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Original Article

Analysis of Brazilian Plant Extracts as Potential Source of Antioxidant Natural Products Using Bench-Top Assays

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ABSTRACT

Introduction: The present work reported the antioxidant and chemical screening of Brazilian plant aqueous and organic extracts. **Methods:** An amount of 895 Brazilian Amazon aqueous and organic plant extracts were tested in thin layer chromatography plates (TLC) using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene, Dragendorff's reagent, Kedde's reagent, and KOH reagent so as to evaluate antioxidant activity and chemical profiles. Antioxidant and DPPH free radical scavenging activities results were submitted to chi-square analyses. **Results:** Only 8.60% of the extracts showed β-carotene/bleaching response, while 96.09% of the extracts responded as a radical scavenger, alkaloids occurred in 8.0% of the extracts whereas anthraquinones occurred in 0.89% of the extracts and cardenolides in 3.89% of the extracts. **Conclusion:** Present findings described that Amazon plant extracts have a huge potential to be a source of antioxidant compounds to be used in preventive medicine, as well as the chemical screening revealed that their plants can be strategically assessed as a source of alkaloids to be tested in further biological assays.

Key words: Amazon Rain Forest, Biodiversity, Plant extracts, Thin layer chromatography, Phytochemistry, Radical scavenge.

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INTRODUCTION

Naturally occurring antioxidants play an important hole in therapeutics. Curcumin, a diarylheptanoid compound, has shown to be effective against some types of cancer due to its antiproliferative effect caused by inhibiting angiogenesis and by inducing apoptosis, *in vitro*.[1] Also, curcumin was shown to have neuroprotective properties that may postpone or even prevent diseases as Alzheimer's, due ti its anti-inflammatory and antioxidant properties.[2] Recent studies described the screening of Brazilian plant extracts for the antioxidant activity, as was made with six plant extracts aiming their antioxidant and photoprotective activity, which showed that the extract from *Dalbergia monetaria* is a potentially source of new antioxidants to be used in *photoprotective* formulations.[3] The alcoholic extract from the rhizomes of *Aristolochia cymbifera* and the aqueous extracts of the leaves of *Caesalpinia pyramidalis* and *Cocos nucifera* were evaluated against micro-organisms related to oral diseases, as well as their antioxidant activity was assessed and showed to be more effective than those observed to *Ziziphus joazeiro*, a well known antibacterial traditional plant.[4]

Amazon rain forest is the biggest in the world, as it is the richest in terms of biodiversity, both for plants and animals,[5] although much of it remains unknown from the pharmacological point-of-view. Such biological richness corresponds to a chemical diversity that is of interest to the prospection of new drugs to be introduced to therapeutics,[6] particularly those aiming the antioxidant preventive and therapeutic potentiality. Still, high-throughput assays are the fastest, inexpensive and easily expanding way of analyzing the vast Amazon rain forest diversity that remains unexploited to today.[7]

Our group aimed at the chemical screening of 895 organic and aqueous extracts obtained from plants found in the Amazon rain forest that have been preciously screened for their biological,[8-10] pharmacological and toxicological activities.[11-16] The extracts were tested for their free radical scavenging and antioxidant activities, as well as screened for the presence of alkaloids, anthraquinones and cardenolides, some of the most active classes of phytochemicals.[12]

MATERIALS AND METHODS

Plant collection

Plant material was collected under Brazilian Government law, according to official documents #14895 [MMA/ICMBio/SISBIO] and #012A-2008 [IBAMA/MMA/CGen]. Plants were collected in the Amazon rain forest, (02° 23' 41" S 60° 55' 14" O); Manaus and Novo Airão, Amazonas, Brazil, and in the Atlantic

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Rain Forest, (24° 35' 30" S 47° 27' 7" O), Southern coast of the State of São Paulo, Brazil. Different parts of the plants were obtained (stem, leaf, flowers, fruits or roots), according to their biomass availability. Botanical material (i.e., *vouchers*) was collected and stored following the standard recommendations for botanical collections. Vouchers numbers are given in Table 1; all vouchers are deposited at the Herbarium UNIP. The botanical determinations were done in laboratory, with aid of taxonomic keys and specialist expertise.

Extract preparation

Each plant part was separatedly dried in an air-circulating stove (Fanem, Diadema, São Paulo, Brazil) at 40°C (i.e., a temperature that is usually used to dry crude plant material). Each plant part has provided an organic and an aqueous extract, made by a 24 h maceration with a 1:1 (v/v) mixture of dichloromethane (DCM) and methanol (MeOH), followed by 24 h maceration with distilled water.[13] Solvents were removed under vacuum or lyophilized. Three hundred milligrams of each extract were weighed in a 5 mL vial and diluted with 3.0 mL of DCM/MeOH or water to obtain a concentration of 100 mg/mL.[14]

Thin layer chromatography analysis

Thin layer chromatography silica gel GF_{254} plates (Merck®) were used in the analysis. Two mobile phases were chosen to be used[12] ethyl acetate: formic acid: acetic acid: water (100:11:11:26) and ethyl acetate: methanol: water (100:35:10). β-carotene,[15] (B) 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used as reagents. The evaluation of the free radical scavenging analyses were made by the establishment of scores as strong (++++), very good (+++), good (++), weak (+) and absence of free radical scavenge activity (0).[16] The following reagents were used in the chemical analysis:[11] (C) Dragendorff's reagent, (D) 5% KOH diluted in ethanol for checking the presence of anthraquinones; (E) Kedde's reagent.

Statistical analyses of antioxidant and free radical scavenging potential activity of extracts

Pearson's $\chi 2$ test was applied to evaluate the occurrence of antioxidant activity and free radical scavenging activity for both the organic and aqueous extracts, with α = 5%.[17] The analysis of free radical scavenging activity was performed to determine different grades of intensities with scores from 0 (absence of free radical scavenging activity) to 4 (strong free radical scavenging activity; Figure 1). The chemical results are expressed as percentages.

RESULTS

In the present work, 895 plant extracts divided in to 450 organic extracts (50.28%) and 445 aqueous extracts (49.72%) were analyzed. It was observed that 77 (8.60%) of 895 plant extracts showed antioxidant activity in the β-carotene/bleaching assay (Table 1). Beta-carotene/bleaching assay tends to identify compounds that can chain-break free radical reactions, particularly initiated by light exposition and that consequently protect β-carotene from suffer radical reaction, such as compounds having phenolic rings and hydroxyl groups.

Moreover, 860 of 895 plant extracts showed radical scavenge (RS) activity in DPPH assay (Table 1), and astonishingly, it represents 96.09% of the tested extracts. Only 35 extracts showed absence of RS activity in the present assay, from those 25 were organic (71.42%) and ten were aqueous (28.57%). Although not possible to be quantified by the adopted TLC method, the level of RS activity of the extracts received scored so as to conduct chi-square analyses. So, 169 out of 860 (19.65%) plant extracts showed strong (++++) RS activity, while 325 (37.79%) showed a very good (+++) RS activity, 229 (26.62%) showed a good (++) RS activity and 137 (15.93%) showed weak (+) RS activity (Figure 1). Among the strong RS extracts scored with (++++), 78 (46.15%) of them are organic extracts and 91 (53.85%) are aqueous extracts. Among the extracts scored with (+++), 206 (63.38%) are organic extracts and 119 (36.62%) are aqueous extracts. Among the extracts scored with (++), 94 (41.05%) are organic extracts, while 135 (58.95%) are aqueous extracts. Finally, among the extracts scored with (+), 47 (34.31%) are organic extracts, and 90 (65.69%) are aqueous extracts. It was possible to observe that the 860 plant extracts with RS activity was split into two groups of 425 organic extracts (49.42%) and 435 AE (50.58%) each. It was also observed that the group of active extracts that has been tested in the present work showed important RS activity, for their responsiveness was mostly scored as (++++) or (+++) (Figure 1).

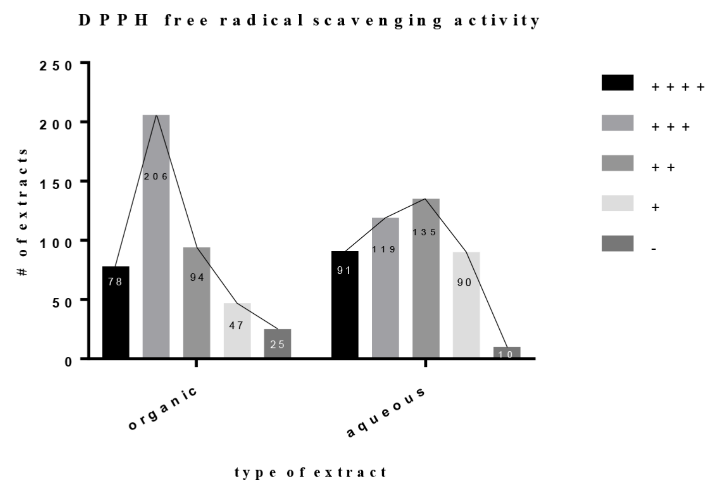

Figure 1: DPPH free radical scavenging activity of the 895 plant extracts tested in by thin layer chromatography analysis.

Table 2 shows $\chi 2$ analyses based on the scores obtained in the anti-oxidant and in the radical scavenge analyses. The null hypothesis (H_0) represents the equality of radical scavenge activity in both organic and aqueous. Pearson's $\chi 2$ test was performed for both organic and aqueous extracts, and the hypothesis H_0, that both extracts would have an equal distribution of (1) activity and (2) no activity in the β-carotene/bleaching assay ($\chi^2_{(1)} = 831.40$, $p = 0.05$; quantiles of the χ^2 distribution with degrees of freedom [df] =1 and α = 5% were 3.84), indicating that hypothesis H_0 could be rejected; so both extracts did not behaved the same. Pearson's $\chi 2$ analysis was performed for both organic and aqueous extracts, 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 ($\chi^2_{(4)} = 527.53$, $p < 0.05$; quantiles of the χ^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 free radical scavenging activity than the aqueous extracts.

Seventy three plant extracts, or 8.16%, presented alkaloids (Table 2). According to a chemosystematic approach, some groups of plants are more likely to biosynthesize alkaloids. So, in the present work alkaloids were found in the following families, which has been shown to present this class of compounds as cited: Annonaceae, Apocynaceae, Bignoniaceae, Capparidaceae, Chrysobalanaceae, Clusiaceae, Convolvulaceae, Euphorbiaceae, Celastraceae, Lauraceae, Fabaceae, Olacaceae, Piperaceae, Rubiaceae, Rutaceae, Sapindaceae and Solanaceae.

Eight (0.89%) plant extracts obtained from plants of the families Apocynaceae, Capparidaceae, Lauraceae, Fabaceae, Rubiaceae and Rutaceae, showed positive reactivity to KOH reagent, indicating the presence of anthraquinones (Table 2), which has been shown to present this class of compounds as cited.

Table 1: **Botanical identification of the plant material used to obtain the extracts tested for alkaloids (Dragendorff's reagent), anthraquinones (KOH reagent) and cardenolides (Kedde's reagent),).Legend: RA=roots; CA=stem; CS=stem bark; FO=leaves; AO=aerial organs; FR=fruits; PL=entire plant; LI=liana; odd numbers= organic extracts; even numbers= aqueous extracts.**

Family	Species	Voucher number	Organs	Extract #	Dragendorff's reagent	KOH ragent	Kedde's reagent	edde's re	DPPH
Rubiaceae	*Psychotria* sp.	PSC250	RA	N23	-	-	-	+	2
Euphorbiaceae	*Croton grandulosus*	PSC398	CA	N87	+	-	-	-	4
Apocynaceae	*Microplumeria anomala*	PSC136	FO	N97	+	-	-	-	3
Apocynaceae	*Microplumeria anomala*	PSC136	FO	N98	+	-	-	-	3
Apocynaceae	*Microplumeria anomala*	PSC136	CA	N127	+	-	-	+	3
Apocynaceae	*Microplumeria anomala*	PSC136	CA	N128	+	-	-	-	3
Apocynaceae	*Aspidosperma excelsum*	PSC360	FO e CA	N131	-	-	-	+	0
Apocynaceae	*Aspidosperma excelsum*	PSC360	CA	N133	+	-	-	+	0
Apocynaceae	*Aspidosperma pachypterum*	AAO3263	FO and CA	N136	+	-	-	-	2
Apocynaceae	*Aspidosperma pachypterum*	AAO3263	CA	N137	+	-	-	+	4
Apocynaceae	*Aspidosperma pachypterum*	1	CA	N138	+	-	-	+	1
Apocynaceae	*Aspidosperma pachypterum*	AAO3263	CS	N139	+	-	-	+	4
Apocynaceae	*Aspidosperma pachypterum*	AAO3263	CS	N140	+	-	-	+	1
Apocynaceae	*Aspidosperma pachypterum*	AAO3263	RA	N141	+	-	-	+	1
Apocynaceae	*Aspidosperma pachypterum*	AAO3263	RA	N142	+	-	-	+	1
Annonaceae	*Duguetia uniflora*	PSC357	CS	N145	+	-	-	-	4
Apocynaceae	*Malouetia tamaquarina*	IBS10	CA	N147	+	-	-	-	3
Annonaceae	*Guatteria riparia*	PSC115	CA	N151	+	-	-	-	3
Rubiaceae	*Palicourea corymbifera*	PSC298	CA	N153	+	-	-	-	3
Rubiaceae	*Palicourea corymbifera*	PSC298	CA	N154	+	-	-	+	2
Rubiaceae	*Remijia tenuiflora*	AAO3264	AO	N163	+	-	-	-	3
Melastomataceae	*Miconia* sp.	AAO3284	OA	N167	-	-	-	+	3
Annonaceae	*Duguetia uniflora*	PSC357	WD	N193	+	-	-	+	4
Calophyllaceae	*Haploclathra paniculata*	AAO3275	CA	N217	+	-	-	-	2
Calophyllaceae	*Haploclathra paniculata*	AAO3275	CA	N218	+	-	-	-	2
Lauraceae	*Ocotea* cf. *cymbarum*	IBS5	CA	N249	+	-	-	+	1
Capparaceae	*Capparidastrum solum*	IBS2	CA	N259	+	-	-	-	2
Fabaceae Faboideae	*Aeschynomene sensitiva*	PSC415	CA	N267	+	-	-	-	2
Rubiaceae	*Sipanea* cf. *pratensis*	AAO3283	OA	N281	-	-	-	+	3
Annonaceae	*Duguetia uniflora*	PSC357	FO e CA	N305	+	-	-	+	3
Annonaceae	*Guatteria foliosa*	AAO3328	FO	N315	+	-	-	+	2
Fabaceae Faboideae	*Dalbergia inundata*	PSC188	OA	N317	+	+	-	-	3
Peraceae	*Pera distichophylla*	PSC403	CA	N319	+	-	-	+	3
Peraceae	*Pera distichophylla*	PSC403	CA	N320	-	-	-	+	2

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Fabaceae Faboideae	*Aldina sp.*	PSC123	CA	N342	-	-	+	-	3

Family	Species	Voucher number	Organs	Extract #	Dragendorff's reagent	KOH ragent	Kedde's reagent	□edde's re	DPPH
Proteaceae	*Roupala montana*	PSC144	FO	N365	-	-	-	+	3
Rubiaceae	*Psychotria* sp.	PSC250	FO e CA	N375	+	-	-	+	1
Euphorbiaceae	*Hevea microphylla*	PSC196	CA	N377	+	-	-	-	3
Celastraceae	*Salacia impressifolia*	PSC125	CA	N389	-	-	-	+	2
Celastraceae	*Salacia impressifolia*	PSC125	CA	N390	-	-	-	+	2
FabaFabaceae Faboideae	*Acosmium* sp.	PSC143	CA	N395	+	-	-	-	3
Fabaceae Faboideae	*Acosmium* sp.	PSC143	CA	N396	+	-	-	-	2
Fabaceae Faboideae	*Ormosia* sp.	PSC116	CA	N400	+	-	-	-	1
Fabaceae Faboideae	*Clathrotropis macrocarpa*	PSC114	CA	N405	+	-	-	-	1
Fabaceae Faboideae	*Ormosia* sp.	PSC205	FO and FR	N433	+	-	+	-	2
Fabaceae Faboideae	*Ormosia* sp.	PSC205	FO and FR	N434	+	-	-	-	2
Fabaceae Faboideae	*Ormosia* sp.	PSC205	CA	N435	+	-	-	-	1
Fabaceae Faboideae	*Ormosia* sp.	PSC205	CA	N436	+	-	-	-	1
Fabaceae Caesalpinioideae	*Macrolobium multijugum*	PSC396	CA	N439	-	-	+	-	3
Fabaceae Caesalpinioideae	*Macrolobium multijugum*	PSC396	CA	N440	-	-	+	-	3
Combretaceae	*Buchenavia suaveolens*	PSC118	OA	N441	-	-	-	+	3
Combretaceae	*Buchenavia suaveolens*	PSC118	OA	N442	-	-	-	+	3
Combretaceae	*Buchenavia suaveolens*	PSC378	OA	N443	-	-	-	+	3
Combretaceae	*Buchenavia suaveolens*	PSC378	OA	N444	-	-	-	+	3
Hypericaceae	*Vismia guianensis*	PSC98	FO e FR	N446	-	-	+	-	3
Lauraceae	*Ocotea cymbarum*	IBS5	FO	N451	-	-	+	-	3
Lauraceae	*Ocotea cymbarum*	IBS5	FO	N452	-	-	+	-	3
Simaroubaceae	*Simaba* cf. *paraenesis*	PSC131	CA	N459	-	-	-	+	1
Sapindaceae	*Toulicia* cf. *pulvinata*	PSC106	CA	N469	+	-	-	+	2
Fabaceae Faboideae	*Ormosia* sp.	PSC135	OA	N471	+	-	-	+	0
Fabaceae Faboideae	*Ormosia* sp.	PSC135	OA	N472	+	-	-	+	0
Fabaceae Faboideae	*Clathrotropis macrocarpa*	PSC114	FO	N479	+	-	+	-	3
Fabaceae Faboideae	*Clathrotropis macrocarpa*	PSC114	FO	N480	-	-	+	-	3
Anacardiaceae	*Tapirira guianensis*	PSC107	CA	N489	-	-	+	-	3
Myrtaceae	*Eugenia* sp.	PSC99	FO	N497	-	-	+	-	3
Fabaceae Faboideae	*Ormosia* sp.	PSC116	FO and FR	N501	+	-	+	-	3
Fabaceae Faboideae	*Ormosia* sp.	PSC116	FO and FR	N502	+	-	+	-	3
Fabaceae Mimosoideae	*Pithecellobium* sp.	PSC204	FO	N509	-	-	+	-	3
Celastraceae	*Salacia* sp.	PSC102	FO	N525	-	-	-	+	2

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Family	Species	Voucher number	Organs	Extract #	Dragendorff's reagent	KOH ragent	Kedde's reagent	edde's re	DPPH
Celastraceae	*Salacia* sp.	PSC102	FO	N526	-	-	-	+	1
Fabaceae Mimosoidae	*Zygia trunciflora*	PSC97-A	CA e FO	N527	-	-	+	-	3
Fabaceae Faboideae	*Dioclea malacocarpa*	PSC82	FO e CA	N533	-	-	+	-	3
Meliaceae	*Trichilia* cf. *pleeana*	PSC92	OA	N535	-	-	+	-	3
Meliaceae	*Trichilia* cf. *pleeana*	PSC92	OA	N536	-	-	+	-	3
Fabaceae Mimosoideae	*Pithecellobium*	PSC267	OA	N537	-	-	+	-	3
Sapotaceae	*Pouteria* sp.	PSC126	FO	N557	-	-	-	+	3
Sapotaceae	*Pouteria* sp.	PSC126	FO	N558	-	-	-	+	4
-		PSC109	FO and CA	N560	+	-	-	-	4
Fabaceae Caesalpinioideae	*Cassia* sp.	AAO3306	FO	N563	-	-	-	+	4
Fabaceae Caesalpinioideae	*Cassia* sp.	AAO3306	FO	N564	-	-	-	+	4
Apocynaceae	*Mandevilla scabra*	AAO3354	OA	N569	-	-	-	+	4
Bignoniaceae	*Pachyptera kerere*	PSC366	FO e CA	N574	-	-	-	+	3
Lauraceae	*Endlicheria* cf. *macrophylla*	AAO3333	FO	N575	-	-	-	+	4
Lauraceae	*Endlicheria* cf. *macrophylla*	AAO3333	FO	N576	-	-	-	+	4
Rubiaceae	*Psychotria lupulina*	AAO3298	FO e CA	N585	+	-	-	+	2
Rubiaceae	*Psychotria lupulina*	AAO3298	FO e CA	N586	+	+	-	-	2
Rubiaceae	*Psychotria lupulina*	AAO3298	OA	N593	-	-	-	+	1
Rubiaceae	*Psychotria lupulina*	AAO3298	OA	N594	+	-	-	-	2
Bignoniaceae	*Amphilophium mansoanum*	PSC402	FO and CA	N595	+	-	-	-	3
Celastraceae	*Salacia* sp.	PSC102	CA	N600	+	-	-	-	2
Rutaceae	*Zanthoxylum compactum*	AAO3299	CA	N631	+	+	-	+	1
Fabaceae Faboideae	*Swartzia macrocarpa*	AAO3347	FO	N643	+	-	-	-	2
Fabaceae Faboideae	*Swartzia macrocarpa*	AAO3347	FO	N644	+	-	-	-	2
Capparaceae	*Capparidastrum solum*	IBS2	CA	N647	+	-	-	-	2
Capparaceae	*Capparidastrum solum*	IBS2	CA	N648	+	-	-	-	0
Annonaceae	*Guatteria foliosa*	AAO3328	CA	N655	+	-	-	-	3
Apocynaceae	*Macoubea sprucei*	AAO3373	FR	N657	+	-	+	-	3
Primulaceae	*Cybianthus spicatus*	AAO3350	FR	N660	-	-	-	+	2
Chrysobalanaceae	*Licania* sp.	PSC89	OA	N661	-	-	-	+	3
Chrysobalanaceae	*Licania* sp.	PSC89	OA	N662	-	-	-	+	2
Fabaceae Caesalpinioideae	*Hymenaea courbaril*	AAO3356	FO	N665	-	-	+	-	3
Clusiaceae	*Clusia columnaris*	AAO3357	PO	N667	-	-	+	-	3
Apocynaceae	*Macoubea sprucei*	AAO3406	FO	N676	-	-	+	-	2

Continued...

Family	Species	Voucher number	Organs	Extract #	Dragendorff's reagent	KOH ragent	Kedde's reagent	edde's re	DPPH
Dilleniaceae	*Tetracera tiguarea*	AAO3361	LI	N677	-	-	-	+	3
Gentianaceae	*Coutoubea ramosa*	AAO3340	PL	N679	-	-	+	-	2
Gentianaceae	*Coutoubea ramosa*	AAO3340	PL	N680	-	-	+	-	2
Ebenaceae	*Diospyros guianensis*	AAO3362	FO	N681	-	-	-	+	1
Fabaceae Caesalpinioideae	*Hymenaea courbaril*	AAO3356	CA	N687	-	-	+	-	4
Fabaceae Caesalpinioideae	*Hymenaea courbaril*	AAO3356	CA	N688	-	-	+	-	4
Fabaceae Mimosoideae	*Abarema auriculata*	AAO3353	CA	N689	+	-	-	+	4
Annonaceae	*Annona sericea*	AAO3405	OA	N695	-	-	+	-	3
Apocynaceae	*Macoubea sprucei*	AAO3402	CA	N697	+	+	-	+	3
Apocynaceae	*Macoubea sprucei*	AAO3402	CA	N698	-	-	-	+	2
Chrysobalanaceae	*Licania lata*	AAO3348	CA	N699	+	-	-	+	3
Chrysobalanaceae	*Licania lata*	AAO3348	CA	N700	-	-	-	+	2
Primulaceae	*Cybianthus spicatus*	AAO3350	CA	N701	-	-	-	+	3
Primulaceae	*Cybianthus spicatus*	AAO3350	CA	N702	-	-	-	+	4
Malpighiaceae	*Burdachia* sp.	PSC405	OA	N703	-	-	-	+	3
Malpighiaceae	*Burdachia* sp.	PSC405	OA	N704	-	-	-	+	2
Apocynaceae	*Mesechites trifidus*	AAO3385	OA	N706	-	-	-	+	2
Apocynaceae	*Forsteronia laurifolia*	AAO3400	FO e CA	N707	-	-	+	+	3
Apocynaceae	*Forsteronia laurifolia*	AAO3400	FO e CA	N708	-	-	+	+	4
Combretaceae	*Buchenavia* sp.	AAO3379	FO e CA	N712	-	-	-	-	3
Olacaceae	*Heisteria spruceana*	AAO3390	OA	N713	+	-	-	-	3
Olacaceae	*Heisteria spruceana*	AAO3390	OA	N714	-	-	-	+	3
Euphorbiaceae	*Croton cuneatus*	AAO3382	FO e CA	N721	-	-	+	-	3
Euphorbiaceae	*Croton cuneatus*	AAO3382	FO e CA	N722	-	-	+	-	3
Convolvulaceae	*Maripa repens*	AAO3384	OA	N723	+	-	-	-	3
Apocynaceae	*Himatanthus attenuatus*	AAO3396	CA	N729	-	-	-	+	3
Apocynaceae	*Microplumeria anomala*	AAO3393	FO	N735	+	-	-	-	2
Apocynaceae	*Microplumeria anomala*	AAO3393	FO	N736	+	-	-	-	2
Fabaceae Faboideae	*Pterocarpus santalinoides*	AAO3429	OA	N749	-	+	-	-	2
Clusiaceae	*Garcinia madruno*	AAO3422	FO	N751	+	-	-	-	2
Apocynaceae	*Himatanthus attenuatus*	AAO3396	FO	N771	-	-	-	+	2
Apocynaceae	*Himatanthus attenuatus*	AAO3396	FO	N772	-	-	-	+	1
Piperaceae	*Piper arboreum*	AAO3454	OA	N783	+	-	-	-	3
Solanaceae	*Brunfelsia* cf. *pauciflora*	AAO3466	PL	N795	+	-	-	+	2

Continued...

Family	Species	Voucher number	Organs	Extract #	Dragendorff's reagent	KOH ragent	Kedde's reagent	edde's re	DPPH
Fabaceae Faboideae	*Degelia negrensis*	PSC133	FO e CA	N808	-	-	+	-	2
Annonaceae	*Unonopsis stipitata*	AAO3449	FO	N817	+	-	-	-	3
Annonaceae	*Unonopsis stipitata*	AAO3449	FO	N818	+	-	-	-	3
Clusiaceae	*Clusia spathulaefolia*	AAO3407	CA	N823	+	-	-	-	2
Rubiaceae	*Pagamea coriacea*	AAO3488	FO	N857	-	+	-	+	2
Rubiaceae	*Pagamea coriacea*	AAO3488	FO	N858	-	-	-	+	2
Lauraceae	*Ocotea cymbarum*	AAO3525	FO e FR	N860	-	+	-	-	3
Fabaceae Faboideae	*Taralea* sp.	AAO3501	LI	N861	-	-	-	+	4
Rubiaceae	*Pagamea coriacea*	AAO3488	CA	N881	-	-	-	+	2
Asteraceae	*Piptocarpha notata*	AAO3455	OA	N891	-	-	-	+	3
Rubiaceae	*Psychotria amplectans*	AAO3494	OA	N897	-	-	-	-	2
Chrysobalanaceae	*Hirtella rodriguesii*	AAO3497	CA	N905	+	-	-	+	2
Rhizophoraceae	*Cassiporea guianensis*	AAO3512	OA	N907	-	-	-	+	2
Rhizophoraceae	*Cassiporea guianensis*	AAO3512	OA	N908	-	-	-	+	2
Apocynaceae	*Ambelania acida*	AAO3510	FO	N910	-	-	+	-	2
Chrysobalanaceae	*Licania lata*	AAO3513	OA	N911	-	-	-	+	3
Fabaceae Faboideae	*Dalbergia riedelii*	AAO3514	OA	N915	-	-	+	-	4
Rubiaceae	*Warszewiczia coccinea*	AAO3500	CA	N917	+	-	-	+	2
Rubiaceae	*Psychotria amplectans*	AAO3494	RA	N921	-	-	-	+	1

Table 2: Contingency table related to Pearson's χ^2 test conducted with plant extracts submitted to the evaluation of antioxidant activity in the β-carotene assay and to the DPPH free radical scavenging assay.

β-carotene assay				DPPH assay				
negative	positive	Total	Extract	0	1+	2+	3+	4+
399	51	450	Organic	25	47	94	206	78
419	26	445	Aqueous	10	90	135	119	91
818	77	895	Total	35	137	229	325	169

Lastly, 35 (3.89%) plant extracts obtained from plants of the families Anacardiaceae, Apocynaceae, Clusiaceae (including *Vismia* - Hypericaceae), Combretaceae, Euphorbiaceae, Gentianaceae, Lauraceae, Fabaceae (subfamily Caesalpinioideae), Fabaceae (subf. Faboideae), Fabaceae (subf. Mimosoideae), Meliaceae and Myrtaceae that showed positive reaction to Kedde's reactive, indicating the possible presence of cardenolides (Table 2), which has been shown to present this class of compounds as cited.

DISCUSSION

The search for new medicines from natural sources has developed rapidly in the last half century due to the introduction of high-throughput biological and phytochemical screening assays that enabled analyses of large amounts of samples bypassing traditional time-consuming techniques.[14] In the present work, plant extracts were obtained from Brazilian plants. The collection strategy was based on chemotaxonomic approach, as well as an eventual collection of a random species, especially if they were in the reproductive phenophase. As a general rule, plants used in traditional medicine were not our main target, but eventually they were collected in the field. Also, plant parts can vary in terms of their chemical constituents, and for that reason, collecting different organs of the same plant was done in order to obtain a significant amount of the chemical substances produced by the plant. Collection of *terra firme* trees are hard to perform as it depends on safety equipments and training in climbing techniques, so it is likely to have aerial parts being collected, once trees canopies can eventually reach 50 m tall. For that reason, aerial parts have been collected for trees. Also, in terms of ethics in accessing the Brazilian genetic patrimony, collections have to be performed in order to keep physiology of the plant as functional as possible, so, a limited portion of each plant material was collected, rarely the roots of the plants.

The Amazon rain forest, one of the tropical rain forests located in Brazil, is under the constant influence of sun radiation all the year around, in contrast, temperate forests perceivethe clear temperature change depending on seasonality. Also, there is an elevated amount of precipitation in tropical forests. As a result, such climate conditions favours a warm and umid climate that prepossess the occurrence of high levels of biodiversity, including all organisms, particularly the plants. It is expected that the constant high levels of sunlight irradiation, including UV wavelengths, particularly UV-B, may produce free radicals within the plant tissues, and in a direct response to this phenomenon the plant organism is stimulated to produce more enzimes related to the production of specific secondary metabolites, as phenolic compounds and flavonoids to work as antioxidant molecules protecting noble molecules as DNA, RNA and lipoproteins.[18] For that reason, the Amazon rain forest is one of the main spots on the planet where chemical and biological screening programs are the easiest and cheap ways of identifying plants as a potential source of new medicines. The present work reports the chemical screening of 895 plant extracts aiming the identification of their antioxidant potential, as well as to look for alkaloids, quinones[19-20] and cardenolides derivatives,[21] which are compounds already related to antioxidant activity.

wo chi-square ($\chi 2$) analyses were performed to evaluate the occurrence of antioxidant and radical scavenge activities in both groups of organic and aqueous extracts. The H_0 was considered as the equal distribution of antioxidant activity or radical scavenge activity in both organic and aqueous extracts. As our results for antioxidant (($\chi^2_{(1)} = 8.58$, $p = 0.05$; quantiles of the χ^2 distribution with degrees of freedom [df] =1 and $\alpha = 5\%$ were 3.84) and radical scavenge activities (($\chi^2_{(4)} = 51.53$, $p < 0.05$; quantiles of the χ^2 distribution with df = 4 and $\alpha = 5\%$ were 9.49) respectively, and when such results are compared to given quantiles ($\chi 2 = 3.84$ and 9.49 respectively, for df = 1 and 4 respectively, and α=0.05% for both analyses), it is easily noticed that the nule hypothesis can not be confirmed, and we can state that the occurrence of antioxidant and radical scavenge activities are different between organic and aqueous extracts. The present findings suggest that plant extracts made with organic solvents are more likely to present a wide range of possible antioxidant and radical scavenging active compounds, and are in accordance with previous results,[22] who evaluated the effect of the use of methanol, ethanol and water in different compositions to extract phenolic compounds from plants and compared the efficacy of these extracts in antioxidant and radical scavenge *in vitro* models.

In the present work, TLC techniques were adopted. Despite limitations of TLC assays being applied to screen for antioxidant and to prospect for the presence of phytochemicals, it is easily established in any laboratory and is approved by the World Health Organization for the quality control of plant-derived drugs.

Alkaloids is a group of substances that can exert antioxidant properties, depending on the presence of a phenolic ring in their structure.[23] In our report, we have found that 8.16% of the tested extracts contained alkaloids, mostly observed in Fabaceae and Apocynaceae, although their occurrence in Annonaceae and in Rubiaceae was somewhat expressive. From the 73 alkaloidic extracts only four did not show radical scavenge activity, but three of them have shown β-carotene/bleaching response. According to our results, families showing alkaloids can indeed be considered as a target group to undergo antioxidant and chemical screening of molecules to be tested in biological screening,

Anthraquinones were found in a few plant extracts belonging to Apocynacae, Lauraceae, Fabaceae, Rubiaceae and Rutaceae. All the extracts have also shown a good radical scavenge activity. Cardenolides were found mainly in the extracts that belong to Fabaceae, followed by the extracts belonging to Apocynaceae. These extracts have also shown significant radical scavenge activity. A β-carotene/bleaching response was found in 77 plant extracts. Eighteen extracts belong to Apocynaceae, and have also showed an expressive radical scavenge activity.

CONCLUSION

Present findings described that Amazon plant extracts have a huge potential to be a source of antioxidant compounds to be used in preventive medicine, as well as the chemical screening revealed that their plants can be strategically assessed as a source of alkaloids to be tested in further biological assays, both aiming treatment and prevention of diseases.

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CONFLICT OF INTEREST

All authors have none to declare.

ABBREVIATIONS

TLC: Thin Layer hromatography; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; KOH: potassium hydroxide; **DCM:** dichloromethane; **MeOH:** methanol; **mg:** milligram(s); mLmilliliter(s); **IBAMA:** Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis; **MMA:** Ministério do Meio Ambiente; **ICMBio:** Instituto Chico Mendes de Conservação da Biodiversidade; **CGen:** Conselho de Gestão do Patrimônio Genético; **v/v:** volume/volume; **UNIP:** Universidade Paulista; **RS:** radical scavenge; **df:** degrees of freedom; **RA:** roots; **CA:** stem; **FO:** leaves; **CS:** stem bark; **FR:** fruits; **UV:** ultraviolet ray; **UVB:** ultraviolet-B ray; **DNA:** desoxyribonucleic acid; **RNA:** ribonucleic acid.

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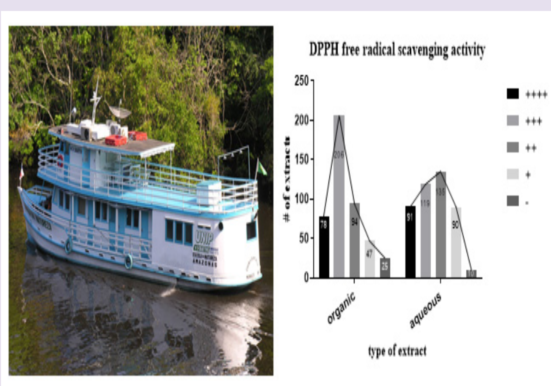
GRAPHICAL ABSTRACT



SUMMARY

- 895 Brazilian Amazon aqueous and organic plant extracts were evaluated by their free radical scavenging and antioxidant activities;-plants were also evaluated for the presence of alkaloids, cardenolides and anthraquinones;-present findings described that Amazon plant extracts have a huge potential to be a source of antioxidant compounds and a source of alkaloids to be tested in further biological assays.

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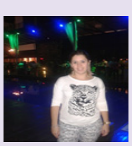

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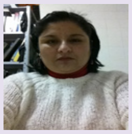
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