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Original article

Plant species used in giardiasis treatment: ethnopharmacology and *in vitro* evaluation of anti-Giardia activity

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ABSTRACT

The aim of this study was to compile the traditional knowledge about plants used for the treatment of giardiasis, and also to carry out experimental research to evaluate the anti-Giardia activity of five species. To reach this objective, 398 interviews were performed using a previously prepared questionnaire, followed by an in vitro evaluation of giardicidal potential of hydroalcoholic leaf extracts of *Anacardium occidentale* L., *Chenopodium ambrosioides* L., *Passiflora edulis* Sims, *Psidium guajava* L., and *Stachytarpheta cayennensis* (Rich.) Vahl. Among the interviewed people, 55.53% reported the use of plants to treat diarrhea, the most severe symptom of giardiasis. The results indicated 36 species used by this population for these problems. The use of leaves (72.50%) of a single plant (64.25%) collected from backyards and gardens (44.34%) and prepared by decoction were predominant. The majority of the interviewees (85.52%) attributed their cure to the use of plants. In the experimental tests, all extracts inhibited the growth of *Giardia lamblia* trophozoites in different intensities: *A. occidentale* and *P. guajava* extracts elicited a moderate activity (250 ≤ IC_{50} ≤ 500 μg/ml), *C. ambrosioides* and *S. cayennensis* extracts evoked a high activity (100 ≤ IC_{50} ≤ 250 μg/ml), and *P. edulis* extract showed very high activity (IC_{50} ≤ 100 μg/ml). This study shows that an ethnopharmacological approach is useful in the selection of plant materials with potential giardicidal activity.

Introduction

Giardiasis is an intestinal infection caused by the flagellate protozoan *Giardia lamblia* (synonyms: *Giardia intestinalis* and *Giardia duodenalis*), with worldwide distribution, high prevalence, and significant morbidity (Rocha, 2003; Arani et al., 2008). Data from the World Health Organization report about 400 million new cases of *G. lamblia* infection per year (WHO, 2009). Since giardiasis is not a notifiable disease in Brazil, few prevalence records are available; however, several

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epidemiological studies document infection rates of up to 70.5%, indicating that it is a serious public health problem (Borges et al., 2011).

Clinical manifestations of giardiasis vary, however it's most predominant symptom, diarrhea, occurrs in 90% of symptomatic patients. Diarrhea can be acute and self-limiting, or chronic and debilitating, associated with abdominal pain, flatulence, dyspepsia, epigastric pain, nausea, vomiting, steatorrhea, low-lipid stools and fat-soluble vitamin absorption, and weight loss. The poor absorption of fats, carbohydrates, iron, and vitamins (A and B12) can delay physical and mental development, especially in younger individuals (Heresi et al., 2000; Lebwohl et al., 2003; Al-Mekhlafi et al., 2005).

The typical treatment for giardiasis is chemotherapy using one or more drugs, predominantly 5-nitroimidazole derivatives such as metronidazole, a first-line drug. Other nitroimidazoles (secnidazole, tinidazole, and ornidazole), benzimidazoles (albendazole, mebendazole), furazolidone, quinacrine, and benzimidazole derivatives have been used for giardiasis treatment. However, chemotherapeutic agents cause adverse reactions such as gastrointestinal disturbances, nausea, headache, leukopenia and parageusia; and may trigger neurotoxic effects as ataxia, seizures, and vertigo, leading to discontinuation of treatment. Moreover, mutagenic and carcinogenic effects have been observed in laboratory animals (Harris et al., 2001; Campanati and Monteiro-Leal, 2002; Petri-Jr, 2003; Andrade et al., 2010). Because of the side effects of conventional drugs and an increased parasite resistance to treatment, it is necessary to identify new effective and safe agents for the treatment of this infection (Upcroft and Upcroft, 2001; Sangster et al., 2002, Hernández and Hernández, 2009).

Natural resources, especially of plant origin, are important sources of new bioactive products, considering the wide variety and complexity of metabolites with potential therapeutic value (Pinto et al., 2002; Anthony et al., 2005; Gurib-Fakim, 2006; Oliveira et al., 2011).

The assessment of therapeutic potential aimed at developing herbal or phytochemical products requires validation of the plant species through ethnobotanical, ethnopharmacological, chemical, biological, pharmacological, and toxicological studies (Gilani and Rahman, 2005; Macêdo and Oliveira, 2006; Klein et al., 2009). Ethnobotanical and ethnopharmacological studies have been shown to provide important findings in the search for new active products of plant origin, effectively contributing to defining the inclusion and exclusion criteria for the selection of plant species and their subsequent validation (Gilani and Rahman, 2005; Patwardhan, 2005; Albuquerque and Hanazaki, 2006).

Considering the high global prevalence of giardiasis and the need for new therapeutic options, taking into account the ethnopharmacological approach in the research and development of drugs of plant origin, an ethnopharmacological survey was conducted to identify the plant species traditionally used to treat giardiasis in the municipality of São Luís, Maranhão State, Brazil. Afterwards, validation studies for anti-Giardia activities of the plant species were conducted.

Materials and methods

The study was approved by the Ethics Committee of the Federal University of Maranhão under protocol number 23115-012975/2008-43. Prior to data collection, the participants were asked to sign an informed consent form authorizing participation and dissemination of the data collected.

Ethnopharmacological study

Type of study

The first stage of the research was a descriptive, observational, and cross-sectional study. Structured and semi-structured interviews were used to collect ethnopharmacological data. The plants used traditionally by the study population were collected and identified. An experimental study was performed to assess the giardicidal activity in vitro of the extracts from the selected plants.

Study population

Data were collected from patients aged over 18 years and caregivers of children treated at a municipal public primary healthcare facility (Unit A) and private institution specializing indigestive system diseases (Unit B), both facilities located in the city of Sao Luis, Maranhão state, Brazil.

São Luís, the capital of Maranhão state, Brazil, located at 2° 33′ 00″ S and 44° 18′ 00″ 19′ W, has an area of 831.7 km2, an estimated 1,053,922 inhabitants, and population density of 1215.69 inhabitants/km² (IBGE, 2013).

On the basis of a previous ethnopharmacological giardiasis survey in São Luis, Maranhão, Brazil (Amaral, 2007), considering 30% prevalence of plant use, 5% error, and 90% confidence interval, we surveyed 398 patients and/or caregivers as the study subjects.

Collection and analysis of ethnopharmacological data

To collect ethnopharmacological data, we used structured and semi-structured interviews with open and closed questions. Interviews were conducted from January to March 2011, and focused on the plant species used to treat diarrhea and dysentery.

This approach was chosen since people find it difficult to recognize giardiasis, but they can easily identify the plants used to treat diarrhea, the predominant giardiasis symptom that occurs in 90% of symptomatic patients (Lebwohl et al., 2003; Al-Mekhlafi et al., 2005). Dysentery was the disorder used in the above approach because it was difficult for interviewees to differentiate diarrhea from dysentery.

The respondents that mentioned the use of plants were also questioned about the form(s) of preparation, plant part(s) used, origin of the plant material, source of information, knowledge about possible side effects and contraindications, as well as their socio-economic characteristics.

Collection and botanical identification

The species cited in the ethnopharmacological survey were collected from areas mentioned by the respondents, including urban landscapes (parks, squares, backyards, and vegetable and medicinal gardens) and the countryside. The voucher specimens were deposited in the Ático Seabra Herbarium (SLS), of the Federal University of Maranhao, São Luis, MA, Brazil. The specimens were authenticated by Prof. Terezinha J. A. Rêgo.

Biological analysis

Selection of plant species

From the ethnopharmacological survey, *Anacardium occidentale* L., Anacardiaceae, *Chenopodium ambrosioides* L., Amaranthaceae, *Passiflora edulis* Sims, Passifloraceae, *Psidium guajava* L., Mystaceae, and *Stachytarpheta cayennensis* (Rich.) Vahl, Verbenaceae, were selected to assess their giardicidal activity *in vitro*. The inclusion criteria were as follows: the plants most frequently used to treat diarrhea and dysentery; whether they were native or exotic, and if they had a wide distribution in the region. The exclusion criteria included: toxic plants, with validated giardicidal activity, endangered and/or not cultivated in the region.

Preparation of extracts

Leaves of *A. occidentale, C. ambrosioides, P. edulis, P. guajava,* and *S. cayennensis* were collected manually from their natural habitats, in the city of São Luís, Maranhão state, from May to July 2011.

The plant material of each species were dried separately in an air circulation oven at a temperature of 38°C, and then ground in a Wiley mill to obtain a moderately coarse powder (250-710 µm). The *A. occidentale* (70 g), *C. ambrosioides* (75 g), *P. edulis* (75 g), *P. guajava* (68 g), and *S. cayennensis* (72 g) plant material was extracted by maceration and percolation using 70% ethanol for a period of 15 days (in 3-day intervals) for both extractive processes.

The extraction solutions were concentrated under reduced pressure in a rotary evaporator. The dry residue was re-suspended in pH 7.2 phosphate buffered solution (PBS) to a final concentration of 5 mg/ml, or previously solubilized in dimethyl sulfoxide (DMSO) with a limit of 0.1% (Gillin et al., 1982). All solutions were sterilized by filtration through a 0.22-µm membrane, and stored in sterile bottles at 4 °C until analysis.

Evaluation of in vitro *anti-Giardia activity*

Axenic strains of G. lamblia (Portland-1; ATCC 30888) were maintained in TYI-S-33 medium in glass tubes, enriched with bovine bile and supplemented with inactivated bovine serum, at 37°C (Diamond et al., 1978; Keister, 1983). For maintenance and preservation of the strains, the cultures were examined daily under an inverted microscope to check the growth, activity, and degree of adherence of trophozoites to the tube walls. Subculturing was performed every 96 h, corresponding to the exponential phase of growth (Rocha, 2003; Amaral, 2007).

The giardicidal activity *in vitro* test was performed as described previously (Cedillo-Rivera and Munoz, 1992; Cedillo-Rivera et al.1992; Calzada et al., 1999), with modifications (Amaral, 2007). Aliquots of the stock solutions of leaf extracts of *A. occidentale, C. ambrosioides, P. edulis, P. guajava*, and *S. cayennensis* (5 mg/ml) were added to 1.5 ml Eppendorf® tubes and serially diluted in TYI-S-33 modified medium to final concentrations of 500, 100, 20, and 4 µg/ml. An inoculum containing 5×10^3 trophozoites/ml from a logarithmic growth phase culture was distributed in 1.5-ml Eppendorf® tubes.

After 72 h of incubation at 37°C quantitative evaluations were performed by indirect method (colorimetric method / MTT). The tests included a positive control (metronidazole) and negative control (TYI-S-33 and/or DMSO).

Statistical analysis

The ethnopharmacological data were formatted and analyzed using SPSS 18.0 for Windows, with descriptive analysis of data and presentation of frequency tables, followed by analysis using Fisher's exact test and χ^2 test of independence, with a significance level (α) of 5%.

In the evaluation of the *in vitro* giardicidal activity, the results were expressed as the growth inhibitory concentration (IC_{50}), calculated by linear regression using the GraphPad Prism 5.0 program. All samples were tested in triplicate and the experiments were performed twice (n = 6).

Results and discussion

In order to gain knowledge about plants widely used to treat *Giardia infection*, we focused our study on plants used against diarrhea and dysentery.

This study included 262 women (65.83%) and 136 men (34.17%). We interviewed 211 participants in Unit A and 187 in Unit B. We noted 55.53% prevalence for the use of plants for medicinal purposes. Although results shows a lower prevalence of use of plants compared to a previous study by Amaral (2007) (76.25%) also in São Luís, MA, the amount of plants observed in our sample is still considered high. It is noteworthy that respondents in Unit B, who were from a private health care institution with accredited/or private health care plans, used medicinal plants even though their prevalence rate was expected to be lower in this study than the reported in the above-mentioned report.

Considering that the area where this study was carried out is classified as a micro-region of a large urban cluster, the high percentage of plant users is surprising. Nevertheless, this finding is in accordance with the study of Camargo (2003), which reports that "medicinal plants" and "herbal remedies" are used by both urban and rural population, to a different extent, depending on the cultural environment.

The sample consisted of an adult population with an average age above 48 years old. The survey showed that the majority of respondents (75.12%) had at least complete secondary education. The economic profile of the respondents was determined using the economic classification criteria adopted by the Brazilian Association of Research Companies (ABEP, 2012), which showed that the majority of individuals belonged to class B (42.71%) and C (42.21%) of this classification (Table 1).

In the studied population sample, gender, education, and socio-economic status were statistically significant factors for the use of plants ($p < 0.05$) (Table 1). The prevalence of plant use by women for treating diarrhea and dysentery in

Table 1
Socio-economic and demographic data (gender, age, education level and social class) of the selected users of healthcare services from São Luis, Maranhão, Brazil, and their knowledge regarding medicinal plants used to treat diarrhea and dysentery.

Variables	Use of plants to treat diarrhea and dysentery				Total	
	No		Yes			
	n	%	n	%	n	%
Gender[a]						
Female	103	39.31	159	60.69	262	65.83
Male	74	54.41	62	45.59	136	34.17
Total	177	44.47	221	55.53	398	100.00
Age[b] (years)						
18–27	35	76.09	11	23.91	46	11.56
28–37	37	63.79	21	36.21	58	14.57
38–47	32	42.67	43	57.33	75	18.84
48–57	40	36.04	71	63.96	111	27.89
> 57	33	30.56	75	69.44	108	27.14
Total	177	44.47	221	55.53	398	100.00
Education						
Incomplete primary school	26	59.09	18	40.91	44	11.06
Complete primary school	21	36.21	37	63.79	58	14.57
Incomplete secondary school	28	38.89	44	61.11	72	18.09
Complete secondary school	51	40.80	74	59.20	125	31.41
Incomplete university	11	37.93	18	62.07	29	7.29
Complete university	35	59.32	24	40.68	59	14.82
Post-graduate	5	45.45	6	54.55	11	2.76
Total	177	44.47	221	55.53	398	100.00
Economic class[a]						
A	19	61.29	12	38.71	31	7.79
B1	40	57.14	30	42.86	70	17.59
B2	33	33.00	67	67.00	100	25.12
C1	46	42.20	63	57.80	109	27.39
C2	28	47.46	31	52.54	59	14.82
D	11	37.93	18	62.07	29	7.29
Total	177	44.47	221	55.53	398	100.00

n = 398, χ^2 Independence test.
[a]$p < 0.05$.
[b]$p < 0.001$.

this study may be explained by their role in the cultural and traditional representation of health and familial disease (Medeiros and Cabral, 2001; Almassy, 2004; Budó et al., 2008), as well as their therapeutic knowledge (Borba and Macedo, 2006) result of their important role as caregivers.

The frequency of plant use by elders demonstrated in this study is in agreement with the results of several national ethnopharmacological studies, reflecting the tendency of the accumulation of knowledge by this group, given the cultural preservation and traditional use of plants as a therapeutic resource. The high percentage of plant use among the elderly deserves attention, and health professionals should be able to guide its safe and rational use, minimizing the risk and dangers of this practice (Gama and Silva, 2006).

With regard to education level, the data of our study show that 31.41% of the respondents completed high school. This high prevalence of plant use in individuals with high education levels differs from other studies (Amaral, 2007; Pessoa and Cartágenes, 2010; Almeida et al., 2012), yet this data agrees with the study performed by Vieira (2011). Nevertheless, a higher education level of the respondents may reflect a greater purchasing power. It must be taken into consideration that the survey was conducted in a private health institution (Unit B), which may be indicative of the increased interest in phytotherapy in recent years (Borba and Macedo, 2006), as well as the implementation of the National Policy on Medicinal Plants and Phytopharmaceuticals (Decree 5.813/06) (Ministério da Saúde, 2006) and the National Policy on Integrative and Complementary Practices at the SUS (Public Health System) (Anvisa, 2006) expanded and globalized their access.

The economic profile of the respondents casts the economy from Maranhão as a Brazilian state with a low per capita income (IBGE, 2013), which is also in agreement with the study by Amaral (2007).

The plant material data indicated that the use of the leaves (72.5%) prepared by decoction predominated (49.32%), followed by infusion (32.58%), steeping (13.13%), and as syrup (4.98%). The predominance of leaf use can be explained by the belief that there is a greater concentration of the active ingredients in this plant part (Gonçalves and Martins, 1998), availability at all times of the year in most biomes and/or ease of harvesting (Castellucci et al., 2000). However, it is important to identify and select the correct part of the plant, since the distribution of active componds responsible for the expected therapeutic effect may vary between organs according to the species (Pinto and Santiago, 2000; Calábria, 2008).

The frequent use of decoction is observed in several ethnopharmacological studies. Oliveira et al. (2010) correlated the predominant mode of preparation and the use of heat, to assess the efficacy of the preparations obtained. Mosca and Loiola (2009) reported the practicality and speed of the preparation methods. Badke (2008) found that people considered baking as the best way to extract the chemicals that elicit healing properties, especially when a dry plant is used. However, the observation that heating is commonly used in homemade plant preparations shows a lack of knowledge about the losses and/or metabolic changes in the plant caused by the rise in temperature (Pascarelli et al., 2006), which demonstrates the need for guidance on the correct preparation of plant extracts.

Backyards and home gardens were the predominant places (44.34%) from which the plants were collected, followed by pharmacies (21.72%), markets and fairs (18.55%), and other areas (15.38%). Our findings are consistent with those of other local ethnopharmacological studies (Amaral et al., 2001; 2002; Pessoa and Cartágenes, 2010; Vieira, 2011; Silva, 2012). Nevertheless, we observed significantly more purchases in pharmacies compared with the results shown by Amaral (2007), which can be explained by a noted increase of commercially available natural products in the past years (unpublished data).

Our data on the number of plant species used during treatment show that the majority of people (64.25%) used single species in their preparations. When asked about plants used along with synthetic drugs, the majority of respondents (53.39%) specified that they used only plants. The result was similar to that by Amaral (2007), showing that although both studies were conducted in urban areas with greater access to allopathic medicines, the use of a single plant species not predominates.

Although studies confirm mixing plants for medicinal use is common practice in different cultures (Ming and Amaral Junior, 2003; Calábria et al., 2008; Veiga Junior, 2008), the scientific consensus points the lack of pharmacological studies regarding plant-plant and plant-synthetic drug interactions, and the harmful effects already observed and attributed to such associations (Albuquerque and Andrade, 2002; Albuquerque and Hanazaki, 2006; Oliveira and Dalla Costa, 2004; Arnous et al., 2005; Nicoletti et al., 2007; Veiga Junior, 2008).

Information obtained from relatives was the primary source of knowledge of plants used to treat diarrhea and dysentery (62.44%), followed by healers (18.55%), the media (15.38%), and health professionals (3.62%).

When asked about the credibility of treating diarrhea and dysentery using plants, the majority (85.52%) attributed the healing to herbal use. When questioned about their knowledge of the hazards or risks of using plants as a therapeutic resource, the majority of respondents (91.40%) indicated that they were not aware of risks.

The lack of knowledge on the hazards and risks of using plants as a therapeutic resource among respondents is worrying. This finding coupled with the fact that most plants available for therapeutic purposes in Brazil have not been validated, the poisoning potential of known toxic plants and poisoning because of the use of plant material of poor quality, either by inadequate dosage, identification, cultivation method, harvesting, storage, preservation, and preparation (Matos, 2000; Araújo and Ohara, 2000); processes that require the involvement of supervisory bodies, with a strong commitment with pharmacovigilance in phytotherapy (Silveira et al., 2008).

Among the plants most frequently mentioned by the respondents, 33 were identified by species and three by genus, and they were distributed in 26 families, predominantly Myrtaceae, Anacardiaceae, Lamiaceae, Verbenaceae, and Asteraceae (Table 2).

The species most frequently cited by the respondents for the treatment of diarrhea and dysentery are *Allium sativum* L., *A. occidentale* L., *Baccharis trimera* (Less) DC., *Jacaranda decurrens* Cham., *Bixa orellana* L., *C. ambrosioides* L., *Momordica charantia* L., *Eleutherine plicata* Herb., *Mentha* spp., *Musa* spp., *P. guajava* L., *Averrhoa carambola* L., *P. edulis* Sims., *Oryza sativa* L., *Malus* spp., and *Citrus sinensis* (L.) Osbeck (Table 2). Regarding these species, we noted that many plants identified in our study were also cited by other ethnobotanical and/or ethnopharmacological studies developed in different regions from Brazil, highlighting *A. occidentale* L. (Calábria et al., 2008; Pinto, 2008; Oliveira et al., 2010), *Eleutherine plicata* Herb. (Martins et al., 2005; Vallinoto et al., 2007), *P. guajava* L. (Vallinoto et al., 2007; Calábria et al., 2008; Oliveira, 2008; Pinto, 2008; Oliveira et al., 2010; Niehues et al., 2011; Nóbrega et al., 2011), *Byrsonima*

Table 2
Plants used by the respondents interviewed in health facilities in São Luís, Maranhão, Brazil, for the treatment of diarrhea and dysentery as classified by family, regional vernacular name, voucher number, part used, and number of citations.

Family	Botanical name	Regional vernacular name	Voucher number	Part used	Citations (N)[a]
Alliaceae	*Allium sativum* L.	alho	0996/SLS[b]	bulb	102
Anacardiaceae	*Myracrodruon urundeuva* Fr. All	aroeira	1056/SLS	bark	45
	Spondias lútea L..	cajá	1500/SLS	fruit/pseudofruit	66
	Anacardium occidentale L.	caju	1050/SLS	bark	105
Annonaceae	*Annona muricata* L.	graviola	0422/SLS	leaf	61
	Annona squamosa L.	ata	1109/SLS	bark	29
Arecaceae	*Orbignya phalerata* Mart.	babaçu	1022/SLS	fruit mesocarp	84
Asteraceae	*accharis trimera* (Less)	carqueja	0826/SLS	aerial part	92
	Matricaria chamomilla L.	camomila	1498/SLS	flower	39
	Achyrocline satureioides (Lam.) DC.	macela	1494/SLS	flower	41
Bignoniaceae	*Jacaranda decurrens* Cham.	carobinha	1140/ SLS	leaf/ bark	141
Bixaceae	*Bixa orellana* L.	urucum	0815/SLS	seed	98
Chenopodiaceae	*Chenopodium ambrosioides* L.	mastruz	1148/SLS	leaf	149
Cucurbitaceae	*Jacaranda decurrens* Cham.	carobinha	1140/ SLS	leaf/ bark	141
Equisetaceae	*Equisetum giganteum* L.	cavalinha	1501/SLS	leaf	53
Euphorbiaceae	*Jatropha gossypiifolia* L.	pião-roxo	1006/SLS	leaf	64
	Julocroton triqueter (Lam.) Didr. var. *triqueter*	velame	1265/SLS	leaf	55
Fabaceae	*Caesalpinia férrea* Mart.	jucá	0834/SLS	stem bark	36
Iridaceae	*Eleutherine plicata* Herb.	coquinho	1131/SLS	leaf/stem	183
Lamiaceae	*Ocimum basilicum* L.	majericão	1008/SLS	leaf	39
	Mentha spp.	hortelã	1062/SLS	leaf	168
Malpighiaceae	*Byrsonima variabilis* A. Juss	murici	1274/SLS	leaf	19
Malvaceae	*Gossypium hirsutum* L.	algodoeiro	1115/SLS	leaf/seed	75
Musaceae	*Musa* spp.	banana	1091/SLS	leaf/fruit	212
Myrtaceae	*Eugenia uniflora* L.	Eugenia uniflora L.	0999/SLS	leaf/fruit	64
	Psidium guajava L.	Psidium guajava L.	0528/SLS	leaf/fruit bud	209
	Syzygium jambolanum DC.	Syzygium jambolanum DC.	1079/SLS	leaf	36
Nyctaginaceae	*Boerhavia diffusa* L.	pega-pinto	1070/SLS	root	56
Oxalidaceae	*Averrhoa carambola* L.	carambola	0561/SLS	leaf	109
Passifloraceae	*Passiflora edulis* Sims.	maracujá	1155/SLS	leaf	115
Poacea	*Oryza sativa* L.	arroz	1095/SLS	seed	202
Rosaceae	*Malus* spp.	maçã	1497/SLS	fruit	206
Rubiaceae	*Spermacoce verticillata* L.	vassourinha-de-botão	1493/SLS	aerial part	54
Rutaceae	*Citrus sinensis* (L.) Osbeck.	laranja	1014/SLS	fruit	101
Verbenaceae	*Lippia alba* (Mill) N. E. Brown.	erva-cidreira	1122/SLS	leaf	89
	Stachytarpheta cayenensis (L.C.Rich.) Vahl	gervão	1081/SLS	leaf	106

[a]The interviewees referred to more than one plant for the treatment of diarrhea and dysentery.
[b]ÁticoSeabra Herbarium (SLS) of the Federal University of Maranhão, São Luis, MA, Brazil.

verbascifolia Rich ex. Juss (Agra et al., 2007; Boscolo and Valle, 2008; Oliveira et al., 2010), *Eugenia uniflora* L. (Calábria et al., 2008; Costa and Nunes, 2010), and *Lippia alba* (Mill) N. E. Brown. (Calábria et al., 2008; Oliveira, 2008; Oliveira et al., 2010; Tavares et al., 2011).

However, we found that some of the referred species such as *Allium sativum* L., *Annona squamosa* L., *Caesalpinia ferrea* Mart., *C. ambrosioides* L., *Hymenaea courbaril* L., *Mentha* spp., *Momordica charantia* L., *Ocimum basilicum* L., and *S. cayenensis* (Rich.) Vahl (Table 2) have been reported for the treatment of intestinal parasites and gastrointestinal disorders. These health hazards are frequently accompanied by symptoms like diarrhea and dysentery, and therefore may represent a use agreement.

The relative IC_{50} values of hydroalcoholic leaf extracts of *A. occidentale, C. ambrosioides, P. edulis, P. guajava*, and *S. cayennensis* obtained by maceration and percolation on the growth of *G. lamblia* trophozoites were determined using an indirect cell quantification method (Table 3).

The results show that all extracts of the selected species had an inhibitory effect on the growth of *G. lamblia* trophozoites. According to the classification criteria established by Amaral et al. (2006), the *in vitro* giardicidal activity was moderate for *A. occidentale* and *P. guajava* ($250 \leq IC_{50} \leq 500$ µg/ml), high for *C. ambrosioides* and *S. cayennensis* ($100 \leq IC_{50} \leq 250$ µg/ml), and very high for *P. edulis* ($IC_{50} \leq 100$ µg/ml). The extraction method did not influence the giardicidal activity IC_{50} values (Table 3).

A study performed using extracts rich in polyphenols showed significant giardicidal activity attributed to these metabolites (Anthony et al., 2011); and, according to Trabulsi Filho et al. (2013), the extract of *A. occidentale* studied in this paper has polyphenols as major constituents. Thus, these compounds may be probably considered responsible for the moderately active giardicidal properties found in this study.

An *in vitro* giardicidal evaluation of a methanolic extract of the aerial parts of *C. ambrosioides* collected in Mexico indicated an IC_{50} of 116.10 µg/ml (Calzada et al., 2006). Our evaluation of the *in vitro* giardicidal activity of a hydroalcoholic extract of the leaves of this species collected in São Luís, Maranhão state, showed an IC_{50} from 198.18 to 214.16 µg/ml. Thus, on the basis of the criteria adopted in this study, our study confirmed the *in vitro* giardicidal activity for the species. The difference in trophozoite growth inhibition in these two studies may be related to variables that interfere with the synthesis of secondary metabolites, the place and time of collection, the age of the plant, the solvent used, the strain of *G. lamblia*, and the test model.

We did not find any studies on the anti-parasitic, especially anti-*Giardia*, activity of *P. edulis* and/or other species of Passifloraceae. However, our results showing IC_{50} from 75.13 to 77.28 µg/ml may result from the presence of flavonoids, especially rutin and luteolin glycosides, chemical constituents with anti-*Giardia* activity, as described in other species (Calzada et al., 2001; 2003; 2005).

The extracts of *P. guajava* assessed in this study showed a moderate activity, but a study by Ponce-Macotela et al. (1994) indicated giardicidal potential of this species. This difference in the cytotoxicity profile may be the result of

Table 3
In vitro activity against *Giardia lamblia trophozoites* (Portland 1 strain, ATCC 30888), expressed as relative growth inhibitory concentration (IC_{50}) values of the hydroalcoholic extracts of the leaves of *Anacardium occidentale* L., *Chenopodium ambrosioides* L., *Passiflora edulis* Sims., *Psidium guajava* L., and *Stachytarpheta cayennensis* (Rich.) Vahl. obtained by maceration and percolation.

Plant species selected/	Extraction procedure	Giardicidal activity IC_{50} (µg/ml)
Anacardium occidentale L.	maceration	378.88 ± 19.63[a]
	percolation	399.23 ± 21.13[a]
Chenopodium ambrosioides L.	maceration	214.16 ± 5.02[a]
	percolation	198.18 ± 4.28[a]
Passiflora edulis Sims.	maceration	77.28 ± 1.52[a]
	percolation	75.13 ± 1.41[a]
Psidiumguajava L.	maceration	457.91 ± 25.06[a]
	percolation	439.83 ± 24.11[a]
Stachytarpheta cayennensis (Rich.) Vahl.	maceration	120.93 ± 2.54[a]
	percolation	118.75 ± 2.50[a]

[a]Results are expressed as the mean number of viable *Giardia lamblia* trophozoites ± standard deviation (SD) in hydroalcoholic extracts of *Anacardium occidentale, Chenopodium ambrosioides, Passiflora edulis, Psidium guajava*, and *Stachytarpheta cayennensis* leaves obtained by maceration and percolation with hydromodule ratios of 1:6, 1:5, 1:8, 1:5, and 1:5, respectively. Metronidazole (IC_{50} 0.22 µg/ml) was used as a positive control. Identical letters indicate equal values.

variables between the studies. Studies performed by Calzada et al. (1999; 2005) showed that the flavonoid quercentin, isolated from different species, has high giardicidal activity, thus , the presence of flavonoids in *P. guajava*, may likely explain the activity found in these studies.

Moreira et al. (2007) showed the leishmanicidal activity of hydroalcoholic extracts of dried leaves of *S. cayennensis* against *Leishmania braziliensis* and *Leishmania amazonensis* promastigotes. Another study by Ordóñez al. (2001) documented the *in vitro* giardicidal activity of hydroalcoholic extract of *Stachytarpheta jamaicensis* (L.) Vahl., with proven growth inhibition of *G. lamblia* trophozoites, compared with other species of the family Verbenaceae (Tapia-Pérez et al., 2003; Calzada et al., 2006; Ponce-Macotela et al., 2006). Our analysis of the giardicidal activity of *S. cayennensis*, along with the evidence of anti-protozoan activity in other *in vitro* studies using species of the same genus and family, is indicative of the contribution of a chemotaxonomic approach as a valid strategy in the search for bioactive substances, which should stimulate further studies.

The selection of plant species for anti-*Giardia* activity validation studies, based on an ethnopharmacological approach is useful in the search for new therapies to combat the disease with a marked morbidity in the region.

Authors' contributions

VAN contributed to the harvest of plant material, conduction and analysis of ethnopharmacological data, as well as carried out all the experimental investigation of the plants selected for this study. FMMA, FRFN e MNSR designed the study, working on the development of it. MSSR worked on the biological evaluation, and data analysis. DFCM contributed to the harvest of plants, taxonomic identification, confection of herbarium vouchers and analyses. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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