igapó* and in *terra firme* forests, municipalities of Manaus and Novo Airão, State of Amazonas, Brazil. In the Atlantic Rain Forest, some plants were also collected within the limits of the APA-Cananeia-Iguape-Peruíbe (24° 35' 30" S 47° 27' 7" O), a natural reserve located at southeast coast of the State of São Paulo.

Plants were randomly collected as they presented flowers or fruits and according to the biomass availability for each plant organ (i.e., stems, leaves, flowers, fruits, and roots). The present approach follows ethical regards indicated by the Brazilian official offices. Vouchers of each species were obtained and deposited at Herbaria UNIP and MG. Each plant part provided an organic extract and an aqueous extract (Younes *et al*., 2007).

Extract preparation

Each plant part was separatedly dried in using air-circulating stove (Fanem, Diadema, São Paulo, Brazil) at 40 ºC (i.e., a temperature that is usually used to dry crude plant material). Crude material was ground in a hammer mill (Holmes, Danville, IL, USA) and the resulting powder was subsequently placed in a glass percolator (Kontes, Vineland, NJ, USA) for 24 h. After, maceration of thes material was performed using a 1:1 (v/v) mixture of dichloromethane (DCM; Synth, Diadema, São Paulo, Brazil) and methanol (MeOH; Synth, Diadema, São Paulo, Brazil), followed by 24 h of maceration using water as solvent (Millipore, Bedford, MA, USA), as described in Suffredini *et al*. (2007b). Solvents in the organic extracts were evaporated under vacuum (Buchii, Flawil, Switzerland), whereas aqueous extracts were lyophilized (Virtis, Stone Ridge, NY, USA). All dried extracts were kept in a freezer (Revco, Waltman, MA, USA) at -20 ºC until use. Three hundred micrograms of each extract were weighed in a 5 mL vial and diluted with 3 mL of DCM/MeOH (for organic extracts) or water (for aqueous extracts) in order to obtain a concentration of 100 µg/mL.

Standard extract preparation

The following plant drugs were used as standard in the present assay: Cortex *Stryphnodendron barbatiman*, cortex *Rhamnus purshiana*, folium *Nerium oleander* and cortex *Quina* sp. Standard drugs were submitted to the same extraction conditions as described previously.

Thin-layer chromatography

Thin-layer chromatography silica gel GF_{254} plates (Merck, Darmstadt, Germany) were used in the analysis. A 200 mm x 200 mm plate was cut into 16 miniplates (50 mm x 50 mm), and 12 different plant extract samples were applied to each miniplate. Two mobile phases were chosen for use (Wagner & Bladt, 1996) and were identified as (x) if composed of ethyl acetate:formic acid:acetic acid:water (100:11:11:26) or (y) if composed of ethyl acetate:methanol:water (100:35:10). The following reagents were used in the experiments:

(A) β-carotene - solution prepared with a mixture of a solution of 20 µL of linoleic acid, 200 µL of Tween 40, 25 µL of β-carotene, and 500 µL of chloroform. Positive reaction was observed if considered when an orange spot appeared after spraying the reagent on the plates that remained under sunlight for 2 h;

(B) 2,2-diphenyl-1-picrylhydrazyl (DPPH; Merck, Darmstadt, Germany) - 20 mg/mL in methanol, for the evaluation of antioxidant and radical scavenging properties (a positive reaction was considered when white spots appeared on the chromatography plates after spraying the reagent, indicating radical scavenging activity of the substances that were present in the plant extract). Scores were given based on the intensity of the white spots resulted from the reaction. Scores were classified as 0, +, ++, +++ or ++++ as they expressed no reaction (0), weak (+), good (++), very good (+++) and excellent (++++) responses, as shown in figure 1B.

(C) 20% H_2SO_4 (Merck, Darmstadt, Germany) diluted in water was used as reagent in the system where mobile phase (x) was used, followed by heat for the general analysis of oxidizing compounds in the extracts (different colored spots indicated the presence of oxidizing compounds);

(D) 20% H_2SO_4 diluted in water was used as reagent in the system where mobile phase (y) was used, followed by heat for the general analysis of oxidizing compounds in the extracts (different colored spots indicated the presence of oxidizing compounds);

(E) 1% diphenylboryloxyethyldiamine (NP reagent, Merck, Darmstadt, Germany) - diluted in methanol followed by 254 nm and 366 nm ultraviolet (UV) light for the analysis of phenolic compounds (purple, green, and orange spots indicated the presence of phenolic compounds);

(F) Dragendorff's reagent - a mixture of a solution of 0.85 g of $4[Bi(NO_3)(OH)_2]BiO(OH)$ or basic bismuth nitrate, diluted in 10 mL glacial acetic acid and 40 mL water, and a solution of 8 g potassium iodide in 50 mL of water in a proportion of 1:1 (orange to brown spots confirmed the presence of alkaloids after spraying the reagent);

(G) 5% KOH - diluted in ethanol (Merck, Darmstadt, Germany; yellow spots indicated the presence of anthraquinones);

(H) Kedde's reagent - 3% 3,5-dinitrobenzoic acid diluted in 2 M potassium hydroxide (Merck, Darmstadt, Germany; purple spots verified the presence of cardenolides).

Analysis of results

Pearson's χ2 test was applied to evaluate the occurrence of antioxidant activity and radical scavenging activity for both the organic and aqueous extracts, with α = 5% (Zar *et al*., 2010). For the analyses, the hypothesis (H0) was that both organic and aqueous extracts would have similar antioxidant and radical scavenging responses. The analysis of antioxidant activity (β-carotene reagent) resulted in a portion of positive (revealed by the presence of orange spots after sunlight dispose) and negative (revealed by the absence of orange spots after sunlight dispose) antioxidant organic plant extracts and a portion of positive and negative antioxidant aqueous plant extracts, generating a 2 x 2 matrix that was subjected to χ^2 analysis. The analysis of radical scavenging activity was performed to determine different grades of intensities for each organic and aqueous plant extract, with scores from 0 to 4, where 0 means the lack of radical scavenging activity observed as absence of white spots; 1=small one or two small defined spots appearing in the run; 2=three to six defined spots appearing in the run; 3=more than six clear or undefined spots appearing in the run and eventual tails; 4=undefined spots across the run (Figure 1). Score definitions were based on results obtained from standard drugs as shown in Figure 1. A 2 x 5 matrix was originated and subjected to χ2 test.

RESULTS

In the present study, 1,260 plant extracts were analyzed. Of these, 636 (50.48%) were organic extracts, and 624 (49.52%) were aqueous extracts (Figure 2). The extracts were obtained from plants that belonged to 476 species and 79 families. The presence of antioxidant activity, based on β-carotene, is depicted in Tables 1 and 2. The DPPH radical scavenging potential of each extract was scored as +, ++, +++, or ++++ according to intensity (Figures 4, 5, and 6, Tables 2 and 3). The presence of alkaloids is shown in Table 2, as it is the presence of anthraquinones and cardenolides.

According to our findings, 837 (66.43%) of the 1,260 plant extracts presented activity in the β-carotene assay, and 423 did not show a response in the assay. These plants belonged to 74 families and 390 different species. Of the 837 extracts, 432 (51.61%) were organic and 405 (48.39%) were aqueous (Figure 2). For some particular families (16 overall), we noticed that all of the extracts had antioxidant activity, including Acanthaceae, Anacardiaceae, Cecropiaceae, Combretaceae, Dennstaedtiaceae, Dilleniaceae, Dryopteridaceae, Elaeocarpaceae, Gnetaceae, Humiriaceae, Nyctaginaceae, Quiinaceae, Rhabdodendraceae, Rhizophoraceae, Verbenaceae, and Vochysiaceae. Extracts from other families did not present antioxidant activity, such as Caryocaraceae, Chenopodiaceae, Commelinaceae, Erythroxilaceae, and Loganiaceae.

The presence of antioxidant activity in organic extracts was observed for the following families: Annonaceae, Burseraceae, Caesalpinioideae, Celastraceae,

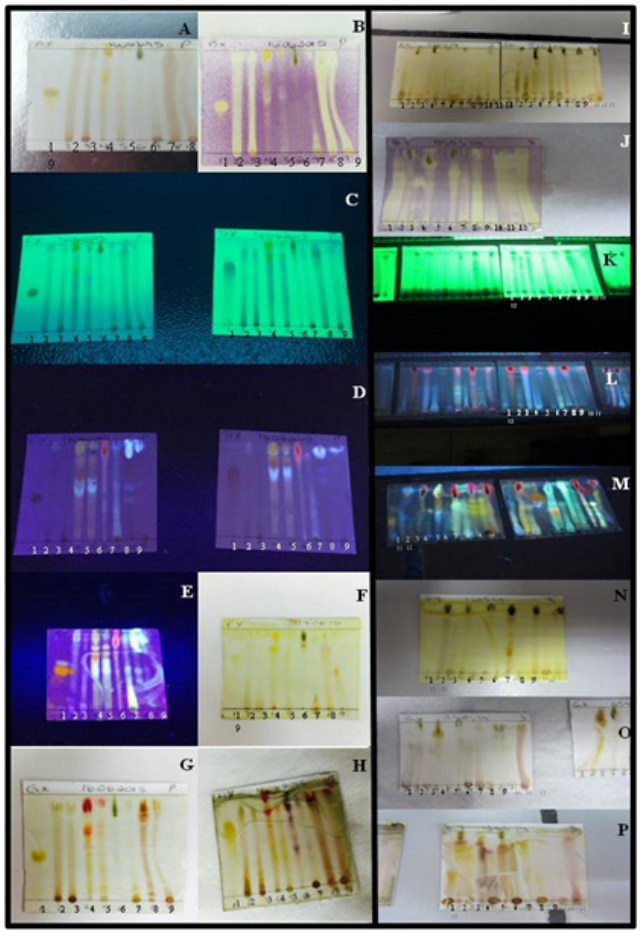

Figure 1. Tin-layer chromatograms made with standard plant drugs (A to H), described as (1) rutin, (2) organic extract (OE) from the bark of *Stryphnodendron barbatiman*, (3) aqueous extract (AE) from the bark of *Stryphnodendron barbatiman*, (4) OE from the bark of Rhamnus pursiana, (5) AE from the bark of Rhamnus pursiana, (6) OE from the leaves of *Nerium oleander*, (7) AE from the leaves of *Nerium oleander*, (8) OE from the bark of *Quina* sp. and (9) AE from the bark of *Quina* sp. I to J – Chromatograms from 12 organic and aqueous extracts from the extract library are shown on the right. Ax - chromatograms revealed with β-carotene; Bx - chromatograms revealed with DPPH; Cx - chromatograms revealed with 20% H_2SO_4; Dy - chromatograms revealed with 20% H_2SO_4; Ex - chromatograms revealed with NP reagent; Fy - chromatograms revealed with Dragendorff's reagent; Gx - chromatograms revealed with KOH; Hy, TLC plates revealed with Kedde's reagent. Bx chromatograms are interpreted according to the intensity of radical scavenging activity, reflected by the presence of white spots on the plates, where scores were assigned from 0 to 4. A score of 0 indicates that no activity was observed, such as in spot 5 from the right on the Bx plate. A score of 1 indicates light radical scavenging activity, such as in spots 4, 5, and 6 from the left. A score of 2 can be observed in spots 3 and 9 from the right. A score of 3 can be observed in spots 2, 6, 7, and 8 from the right. A score of 4 can be observed in spots 1, 11, and 12 from the right and spot 1 from the left (rutin).

Table 2. Plant extracts that were obtained from Amazon plants that were responsive to Dragendorff's reagent (Fy), KOH reagent (Gx), and Kedde's reagent (Hx) in thin-layer chromatography using a stationary phase that was composed of silica gel GF_{254} and a mobile phase that was composed of ethyl acetate:formic acid:acetic acid:water (100:11:11:26). Odd numbers are given for the organic extract obtained from a mixture of dichloromethane and methanol (1:1) and even numbers were given for the aqueous extracts.

Family	Species	Colletor#	Plant parts	Extract#	Dragendorff	KOH reag.	Kedde's reag.	β-caroten	DPPH
Anacardiaceae	Tapirira guianensis	AAO4129	AO	N1630	+	-	-	+	+++
Annonaceae	Annona hypoglauca	MPB768	ST	N1673	+	-	-	+	++++
Annonaceae	Guatteria schomburgkiana	AAO3589	ST	N1089	+	-	-	+	+++
Annonaceae	Guatteria riparia	AAO3562	LF	N1091	+	-	-	+	+++
Annonaceae	Guatteria riparia	AAO3562	ST	N1093	+	-	-	+	+++
Annonaceae	Xylopia emarginata	AAO3558	LF	N1103	+	-	-	+	+++
Annonaceae	Annona hypoglauca	AAO3577	AO	N1107	+	-	-	+	+++
Annonaceae	Annona hypoglauca	AAO3577	ST	N1109	+	-	-	+	+++
Annonaceae	Duguetia uniflora	AAO3689 II	ST	N1193	+	-	-	+	+++
Annonaceae	Unonopsis guatterioides	IBS42	LF	N1253	+	-	-	+	+++
Annonaceae	Unonopsis guatterioides	IBS42	ST	N1331	+	-	-	+	+++
Annonaceae	Guatteria spp.1	IBS82	AO	N1335	+	-	-	+	+++
Annonaceae	spp.2	IBS94	AO	N1353	+	-	-	+	+++
Annonaceae	spp.1	IBS115	ST	N1433	+	-	-	+	+++
Annonaceae	Guatteria spp.2	IBS196	AO	N1711	+	-	-	+	+++
Annonaceae	Unonopsis duckei	AAO4072	AO	N1903	+	-	-	+	+++
Annonaceae	spp.3	AAO3809	ST	N2035	+	+	-	+	+++
Annonaceae	Unonopsis duckei	AAO4072	AO	N2141	+	-	-	+	+++
Annonaceae	Guatteria schomburgkiana	AAO3589	ST	N1090	+	-	-	+	++
Annonaceae	Guatteria riparia	AAO3562	ST	N1094	+	-	-	+	++
Annonaceae	Xylopia emarginata	AAO3558	LF	N1104	+	-	-	+	++
Annonaceae	Xylopia aromatic	AAO3548	ST	N1139	+	-	-	+	++
Annonaceae	Onychopetalum	AAO4021	AO	N1427	+	-	-	+	++
Annonaceae	spp.1	IBS115	FR	N1763	+	-	-	+	+
Apocynaceae	Malouetia tamaquarina	AAO3415	LF	N1166	+	-	-	+	+++
Apocynaceae	spp.1	IBS69	ST	N1269	+	-	-	+	+++
Apocynaceae	Malouetia spp.	IBS86	AO	N1273	+	-	-	+	+++
Apocynaceae	spp.3	IBS209	AO	N1737	+	-	-	+	+++
Apocynaceae	Tabernaemontana rupicula	AAO3392	ST	N1167	+	-	-	+	++
Apocynaceae	Aspidosperma pachypterum	AAO3691	ST	N1191	+	-	-	+	++
Apocynaceae	Malouetia spp.	IBS86	ST	N1311	+	-	-	+	++
Apocynaceae	Tabernaemontana spp. angulata	IBS109	FL	N1369	+	-	-	+	++
Apocynaceae	Malouetia tamaquarina	AAO3395	ST	N1939	+	-	-	+	++
Apocynaceae	Tabernaemontana angulata	IBS69	ST	N1941	+	-	-	+	++
Apocynaceae	Rauvolfia sprucei	MPB1034	AO	N2003	+	-	-	+	++
Apocynaceae	Aspidosperma pachypterum	AAO3691	AO	N1214	+	-	-	+	+

Boraginaceae	Cordia spp. Exaltata	AAO3543	ST	N1119	+	-	-	+	++
Caesalpinioideae	Hymenaeae parvifolia	IBS143	ST	N1988	+	-	-	+	+++
Caesalpinioideae	Synometra spp.	MPB2682	AO	N2079	+	-	-	+	+++
Cecropiaceae	Pourouma ovata	AAO4130	AO	N1609	+	-	-	+	+++
Cecropiaceae	Pourouma spp.	AAO4140	AO	N1580	+	-	-	+	++
Chrysobalanaceae	Licania rodriguesii	MPB748	AO	N1798	+	-	-	+	++
Clusiaceae	Tovomita brasiliensis	AAO3532	ST	N1095	+	-	-	+	+++
Clusiaceae	Caraipa grandifolia	IBS40	ST	N1367	+	-	-	+	+++
Clusiaceae	Clusia spp.	IBS194	ST	N1727	+	-	-	+	+++
Clusiaceae	Tovomita spp.	IBS201	AO	N1735	+	-	-	+	+++
Clusiaceae	Clusia spp.	IBS194	ST	N1728	+	-	-	+	++
Clusiaceae	Clusia spp.	IBS194	LF	N1731	+	+	-	+	++
Clusiaceae	Moronobea spp.	IBS142	FL	N1765	+	-	-	+	++
Combretaceae	spp.1	IBS66	AO	N1327	+	-	-	+	+++
Combretaceae	Combretum spp.1	IBS120	ST	N2011	+	-	-	+	+++
Elaeocarpaceae	Sloanea spp.2	AAO4166	AO	N1634	+	-	-	+	++++
Euphorbiaceae	Phyllanthus spp.	AAO4011	AO	N1537	+	-	-	+	++
Fabaceae	Ormosia coarctata	AAO3704	ST	N1223	+	-	-	+	+++
Fabaceae	Ormosia coarctata	AAO3704	AO	N1225	+	-	-	+	+++
Fabaceae	Ormosia coarctata	AAO3704	AO	N1226	+	-	-	+	+++
Fabaceae	Acosmium nitens	AAO3707	AO	N1237	+	-	-	+	+++
Fabaceae	Abarema spp.1	AAO3708	ST	N1240	+	-	-	+	+++
Fabaceae	Acosmium nitens	AAO3707	AO	N1267	+	-	-	+	+++
Fabaceae	spp.2	IBS176	AO	N1451	+	-	-	+	+++
Fabaceae	Ormosia coarctata	AAO3704	ST	N1224	+	-	-	+	++
Fabaceae	Acosmium nitens	AAO3707	AO	N1238	+	-	-	+	++
Fabaceae	spp.3	IBS200	AO	N1734	+	-	-	+	++
Fabaceae	Dalbergia inundata	AAO3802	FR	N1770	+	-	-	+	++
Lauraceae	Endlicheria macrophylla	IBS54	ST	N1235	+	-	-	+	++++
Lauraceae	Licaria spp.	IBS177	ST	N1653	+	-	-	+	++++
Lauraceae	Licaria canella	AAO3525	ST	N1117	+	-	-	+	+++
Lauraceae	Ocotea amazonica	AAO3703	LF	N1243	+	-	-	+	+++
Lauraceae	Ocotea myriantha	AAO3721	LF	N1283	+	-	-	+	+++
Lauraceae	Endlicheria macrophylla	IBS54	AO	N1307	+	-	-	+	+++
Lauraceae	spp.2	SAF1	AO	N1603	+	-	-	+	+++
Lauraceae	spp.6	AAO4250	AO	N1893	+	-	-	+	+++
Lauraceae	Ocotea amazonica	AAO3703	LF	N1244	+	-	-	+	++
Lauraceae	Ocotea amazonica	AAO3703	ST	N1265	+	-	-	+	++
Lauraceae	Endlicheria spp.	IBS207	AO	N1718	+	-	-	+	++
Lauraceae	Ocotea amazonica	AAO3703	ST	N1266	+	-	-	+	+
Lecythidaceae	Eschweilera spp.3	AAO4131	AO	N1622	+	-	-	+	+++
Lecythidaceae	Eschweilera spp. atropetiolata	AAO4059	AO	N1759	+	-	-	+	+++
Lecythidaceae	Eschweilera spp.4	AAO4124	ST	N1682	+	-	-	+	++
Loranthaceae	Struthanthus spp.	AAO3705	AO	N1203	+	-	-	+	+++
Loranthaceae	spp.1	AAO4002	AO	N1995	+	-	-	+	+++
Malpighiaceae	Byrsonima incarnata	IBS59	AO	N1250	+	-	-	+	++

Meliaceae	Trichilia spp.2	AAO4012	AO	N1754	+	-	-	+	+++
Meliaceae	Guarea humaitensis	MPB725	AO	N1808	+	-	-	+	+++
Meliaceae	Guarea spp.1	AAO4169	LF	N1620	+	-	-	+	++
Menispermaceae	Abuta spp.	MPB804	AO	N1707	+	-	-	+	+++
Menispermaceae	Curarea spp.2	AAO4023	LF/ST	N1541	+	-	-	+	++
Menispermaceae	Abuta spp.	MPB804	AO	N1708	+	-	-	+	++
Menispermaceae	Abuta imene	MPB2624	AO	N2155	+	-	-	+	++
Menispermaceae	Abuta imene	MPB2624	AO	N2156	+	-	-	+	++
Menispermaceae	Curarea spp.1	AAO4001	AO	N1465	+	-	-	+	+
Mimosoideae	Inga spp.3	MPB765	AO	N2063	+	-	-	+	+++
Mimosoideae	spp.1	IBS136	ST	N1435	+	-	-	+	++
Moraceae	Pseudolmedia laevigata	AAO4103	AO	N1632	+	-	-	+	++
Moraceae	Helianthostylis sprucei	MPB2625	AO	N2132	+	-	-	+	++
Olacaceae	Dullacia spp.	AAO4127	AO	N1577	+	-	-	+	+++
Olacaceae	Minquartia guianensis	AAO4201	AO	N1994	+	-	-	+	+++
Piperaceae	Piper arboreum	IBS56	ST	N1305	+	-	-	+	++
Rubiaceae	spp.7	AAO3578	ST	N1053	+	-	-	+	+++
Rubiaceae	Pagamea spp. puberula	AAO3719	AO	N1287	+	-	-	+	+++
Rubiaceae	Calycophyllum spp.	AAO3800	AO	N1755	+	-	-	+	+++
Rubiaceae	Duroia spp.1 gransabanensis	MPB777	AO	N1859	+	-	-	+	+++
Rubiaceae	Palicourea corymbifera	AAO3584	LF	N1045	+	-	-	+	++
Rubiaceae	Palicourea corymbifera	AAO3584	LF	N1046	+	-	-	+	++
Rubiaceae	Palicourea corymbifera	PSC298	LF	N1159	+	-	-	+	++
Rubiaceae	Palicourea corymbifera	PSC298	LF	N1160	+	-	-	+	++
Rubiaceae	Palicourea guianensis	AAO3717	ST	N1303	+	-	-	+	++
Rubiaceae	Palicourea spp.2	IBS72	FL	N1319	+	-	-	+	++
Rubiaceae	Palicourea corymbifera	IBS96	LF/ST	N1341	+	-	-	+	++
Rubiaceae	Palicourea corymbifera	IBS96	LF/ST	N1342	+	-	-	+	++
Rubiaceae	Remijia spp.	IBS93	FL	N1363	+	-	-	+	++
Rubiaceae	Remijia spp.	IBS93	ST	N1419	+	-	-	+	++
Rubiaceae	Palicourea corymbifera	AAO3584	ST	N1421	+	-	-	+	++
Rubiaceae	Palicourea spp.4	AAO4115	LF	N1590	+	-	-	+	++
Rubiaceae	Psychotria prancei	MPB794	AO	N1747	+	-	-	+	++
Rubiaceae	Psychotria prancei	MPB794	AO	N1748	+	-	-	+	++
Rubiaceae	Bothriospora spp.	AAO3712	FR	N1217	+	-	-	+	+
Rubiaceae	Psychotria spp.2	IBS111	PL	N1347	+	-	-	+	+
Rubiaceae	Palicourea grandifolia	IBS95	LF/ST	N1355	+	-	-	+	+
Rubiaceae	Palicourea spp.3	IBS100	AO	N1401	+	-	-	+	+
Rubiaceae	Psychotria spp.3	IBS132	PL	N1405	+	-	-	+	+
Rubiaceae	Palicourea corymbifera	AAO3584	ST	N1422	+	-	-	+	+

Rubiaceae	Palicourea spp. marcgravii	AAO3507	ST	N955	+	-	-	+	+
Rutaceae	Adiscanthus fusciflorus	AAO3591	ST	N1105	+	-	-	+	++
Salicaceae	Ryania speciosa	AAO3698	LF	N1177	+	-	-	+	+++
Salicaceae	Homalium spp.	AAO4019	LF	N1484	+	-	-	+	+++
Salicaceae	Laetia spp.	IBS169	AO	N1528	+	-	-	+	++
Salicaceae	Casearia spp.	AAO4163	AO	N1606	+	-	-	+	+
Sapotaceae	spp.1	AAO4137	AO	N1639	+	-	-	+	+++
Sapotaceae	Micropholis spp.1	AAO4266	AO	N1677	+	-	-	+	+++
Sapotaceae	Pouteria spp.3	AAO4043	AO	N1725	+	-	-	+	+++
Sapotaceae	Chromolucuma rubiflora	AAO4219	AO	N1843	+	-	-	+	+++
Sapotaceae	Pouteria spp.1	AAO4044	AO	N1535	+	-	-	+	++
Smilacaceae	Smilax rufescens	AAO3811	AO	N1776	+	-	-	+	+++
Smilacaceae	Smilax spp.2	AAO3812	AO	N1780	+	-	-	+	+
Solanaceae	Solanum spp.1	AAO3544	AO	N1147	+	-	-	+	++
Solanaceae	Solanum spp.2	IBS39	AO	N1241	+	-	-	+	++
Solanaceae	Solanum spp.5	AAO4038	AO	N1565	+	-	-	+	++
Solanaceae	Solanum spp.5	AAO4038	ST	N1664	+	-	-	+	+
Violaceae	Rinorea spp.	AAO3585	ST	N1133	+	+	-	+	+++
Annonaceae	Guatteria riparia	AAO3520	ST	N1041	+	-	-	-	+++
Annonaceae	Guatteria spp.1	IBS82	ST	N1375	+	-	-	-	+++
Annonaceae	Duguetia uniflora	MPB769	ST	N1687	+	+	-	-	+++
Annonaceae	spp.1	IBS115	LF	N1339	+	-	-	-	++
Annonaceae	spp.3	AAO3809	LF	N1665	+	-	-	-	++
Annonaceae	Guatteria spp.1	IBS82	ST	N1376	+	-	-	-	+
Apocynaceae	Tabernaemontana angulata	AAO3700	ST	N1199	+	-	-	-	++
Apocynaceae	Ambelania acida	AAO3684 II	ST	N1189	+	-	-	-	+
Apocynaceae	Prestonia spp. megagross	AAO4114	AO	N1587	+	-	-	-	-
Apocynaceae	Prestonia spp. megagross	AAO4114	AO	N1588	+	-	-	-	-
Apocynaceae	Ambelania acida	AAO3510	ST	N959	+	-	-	-	-
Bignoniaceae	Anemopaegma chrysoleucum	AAO3492	AO	N961	+	-	-	-	+++
Bignoniaceae	Arrabidaea chibata	AAO4041	AO	N1479	+	-	-	-	++
Boraginaceae	Cordia spp.	AAO4005	AO	N1778	+	-	-	-	++
Boraginaceae	Cordia nodosa	IBS61	AO	N1383	+	-	-	-	+
Caesalpinioideae	Dicorynia paraensis	AAO3546	AO	N1024	+	-	-	-	++++
Caesalpinioideae	Hymenaeae parvifolia	IBS143	LF	N1395	+	-	-	-	+++
Caesalpinioideae	Macrolobium acaciifolium	MPB2792	AO	N2085	+	-	-	-	+++
Chrysobalanaceae	Licania lata	AAO3498	ST	N964	+	-	-	-	++
Clusiaceae	Clusia spathulaefolia	AAO3551	AO	N1082	+	-	-	-	++
Convolvulaceae	Ipomoea spp.1	AAO4035	AO	N1490	+	-	-	+	-
Fabaceae	Machaerium ferrugineum	AAO3550	AO	N1088	+	-	-	-	+
Gentianaceae	Tachia grandiflora	IBS99	LF/ST	N1351	+	-	-	-	++
Gentianaceae	Tachia grandiflora	IBS99	LF/ST	N1352	+	-	-	-	+
Icacinaceae	Poraqueiba sericea	IBS108	LF	N1381	+	-	-	-	+++
Icacinaceae	Poraqueiba sericea	IBS108	LF	N1382	+	-	-	-	++

Lauraceae	spp.7	MPB1030	AO	N2136	+	-	-	-	++++
Lauraceae	Ocotea spp.2	MPB2791	AO	N2087	+	-	-	-	+++
Lauraceae	Ocotea spp.1	MPB734	AO	N1792	+	-	-	-	+
Lauraceae	spp.8	AAO4211	AO	N1969	+	-	-	+	-
Loganiaceae	Strichnos spp.2	MPB2800	AO	N2089	+	-	-	-	+++
Loranthaceae	Struthanthus spp.	AAO3705	AO	N1204	+	-	-	-	++
Malpighiaceae	Byrsonima spp.	AAO4020	ST	N1949	+	-	-	+	-
Menispermaceae	Curarea spp.2	AAO4023	LF/ST	N1542	+	-	-	-	+
Mimosoideae	spp.1	IBS136	FL	N1377	+	-	-	-	++++
Mimosoideae	spp.1	IBS136	FL	N1378	+	-	-	-	+++
Piperaceae	spp.1	IBS165	AO	N1505	+	-	-	-	+++
Piperaceae	Piper spp.2	MPB2257	AO	N1989	+	+	-	-	+++
Piperaceae	Piper spp.1	IBS38	AO	N1245	+	-	-	-	++
Polygalaceae	Moutabea guianensis	MPB1026	ST	N2187	+	-	-	-	+
Polygalaceae	Polygala spectabilis	AAO3519	PL	N994	+	-	-	-	-
Rubiaceae	spp.7	AAO3578	FR	N1057	+	-	-	-	+++
Rubiaceae	Duroia spp.1	AAO3583	ST	N1061	+	-	-	-	+++
Rubiaceae	Palicourea guianensis	AAO3717	LF	N1201	+	-	-	-	++
Rubiaceae	spp.4	IBS141	PL	N1392	+	-	-	-	++
Rubiaceae	Psychotria spp.3	IBS132	PL	N1406	+	-	-	-	++
Rubiaceae	Palicourea spp.5	MPB2774	AO	N2133	+	-	-	-	++
Rubiaceae	Palicourea longiflora	AAO3495	AO	N966	+	-	-	-	++
Rubiaceae	spp.3	IBS112	AO	N1379	+	-	-	-	+
Rubiaceae	Rudgea graciliflora	AAO4057	AO	N1569	+	-	-	-	-
Rubiaceae	Rudgea graciliflora	AAO4057	AO	N1570	+	-	-	+	-
Rubiaceae	spp.8	AAO4145	AO	N1573	+	-	-	-	-
Rutaceae	Adiscanthus fusciflorus	AAO3591	LF	N1077	+	-	-	-	++
Salicaceae	Casearia aculeata	AAO4022	AO	N1850	+	-	-	-	+
Sapotaceae	Micropholis spp.3	AAO4189	AO	N2031	+	-	-	-	+++
Sapotaceae	Micropholis spp.3	AAO4189	AO	N2032	+	-	-	-	+
Sapotaceae	Micropholis spp.2 guyanensis	AAO4261	AO	N1973	+	-	-	+	-
Solanaceae	Solanum spp.1	AAO3544	AO	N1148	+	-	-	-	++
Solanaceae	Solanum spp.3	IBS78	PL	N1291	+	-	-	-	++
Solanaceae	Solanum spp.5	AAO4038	ST	N1663	+	-	-	-	++
Solanaceae	Solanum spp.4	IBS121	AO	N1407	+	-	-	-	+
Solanaceae	Solanum spp.4	IBS121	AO	N1408	+	-	-	-	+
Violaceae	Leonia cymosa	AAO4073	LF/ST	N1504	+	-	-	-	+

Legend: ST=stem; LF=leaves; AO=aerial organs; FR=fruits; PL=entire plant; BK=bark.

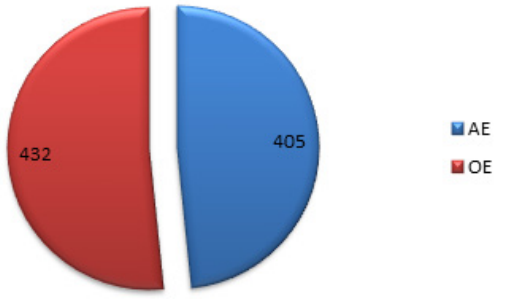

Figure 2. Percentage of organic and aqueous extracts that presented antioxidant activity in the β-carotene assay (ntotal = 837). The results were obtained from positive and negative values in the β-carotene assay, indicated by orange spots on the TLC chromatogram, developed with mobile phase (x) and revealed with β-carotene. AO, aqueous extract; OE, organic extract.

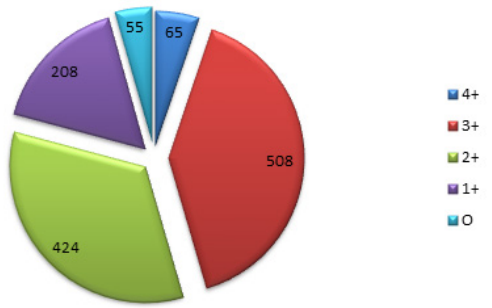

Figure 3. Representation of the intensity of radical scavenging activity in 1,205 (95.63%) of 1,260 plant extracts that were tested in the DPPH/TLC assay, in which white spots revealed the presence of active compounds in the extracts. The radical scavenging activity of the plant extracts was scored from 0 (no activity) to 4+ (more activity) according to the intensity of white spots.

Clusiaceae, Combretaceae, Connaraceae, Fabaceae, Heliconiaceae, Lauraceae, Loranthaceae, Malpighiaceae, Malvaceae, Menispermaceae, Mimosoideae, Myristicaceae, Myrtaceae, Polygonaceae, Polypodiaceae, Rutaceae, Sapotaceae and Simaroubaceae.

The presence of antioxidant activity in aqueous extracts was observed for the following families: Apocynaceae, Asteraceae, Bignoniaceae, Boraginaceae, Chloranthaceae, Chrysobalanaceae, Convolvulaceae, Cucurbitaceae, Cyatheaceae, Dioscoreaceae, Ebenaceae, Icacinaceae, Lecythidaceae, Lythraceae, Moraceae, Orchidaceae, Passifloraceae, Piperaceae, Polygalaceae, Salicaceae, Smilacaceae, Turneraceae, and Violaceae.

For some families, equal amounts of organic and aqueous extracts among those that presented antioxidant activity were observed for the following families: Acanthaceae, Anacardiaceae, Araceae, Bombacaceae, Cecropiaceae, Dennstaedtiaceae, Dilleniaceae, Dryopteridaceae, Elaeocarpaceae, Euphorbiaceae, Gentianaceae, Gnetaceae, Humiriaceae, Lacistemataceae, Melastomataceae, Meliaceae, Memecylaceae, Myrsinaceae, Nyctaginaceae, Olacaceae, Quiinaceae, Rubiaceae, Rhabdodendraceae, Rhizophoraceae, Sapindaceae, Solanaceae, Theaceae, Verbenaceae, and Vochysiaceae.

Pearson's $\chi 2$ test was performed for both organic and aqueous extracts (Table 1), supporting hypothesis H_0 that both extracts would have an equal distribution of (1) activity and (2) no activity in the β-carotene/bleaching assay ($\chi 2(1) = 1.289$, $p = 0.256$; quantiles of the $\chi 2$ distribution with degrees of freedom [df] =1 and $\alpha = 5\%$ were 3.84), indicating that hypothesis H_0 could not be rejected, meaning that both extracts behaved similarly.

Table 1. Contingency table related to evaluation of the occurrence of antioxidant activity related to both organic and aqueous extracts. Pearson's $\chi 2$ analyses were performed by considering df = 1, $\alpha = 0.5\%$, and n = 1,260 (quantiles of the $\chi 2$ distribution with df = 1 and $\alpha = 5\%$ were 3.84).

Type of extract	Lack of activity	Activity	Total
Organic	204	432	636
Aqueous	219	405	624
Total (groups)	423	837	1260

Radical scavenging potential was also analyzed. Over the 1,260 plant extracts, 1,205 have shown radical scavenging activity in the DPPH assay. Remarkably, this represented 95.63% of the tested extracts (Figure 3). Although it was not possible to quantify antioxidant activity using the present TLC method, we scored the antioxidant activity of the extracts. Figure 3 shows that 65 (5.39%) of the 1,205 plant extracts had excellent (++++) radical scavenging activity, 508 (42.16%) had very good (+++) radical scavenging activity, 424 (35.19%) had good (++) radical scavenging activity, and 208 (17.26%) had weak (+) radical scavenging activity. As shown in Figure 4, the 1,205 plant extracts with radical scavenging activity were split into two groups. The organic extract group consisted of 608 (50.46%) extracts, and the aqueous extract group consisted of 597 (49.54%) extracts. We found that most of the extracts in the group that tested positive for radical scavenging activity had scores of +++ or ++ (Figure 5). Among the extracts with scores of ++++, 56.92% were organic extracts, and 43.08% were aqueous extracts. Among the extracts with very good radical scavenging activity (+++), 70.87% were organic extracts, and 29.13% were aqueous extracts. Among the extracts with good radical scavenging activity (++), 37.5% were organic extracts, and 62.5% were aqueous extracts. Among the extracts with weak radical scavenging activity (+), 25.00% were organic extracts, and 75.00% were aqueous extracts.

Pearson's $\chi 2$ analysis was performed for both

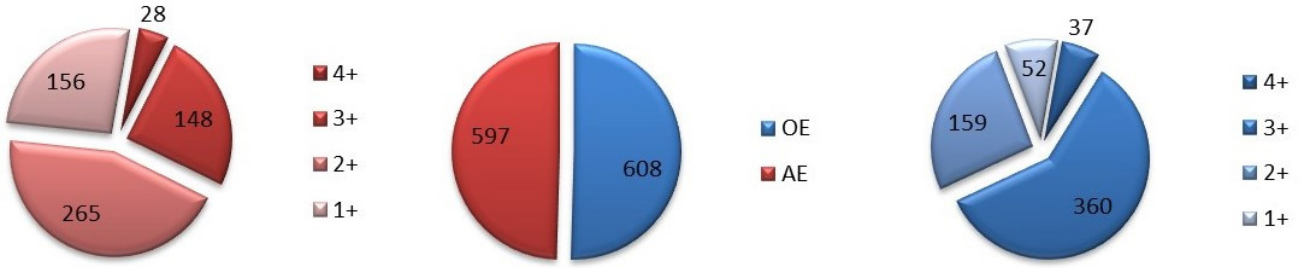

Figure 4. Representation of the intensity of radical scavenging activity of organic (OE) and aqueous (AE) plant extracts that presented antioxidant activity in the DPPH/TLC assay. OE, organic extract; AE, aqueous extract; 4+, 3+, 2+ and 1+, scores related to the intensity of radical scavenging activity when compared to control plant extracts.

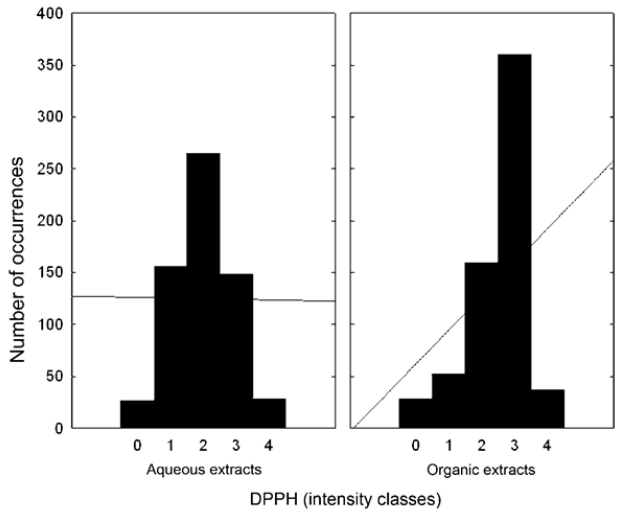

Figure 5. Graphic representation of Pearson's □2 analysis that was performed with both organic and aqueous extracts in the evaluation of radical scavenging activity, expressed as intensity classes of 0, 1+, 2+, 3+, and 4+ related to the intensity of activity (quantiles of the $\chi 2$ distribution with df = 4 and α = 5% were 9.49).

organic and aqueous extracts (Table 3), showing that although the hypothesis was that both extracts would show an equal distribution of activity and no activity in the DPPH assay, a significant difference was found between these types of extracts ($\chi 2_{(4)}$ = 168,138, $p < 0.001$; quantiles of the $\chi 2$ distribution with df = 1 and α = 5% were 9.49), indicating that hypothesis H_0 could be rejected, meaning that the organic and aqueous extracts behaved differently, and the organic extracts were more likely to present radical scavenging activity than the aqueous extracts (Figure 5).

Seventy-eight plant families and 458 species of plants presented radical scavenging activity in the DPPH/TLC assay.

Considering the results of both antioxidant and radical scavenging activity, 797 (63.25%) of the extracts presented activity in both tests, and these extracts were obtained from plants that belongs to Rubiaceae (65 extracts), Fabaceae (51 extracts), Annonaceae (45 extracts), Burseraceae (41 extracts), Apocynaceae (39 extracts), Lauraceae (34 extracts), Moraceae (33 extracts), Chrysobalanaceae (31 extracts), and Sapotaceae (30 extracts). Additionally, 408 extracts (32.38%) presented radical scavenging activity in the DPPH assay and no antioxidant activity in the β-carotene assay. Notwithstanding, 16 extracts did not show antioxidant or radical scavenging activity; these belonged to Apocynaceae, Polygalaceae, Moraceae, and Lecythidaceae.

The presence of alkaloids was evaluated, and 203 (16.11%) were positive to Dragendorff's reagent. Table 2 lists the botanical and collection information related to the alkaloidal extracts. In the present study, alkaloids were found in the following families: Anacardiaceae (Li *et al*., 2014), Annonaceae (Sesang *et al*., 2014), Apocynaceae (Zhang *et al*., 2015; Henrique *et al*., 2014), Bignoniaceae (Roy *et al*., 2011), Boraginaceae (Mandic *et al*., 2013), Caesalpiniaceae (Gupta *et al*., 2009), Cecropiaceae (Adeneye, 2006), Chrysobalanaceae (Barbosa *et al*., 2013), Clusiaceae (Moulari *et al*., 2007), Combretaceae (Manosroi *et al*., 2011), Convolvulaceae (Chen *et al*., 2014), Elaeocarpaceae (Muñoz *et al*., 2011), Euphorbiaceae (Johnson-Ajinwo *et al*., 2015), Fabaceae (Rastogi *et al*., 2011), Gentianaceae (Mahmood *et al*., 2014), Icacinaceae (Shweta *et al*., 2013), Lauraceae (Zhang *et al*., 2014), Lecythidaceae (Adiele *et al*., 2014), Loganiaceae (Tchinda *et al*., 2014), Loranthaceae (Omeje *et al*., 2011), Malpighiaceae (Zhao *et al*., 2012), Meliaceae (Jain *et al*., 2014), Menispermaceae (Thavamani *et al*., 2013), Mimosaceae (Ayanwui *et al*., 2010), Moraceae (Ishola *et al*., 2013), Olacaceae (Lorence *et al*., 2004), Piperaceae (Han *et al*., 2014), Polygalaceae (Egashira *et al*., 2006), Rubiaceae (Ashihara & Watanabe, 2014), Rutaceae (Zhao *et al*., 2015), Salicaceae (Rasmussen *et al*., 2006), Sapotaceae (Kuete *et al*., 2006), Smilacaceae (Xu *et al*., 2013), Solanaceae (Gutiérrez *et al*., 2014), and Violaceae (Patel *et al*., 2013), families in which alkaloids are generally expected to occur (Evans, 2009).

Only three plant extracts (0.24%) that were obtained from plants of the Annonaceae and Cucurbitaceae families showed the presence of anthraquinones (Table 3).

Table 3. Contingency table related to evaluation of the occurrence of radical scavenging activity related to both organic and aqueous extracts, expressed as intensity classes of 0, 1+, 2+, 3+, and 4+ related to the intensity of activity. Pearson's $\chi 2$ analyses were performed by considering df = 4, $\alpha = 0.5\%$, and n = 1,260 (quantiles of the $\chi 2$ distribution with df = 4 and $\alpha = 5\%$ were 9.49).

Type of extract	0	1+	2+	3+	4+	Total
Aqueous	27	156	265	148	28	624
Organic	28	52	159	360	37	636
All Groups	55	208	424	508	65	1260

Lastly, eight plant extracts (0.63%) that were obtained from Clusiaceae (Policegoudra *et al*., 2012), Caesalpiniaceae (Mbagwu & Adeyemi, 2008), Apocynaceae (Dai *et al*., 2014), Nyctaginaceae (Abbasi *et al*., 2012), Mimosaceae (Ayanwuyi *et al*., 2010), and Myrtaceae (Vaghasiya *et al*., 2008) showed a positive reaction to Kedde's reagent, indicating the possible presence of cardenolides (Table 2).

DISCUSSION

The search for new medicines from natural sources has developed rapidly in recent decades through the introduction of high-throughput biological and chemical screening assays that enable analyses of large amounts of samples that bypass traditional time-consuming techniques (Younes *et al*., 2007) that require large amounts of plant material, solvents, reagents, and personnel. As studies of the chemistry and biology of plants spread to tropical countries and as tropical forests became endangered in the last 50 years, an urgent need exists to improve the chemical, biological, and pharmacological knowledge of tropical plants, particularly those in the Brazilian Amazon rain forest and southern Brazilian Atlantic rain forest.

The structure of biological and chemical high-throughput screening (HTS) techniques is being widely adopted in the search for new lead compounds, particularly those that originate from nature (Klausmeyer *et al*., 2012). High-throughput screening is usually based on the use of high-tech equipment (Eldridge *et al*., 2002; Tian *et al*., 2007), with significant advances in analytical techniques for the achievement of natural product chemistry information, such as high performance thin layer chromatography, gas chromatography, high-performance liquid chromatography, nuclear magnetic resonance, and mass spectrometry (Phillipson, 2007). Unfortunately, such advanced techniques are not available to all research facilities. For this reason, simple techniques that are more readily accessible, such as TLC, can prove to be useful for the analysis of a wide range of phytochemical information that is generated from extract libraries. The limitations of using TLC as a chemical screening technique include the risk of obtaining false negative and/or false positive results, the adaptation of specific reactions of medicinal plants to more generalized analyses of wild plants, the sensitivity of the method, and environmental conditions that are required to develop TLC analysis. Once the results are supported by chemosystematics, they can be considered more reliable and likely to support biological assays. Besides, World Health Organization recommends TLC technique for the quality control of plant-derived drugs.

The TLC methodology that was used in the present assay demonstrated limited number of mobile phase (two, named x and y) due to the large amount of extracts to be submitted to the analysis. Is is possible to observe that TLC methods to evaluate constituents are commonly used, as it was made to the evaluation of Camelia sp. profiles (Bashir *et al*., 2014), and can be used as a preliminary evaluation of the presence of antioxidant potential with the use of DPPH, as seen in the evaluation of 15 bamboo species (Wang *et al*., 2012) or in the introduction of the quality control technique described for selected pharmaceutical preparations containing Salvia officinalis (Cieśla & Waksmundska-Hajnos, 2010). The elevated number of TLC assays that were developed to assess the antioxidant potential of plant extracts (Jasprica *et al*., 2007) and can be considered a method of choice due to its flexibility, simplicity (Cimpoiu, 2006), low-cost and the a reliable throughput methodology that can also be associated to other techniques as mass spectroscopy and nuclear magnetic resonance (Grzelak, Hwang and Jaki, 2016) and bioauthography assays (Bashir *et al*., 2014).

TLC analyses were used for the assessment of antioxidant and radical scavenging activity of 1,260 plant extracts. The β-carotene/bleaching assay tends to identify compounds that can chain-break free radical reactions, particularly those that are initiated by light exposure and consequently protect β-carotene from suffering radical reactions, such as compounds that have phenolic rings and hydroxyl groups. In the present work, it was observed that of the 1,260 extracts, 837 (66.43%) presented a β-carotene/bleaching response, while 1,205 (95.63%) presented radical scavenging activity.

Present findings related to antioxidant activity of the 1,205 plant extracts made with plants belonging to different families are preliminary, and will support further assays on the evaluation of phenolic content, terpenes and cardenolides, as our present temptatives were not conclusive, and for that reason, not discussed in the present work.

The actual number of known alkaloids is controversial, but authors agree that there are between 10,000 and 16,000 known alkaloids (Croteau *et al*., 2000) that have been isolated from plants and other living organisms, such as fungi (Miedaner & Geiger, 2015), the skin of toads (Clarke, 1997), and some marine organisms (Huang *et al*., 2014). Alkoaloids were found in 16.11% of our extracts and the present findings are consistent with the overall number of alkaloids that are expected to be found in the plant kingdom, which is reported to occur in 10-20% of plants, according to some authors (Croteau *et al*., 2000; Wink *et al*., 2010).

According to the literature, anthraquinones are common secondary metabolites that are found in fungi, liquens, and Angiosperms (both in Monocotyledons, such as Liliaceae, and Dicotyledons, such as Polygonaceae, Rhamnaceae, Leguminosae, and Rubiaceae). They are not found in Bryophytes, Pteridophytes, or Gnetaceaes (Swain, 1965). Finally, cardenolides were also found to be present in some of the extracts, but less dispersed, if compared to results obtained for alkaloids.

CONCLUSIONS

The present findings report achievements on the potential antioxidant and radical scavenge of Brazilian plants, as reports the presence of alkaloids and anthraquinones, relfecting the importance of the Brazilian tropical forests in the search for new active molecules.

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Conflict of interest: All authors have none to declare.

RESUMO

Análise preliminar de extratos vegetais de plantas da Amazônia brasileira usando ensaios de bancada em larga escala direcionada à identificação de produtos naturais antioxidantes.

Plantas são fontes de compostos usados para o tratamento de doenças humanas e veterinárias. O Brasil é um dos países mais ricos, em termos de biodiversidade. O presente estudo avaliou extratos vegetais usando cromatografia em camada delgada para acessar atividade antioxidante e para determinar a presença de determinadas classes químicas de compostos secundários. Um total de 1.260 extratos foram obtidos de plantas coletadas na floresta Amazônica e na Mata Atlântica. Cromatografia em camada delgada foi realizada para avaliar a presença de alcaloides, antraquinonas, glicosídeos cardioativos, terpenos e compostos fenólicos, e para determinar a atividade antioxidante e sequestradora de radicais livres usando os seguintes reagentes: reagente de Dragendorff, KOH, reagente de Kedde, ácido sulfúrico, reagente NP, β-caroteno e 2,2-difenila-1-picrilhidrazila (DPPH). Alcaloides foram encontrados em 203 extratos (16,11%). Antraquinonas foram encontradas em 14 extratos (1,11%). Cardenolídeos foram encontrados em apenas oito extratos (0,63%). De 1.260 extratos, 837 (66,43%) apresentaram resposta ao β-caroteno, e 1.205 (95,63%) apresentaram capacidade sequestradora de radical livre. Os resultados encontrados demonstraram que a família das Annonaceae pode ser considerada uma importante fonte potencial de compostos antioxidantes.
Palavras-chave: Extratos Vegetais. Biodiversidade. Annonaceae. Alcaloides. Antioxidantes.

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