

QUANTIFICATION OF THE ANTIOXIDANT ACTIVITY OF *COUSSAPOA ASPERIFOLIA MAGNIFOLIA* (TRÉCUL) AKKERMANS & C.C. BERG (CECROPIACEAE) AND *BROSIMUM PARINARIOIDES* DUCKE (MORACEAE)

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Summary

Leaves and fruits of *Coussapoa asperifolia magnifolia* (Trécul) Akkermans & C.C. Berg (Cecropiaceae) and leaves of *Brosimum parinarioides* Ducke (Moraceae) were collected in order to conduct a systematic study on Amazonian plants. Plant materials were dried at room temperature (leaves) or liophilized (fruits) then extracted with dichloromethane, methanol and water. The antioxidant analyses were done by comparing both extracts and ascorbic acid inhibition percent using DPPH. The results obtained show *Coussapoa asperifolia magnifolia* extracts to be extremely active when assayed with DPPH (values ranging from 61.1 to 96.5 %). On the other hand, *Brosimum parinarioides* leaf dichloromethanic and aqueous extracts showed to be less active (values ranging from 12.8 to 34.7 %) whereas the methanolic extract to be more active (values ranging from 64.5 to 92.9 %), on assays using DPPH. All these analyses were compared with the ascorbic acid inhibition ability which showed values ranging from 80 to 94.7%. These results showed the huge antioxidant potential of the studied plants, since the whole extract presented percent inhibition comparable to those of ascorbic acid.

Keywords: antioxidant activity, Amazonian plants, Cecropiaceae, Moraceae, 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent.

Moraceae and Cecropiaceae families show similar botanical features (1) and some experts place Cecropiaceae genera, such as *Pourouma*, into the Moraceae family (2). However, the most acceptable classification is that of *Morus*, *Maclura* and *Trophis* genera belonging to the Moraceae and *Cecropia*, *Coussapoa* and *Pourouma* genera to Cecropiaceae (3). One of the features of Cecropiaceae is interacting with ants, some of which are very aggressive.

To our knowledge, no chemical study on *Coussapoa asperifolia magnifolia* or *Brosimum parinarioides* is available in literature as of yet. However, flavonoids (4) and triterpenoids (5) were isolated or identified from other species of Cecropiaceae and flavonoids (6,7), chalcones (8), triterpenoids (9), alkaloids (10) from Moraceae.

When carrying out a systematic study on plants from the Amazonian region in the vicinity of Manaus (AM), two species were collected: *Coussapoa asperifolia magnifolia* (Trécul) Akkermans & C.C. Berg (Cecropiaceae) and *Brosimum parinarioides* Ducke (Moraceae) the extracts of which were assayed as to their antioxidant ability using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent.

Methods

The studied leaves were collected at INPA's Ducke Reserve, Manaus (AM). Plant species were identified by MSc. Ieda Leão do Amaral and the voucher specimens are kept in the Herbarium at the National Research Institute of Amazon (Instituto Nacional de Pesquisas da Amazônia - INPA), Manaus (AM).

Following their collection, *Coussapoa asperifolia magnifolia* and *Brosimum parinarioides* leaves were dried at room temperature, ground in an electric grinder and weighed. *Coussapoa asperifolia magnifolia* fruits were collected twice, the ones from the first collection were lyophilized whole and those from the second collection were separated into skins and pulps and mashed with solvent. Plant materials were extracted with dichloromethane, by using ultra-sound for 20 minutes, which was repeated twice, then with methanol and later with water. These procedures were all repeated twice. Reduced-pressure evaporator or lyophilizer were used to obtain the extracts through solvent evaporation.

Antioxidant Activity

The acquired plant extracts were tested with 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.

The DPPH solution was prepared in a 30 µg/mL concentration and 1000 µL out of the solution were added to 100 µL of the samples (0,3 mg/mL) in order to obtain the 40 µg/mL solutions, which were measured in triplicate with 517 nm readings at 2 and 20 minutes.

The color fading ability was calculated through the following equation:

$$CD = (ABS_{DPPH} - ABS_{sample}) / ABS_{DPPH} * 100$$

Where:

ABS_{DPPH} = absorbancy measured with a DPPH solution;

ABS_{sample} = absorbancy obtained from the extract sample;

Results

The results were expressed as DPPH color fading ability and compared to those of ascorbic acid (Table 1), which was chosen to be the standard antioxidant on account of it being biologically active by participating in organism reactions.

Table 1. DPPH color fading ability of *Coussapoa asperifolia magnifolia* and *Brosimum parinarioides* plant extracts as compared to that of ascorbic acid.

Plant species/ studied part	Extract (abbreviation)	Concentration ($\mu\text{g/mL}$)	DPPH color fading ability (%)		ascorbic acid color fading ability (%) Conc. 5 $\mu\text{g/mL}$
			2 min	20 min	
<i>Coussapoa asperifolia magnifolia</i> Leaves	DCM (a)	40	61,1	92,3	94,7
	MeOH (b)	40	95,4	95,2	94,7
<i>Coussapoa asperifolia magnifolia</i> Liophylized Whole Fruits	DCM (c)	40	67,4	92,6	94,7
	MeOH (d)	40	95,4	95,7	94,7
	H ₂ O (e)	40	92,8	95,6	94,7
<i>Coussapoa asperifolia magnifolia</i> Fruits (skins)	MeOH (f)	40	94,7	95,5	94,7
	H ₂ O (g)	40	95,2	96,3	94,7
<i>Coussapoa asperifolia magnifolia</i> Fruits (pulpes)	MeOH (h)	40	95,9	96,5	94,7
	H ₂ O (i)	40	95,4	95,2	94,7
<i>Brosimum parinarioides</i> Leaves	DCM (j)	40	12,8	14,8	80,0
	MeOH (k)	40	64,5	92,9	80,0
	H ₂ O (l)	40	22,9	34,7	80,0

Discussion

Table 1 analyses show that the color fading percentile of 3 extracts (a, c e k) increased remarkably as time went by, which points out in addition to there being antioxidant substances in the tested extracts, it could be that a significant amount of these compounds is being or that the same regenerated during the process thus enhancing their antioxidant ability (synergy, as it occurs between ascorbic acid and vitamin E?). Other 7 extract (b, d, e, f, g, h, i) presented high antioxidant activity which remained practically constant throughout the assay which might mean that the extant antioxidants are permanently capturing or stabilizing the DPPH oxidant molecules impeding them from returning to the medium. Just two extracts (j e l) were totally inactive throughout the test. The extract were compared as to their ascorbic acid color fading ability which in the concentration of 5 $\mu\text{g/mL}$ showed that all *Coussapoa asperifolia magnifolia* extracts presented similar activity, pointing out there may be substances with higher antioxidant ability on account of then being raw extracts, with no treatment at all. The k extract draws attentions since it presented higher than standard activity, measured at 20 min.

The active extracts chromatographic fractioning is underway, but there are no isolated active substances as of yet. A few of the tested extracts (d, e, f, g and i) were assayed by Nuclear Magnetic Resonance (NMR) and the most active extracts spectra show signals of presenting aromatic substances, probably flavonoids and glycosylated flavonoids.

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