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In vitro screening of Amazonian plants for hemolytic activity and inhibition of platelet aggregation in human blood

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ABSTRACT
In the present study, different aerial parts from twelve Amazonian plant species found in the National Institute for Amazon Research's (INPA's) Adolpho Ducke Forest Reserve (in Manaus, Amazonas, Brazil) were collected. Separate portions of dried, ground plant materials were extracted with water (by infusion), methanol and chloroform (by continuous liquid-solid extraction) and solvents were removed first by rotary evaporation, and finally by freeze-drying which yielded a total of seventy-one freeze-dried extracts for evaluation. These extracts were evaluated initially at concentrations of 500 and 100 µg/mL for *in vitro* hemolytic activity and *in vitro* inhibition of platelet aggregation in human blood, respectively. Sixteen extracts (23 % of all extracts tested, 42 % of all plant species), representing the following plants: *Chaunochiton kappleri* (Olacaceae), *Diclinanona calycina* (Annonaceae), *Paypayrola grandiflora* (Violaceae), *Pleurisanthes parviflora* (Icacinaceae), *Sarcaulus brasiliensis* (Sapotaceae), exhibited significant inhibitory activity towards human platelet aggregation. A group of extracts with antiplatelet aggregation activity having no *in vitro* hemolytic activity has therefore been identified. Three extracts (4 %), all derived from *Elaeoluma nuda* (Sapotaceae), exhibited hemolytic activity. None of the plant species in this study has known use in traditional medicine. So, these data serve as a baseline or minimum of antiplatelet and hemolytic activities (and potential usefulness) of non-medicinal plants from the Amazon forest. Finally, in general, these are the first data on hemolytic and inhibitory activity on platelet aggregation for the genera which these plant species represent.

KEYWORDS: Adolpho Ducke Reserve, *Chaunochiton kappleri*, *Diclinanona calycina, Elaeoluma nuda*.

Testes *in vitro* de plantas Amazônicas para atividade hemolítica e inibição da agregação plaquetária em sangue humano

RESUMO
No presente estudo, partes aéreas obtidas de doze (12) espécies vegetais da Amazônia encontradas na Reserva Florestal Adolpho Ducke (localizada na cidade de Manaus, Estado do Amazonas, Brasil) do Instituto Nacional de Pesquisas da Amazônia foram coletadas, secadas e moídas. Porções dos materiais vegetais em pó foram extraídas com água (por infusão), metanol e clorofórmio (por extração líquido-sólido contínua) e os solventes foram removidos por evaporação rotatória e finalmente liofilização, forneceram um total de setenta e one (71) extratos liofilizados. Esses extratos foram avaliados inicialmente para atividade hemolítica *in vitro* e atividade inibitória da agregação plaquetária em sangue humano *in vitro* em concentrações de 500 e 100 µg/mL, respectivamente. Dezesseis (16) extratos (23 % dos extratos testados, 42 % das espécies vegetais) representando as seguintes plantas, apresentaram inibição significativa frente a agregação de plaquetas humanas *in vitro*: *Chaunochiton kappleri* (Olacaceae), *Diclinanona calycina* (Annonaceae), *Paypayrola grandiflora* (Violaceae), *Pleurisanthes parviflora* (Icacinaceae), *Sarcaulus brasiliensis* (Sapotaceae). Como principal resultado, um grupo de extratos apresentando atividade inibitória da agregação plaquetária e em que não há atividade hemolítica *in vitro* foi identificado. Três (3) extratos (4 % do total de extratos testados), todos obtidos a partir de *Elaeoluma nuda* (Sapotaceae), apresentaram atividade hemolítica. Nenhuma das espécies vegetais nesse estudo tem uso medicinal conhecido. Assim, esses dados servem de linha base ou mínimas das atividades antiplaquetária e hemolítica (e utilidade potencial) de plantas da floresta Amazônica. Finalmente, em geral, esses dados são os primeiros disponíveis sobre ação hemolítica e inibição da agregação plaquetária dos gêneros representados por essas espécies de plantas.

PALAVRAS-CHAVE: Reserva Florestal Adolpho Ducke, *Chaunochiton kappleri*, *Diclinanona calycina, Elaeoluma nuda*.

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INTRODUCTION

The Amazon region represents the most extensive tropical forest ecosystem in the world and is considered to be the largest single reserve in terms of medicinal plants. Estimates put the total number of plant species in this region at as many as 125,000 and the number of traditionally-used medicinal plant species at between 5,000 and 10,000 (Gurib-Fakim, 2006). A large number of medicinal plants owe their discovery to Amerindians who detain and associate an ever-richer traditional knowledge to these plant species with the passing of time. Only about 5-10 % of all Amazonian plants have been the object of at least one study on pharmacological activity or chemical composition (Gurib-Fakim, 2006). Besides traditional knowledge as an important source of medicinal and pharmacological information on the utility, both potential and direct, of plant biodiversity, it is important to recognize a complementary or unique niche for bioprospection based on random sampling techniques (that is, sampling which does not rely on traditional medicine, chemosystematics or specific, prior knowledge of plant properties).

This method can be used to locate, evaluate, and systematically exploit the biodiversity of a given location, especially in the pursuit of genetic, biochemical and pharmaceutical resources for commercial ends. It should also be recognized that traditional knowledge may not always be easy to access for a number of reasons and its interpretation is not always straight forward in light of modern pharmacological and biochemical understanding of disease and normal functioning of healthy organisms.

Among the most important and costly causes of hospitalization today are heart disease and stroke. The evaluation of platelet function is a crucial datum in establishing necessary therapeutic measures associated with each clinical case. Platelets are important players in the processes responsible for the control of bleeding (hemostasis) and the formation of clots in injured blood vessels (thrombosis). For this reason, the pharmacological basis for therapy and prevention of cardiovascular disturbances is platelet structure and specific function (Jensen & Gordon, 1992).

The use of drugs having the capability to reduce platelet activity, such as acetyl salicylic acid (aspirin), requires periodic check-ups on the impact of these pharmaceuticals on platelet aggregation. Laboratory evaluation of platelet aggregation permits the measurement of qualitative and semi-quantitative temporal parameters of platelet function in the presence of several aggregation-provoking agents such as adenosine diphosphate (ADP), collagen (COL), adrenalin (ADR), thrombin and arachidonic acid (AA). Quantification can be performed on platelet-rich fractions or on whole blood samples. In comparison to other tests which evaluate primary hemostasis, this technique seems to represent the best auxiliary laboratory diagnostic test for the verification of acquired or congenital qualitative disturbances of platelet function (Zucker, 1989).

Many *in vitro* and *in vivo* studies have been performed to evaluate the antiaggregant action of natural products as a means to drug lead discovery. These studies are important since some patients present resistance to aspirin (5-45 % of users) (Undas et al., 2007) and/or use conventional medication (warfarin, aspirin) in association with medicinal plant formulations (*Panax ginseng* C.A. Mey., *Allium sativum* L., *Ginkgo biloba* L., *Matricaria chamomilla* L., *Angelica sinensis* (Oliv.) Diels, *Camellia sinensis* L., *Salvia miliorrhiza* Bunge, *Zingiber officinale* Roscoe). These are the most studied species because they can inhibit or increase the anticoagulant or antiplatelet aggregation effects (Basila & Yuan, 2005; Saw *et al*., 2006).

In recent years, many antiplatelet aggregating agents have been isolated from plants and have demonstrated potent activity (Dong *et al*., 1998). Important classes of natural antiaggregant compounds are flavonoids such as quercetin and myricetin (Hommam *et al*., 2000), and flavones chrysin and apigenin (Landolfi *et al*., 1984). In the investigation of the action of plant extracts on human blood, besides platelet function, it is important to determine any hemolytic activity as this is an indicator of general cytotoxicity and bioactivity. *In vitro* testing for hemolytic action has been used as one of the methods of triage for different toxic agents (Kublik *et al*., 1996). The *in vitro* hemolysis test has also been employed by many different groups for the toxicological evaluation of different plants (Gandhi & Cherian, 2000).

Considering the richness and potential of the Amazon flora, the present study has as its primary goal the prospection of extracts prepared from the following twelve plant species for hemolytic activity and inhibition of platelet aggregation in human blood: *Moutabea guianensis* Aubl. (Polygalaceae), *Thyrsodium spruceanum* Benth. (Anacardiaceae), *Sarcaulus brasiliensis* (A.DC.) Eyma (Sapotaceae), *Chaunochiton kappleri* (Sagot ex Engl.) Ducke (Olacaceae), *Paypayrola grandiflora* Tul. (Violaceae), *Schoenobiblus daphnoides* Mart. (Thymelaeaceae), *Elaeoluma nuda* (Baehni) Aubrév. (Sapotaceae), *Pleurisanthes parviflora* (Ducke) Howard (Icacinaceae), *Diclinanona calycina* Benoiste (Annonaceae), *Lacmellea gracilis* (Mull. Arg.) Markgr. (Apocynaceae), *Dilkea johannesii* Barb. Rodr. (Passifloraceae) and *Sterigmapetalum obovatum* Kuhlm. (Rhizophoraceae).

What these plant species have in common is that they are found in the western Brazilian Amazon (in the National Institute for Amazon Research's Adolpho Ducke Forest Reserve) (Ducke Reserve, 2004) in Manaus, Amazonas, Brazil. In an earlier study, antibacterial and antifungal properties of these twelve species were investigated and several of these species proved to have significant inhibitory activity towards

strains of bacteria and fungi associated with human diseases (Carneiro *et al.*, 2008). The present study therefore represents a further expansion on the available knowledge of the in vitro biological activity of these non-medicinal species from the western Amazon forest.

MATERIAL AND METHODS

PLANT MATERIALS

The plants studied are from non-flooding, or terra firme, regions and were catalogued during INPA's Flora Project at the Adolpho Ducke Forest Reserve (Ducke Reserve, 2004; Table 1). Starting from a complete list of families and genera of plants found in the Ducke Reserve, systematic literature searching was performed to identify a group of genera having few or no previous chemical composition or pharmacological studies. From this larger group of genera, a sub-group of twelve species having no relevant prior study, availability in the Reserve, and a previously identified Flora Project fertile voucher on deposit at the INPA Herbarium were chosen to represent 12 plant families found in this forest area. Collection was performed from February to June, 2004. Plant specimens (Table 1) were located based on polar coordinates and field tags on individual specimens as provided by the Flora Project. Freshly collected leaves and branches were weighed and then dried at less than 50 °C in an oven under incandescent lamps. Dried samples were weighed and then each plant part was separately ground. Pulverized plant materials were stored in impermeable plastic bags under incandescent lighting at room temperature (ca. 27 °C).

CALCULATION OF PERCENT WEIGHT LOSS UPON DRYING

From the difference in weight of each plant material at the time of collection (fresh, FW) and the weight after drying (DW), the weight-loss upon drying was calculated as a percentage (WLD%) using the following formula: WLD% = 100 × [1 – (DW/FW)]

PREPARATION OF EXTRACTS

Each dried, ground plant material was divided into three portions which were separately extracted with methanol, chloroform (continuous liquid-solid extraction) and water (infusion). Rotary evaporation under reduced pressure was used to remove solvents followed by freeze-drying. This procedure resulted in seventy-one freeze-dried extracts which were sealed in glass vessels and stored at -20 °C.

HUMAN BLOOD SAMPLES

\Venous blood samples (5 mL) were collected using plastic or silicone-coated materials from volunteer donors after obtaining prior consent. Donors were screened to guarantee that none had ingested drugs such as aspirin or other anti-inflammatory agents for a period of at least 10 days prior to donation, since these compounds could be a source of non-systematic alteration in platelet function.

Anticoagulant human blood was centrifuged (1000 rpm for 5 min at room temperature). The supernatant was collected as platelet-rich plasma (PRP). After PRP was removed, residual plasma was centrifuged at 3000 rpm for 15 min and the supernatant was collected as platelet-poor plasma (PPP). PRP was stabilized with 3.8 % sodium citrate (anticoagulant) at a proportion of 1:9. The platelet number was determined (manual chamber method – hemolyzing 1 % ammonium oxalate solution) and adjusted to 3 × 105/mm^3 by mixing PRP and PPP with the aid of a platelet counter.

IN VITRO HEMOLYTIC ACTIVITY

For this experiment, 1% red-blood cell suspension in pH 7.4 phosphate buffer was used throughout in the preparation of experimental (test) and control tubes (WHO, 1998). Extracts prepared as described above were initially dissolved in dimethylsulphoxide (DMSO), ethyl alcohol or distilled water at known sample concentrations. These sample solutions were transferred by micropipette to test tubes containing a fixed volume of red-blood cell suspension. The extracts were screened at test concentrations of 500, 250, 100 and 50 µg/mL. Hemolytic activity of extracts was evaluated by comparison with the effects of the commercially available positive control saponin from quillaja bark (Sigma, U.S.A.). This control provides total hemolysis of red-blood cell suspensions at concentrations of 10-20 µg/mL. Negative controls (blanks) contained 1 % solvent (DMSO, ethanol or distilled water) in red-blood cell suspension.

The result for each test concentration of extract was interpreted qualitatively *in vitro* hemolytic action either being present or absent. Overall, the result was a semi-quantitative evaluation of hemolytic activity for each extract in accordance with international guidelines for the evaluation of this activity in medicinal plant materials (WHO, 1998).

IN VITRO PLATELET AGGREGATION MEASUREMENT

In the platelet aggregation assay, PRP activity/aggregation was determined spectrophotometrically using a multi-channel automated aggregometer (Chronolog Corporation, Model 400, U.S.A.) according to the method described by Born (1962). This apparatus can furnish data on the platelet activity of several samples simultaneously. 10 µL of agonists ADP (10 µM), ADR (10 mg/L) and COL (5 mg/L), which induce platelet aggregation, were added to PRP (400 µL) and the variation of light transmittance was registered over time. Increase in transmittance is associated with the degree of platelet aggregation (Born, 1962). Acetylsalicylic acid (30 mg/L) (Pharma Nostra, Anápolis, Brasil) was used as positive control for platelet aggregation (Sanabria *et al.*, 1997)

and 0.5 % DMSO was used as negative control. Extracts were initially screened at a concentration of 100 μg/mL for inhibition-inducing activity towards the above-mentioned agonists. Those extracts presenting inhibitory activity of platelet aggregation > 97 % (relative to the inhibition induced by the aspirin controls) were next tested at different dilutions to establish minimum inhibitory concentrations (MIC). Percent inhibition (I%) of platelet aggregation was calculated according to the equation: $I\% = 100 \times [1 - (SA\%/CA\%)]$, where SA% is the measured percentage aggregation observed for the sample (extract) and CA% is the measured percentage aggregation observed for the control.

TESTS FOR PHENOLS, TANNINS AND SAPONINS

Each extract (10 mg) was dissolved in solvent (5 mL) in a test tube and 10 % iron (III) chloride solution (100 μL) was added. Color change or precipitate formation is indicative of a positive result as compared to blank (solvent + iron (III) chloride solution). Blue, blue-green and red initial coloration is indicative of the presence of phenolic compounds after complex formation. Dark, blue- or green-colored precipitate indicated the presence of tannins. Saponin containing extracts presented long-lasting foam heads after shaking of the extract dissolved in water.

TEST FOR FLAVONOIDS

Methanol solution of each extract (3 mL) was placed in a test tube together with three pieces of magnesium metal. Concentrated hydrochloric acid (1 mL) was added slowly. After effervescence had seized, a rose or red-colored solution was considered a positive result. This reaction is known as the cyanidin, Shinoda or hydrogenation (III) reaction (Matos, 1997).

RESULTS AND DISCUSSION

Twelve Amazonian plant species representing twelve families were screened in the present work. Herbarium voucher numbers, parts of each plant species studied and drying and extraction data are presented in Table 1.

Of a total of seventy-one extracts tested only three extracts (4 % of the total tested) from *E. nuda* (8 % of all species tested) presented significant hemolytic activity. Thus, *E. nuda* leaf methanol extracts presented hemolytic action at a concentration of 500 μg/mL while branch methanol and water extracts presented hemolytic action at concentrations of 250 μg/mL.

A large number of compounds from different sources have been found to be responsible for *in vitro* hemolysis. Among these are substances derived from plants (Gandhi and Cherian, 2000), heavy metals (Ribarov & Bemov, 1981) and pharmaceuticals (Yamamoto *et al.*, 2001). The direct hemolytic effect of different toxic agents is due to a variety of non-specific mechanisms. For example, surfactants cause hemolysis through dissolution of the erythrocyte plasma membrane which ruptures due to increased fragility or due to osmotic lysis caused by increased permeability of the plasma membrane (Aparicio *et al.*, 2005). On the other hand, reduced xenobiotic compounds, such as phenols, are capable of promoting hemolysis through oxidation of hemoglobin, forming metahemoglobin (Bukowska & Kowalska, 2004). Bilirubin promotes the loss of lipids in the erythrocyte plasma

Table 1 - Species and family names, herbarium voucher numbers, drying and extraction data for the twelve plants screened in this study.

Species	Family	Voucher No.	Weight loss on drying (%)		Extract Yield (w/w, %) Leaves			Extract Yield (w/w, %) Branches / Vines		
			Leaves	Branches/Vines	CH_3OH	$CHCl_3$	H_2O	CH_3OH	$CHCl_3$	H_2O
Chaunochiton kappleri	Olacaceae	181856	66,6	22.8	19.4	4.1	18.6	7.8	1.3	5.9
Diclinanona calycina	Annonaceae	179085	55.3	46.0	36.8	12.2	16.3	5.2	1.8	6.0
Dilkea johannesii	Passifloraceae	206552	62.4	50.5	10.3	2.6	7.9	4.4	0.7	2.4
Elaeoluma nuda	Sapotaceae	179316	47.0	56.5	32.2	7.8	15.9	12.3	3.0	7.2
Lacmellea gracilis	Apocynaceae	189617	70.9	44.9	30.3	8.8	*	5.3	1.2	4.2
Moutabea guianensis	Polygalaceae	179914	58.2	46.3	8.3	3.0	10.8	4.9	1.3	8.4
Paypayrola grandiflora	Violaceae	185700	42.9	45.9	17.6	3.8	7.7	3.7	1.2	3.5
Pleurisanthes parviflora	Icacinaceae	195327	55.2	42.2	14.7	4.6	13.2	4.8	1.5	7.2
Sarcaulus brasiliensis	Sapotaceae	183442	44.5	40.7	17.0	3.4	7.7	7.1	2.7	8.2
Schoenobiblus daphnoides	Thymelaeaceae	191794	21.8	25.3	18.7	4.1	19.0	13.9	2.3	5.4
Sterigmapetalum obovatum	Rhizophoraceae	183424	52.1	56.0	17.1	5.7	14.2	7.8	1.4	6.9
Thyrsodium spruceanum	Anacardiaceae	178282	55.0	49.0	19.2	4.1	10.5	10.2	2.7	3.5

Notes. * - formed solid emulsion, yield data could not be calculated. This extract was not tested.

membrane, with exposure of residues of phosphatidyl serine (Brito *et al.*, 2002). Saponins, used in the present study as positive control for evaluation of hemolytic action, produce changes in the erythrocyte membrane, causing rupture and release of characteristic hemoglobin pigments.

A total of seventy-one extracts were tested for inhibition of platelet aggregation caused by three different agonists. Sixteen extracts (23 % of all extracts tested) proved to be active inhibitors of platelet aggregation (≥ 75 % inhibition) at a concentration of 100 mg/L (Table 2). All experimental values were referenced to the inhibitory activity of the aspirin standard. Extracts (five in all) presenting ≥ 97 % inhibitory activity were considered highly active and were diluted and further screened to establish minimum inhibitory concentrations (MIC, Table 3). Branch chloroform extracts of *C. kappleri* presented very good inhibitory potential towards platelet aggregation promoted by agonists ADR and COL (MIC 25.0 and 16.6 µg/mL, respectively). Leaf chloroform extract of *P. grandiflora* presented MIC of 25 µg/mL against the effects of ADR. Leaf methanol extract of *P. parviflora* (MIC 16.6 µg/mL) and branch methanol extract of *D. calycina* (MIC 25 µg/mL) inhibited platelet-aggregation promoted by COL. In general, the studied extracts presented a greater tendency towards inhibition of platelet-aggregation promoted by COL.

Antiaggregant activity has previously been demonstrated for some known medicinal plants. For example, Bydlowski *et al.* (1988) demonstrated platelet antiaggregant properties *in vivo* and *in vitro* for the whole fruit of the Amazonian tree-vine guaraná (*Paullinia cupana*). In an *in vitro* and *ex vivo* study, Son *et al.* (2004) observed that green tea (*Camellia sinensis*) catechins inhibit *in vitro* platelet aggregation induced by COL, AA and U46619 (9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$) and *ex vivo* induced by AA. *Taxus baccata* presented a significant inhibitory effect on platelet aggregation induced by AA, COL, and platelet activating factor at a concentration of 400 µg/mL (Erdemoglu *et al.*, 2004).

According to Dong *et al.* (1998), substances with antiaggregant activity are found in plants and the flavonoids are the most important class. Mesa *et al.* (2002) have demonstrated the capacity of the flavonoids naringin, naringenin, hesperidin e hesperetin from citric plants to inhibit platelet-aggregation stimulated by COL in human PRP. Flavonoids are capable of inhibiting lipid peroxidation and platelet aggregation by acting on enzyme systems such as cyclooxygenases and lipoxygenases and interfering in the production of metabolites of AA (Silva

Table 2 - Average inhibition of platelet aggregation [as percentage of effect of positive control (acetylsalicylic acid) in three different agonist systems] for studied Amazonian plant extracts at concentrations of 100 mg/L.

Species	Part	Extraction Solvent	Platelet Aggregating Agents (Avg % inhibition)		
			ADP	ADR	COL
Chaunochiton kappleri	Leaf	$CHCl_3$	9	3	6
		CH_3OH	61	49	66
		H_2O	61	86	84
	Branch	$CHCl_3$	75	98	99
		CH_3OH	12	20	39
		H_2O	59	78	72
Diclinanona calycina	Leaf	$CHCl_3$	11	5	21
		CH_3OH	56	30	89
		H_2O	18	33	36
	Branch	$CHCl_3$	9	35	95
		CH_3OH	33	88	97
		H_2O	22	19	31
Dilkea johannesii	Leaf	$CHCl_3$	11	9	31
		CH_3OH	0	0	18
		H_2O	2	0	7
	Branch	$CHCl_3$	4	0	0
		CH_3OH	17	6	0
		H_2O	15	4	0

Table 2 - Continuation.

Species	Part	Extraction Solvent	Platelet Aggregating Agents (Avg % inhibition)		
			ADP	ADR	COL
Elaeoluma nuda	Leaf	$CHCl_3$	25	11	10
		CH_3OH	16	12	11
		H_2O	5	4	0
	Branch	$CHCl_3$	0	2	22
		CH_3OH	11	38	47
		H_2O	19	8	28
Lacmellea gracilis	Leaf	$CHCl_3$	14	13	34
		CH_3OH	15	11	12
		H_2O	nt	nt	nt
	Branch	$CHCl_3$	6	11	63
		CH_3OH	0	0	18
		H_2O	2	16	16
Moutabea guianensis	Leaf	$CHCl_3$	48	21	23
		CH_3OH	3	6	1
		H_2O	0	0	1
	Branch	$CHCl_3$	19	22	33
		CH_3OH	3	4	0
		H_2O	4	0	0
Paypayrola grandiflora	Leaf	$CHCl_3$	72	98	95
		CH_3OH	30	12	29
		H_2O	51	63	57
	Branch	$CHCl_3$	7	23	18
		CH_3OH	27	6	3
		H_2O	8	2	8

Table 2 - Continuation.

Species	Part	Extraction Solvent	Platelet Aggregating Agents (Avg % inhibition)		
			ADP	ADR	COL
Pleurisanthes parviflora	Leaf	$CHCl_3$	12	28	10
		CH_3OH	5	4	98
		H_2O	0	2	0
	Vine	$CHCl_3$	0	7	13
		CH_3OH	20	0	95
		H_2O	0	0	0
Sarcaulus brasiliensis	Leaf	$CHCl_3$	7	10	18
		CH_3OH	55	36	54
		H_2O	46	53	44
	Branch	$CHCl_3$	23	9	13
		CH_3OH	46	59	50
		H_2O	65	2	85
Schoenobiblus daphnoides	Leaf	$CHCl_3$	33	20	25
		CH_3OH	35	28	23
		H_2O	0	1	0
	Branch	$CHCl_3$	1	22	10
		CH_3OH	43	69	43
		H_2O	4	0	5
Sterigmapetalum obovatum	Leaf	$CHCl_3$	9	6	21
		CH_3OH	10	2	12
		H_2O	2	3	0
	Branch	$CHCl_3$	5	12	18
		CH_3OH	12	1	94
		H_2O	8	2	8
Thyrsodium sprucenum	Leaf	$CHCl_3$	11	12	8
		CH_3OH	42	32	37
		H_2O	2	10	13
	Branch	$CHCl_3$	8	0	7
		CH_3OH	15	35	39
		H_2O	62	59	39
AAS (C +)			79	98	99
DMSO (C -)			0	0	0

C + = Positive control; C - = Negative control, ADP = adenosine diphosphate, ADR = adrenalin, COL = collagen, nt = not tested.

et al., 2002). Flavonoids quercetin and myricetin strongly inhibit human platelet aggregation induced by AA and ADP (Hommam *et al.*, 2000). According to Landolfi *et al.* (1984), flavones chrysin and apigenin inhibit platelet aggregation by inhibiting cyclooxygenase which may lead to increased cAMP (cyclic adenosine monophosphate) levels.

Preliminary phytochemical screening revealed that *E. nuda* leaves and branches contain saponins. These compounds are presumably the source of the hemolytic activity of extracts prepared from this plant. Phenols, flavonoids, and tannins were detected in *D. calycina* and *E. nuda* methanol extracts. *P. grandiflora* was shown to have phenolic compounds, but no tannins or saponins. Plants rich in tannins are employed in traditional medicine as adstringents with internal and external action in the treatment of microbial infections and as hemostatic agents (Longuefosse, 2003, Gurib-Fakim, 2006). The presence of flavonoids, tannins and/or phenolic compounds in *D. calycina* and *P. grandiflora* is probably related to the inhibition of platelet aggregation observed for the extracts of these plants.

It is important to correlate inhibition of platelet aggregation with the absence of hemolytic activity which was in general observed for the plant extracts tested. This correlation indicates in general that the antiaggregant extracts identified in this study present no toxicity towards erythrocyte membrane which favors them for further research aimed at establishing their chemical composition, among other properties.

D. calycina (Annonaceae) is a promising antimicrobial plant (Carneiro et al., 2008) which needs further study to reveal the identity of several antimicrobial substances present in its extracts according autobiography performed on thin-layer chromatographic plates.

In short, several plants from the Amazon forest having no known history of medicinal use have been shown to have inhibitory effects on platelet aggregation or hemolytic action. These data serve as a baseline or minimum of antiplatelet and hemolytic activities (and potential usefulness) of non-

Table 3 - Average percentage inhibition and minimum inhibition concentrations (MIC) of platelet aggregation for the most active extracts from the studied Amazonian plants. For each MIC determination, the inhibition is expressed as an average relative to that of the positive control (acetylsalicylic acid) in each agonist system.

Species	Part	Extract	Aggregation Agents (Avg % inhibition/MIC μg/mL)	
			ADR	COL
Chaunochiton kappleri	Branch	$CHCl_3$	98 / 25±4	99 / 17±7
Diclinanona calycina	Branch	CH_3OH	nd	97 / 25±4
Paypayrola grandiflora	Leaf	$CHCl_3$	98 / 25±6	nd
Pleurisanthes parviflora	Leaf	CH_3OH	nd	98 / 17±7

nd= not determined. ADR=adrenalin, COL=collagen.

medicinal plants from the Amazon forest. Additionally, these data may have use for comparison with results of planned studies on these activities in traditionally-used medicinal plants from the Amazon.

These and other plants are being screened by our lab in collaboration with others as a means to evaluate the unknown potential of the flora of the Amazon region. Chemical fractionation of the more active extracts is underway and is expected to reveal the chemical agents responsible for these biological activities.

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