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Full Length Research Paper

In vivo antitumor effect of the aqueous extract of *Punica granatum* in the sarcoma 180 murine model

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Punica granatum, **specifically the fruit, has a long ethno medical history and is a phytochemical reservoir of great medicinal value. The phytochemistry and pharmacological actions of all *P. granatum* components suggest a wide range of clinical applications. The aim of the present study is to investigate the anticancer potential of aqueous extract of *P. granatum* (AEPG) in experimental models. The chemical composition of the AEPG was assessed by HPLC-DAD. In vivo antitumor activity was assessed in sarcoma 180 bearing mice. To evaluate the toxicological aspects related to the AEPG treatment, hematological, biochemical, histopathological and morphological analyses of treated animals were performed. Gallic acid, punicalagin α, punicalagin β, and ellagic acid were identified as the major phytochemical compounds of the extract. AEPG and 5-fluorouracil (5-FU) induced significant inhibition of tumor growth when compared with saline ($p < 0.05$). The percentage of apoptotic cells was significantly increased in 5-FU ($p < 0.01$) and AEPG treated groups ($p < 0.01$). No significant difference was observed between 5-FU and the three doses of AEPG. 5-FU induced toxic effects, such as decrease of body weight, splenic atrophy, and leukopenia, but these effects were not found in AEPG treated groups. The results provide evidence that AEPG exhibits comparable antitumor effects as 5-FU in a murine model, likely the result of increased apoptotic rate, but with no remarkable side effects presented by 5-FU.**

Key words: Cancer, chemotherapy, *Punica granatum*, sarcoma 180.

INTRODUCTION

Cancer is a disease characterized by uncontrolled multiplication of subtly modified normal human cells (Mubeen et al., 2012). An exceptionally difficult problem in cancer treatment is multidrug resistance, when cancer

cells lose their sensitivity to multiple structurally different chemotherapeutics, leading to the search for alternative treatments, such a medicinal plants (Amaral et al., 2015). Plants have a long history of use in the treatment of cancer and the interest in nature as a source of potential chemotherapeutic agents continues. The present day research and development tailored towards the discovery of new antiproliferative agents from natural products have been buoyed by improvement in the science and technology of anticancer drug discovery (Akindele et al., 2015).

Punica granatum is a deciduous tree belonging to the family Punicaceae and contains hydrolysable tannins as major active chemical constituents, that is, punicalagin, punicalin, gallic acid, ellagic acid, and ellagic acid derivative (Akhtar et al., 2015; Sing et al., 2018). There is extensive literature about bioactive compounds from its fruit (pomegranate), showing important biologic activities, such as healing (Zekavat et al., 2016), antimicrobial (Mohammad et al., 2016), chemopreventive (Bishayee et al., 2011) and antitumor effects (Panth et al., 2017).

Several studies have demonstrated that natural compounds such as tannins have a wide variety of biologic functions that are mainly related to modulation of carcinogenesis and antiproliferative effects, such as antioxidant and proapoptotic activities (Dilkmen et al., 2011). Furthermore, these compounds are generally safe, with low toxicity, and receive general acceptance (Fresco et al., 2006).

Apoptosis plays an important role in elimination of tumor cells by chemotherapeutic agents (Hassan et al., 2014). According to Dai and Mumper (2010) apoptosis-inducing compounds are expected to be ideal anticancer drugs due to their ability to promote DNA damage in tumor cells, which are then rapidly recognized by macrophages and removed without inducing an inflammatory response.

In vitro studies have already described the positive results of treatment with *P. granatum* extract, with apoptosis in many cell lines, such as colon cells-SW620, HT-29, and HCT-116 (Joseph et al., 2013), prostate cells-DU145, PC3, mouse prostate cancer cell TRAMP-C1 (Deng et al., 2017; Deng et al., 2018), lung cells-A549, H1299 (Li et al., 2016), and breast cells-MCF-7 (Shirode et al., 2014; Chen et al., 2015). However, in these models, the systemic effect of these compounds could not be assessed.

The aim of this study was to evaluate the antitumor activity of the aqueous extract of *P. granatum* (AEPG) in mice transplanted with sarcoma 180. Hematological, biochemical, histopathological and morphological analyses of the tumor and the organs, including liver, spleen and kidney, were performed to evaluate the toxicological aspects of the treatment.

MATERIALS AND METHODS

Plant

Fruits of *P. granatum* were collected in Petrolina, PE, Brazil (09°23'34"S, 40°30'28"W) in September 2011. Samples were identified and a voucher specimen (ASE 20881) has been deposited in the herbarium of the Department of Biology, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil.

Extraction procedure and sample preparation

The fruits were washed with tap water and pulp samples were obtained. The peel was dried at 55°C and uniformly powdered. The extraction was carried out by dynamic maceration using boiling water 1:100 (w/v) as a solvent for 2 h. The suspension was filtered and the solvent was removed in a circulating air stove at 50 ± 5°C for 48 to 72 h. The percentage of extraction yield was 63.4%, calculated in terms of dry weight.

For HPLC analysis, the crude extract of *P. granatum* (AEPG) was solubilized in a mixture of water: methanol (1:1 v/v) (1 mg/mL), filtered through a 0.45 µm membrane (Millipore, Merck-Billerica, MA, USA) and an aliquot of 10 µL was injected into the chromatographic system.

Apparatus and chromatographic conditions

The HPLC analyses were performed on a Shimadzu liquid chromatograph (Tokyo, Japan), equipped with a LC-6AD pump, an SPD-M20A diode array detector (DAD), and operated with the LC Solution data station software (Shimadzu, Tokyo, Japan). The water used in experiments was obtained with the Millipore (São Paulo, Brazil) Milli-Q purification system. Analysis was carried out on the analytical C18 Luna column (250 × 4.6 mm, 5 µm, Phenomenex, Torrance, CA, USA) with the following conditions: flow rate 1 mL/min and mobile phase consisting of 0.1% aqueous phosphoric acid (v/v, A) and acetonitrile (B). The gradient program was: 1 to 5% B at 0 to 5 min, 5 to 8% B at 5 to 10 min, 8% B at 10 to 16 min, 8 to 25% B at 16 to 22 min, 25 to 90% B at 22 to 27 min, 90 to 1% B at 27 to 33 min. The chromatogram was monitored at 260 nm. Quantification was achieved using the linear calibration curves of gallic acid (1 to 10 µg/mL) and ellagic acid (1 to 5 µg/mL) standards.

In vivo antitumoural assay

Animals

Sixty Swiss mice (male, 20 ± 2 g) were obtained from the central biotery of the Tiradents University (Aracaju, Brazil). The animals were housed in cages with free access to food and water. All animals were maintained under controlled temperature (25 ± 2°C) and relative humidity (50 ± 5%), with a 12h:12 h light-dark cycle (lights on at 6:00 a.m.). The experiments were conducted after approval of the protocols by the Institutional Ethics Committee

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Table 1. Distribution of the animals into the experimental groups according to treatment after transplantation of 2 x 10^6 sarcoma 180 cells/0.5 mL.

Group	Treatment (0.2 mL i.p. administration)
Saline	Saline
5-FU	25 mg/kg of 5-fluorouracil
PG10	10 mg/kg of AEPG
PG25	25 mg/kg of AEPG
PG50	50 mg/kg of AEPG

i.p.: Intraperitoneal administration; AEPG: aqueous extract of *P. granatum* at 10 mg/mL.

(021113) of the Tiradents University (Aracaju, Brazil) and were carried out in accordance with the current guidelines for the care of laboratory animals.

Determination of the effect of the AEPG on the growth of solid tumors in mice

The in vivo antitumor effect was evaluated using sarcoma 180 ascites tumor cells according to the method described by Bezerra et al. (2006). Ten-day-old sarcoma 180 ascites tumor cells (2 × 106 cells per 500 µL) were implanted subcutaneously into the left hind groin of the experimental mice. One day after inoculation, the AEPG was suspended in saline (vehicle) at final concentration of 10 mg/mL and administered intraperitoneally (10, 25 and 50 mg/kg) once a day for seven consecutive days. The negative control was injected with saline solution and the positive control was injected with 5-fluorouracil (5-FU, purity > 99%; Sigma Chemical Co., 25 mg/kg). At the beginning of the experiment, the mice were divided into five groups (n = 12 animals/group) shown in Table 1. Body weight and food and water intakes were measured daily over the time course of the experiment. On the 8th day, peripheral blood samples were collected from the orbital plexus of the mice while under light ether anesthesia and submitted to further hematological and biochemical analyses. The animals were then sacrificed in a CO2 chamber. The tumors, livers, spleens, and kidneys were excised, weighed, and examined for morphology. Then, they were fixed in 10% formaldehyde for histological analysis. The inhibition ratio (%) was calculated by the following formula: inhibition ratio (%) = ((A – B) / A) × 100, where A is the average tumor weight of the vehicle group and B is the average tumor weight of the treated group.

Systemic toxicology analysis

Determination of the effect of the AEPG on body and organ weight: Body weights were determined at the start and on the last day of treatment, and the animals were observed for signs of abnormalities throughout the study.

Tumor, livers, kidneys and spleens were dissected, weighed and observed for any signs of gross lesions or color changes and hemorrhages.

Determination of the effect of the APEG on biochemical parameters: After fasting for 6 to 8 h, the animals were submitted to blood collection from the orbital plexus for biochemical analysis (urea and creatinine to investigate any renal function alterations; AST and ALT as liver parameter). The analysis was carried out in semi-automatic equipment (Bioplus 200®), using enzymatic colourimetric kits (Labtest®).

Determination of the effect of the APEG on hematological parameters: After fasting for 6 to 8 h, the animals were submitted to blood collection from the orbital plexus for hematological analyses. To determine hematological parameter, an automated blood cell counter was used (Sysmex America, Inc., USA). The total count as well as differential counts of leukocytes, including eosinophils, lymphocytes, neutrophils and monocytes was performed using optical light microscopy after staining with Pappenheim's method.

Histopathology and morphological observations

The tumors, spleens, liver and kidneys were fixed in 10% formaldehyde (pH 7.4), dehydrated in alcohol, and diaphanized in xylene and paraffin-embedded.

Subsequently, 7 µm thick histological sections were obtained and stained with hematoxylin and eosin. Histological analyses were performed under light microscopy.

Terminal deoxyuridine nick-end labeling (TUNEL) staining

The number of apoptotic cells was assessed by the TUNEL technique described by Woodside et al. (2003). Histological sections (7 µm thick, n = 3) were obtained from the paraffin-embedded tissue and incubated using an in situ cell death detection kit, POD (Roche Diagnostics, Indianapolis, IN, USA). At first, the sections were deparaffinized in xylene (three changes at 3 min intervals with air-drying in between each change for better section adherence), rehydrated in graded alcohol (99, 95 and 70%) for 3 min each, and washed with deionized water. Then, the samples were treated with proteinase K (20 µl/ml in PBS) to digest the proteins, and endogenous peroxidase activity was quenched with 2% H2O2 in PBS for 10 min at room temperature. Thereafter, sections were washed with 50 µl PBS buffer, diluted TdT enzyme solution was applied, and the sections were incubated at 37°C in a humidified chamber for 1 h. After incubation, the sections were washed again with PBS buffer. Subsequently, 50 µl of antidigoxygenin peroxidase was added, and the sections were incubated in a humidified chamber for 30 min at room temperature. Once more, the sections were washed with PBS, and diaminobenzidine (DAB)-hydrogen peroxide was used for color development. For negative controls, the TdT enzyme was replaced with PBS on one section on each slide and was processed in parallel. Counterstaining of nuclei was performed with 2% Meyer's hematoxylin and mounted for examination. Apoptotic cells were identified as cells with brown-stained nuclei or as apoptotic bodies (fragments of apoptotic cells engulfed by neighboring cells). The number of TUNEL-positive cells was determined in 1000 counted cells, and the apoptosis ratio (AR) was calculated according to the

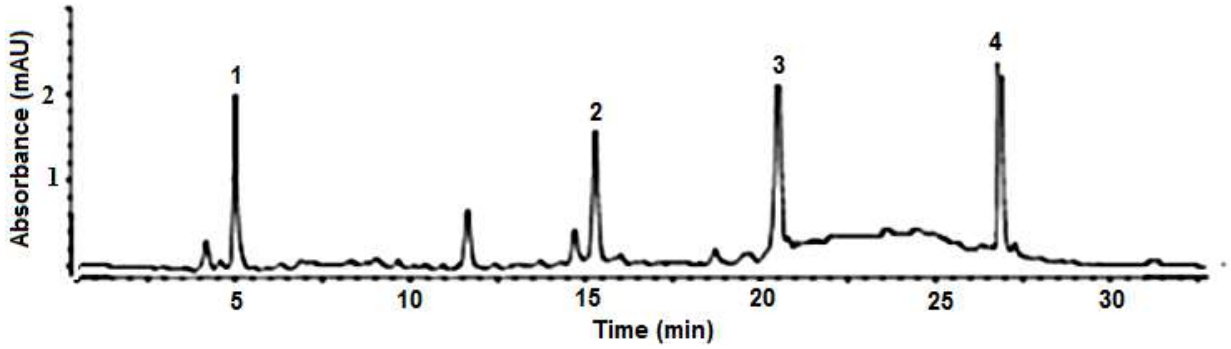

Figure 1. Chromatography of the aqueous extract of *P. granatum*. The peaks represent gallic acid [1], punicalagin α [2], punicalagin β [3], and ellagic acid [4] at 260 nm.

following equation: AR (%) = (TnP/1000) × 100, where AR is the apoptosis ratio and TnP is the number of TUNEL-positive cells.

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM) or as a percentage of the saline group. Subsequently, data were assessed by one-way analysis of variance (ANOVA) followed by post hoc Tukey-Kramer multiple comparison test. The values at $p < 0.05$ were considered to be statistically significant.

RESULTS

HPLC-DAD analysis

The HPLC-DAD analysis of the AEPG revealed the presence of four major peaks (Figure 1) which were identified according to retention times, UV and comparison with authentic samples as gallic acid [1], punicalagin α [2], punicalagin β [3], and ellagic acid [4]. The ellagic and gallic acids were quantified using external standard method, which presented concentrations of gallic acid and ellagic acid in the sample were 32.24 and 41.67 mg/g, respectively.

In vivo anti-tumor activity of the AEPG

The effects of the AEPG on mice transplanted with sarcoma 180 tumors cells are as shown in Figure 2. As shown in Figure 2A and B, the tumor weight and volume of 5-FU-treated animals were significantly lower than those of the saline group ($p < 0.001$). Similarly, intraperitoneal administration of AEPG (10, 25, and 50 mg/kg) also reduced significantly the average weight and volume of the tumors ($p < 0.001$). The reductions of tumor weight and volume obtained with the treatment with AEPG at 10 and 50 mg/kg were statistically similar to that obtained with 5-FU ($p > 0.05$). Moreover, there was no significant difference in the average tumor weight and volume between the AEPG-treated groups ($p > 0.05$). No significant difference in the tumor growth IR was observed between 5-FU and treatment with 10 and 50 mg/kg AEPG ($p > 0.05$) (Figure 2C). However, the IR for 25 mg/kg AEPG was significantly lower than that for 5-FU ($p < 0.05$). Assessment of the average tumor weight and tumor growth IR indicated that the response of sarcoma 180 growth to treatment with AEPG was not dose-dependent.

Post-mortem analysis of the tumors revealed similar histopathologic features in all groups (Figure 3). The tumors were characterized by neoplastic sheets of small polygonal and ovoid cells compactly arranged in some areas but loosely disposed in others. Tumor cells often invaded and dissociated lobules of adipose tissue and striated skeletal muscle bundles. Most of the tumor cells exhibited strongly eosinophilic cytoplasm and round-shaped hyperchromatic and moderately pleomorphic nuclei, but sometimes the nuclear chromatin was disperse and presented prominent nucleoli. Typical and atypical mitotic figures were often found (2 to 3 mitoses/histologic field at 400× magnification) among the neoplastic parenchyma. In addition, extensive areas of coagulative necrosis and a mild to moderate inflammatory response composed of lymphocytes andneutrophils were also observed. Vascular and perineural invasion were rare histologic findings in all groups, regardless of the treatment applied to the animals.

Apoptotic cell death was also detected using TUNEL assay. Positive labeling was identified by brownish color of the nucleus, regardless of the intensity of the staining (Figure 4). TUNEL-positive cell count varied considerably in all groups (Figure 5), but the average percentage was significantly increased in the groups treated with 5-FU ($p < 0.01$) and AEPG at the doses of 10 mg/kg ($p < 0.01$), 25 mg/kg ($p < 0.05$), and 50 mg/kg ($p < 0.001$). No significant difference was observed either between 5-FU and the three doses of AEPG or between the groups treated with AEPG.

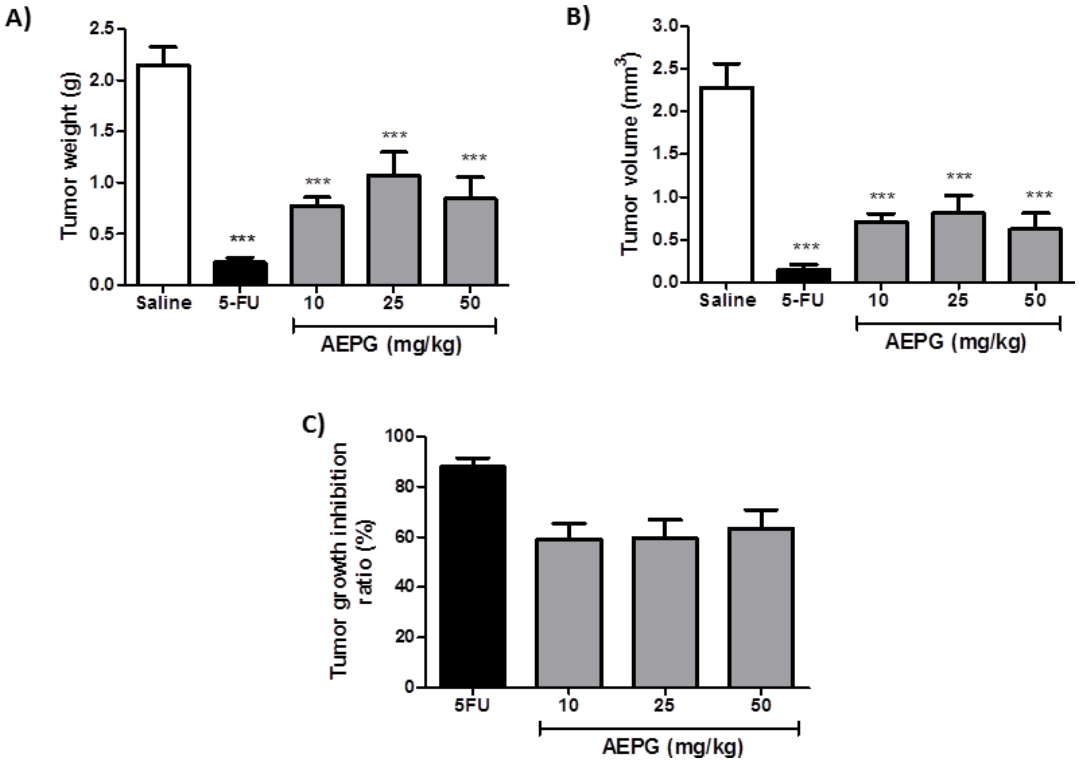

Figure 2. Assessment of the (A) average tumor weight and (B) tumor volume of the different test groups. (C) Assessment of the average tumor growth inhibition ratio of the different test groups (in relation to tumor weight of the saline-treated group). Data are expressed as mean ± standard error of the mean (SEM). *** Statistically significant difference ($p < 0.001$) in comparison with the saline-treated group; # statistically significant difference ($p < 0.05$) in comparison with the 5-fluorouracil-treated group; * statistically significant difference ($p < 0.05$) in comparison with the 5- fluorouracil-treated group (ANOVA and Tukey test).

Systemic toxicological evaluation

No behavioral changes were observed in the animals treated either with AEPG or 5-FU, and no remarkable changes in the intake of food and water were observed, regardless of treatment. Figure 6A shows the body weight of the animals at the beginning and end of the experiment. No significant differences in body weight between the groups were observed at the beginning of the experiment ($p > 0.05$), but at the eighth day, the body weight of the mice treated with 5-FU was significantly lower than that of the other groups ($p < 0.05$). Furthermore, as shown in Figure 6B, the average percentage of weight loss (in relation to the initial body weight) was significantly greater in the group treated with 5-FU than in the others ($p < 0.05$).

Table 2 shows the results of the biochemical parameters. Serum levels of aspartate aminotransferase (ALT) significantly increased in the group treated with 50 mg/kg AEPG in comparison with the saline-treated group ($p < 0.001$). Creatinine levels were significantly decreased in the groups treated with 25 mg/kg AEPG ($p < 0.001$) and 50 mg/kg AEPG ($p < 0.01$), as well as in the 5-FU-treated group ($p < 0.05$), again in comparison with the group treated with saline. Despite the significant differences found in these data, the values were within the normal interval established by the experimental research support database of the Biotery of the Tiradentes University.

Analysis of the hematologic parameters revealed no significant changes in the erythrogram data (Table 3, $p > 0.05$). However, as shown in Table 4, treatment with 5-FU induced a significant decrease in the total leukocyte count ($p < 0.01$) and relative neutrophil count ($p < 0.001$).

In addition, a relative increase in the differential count of eosinophils was observed ($p < 0.001$). In contrast, the administration of AEPG induced an increased neutrophil count at the three doses of 10 mg/kg ($p < 0.05$), 25 mg/kg ($p < 0.001$), and 50 mg/kg ($p < 0.05$), without causing expressive leukocytosis.

Pathological examination of the organs removed on a gross basis revealed that livers and spleens of the 5-FU-treated group showed an opaque surface tissue that was not present in the other groups, but no gross difference was detected between the experimental groups regarding the shape and consistency. Furthermore, a significant reduction of the average weight of the spleen in relation to that of the saline group was observed in the 5-FU-

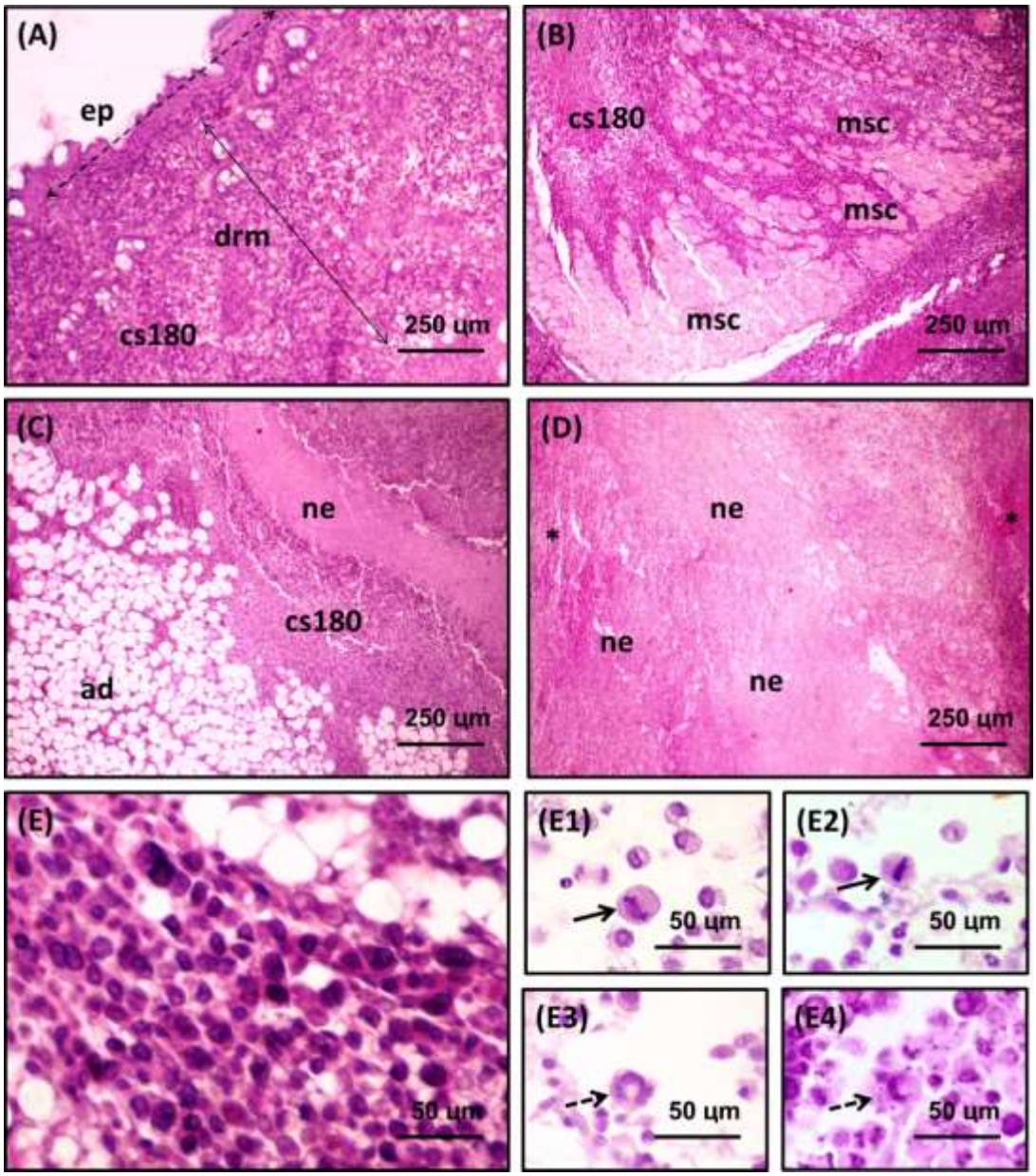

Figure 3. Photomicrographs of sarcoma 180 histologic sections stained with HE. (A) Tumor cells of sarcoma-180 (cs180) proliferating below the epidermis (ep, dashed double-headed arrow), deeply invading the underlying dermis (drm, solid double-headed arrow). (B) Tumor cells invading and dissociating bundles of skeletal muscle fibers (msc). (C) cs180 invading the hypodermic adipose tissue (ad), and causing foci of parenchymal coagulative necrosis (ne). (D) Extensive areas of coagulative necrosis (ne) limited by viable tumor cells (*). (E) Detail of tumor parenchymal component showing intense cell pleomorphism and hyperchromatic nuclei. (F1 and F2) typical and (F3 and F4) atypical mitotic figures (HE, A–D, 100× magnification, E–F, 800× magnification).

treated animals ($p < 0.05$), but not in the AEPG groups ($p > 0.05$) (Table 5).

Histologic analysis revealed that the architectural and cellular appearances of the organ tissues were comparatively unremarkable in all groups (Figure 7), except for the spleen samples from the 5-FU-treated group, which displayed atrophy of the white pulp (Figure 8).

DISCUSSION

Natural products play a relevant role in cancer therapy, with a substantial number of natural anticancer agents

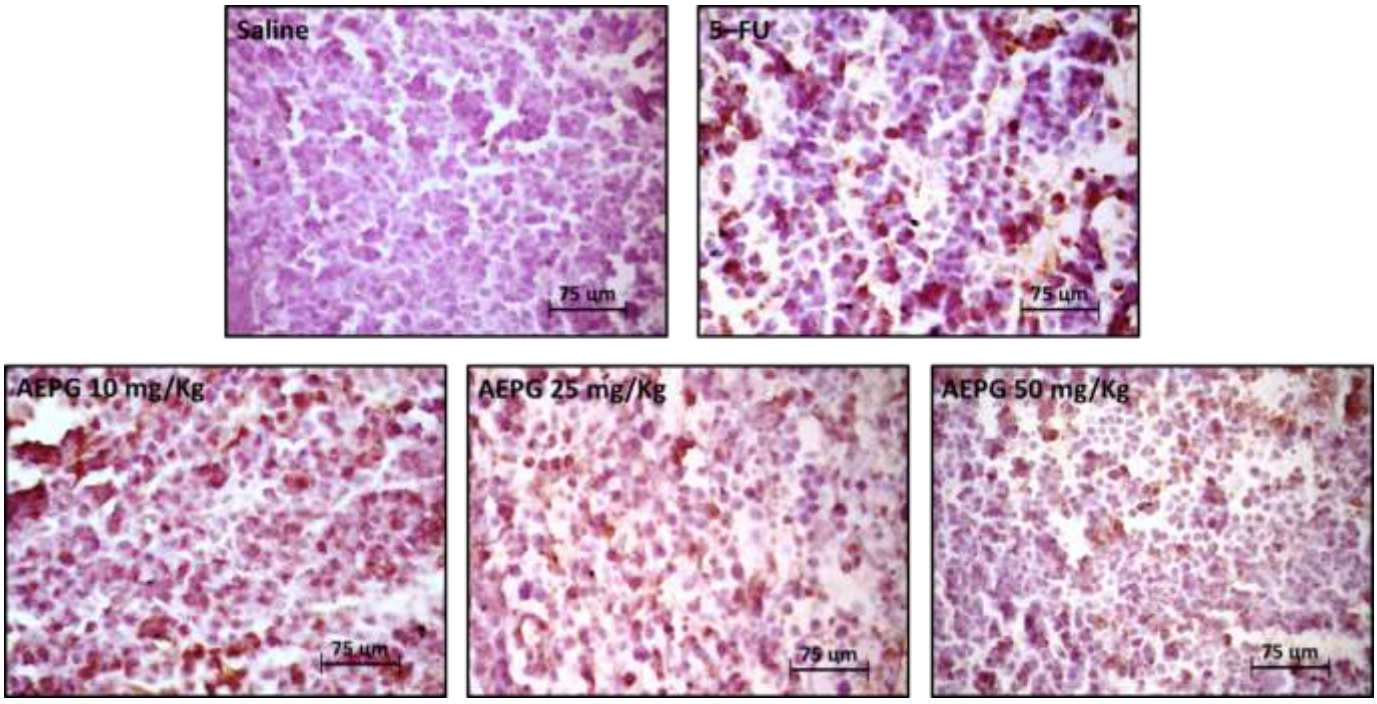

Figure 4. *In situ* apoptotic cell labeling by the terminal desoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method of paraffin-embedded histologic sections of sarcoma 180 tumors treated with saline, 5-fluorouracil (5-FU), and 10, 25, and 50 mg/kg aqueous extract of *P. granatum* (AEPG10, AEPG25, and AEPG50, respectively). TUNEL-positive apoptotic tumor cells show brown-stained nuclei. Note the scarce labeling in the saline-treated group and intense staining in the 5-FU and AEPG-treated groups (original magnification 800×).

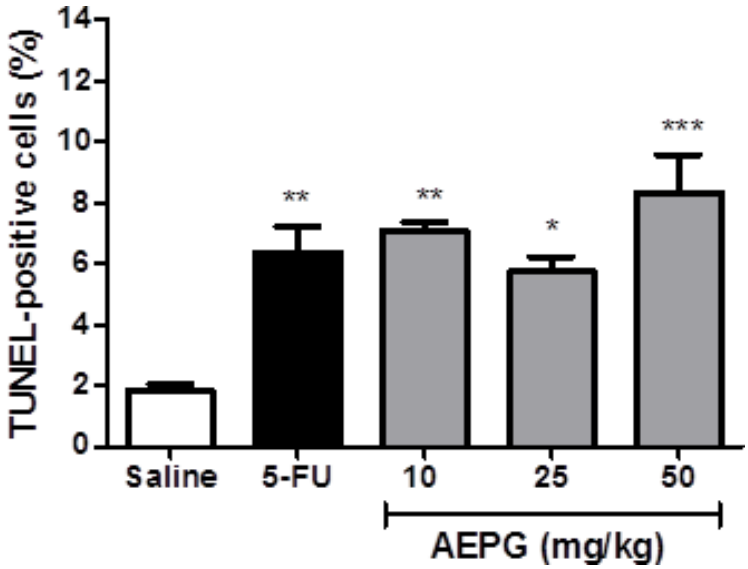

Figure 5. Assessment of the average percentage of TUNEL-positive cells. Data are expressed as mean ± standard error of the mean (SEM). Statistically significant differences between the values are expressed as *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$), in comparison with the saline-treated group (ANOVA and Tukey test).

(Rayan et al., 2017; Blowman et al., 2018; Cui et al., 2018). Furthermore, because cancer treatments have become more aggressive during the last 20 years, the need for new methods to manage adverse and/or side

Table 2. Effect of an aqueous extract of *P. granatum* (AEPG) and 5-fluorouracil (5-FU) on the biochemical parameters of mice subjected to sarcoma 180 cell transplantation.

Drug	Dose (mg/kg/day)	ALT (U/l)	AST (U/l)	Urea (mg/dL)	Creatinine (mg/dL)
Saline	-	37.2 ± 2.1	217.4 ± 13.4	46.5 ± 3.0	0.41 ± 0.03
AEPG	10	42.8 ± 2.2	228.5 ± 10.3	54.4 ± 4.4	0.36 ± 0.05
	25	43.7 ± 4.3	219.7 ± 11.8	37.9 ± 3.4	0.12 ± 0.02****
	50	56.8 ± 6.4***	276.2 ± 31.7	43.0 ± 3.3	0.23 ± 0.07**
5-FU	25	30.5 ± 5.3	201.0 ± 9.3	34.3 ± 4.2	0.32 ± 0.01*
Reference Values[#]	–	17.0 – 77.0	54.0 – 298.0	42.0 – 61.0	0.10 – 0.90

Data are expressed as mean ± standard error of the mean (SEM). Statistically significant differences between the values are expressed as * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$), in comparison with the saline-treated group (ANOVA and Tukey test). [#] Values obtained from the experimental research support database of the Tiradentes University Biotherium.

Table 3. Effect of an aqueous extract of *P. granatum* (AEPG) and 5-fluorouracil (5-FU) on erythrocyte parameters in peripheral blood of mice subjected to sarcoma-180 cell transplantation.

Drug	Dose (mg/kg/day)	Red blood cell ($\times 10^6$)	Hemoglobin (g/dL)	Hematocrit (%)
Saline	-	9.08 ± 0.18	12.99 ± 0.32	40.58 ± 0.68
	10	9.78 ± 0.15	14.24 ± 0.23	44.03 ± 0.63
AEPG	25	8.57 ± 0.66	12.74 ± 0.95	39.46 ± 2.91
	50	9.25 ± 0.25	12.94 ± 0.41	40.42 ± 1.09
5-FU	25	8.30 ± 0.56	11.19 ± 0.81	34.80 ± 2.55
Reference Values[#]	-	6.36 – 9.82	10.20 – 16.60	39.00 – 49.00

Data are expressed as mean ± standard error of the mean (SEM). No statistically significant difference between the groups was observed (ANOVA and Tukey test). [#] Values obtained from the experimental research support database of the Tiradentes University Biotherium.

effects of such therapy has become apparent (Redd et al., 2001).

In this study, treatment with an aqueous extract of *P. granatum* at doses of 10, 25, and 50 mg/kg significantly inhibited the growth of sarcoma 180 tumors in mice, but not in a dose-dependent manner. These data suggest antitumor activity of the extract, which is similar to the effects of 5-FU, a chemotherapeutic agent widely used in experimental models (Mousinho et al., 2011). Therefore, this study provides data indicating the promise of AEPG in anticancer therapy, which is consistent with previous in vitro research (Jayakumar and Haridass, 2012; Joseph et al., 2013). It is possible that such antitumor effects are related to the chemical compounds present in the extract. It has been demonstrated that phytochemical constituents found in *P. granatum* present cytotoxic effects on tumor cell lines, particularly tannic compounds such as ellagic acid (Zhao et al., 2013; Zahin et al., 2014; Zhang et al., 2014), gallic acid (Liang et al., 2012; Locatelli et al., 2013) and punicalagin (Zahin et al., 2014). As reported by Qu et al. (2012), high contents of these tannic compounds were found in the AEPG using the HPLC-MS method, suggesting that ellagic and gallic acids may play an important role in the inhibition of murine sarcoma 180 growth. The reason why the antitumor activity was not dose-dependent is not fully understood, but it is possible that all receptors activated by the chemical compounds present in the EAPG are already saturated at 25 mg/kg, and therefore increasing it provided no additional biological effect. However, further investigations are necessary to clarify the precise biochemical mechanisms underlying these biological effects. In addition, as the effect is not dose-dependent, and therefore does not require dose adjustments to body weight.

Since tissue homeostasis is the result of the balance between proliferation and cell death, the apoptotic rate plays a key role in tumor formation and progression (Evan and Vousden, 2001; Fulda, 2009).

Apoptosis is the process of highly controlled

Table 4. Effect of an aqueous extract of *P. granatum* (AEPG) and 5-fluorouracil (5-FU) on the total leukocytes in peripheral blood of mice subjected to sarcoma-180 cell transplantation.

Drug	Dose (mg/kg)	Total leukocytes (10^3 cells/mL)	Differential count of leukocytes (%)			
			Neutrophil	Lymphocyte	Monocyte	Eosinophil
Saline	-	7.8 ± 0.6	25.7 ± 2.0	63.2 ± 4.8	2.3 ± 0.4	3.3 ± 0.7
AEPG	10	9.6 ± 0.9	37.9 ± 3.1*	56.1 ± 3.2	2.6 ± 0.4	3.3 ± 0.8
	25	9.9 ± 1.0	38.2 ± 5.8***	55.8 ± 3.6	4.3 ± 0.8*	2.8 ± 0.4
	50	8.1 ± 0.7	32.4 ± 2.4*	59.1 ± 3.3	2.8 ± 0.4	5.8 ± 1.4
5-FU	25	2.1 ± 0.3**	8.8 ± 1.4***	70.4 ± 4.8	2.4 ± 0.5	9.9 ± 1.6***
Reference Values[#]		6.0 – 15.0	10.0 – 40.0	55.0 – 95.0	1.0 – 4.0	0.0 – 4.0

Data are expressed as mean ± standard error of the mean (SEM). Statistically significant difference between the values are expressed as * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$) in comparison with the saline-treated group (ANOVA and Tukey test). [#] Values obtained from the experimental research support database of the Tiradentes University Biotherium.

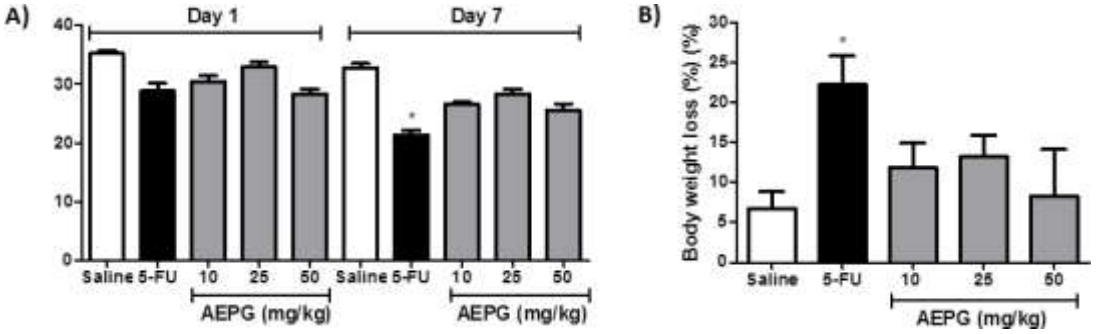

Figure 6. (A) Assessment of the body weight and (B) body weight loss ratio of the animals of the different groups at the first and eighth days of experiment. Data are expressed as mean ± standard error of the mean (SEM). *Statistically significant difference ($p<0.05$) in comparison with the saline-treated group (ANOVA and Tukey test).

Table 5. Effect of an aqueous extract of *P. granatum* (AEPG) and 5-fluorouracil (5-FU) on the average weight of the organs removed from mice subjected to sarcoma 180 cell transplantation.

Drug	Dose (mg/kg)	Organs (g/100 g body weight)		
		Liver	Kidney	Spleen
Saline	-	4.86 ± 0.18	1.22 ± 0.04	0.43 ± 0.05
AEPG	10	4.25 ± 0.18	1.22 ± 0.03	0.39 ± 0.06
	25	5.01 ± 0.28	1.39 ± 0.08	0.49 ± 0.08
	50	4.62 ± 0.42	1.38 ± 0.04	0.43 ± 0.03
5-FU	25	4.45 ± 0.55	1.36 ± 0.04	0.30 ± 0.04**

Data are expressed as mean ± standard error of the mean (SEM). Statistically significant difference between the values are expressed as ** ($p < 0.01$), in comparison with the saline-treated group (ANOVA and Tukey test).

programmed cell death triggered by intrinsic biochemical signaling pathways. The mechanisms of apoptosis involve an energy-dependent cascade of molecular events that includes activation of cysteine proteases such as interleukin-1β-converting enzyme (ICE), Fas signaling, cell cycle interfaces, stress responses, the B-cell

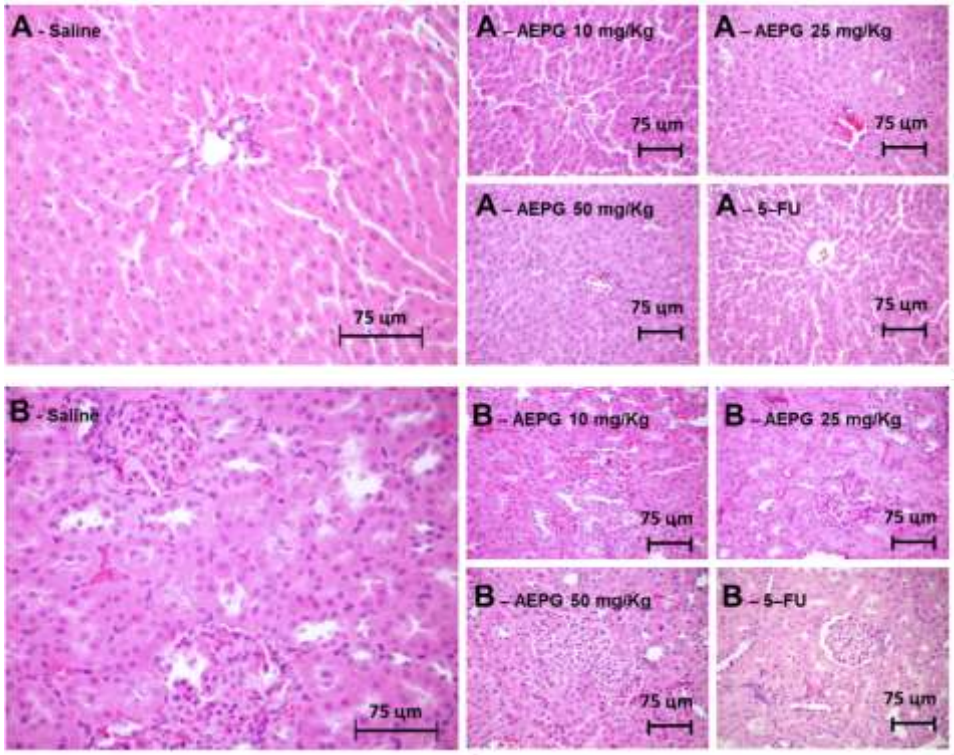

Figure 7. Photomicrographs of (A) liver and (B) kidney samples showing comparatively unremarkable architectural and cellular appearance of the organ tissues in all groups (HE, 400× magnification).

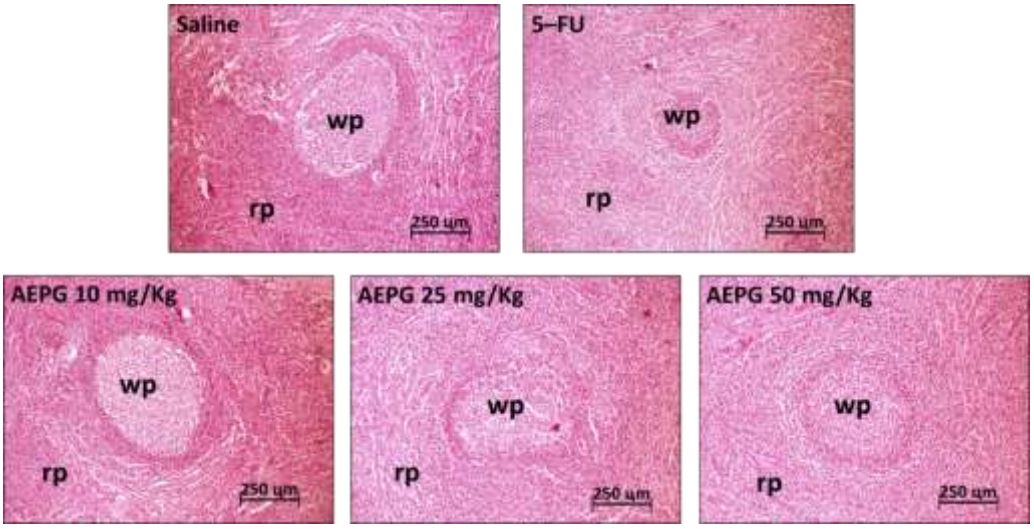

Figure 8. Photomicrographs of spleen samples. The white pulp (wp) presented signs of atrophy in 5-FU-treated group, but presented the usual architecture and morphologic appearance in the other groups. The red pulp (rp) was unremarkable and similar in all groups (HE, 100× magnification).

lymphoma 2 family, and the tumor suppressor gene p53.

This process leads to the cleavage of caspase-3 and results in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors, and finally uptake by phagocytic cells, with

no secondary inflammation (Elmore, 2007).

Thus, to explore the antitumor effect of AEPG on sarcoma 180 tumor in the present study, apoptosis was detected by in situ TUNEL staining. It was found that treatment with AEPG significantly increased the number of TUNEL-positive cells in comparison with saline control. The increased number of TUNEL-positive cells observed in AEPG-treated groups was statistically comparable to that resulting from 5-FU treatment. Thus, the results strongly suggest that the antitumor effects of the extract are related to increased apoptosis-mediated tumor cell death. It is possible that the major tannic compounds present in the extract, such as ellagic and gallic acids, are involved in the proapoptotic effects of AEPG. It has been demonstrated that ellagic acid is able to stimulate apoptosis in poorly differentiated MIAPaCa-2 and moderately differentiated PANC-1 human pancreatic carcinoma cell lines, as a response of inhibition of the transcription factor NF-κB. The decrease in NF-κB leads to activation of the mitochondrial proapoptotic pathway, resulting in cytochrome C release and caspase activation (Edder Kaoui et al., 2008).

In addition, gallic acid has been shown to induce apoptosis of HL-60 human promyelocytic leukemia cells (Yeah et al., 2011) and A375.S2 human melanoma cells (Lo et al., 2010) through caspase-dependent and -independent pathways. However, further investigations are necessary in order to clarify the precise mechanisms underlying the proapoptotic effect of AEPG on tumor cells.

One of the most important challenges regarding chemotherapy against cancer are the minimization of the adverse/side effects of the drugs. As previously demonstrated by Gonzaga et al. (2009), treatment with 5-FU promotes a variety of undesirable adverse effects, such as body weight loss, severe myelosuppression, and spleen atrophy.

Herein, no remarkable changes were observed in the total leukocyte count of the AEPG-treated groups, indicating no suppressive effects on peripheral blood white cells. In fact, AEPG induced the increase of neutrophils, suggesting that the extract might exert a possible stimulatory effect on the bone marrow. In addition, although significant differences were observed in biochemical parameters of liver and renal function in the AEPG-treated groups, all the serum values remained within the physiologic reference range, suggesting that those changes might be considered irrelevant. The fact that the weight, gross appearance, and histologic features of the organs in the AEPG treated groups were unremarkable and comparable to those of the saline group seems to support the hypothesis that the biochemical changes were not severe enough to cause real functional damage. Thus, as these hematologic, biochemical, and gross/histologic parameters have been used to assess the toxicity of *P. granatum* fruit extracts (Vidal et al., 2003), the present data seem to point to the safety of AEPG at the studied doses.

Conclusion

This study demonstrated that intraperitoneal administration of AEPG inhibited the growth of transplanted sarcoma 180 cells in a murine model, and that the antitumor effects were likely related to increased apoptosis rates. In addition, the use of the extract was proven to be safe, with none of the adverse/side effects associated with the use of chemotherapeutics.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Journal of Medicinal Plants Research

Full Length Research Paper

Effects of methanolic calyx extract of *Hibiscus sabdariffa* on body weight, blood cholesterol and liver marker enzymes in Wistar rats

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Eighty male albino rats were used to investigate the effects of methanolic calyx extract of *Hibiscus sabdariffa* on body weight, blood cholesterol and liver enzymes markers. Twenty-eight days oral administration of 100, 200, 400 and 800 mg/kg body weight of the extract showed a significant ($p<0.05$) time-dependent decrease in the body weights of all the treated groups when compared with the control, with 100 mg/kg causing significant ($p<0.05$) decrease at weeks 2 and 4, respectively when compared with other treatment doses. The extract significantly ($p>0.05$) decreased the serum cholesterol and increased the liver marker enzymes (ALP, ALT and AST) in dose-dependent and time-dependent manner, when compared with the control. However, on day 21, the group treated with 400 mg/kg showed a significant ($p<0.05$) increase the serum cholesterol, and decrease in liver marker enzymes when compared with the rest of the treatment groups. Histopathology from all the treatment groups revealed graded degrees of vacuolar degeneration of the hepatocytes and peri-portal infiltration of mononuclear leucocytes. The results of this present study suggests that the methanolic calyx extract of *H. sabdariffa* possesses anti-obesity and hypocholesterolemic potentials which should be harnessed with caution due to its tendency to adversely affect the liver.

Key words: *Hibiscus sabdariffa*, hypocholesterolemia, anti-obesity, liver maker enzymes, Wistar rats.

INTRODUCTION

Hibiscus sabdariffa (family Malvaceae) is an herb cultivated for its leaf, fleshy calyx, seed or fibre (Dalziel, 1973). The dried flowers of *H. sabdariffa* are used as a local juice by Nigerians (Usoh et al., 2005) while its floral parts serve as colourant in food industries (El-Meleigy, 1989). The broad usefulness of this plant as a food agents and herb has attracted the interest of researchers in the last two decades.

In most body tissues such as blood, bile and brain tissues, cholesterol is the main lipid found which according to research is the key lipid linked with arteriosclerotic vascular diseases. It is also required for steroids and cellular membranes formation. Although the liver metabolizes, the cholesterol increased levels are

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found in hypercholesterolaemia, hyperlipidaemia, hypothyroidism, uncontrolled diabetes, nephritic syndrome, and cirrhosis. Whereas decreased levels are found in malabsorption, malnutrition, hyperthyroidism, anaemia and liver diseases (Sood, 2006).

Research has revealed that aqueous extract of red and green *H. sabdariffa* petals caused a significant decrease in the LDL-cholesterol levels while no significant effect was observed on HDL-cholesterol and triglycerides (Olatunji et al., 2005). Carvaja-Zarrabal et al. (2009) and Nnamonu et al. (2013) research findings showed that *H. sabdariffa* calyx aqueous extract at intermediate and greater concentrations could be considered possible anti-obesity agents. Chen et al. (2003) reported of its anti-atherosclerotic property.

As reported by Wang et al. (2000), anthocyanin performs more activities than other antioxidants like ascorbate. In related researches, Olaleye (2007), Powers (1999) and Jonadet et al. (1990) reported that anthocynin were cardioprotective, while Olaleye (2007) Chen et al. (2003), Powers (1999) and Nnamonu et al. (2013) opined that *H. sabdariffa* possesses hypocholesterolemic property. The results of Amin and Hamza (2005) and Wang et al. (2000) revealed that *H. sabdariffa* possesses hepatoprotective and anti-oxidative effects on experimental animals. It has also been reported that, a *Hibiscus* anthocyanin induced apoptosis in human leukemia cells through oxygen reactive species-mediated mitochondrial pathway. Hou et al. (2005) reported apoptosis was induced in human leukemia cells by one anthocynin in Hibiscus (Delphinidin-3-sambubioside), Brunold et al. (2004) discovered differentiation and proliferation of human keratinocytes was initiated by polysaccharides taken from the flowers *H. sabdariffa.*

The aim of this present study was to evaluate the effects of methanolic calyx extract of *H. sabdariffa* on body weight blood cholesterol and liver marker enzymes of albino rats.

MATERIALS AND METHODS

Collection and preparation of *H. sabdariffa* calyx extract

Fresh calyces of *H. sabdariffa* were bought directly from Kalaah farm at Mubi, Adamawa State, Nigeria. The calyces were identified and validated by a botanist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, using the identification key of Morton (1987).

Preparation of *H. sabdariffa* calyx extract

The plant materials were air-dried at room temperature. The dried plant materials were subsequently pulverized into a fine powder. One hundred and thirty-five grams of the powdered plant material was extracted with 500 ml of 80% methanol for 72 h. The set up was agitated every 2 h in order to ensure thorough and homogeneous extraction. The filtrate was dried in a Rotary evaporator and the resultant extract stored at 4°C throughout the experiment. Percentage yield was calculated using the formula:

$$\% \text{ Yield (w/w)} = a / b \times 100$$

where a is the weight of extract and b is the weight of the plant material.

Procurement and management of experimental animals

Eighty adult male albino rats weighing between 120 and 295 g were procured from the Genetics and Experimental Animal Breeding Laboratory of Zoology and Environmental Biology Department, University of Nigeria, Nsukka. The rats had no history of drug consumption (that is, they have not been used for any investigation). They were kept in stainless wire rat cages equipped with drinkers and fecal collecting trays, in a clean and fly proof experimental animal house. The rats were fed with commercial growers chick mash (18 % crude protein) made by Vital Feeds, Nigeria Limited and clean drinking water. They were allowed to acclimatize 14 days before the start of the experiment. The rats were allowed unhindered access to food and water. The fecal droppings in the tray were removed daily.

Experimental design:

Eighty adult male albino rats were randomly assigned to five groups of sixteen rats each and kept according to their groups in stainless wire rat cages. Group I served as the control. They received commercial growers chick mash (18% crude protein) and water. Groups II to V represented the experimental groups. They were fed commercial growers chick mash (18% crude protein), water and the extract daily. Groups II, III, IV and V were orally administered 100, 200, 400 and 800 mg/kg body weight of the methanolic calyx extract of *H. sabdariffa*, respectively for 28 days.

Determination of body weight and weight loss

All the rats were weighed using a Mettler, electronic balance PC 2000 at day 0. Four rats from each group were randomly selected and weighed before collection of blood samples at days 7, 14, 21 and 28. The weight values obtained at days 7, 14, 21 and 28 of the experiment were subtracted from the weight of the each rat at day 0 in order to enable us monitor the effect of the extract on the experimental animals.

Collection of blood sample

About 5 ml of the blood samples was collected from each of the anaesthetized rats using the ocular puncture method described by Hoff (2000). The samples were allowed to clot for about 30 min and subsequently centrifuged at 2000 rpm for 10 min. The sera obtained were used to estimate the levels of total cholesterol, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Determination of liver marker enzymes parameters

Serum AST and ALT were determined using method of Reitman and Frankel (1957). The serum ALP was measured using the method of King and Armstrong (1934) and total cholesterol determined using the method of Roeschlau et al. (1974). All liver marker enzymes were measured using Randox commercial enzyme kit.

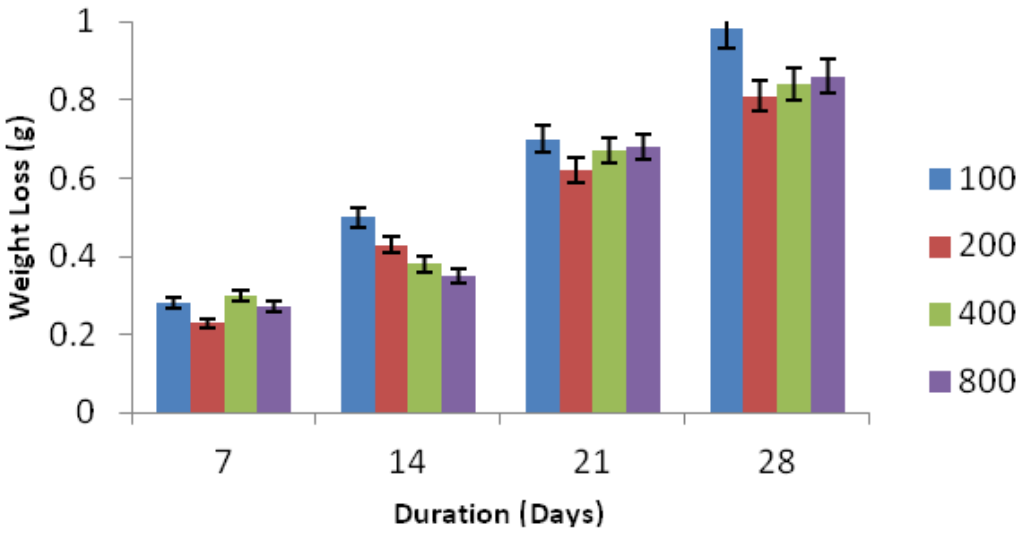

Figure 1. Effects of *H. sabdariffa* on the body weight showing time-dependent decrease in the body weights of all the treated groups when compared to the control.

Statistical analysis

The computer software, statistical package for social sciences (SPSS) version 20.0 for windows was used for the statistical analysis. The data obtained were subjected to one-way analysis of variance (ANOVA) and the differences in means between groups separated using Duncan's New Multiple Range Test. The results were presented as the mean ± standard error of the mean (SEM). Differences in the means were considered significant at the probability values less than 5% ($p<0.05$).

RESULTS

The effect of methanolic extract of *H. sabdariffa* on body weight was presented in Figure 1. The result showed a significant ($p<0.05$) time-dependent decrease in the body weights of all the treated groups when compared with the control, with 100 mg/kg causing significant ($p < 0.05$) decrease at weeks 2 and 4, respectively when compared with other treatment doses.

Figure 2 shows the results the effects of the extract on serum cholesterol. It was observed that the extract significantly ($p<0.05$) precipitated both dose-dependent
The effect of methanolic extract of *H. sabdariffa* on body and time-dependent decrease in the serum cholesterol as compared to the control, except at the dose of 400 mg/kg by day 21 where a significant ($p<0.05$) increase in the serum cholesterol was observed.

The effect of methanolic extract of *H. sabdariffa* on the liver marker enzymes is shown in Table 1. The result showed that beyond day 7, the extract elicited a concentration and time dependent significant ($p>0.05$) increase in the liver enzyme markers as compared to the control, except a slight variation at day 21, where 400 mg/kg of the extract significantly ($p>0.05$) reduced the ALT and AST values.

Further evidence from the histopathology of the liver showed that sections from the untreated group (control) had normal hepatic lobules with cords of normal hepatocytes radiating around a central vein and the bile duct, hepatic artery and the portal vein situated at the periphery of the hepatic lobules (Figure 3). In the treatment groups, histopathological changes were observed in all the different dose groups from the first week to the fourth week. In the groups treated with 100 and 200 mg/kg body weight of the extract, a moderate multifocal hepatocellular vacuolar degeneration with infiltration of mononuclear and polymorphonuclear leucocytes into the periportal areas were observed at the end of the first week (Figure 4). Similar histopathological lesions with a consistency in severity was observed at the ends of the second, third and fourth week post treatment. However, there seemed to be a change in the inflammatory cell population, from polymorphonuclear to mononuclear cell dominated population.

In the groups treated with 400 and 800 mg/kg body weight of the extract, widespread vacuolar hepatocellular degeneration was observed, with multifocal areas of hepatocellular coagulative necrosis and infiltration of mononuclear and polymorphonuclear leucocytes were observed from week one to week four (Figure 5). A summary of the histopathological studies of the liver of rats treated with methanolic calyx extract of *H. sabdariffa* is shown in Table 2.

DISCUSSION

This study evaluated the effects of *H. sabdariffa* methanolic calyx extract on the body weight, cholesterol and liver marker enzymes activities of normal male albino rats. The decrease in the values on body weight when

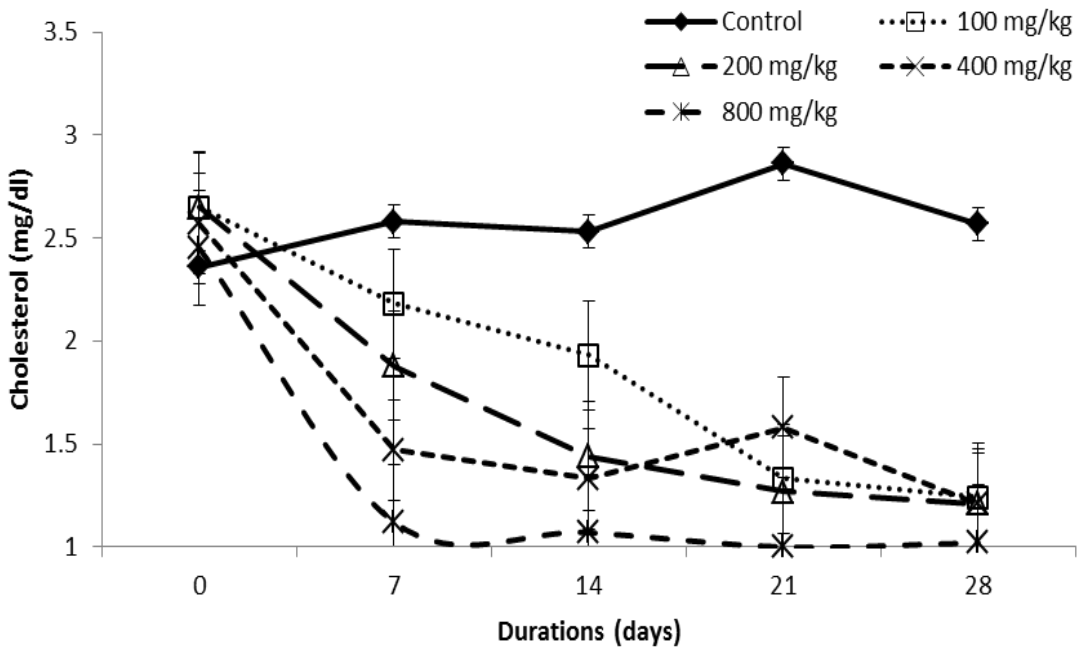

Figure 2. Effects of *H. sabdariffa* on the serum cholesterol showing dose-dependent and time-dependent decrease in the serum cholesterol as compared with the control.

Table 1. Effects of the methanolic extracts of *H. sabdariffa* on ALT, AST and ALP of albino rats.

Parameter	Concentrations (mg/kg)	Duration (Days)				
		0	7	14	21	28
ALT(U/L)	Control	4.00±0.99^{a1}	9.55±0.33^{a2}	9.00±2.31^{a2}	11.33±1.76^{a23}	14.67±1.45^{a3}
	100	4.00±0.99^{a1}	9.67±0.33^{a12}	13.67±3.84^{b2}	26.33±1.86^{b3}	31.00±3.51^{b3}
	200	4.01±0.99^{a1}	9.33±0.33^{a1}	20.33±3.33^{bc2}	35.33±1.20^{c3}	39.33±1.76^{bc3}
	400	4.01±0.99^{a1}	9.67±0.33^{a2}	25.00±2.31^{cd3}	9.67±0.33^{a2}	41.67±0.88^{c4}
	800	4.02±0.99^{a1}	9.33±0.33^{a1}	31.33±1.86^{d12}	43.67±0.88^{d3}	54.33±4.81^{d4}
AST(U/L)	Control	5.00±0.00^{a1}	7.67±0.33^{a12}	8.00±0.38^{a23}	10.00±1.73^{a23}	12.00±1.16^{a3}
	100	5.00±0.00^{a1}	7.33±0.33^{a12}	10.00±0.58^{b2}	16.33±1.20^{b3}	23.67±1.86^{b4}
	200	5.02±0.00^{a1}	7.67±0.33^{a1}	15.33±0.88^{c2}	27.33±0.88^{c3}	35.67±1.45^{c4}
	400	5.00±0.00^{a1}	7.33±0.33^{a2}	18.33±0.67^{c3}	8.33±0.33^{a2}	40.33±0.67^{d4}
	800	5.01±0.00^{a1}	7.67±0.33^{a2}	25.00±0.58^{d3}	37.00±1.00^{d4}	42.67±1.20^{d4}
ALP(U/L)	Control	20.01±0.01^{a1}	47.67±0.33^{a3}	26.67±0.33^{a2}	23.67±2.03^{a12}	26.67±3.33^{a2}
	100	20.01±0.01^{a1}	47.00±0.00^{a2}	33.33±1.76^{b2}	43.33±2.76^{b3}	58.33±6.39^{b4}
	200	20.01±0.01^{a1}	47.00±0.00^{a2}	45.33±1.73^{c2}	46.67±1.20^{b2}	67.67±4.33^{bc3}
	400	20.02±0.01^{a1}	47.67±0.33^{a2}	46.33±1.03^{c2}	47.67±1.33^{b2}	79.33±1.45^{cd3}
	800	20.01±0.01^{a1}	47.33±0.33^{a2}	53.00±3.51^{d23}	60.33±4.84^{c3}	86.33±1.20^{d4}

Values expressed as Mean ± SEM. Mean values in a column with different alphabets are significantly different (p=0.05). Mean values in a row with different figures are significantly different ($p < 0.05$).

compared with the treated and control groups suggests that *H. sabdariffa* possesses anti-obesity property. This result agrees with that of Carvajal-Zarrabal et al. (2009), who observed a drastic loss of weight among animals treated with various concentrations of *H. sabdariffa* extracts. In contrast however, Olatunji et al. (2005) observed no significant decrease in body weight among rats that were chronically treated with 25 and 50 mg/kg

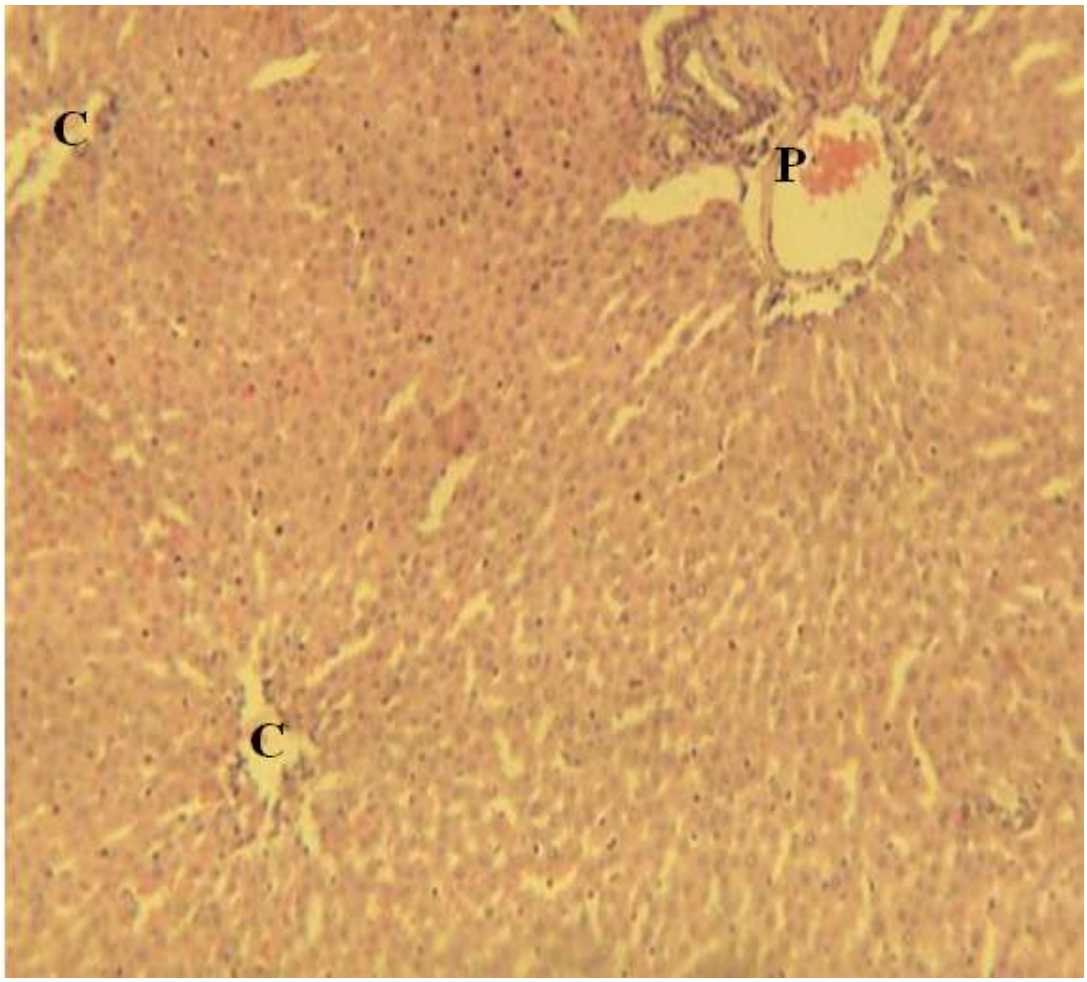

Figure 3. A photomicrograph of the liver from the control group with normal liver architecture. Central vein (C), Portal triad (P), H&E X 40.

body weight of *H. sabdariffa* extracts. Carvajal-Zarrabal et al. (2009) noted that such weight decreases might have been as a result of dietary palatability problem when *H sabariffa* concentration was increases. The loss of appetite in the treated animal models due to daily administration of *H. sabdariffa* extracts had previously been suggested (Abubakar et al., 2010; Orisakwe et al., 2003). This reduction in the body weight could also be directly attributed to the observed hypocholesterolemic property of this extract, owing to the fact that cholesterol significantly contributes to the total fat content of an individual.

The result on the effects of the extracts of *H. sabdariffa* on serum cholesterol showed that the extracts of *H. sabdariffa* are capable of dropping the serum cholesterol levels of treated rats on a dose and time dependent fashion. This observation on the hypocholesterolemic ability of *H. sabdariffa* is consistent with the report of Tzu-Li et al. (2007) who observed that the consumption of *H. sabdariffa* extracts can significantly decrease serum cholesterol levels in human beings. This view was corroborated by the results of Olatunji et al., (2005). Lin et al., (2007) also observed that the level of serum cholesterol among treated individuals decreased significantly. Similarly, Chen et al. (2003) reported on its anti-atherosclerotic property. This hypocholesterolemic effect has been attributed to its abundant antioxidant composition (Andersen et al., 2002).

Study of enzymological and biochemical profile of blood are commonly used as indicators to access the functional status of the animal health and the internal environment of the organism (Rehman et al., 2006). According to Sood (2006) an elevation above normal in the level of liver marker enzymes indicates damage or inflammation of the hepatocytes. The significant increase obtained on the liver marker enzyme tends to suggest liver dysfunction in the experimental animals (Wells et al., 1986). The observed elevation in the liver marker enzymes after day 7 is consistent with earlier reports which showed that a prolonged usage of *H. sabdariffa*

Figure 4. A section of the liver from group 2 and 3 (100 and 200 mg/kg) showing a moderate widespread vacuolar degeneration of the hepatocytes and moderate periportal infiltration of mononuclear leucocytes (arrow). P: Portal area; H&E x100

Table 2. A summary of the histopathological studies of the liver of rats treated with methanolic calyx extract of *Hibiscus sabdariffa*.

Week	Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
1	-	+	+	++	++
2	-	+	+	++	++
3	-	+	+	++	++
4	-	+	+	++	++

+: Mild lesion; ++: Moderate lesion; -: No visible lesion.

aqueous and methanolic calyx extracts could cause liver injury in experimental animals even at dose levels as low as 150 to 180 mg/kg (Akindahunsi and Olaleye, 2003; Ali et al., 2005). This supports the findings of Morton (1987) who reported that *H. sabdariffa* calyx had been analyzed to contain phytic acid, tannin and glycosides such as delphinidin-3-monoglucoside and delphinidin which are toxic to animal and human tissues at high doses. The present observation however is at variance with report of Prommetta et al. (2006) who observed that doses of *H. sabdariffa* ranging from 250 to 1000 mg/kg/day, did not elicit any adverse effect on several important organs such as liver, kidney and the blood system. Reports abound on the hepato-protective effects of *H. sabdariffa* extracts (Tseng et al., 1997; Farombi, 2003). The observed increase in the activities of these marker enzymes following the administration of *H. sabdariffa* extracts may be a unique adaptation by the liver to the assault from the plant extract or as a result of fresh synthesis of the enzyme molecules following extract administration (Yakubu et al., 2007). It seems also plausible that the effect of the extracts on the activities of Aspartate and

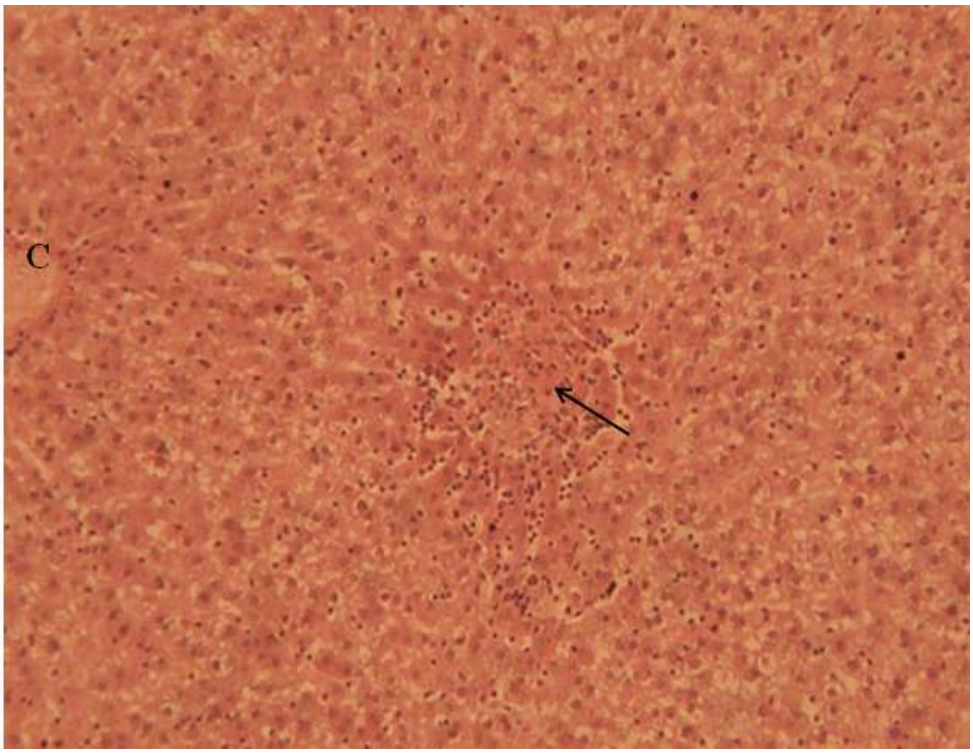

Figure 5. A section of the liver from group 4 and 5 (400 and 800 mg/kg) showing a widespread vacuolar degeneration of the hepatocytes and a focus of coagulative necrosis infiltrated by mononuclear leucocytes (arrow) in the midzonal area. C: Central vein. H&E X100

Alanine Aminotransferase may be the case of organ chain reactions. This is more so as the two enzymes have been found to be localized normally within the cells of the liver, heart, kidney, gill, muscle and other organs (Orisakwe et al., 2003). Regardless of the fact that these enzymes have been reported as important markers in assessing and monitoring liver damage, there is the need to correlate the mechanisms of the extract in the different tissues. This is a vital research area because major toxic effects of *H. sabdariffa* extracts have been reported in the kidneys and reproductive organs of male rats (Orisakwe et al., 2003).

The liver showed varying histopathological changes in hepatic histo-architecture ranging from inflammatory to degenerative and necrotic changes. Groups treated with 100 and 200 mg/kg body weight showed mild lesions from the first week to the fourth week; while the groups treated with 400 and 800 mg/kg body weight showed more severe lesions from the first week to the last week of the study. This revelation explains the basis for the increase in the serum ALT, AST and ALP.

Conclusion

The ability *of H. sabdariffa* extract to lower the total cholesterol level and body weight suggests its usefulness as a potential hypocholesterolemic and anti-obesity agent. However, its ability to cause an increase in the levels of liver marker enzymes (ALP, AST and ALT) tends to suggest a dysfunction in the coordinating physiology of the liver; hence, caution should be applied in its consumption. Alternatively, purification and extraction of the active hypocholesterolemic and anti-obesity principles can be made in order to exclude the hepatotoxic principle(s).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Knowledge of medicinal plants used by residents in two peripheral districts of Boa Vista, Roraima, Northern Brazilian Amazon: Phytotherapy as a new strategy in collective health

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The aim of the study was to describe local knowledge about the use of medicinal plants cited by study participants, as well as to examine how phytotherapy serves as an important strategy of integrative and complementary practice in coping with health problems. The research is characterized as an exploratory study of the descriptive type. Non-probabilistic snowball sampling generated a sample of one hundred and ten participants (n = 110). The study instruments were semi-structured interviews and questionnaires. The data collected were analyzed using Microsoft Excel 2007 and summarized with descriptive statistical methods. The study informants presented knowledge of 77 species and 46 plant families, showing the rich variety of medicinal flora present in the Northern Brazilian Amazon. Among the respondents, elderly women had the most diversified knowledge. Preserving this knowledge is essential and can help in the implementation of public health policies. The results showed high richness of the medicinal flora present in Northern Brazilian Amazon.

Key words: Medicinal plants, phytotherapy, local knowledge, collective health, Brazilian Amazon

INTRODUCTION

Brazil is the most biodiverse country in the world, associated with rich ethnic and socio-cultural diversity, including valuable traditional knowledge about the use of medicinal plants (National Policy of Medicinal Plants and Phytotherapy, 2006). In addition to its recognized natural group of indigenous peoples and traditional populations who have learned, over time, how to live in diverse environments. These groups (Indians, caboclos, riparians, rubber tappers, quilombolas, fishermen, small farmers and extractivists) have vast knowledge about

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plants and understanding of their environment (Vasquez et al., 2014).

According to the World Health Organization (WHO), 80% of the world's population, especially in developing countries such as Brazil, still treat diseases with herbal products and thus use traditional medicine for primary healthcare (Rahman and Singhal, 2002). Medicinal plants are already a significant part of Brazilian popular culture, and in recent decades, interest in phytotherapy has increased considerably among users, researchers and health service providers.

The WHO has also expressed its position on the need to value the use of medicinal plants in health and basic healthcare (Rosa et al., 2011). Given the importance of the subject, interest in conducting this study emerged after understanding and recognizing that healthcare programs should be designed to address not only the medical concerns of the health system but also community participation. Thus, planning must be oriented and constructed according to the needs of individuals, incorporating their social, cultural, environmental and economic characteristics, and with the participation of the people in the communities under study. In recent decades, the use of medicinal plants has grown considerably; this recognition and popular knowledge also contribute to the need for research to clarify and confirm information on the actions of the plants in order to include their use as another form of healthcare.

To better make use of phytotherapy, the Brazilian Ministry of Health implemented the National Policy of Medicinal Plants and Phytotherapy (PNPMF), created in 2006, whose premises concern the principles of safety and efficacy in public health and reconciling socio-economic development and environmental conservation, both locally and nationally. In addition, respect for regional and environmental diversity and particularities are also the guiding principle of this policy. The policy's model of development is aimed at recognizing and promoting proven practices and maintaining the great diversity of usage forms of medicinal plants, ranging from home and community use, through the pharmaceutical manipulation of medicines, to the use and manufacture of industrialized medicines (National Policy of Medicinal Plants and Phytotherapics, 2006).

According to Moraes (2011), the cultural richness in the Amazon constitutes an important component of the social identity of this region and, because it is dynamic, it changes constantly. In many communities, traditional knowledge is the only therapeutic and medicinal resource. Therefore, the objectives of this work are to describe local knowledge and use of medicinal plants mentioned by the participants of the study and to utilize phytotherapy to analyze how strategies used to confront health problems align with the needs and conceptions of the population's health.

The relevance of traditional medicines both in the world and as represented in the National Policy of Medicinal and Phytotherapeutic Plants in Brazil reflects the recognition of scientific evidence of the efficacy and safety of medicinal plants and herbal medicines. The use of synthetic drugs has failed to fulfill the implicit and explicit promises of health systems to address the treatment of diseases. Problems include high costs and the significant adverse effects of synthetic drugs, and the results that are not always satisfactory, which has led to a large number of people seeking less aggressive alternative forms of treatment (Bruning et al., 2012). Medicinal plants can form a new component in healthcare and play an essential role in the treatment of various diseases; this is especially true for developing countries, where phytotherapy is often the only option for primary treatment of diseases.

MATERIALS AND METHODS

Study area

In the Brazilian Amazon, approximately 15 million people live in urban areas according to data from the Brazilian Institute of Geography and Statistics (IBGE, 2010).

The State of Roraima, created by the Federal Constitution of 1988, is part of the Brazilian Amazon located in the extreme north of Brazil and is, therefore, a constituent of the Brazilian Amazon. The characteristics of its biome are well diversified and, thus, particularized from the rest of the Amazon Region. According to the IBGE (2010), the population of Roraima is composed of 450,479 people, distributed in fifteen municipalities, in an area of 224,303,187 km^2. The state has international borders with the Cooperative Republic of Guyana (East and Northeast) and the Bolivarian Republic of Venezuela (North and Northwest), and national borders with the state of Amazonas (South and Southwest) and the state of Pará (Southeast).

The study area, located in the municipality of Boa Vista (229,454 inhabitants), was in the neighborhood of Nova Cidade and Raiar do Sol in the west of the capital, with 5,708 inhabitants and 5,863 inhabitants, respectively (IBGE, 2010). The population is quite heterogeneous, consisting essentially of roraimenses (including Indians and descendants of the pioneers of the colonization of the State) and by smaller numbers of afrodescendants, southerners, northeasterners and northerners. This heterogeneity is important when analyzing the relationship between the use of medicinal plants and migration in the state of Roraima, since many plants were brought from elsewhere to the state, and their use remains as a local health practice. The city of Boa Vista, capital of the state of Roraima, is part of the subdivision Fronteira de Integração in the western portion of the Amazon. The city has an equatorial climate with an average temperature of 27.4ºC and typical savannah vegetation. It is located on the right bank of the Branco River within the hydrographic basin of the same name. The Raiar do Sol district, which is located near the Paca Creek in the western part of the municipality of Boa Vista, has the largest indigenous population in the city of Boa Vista with 287 self-declared indigenous inhabitants, according to data from the Brazilian Institute of Geography (IBGE, 2010). The Nova Cidade region is also located in the western zone of Boa Vista. The western zone is larger than all the other zones together (east, north and south) and is characterized by being peripheral, encompassing the poorer districts of the city, and presenting poor health indicators (Figure 1).

Data collection and analysis procedures

This study respected the ethical principles that govern research

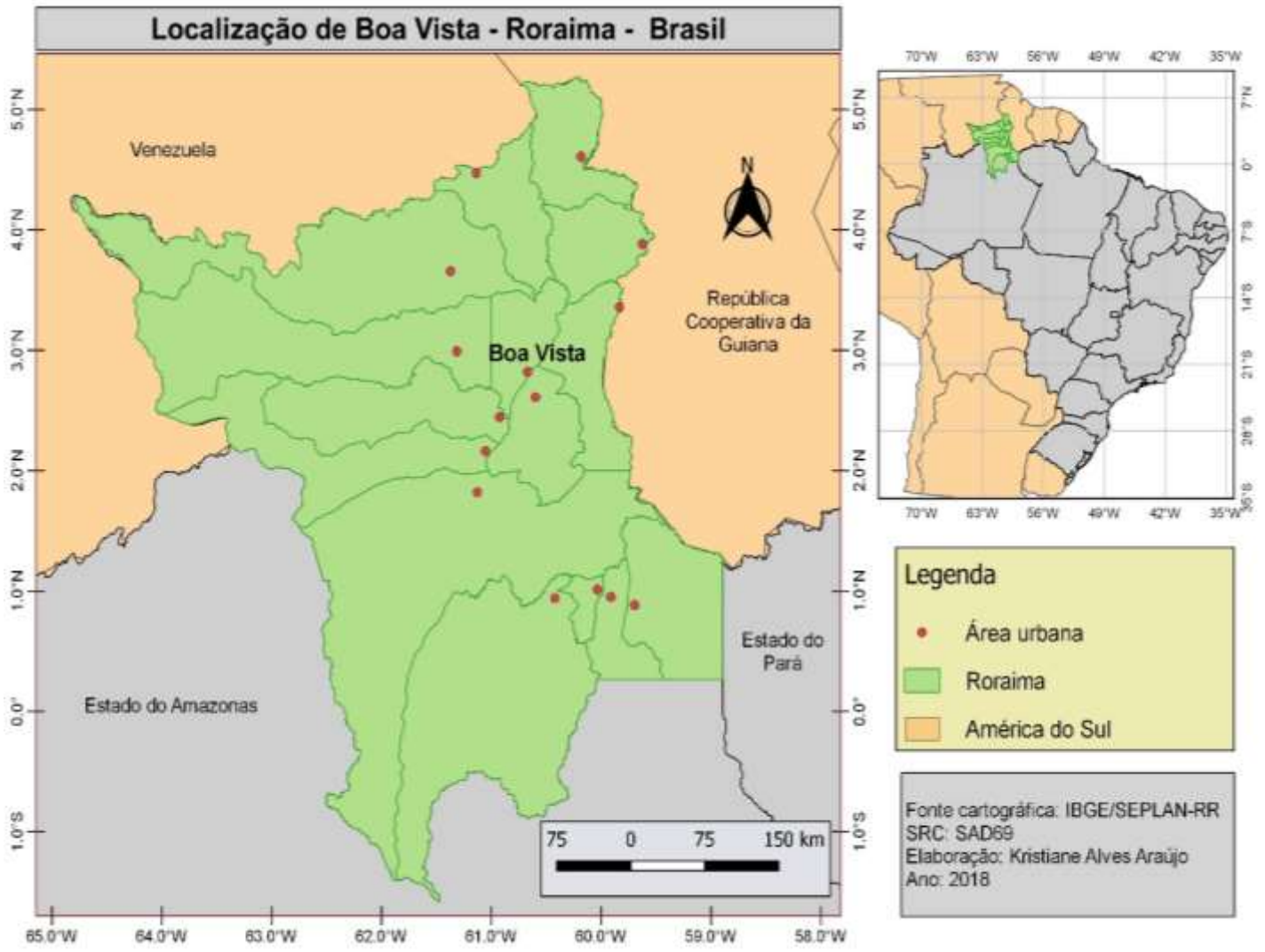

Figure 1. Map of Brazil with the State of Roraima and the municipality of Boa Vista.
Source: https://portugaldigital.com.br/wp-content/uploads/2018/04/MapaBrasilFotoViajero.jpg

involving human beings as defined by Resolution 466/12 of the National Health Council and used the Informed Consent Form (TCLE). The research was approved by the Research Ethics Committee (CEP) of the Federal University of Roraima (UFRR), under protocol numbers 2,288,847 and 9/21/2017 (PRPPG/UFRR) and registration number 1232017. It also had authorization, with letters of agreement, from the representatives of the Nova Cidade and Raiar do Sol districts and the Municipal Health Department of Boa Vista / RR.

Initial visits, mediated by representatives of the two neighborhoods and some community leaders, were made to the research neighborhoods. During the initial visits, the objectives and relevance of the research were presented in general lines, and residents were invited to participate. Study participants were asked to authorize monitoring and observation of their daily activities at home, in religious institutions and at health posts in order to be able to follow all the paths traveled by local residents in search of health. Once authorization was obtained, participant observation was performed in periods and activities as agreed with the community leaders and members of the Family Health Strategy team. During observation periods, the researcher's impressions were recorded in the form of notes in a field diary.

The participant-observation technique enables a researcher to become involved in the daily life of a population. Boiteux (2017) writes that participant observation presupposes interactivity between the researcher, the observed subjects and the context in which they live (and, in this case, in which they work). Participant observation was undertaken by the researchers in various moments that occurred in the houses of residents and in visits to fairs and markets where herbal medicine was sold.

Sites for the present research were selected according to several criteria: a) a region already studied by the researcher during her master's degree (on another topic) and, b) where the researcher had existing contacts with community leaders and with some former residents of the neighborhoods. The initial interview questionnaire solicited the socio-demographic profile of each interviewee. Responses were obtained through the use of a tape recorder. A 20-question elaborated script was used for the semi-structured interviews.

Data collection was carried out between September 2017 and

sample consisted of intentional non-probabilistic sampling in the search for individuals identified as holders of local knowledge on medicinal plants, including those who cultivated or used plants purchased locally. The topics of the research were randomly determined; in each interview, when it became clear which topic the subject was the most knowledgeable about, they were asked to indicate others that had the same domain. Further recommendations were sought until no additional relevant information was being added to the research. This finding indicated that the research had found a saturation point, when information begins to repeat itself, characterizing the "snowball" method. This type of sampling, referred to as “snowball,” is a non-probabilistic sample form, which uses reference chains (Vinuto, 2014).

Data were collected from 110 local residents in the two neighborhoods of the study. Of these, 100 participants responded to the questionnaires with 20 open-and-closed questions on local knowledge of medicinal plants and local basic sanitation. The rest (n = 10) of the interviews used semi-structured interview guides; these targeted the people considered to be legal representatives or leaders and articulators in the research sites.

Guided tours (Albuquerque et al., 2010; Albuquerque et al., 2014) were carried out around their residences, usually in backyards and at free fairs in the city of Boa Vista, Roraima, Brazil. All plants cited in the interviews were photographed, collected or bought at the fairs during guided tours or on subsequent visits. Some domesticated plants were already known or obtained through purchase and were identified based on specialized literature (Lorenzi and Matos, 2002; Souza and Lorenzi, 2012). The spelling of scientific names was checked using the Tropics database, available at http://www.tropicos.org. The list of taxa followed the APG III (2009). The diseases (or symptoms) cited by informants as responding to plant-based remedies were categorized according to the International Statistical Classification of Diseases and health-related problems (ICD-10 2008).

For the interpretation of the data, several independent variables were considered, including age, gender, schooling, income, marital status, and place of residence, among others. The data collected were analyzed using descriptive statistical methods, such as tables, graphs and percentages. Descriptive analyses, including means with standard deviations, were performed. The comparison between sample means was performed using Student’s t-test. The chi-square test was used to compare proportions. The estimation of risk quantification was performed by odds ratio with a 95% confidence interval (Newcombe-Wilson method). The statistical program used was the SPSS: Statistical Package for the Social Sciences / IBM SPSS Statistics Version 24. The level of significance was set at 5% for rejection of the null hypothesis.

RESULTS AND DISCUSSION

Socio-demographic profile

The socio-economic and demographic data of the two communities studied were analyzed using the questionnaires and interviews with 110 participants aged between 20 and 74 years. The average age of the interviewees was 49 years for women and 46 years for men. Of all the interviewees, 80% were over 40 years of age. Regarding gender, 26.6% (n = 29) declared themselves men and 73.4% (n = 71) women. Female predominance can be explained by the greater willingness of women to respond to the questionnaire, and because women were reported to take up more responsibility in caring for children; leading them to learn more about using plants to treat major diseases and symptoms that family members present, and may also be justified by the greater willingness of women to respond to the questionnaire. A similar profile of respondents was found by Oliveira et al. (2014) in a study on the survey on the use of medicinal plants with anticancer therapy among patients of the Annapolis Oncology Unit.

Several studies have shown that there is a high correlation between the number of species and the number of subspecies that can be found in species (Oliveira et al., 2008; Silva et al., 2008). In addition, the attribution of knowledge on the medicinal use of plant species to women, noting that even in urban areas with interior characteristics, such as the present research in Boa Vista/RR, women can demonstrate strong interest and knowledge about medicinal plants.

Figure 2 shows the age groups of the interviewees with their respective percentages. The chi-square test showed that the difference between the age groups of the informants is generally very significant ($\chi^2 = 19.33$, gl = 7, $p < 0.01$) and continues to be significant when gender-specific analyses are performed ($\chi^2 = 6.30$, gl = 6, $p > 0.05$). Thus, the most frequent age group was 40-59 years (49%), followed by 60-74 years (31%) and then 20-39 years (20%). These results indicate that most of the informants are of mature age (40-69 years), making up a good part of this sample. In this case, there is a great deal of evidence that young people (aged 20-39) have less interest in the use and knowledge of medicinal plants, preferring to use industrialized medicines.

According to the interview findings, the 40-59-year age group has greater knowledge about medicinal plants than other classes, and the number of citations of plant species was similar to the number mentioned by the oldest age group (60-74 years).

Informants in both neighborhoods knew an average of 77 (± 8) useful plant species. The 20-39-year age group knew fewer species than the other two age categories ($H = 16.13674$; $p = 0.0003$). There were significant gender differences in the number of species known, with female informants knowing a larger number of species as compared to male informants ($U = 222.5$; $p = 0.001571$). Between male and female informants in the 60-74 yearage group, there was a statistically insignificant difference in the knowledge of species.

Schooling

The traditional medicine professionals had limited advanced education. None of the study participants had a university education; 39.7% were illiterate; 33% fundamental; 15.5% high school; and 12.3% incomplete higher education. As for schooling of traditional medicine practitioners, 14.70% had university education; 44.7%, illiterate; 27%, fundamental; 18.3%, elementary school; 10.0%, high school. Thus, this study observed that knowledge about medicinal plants by both traditional

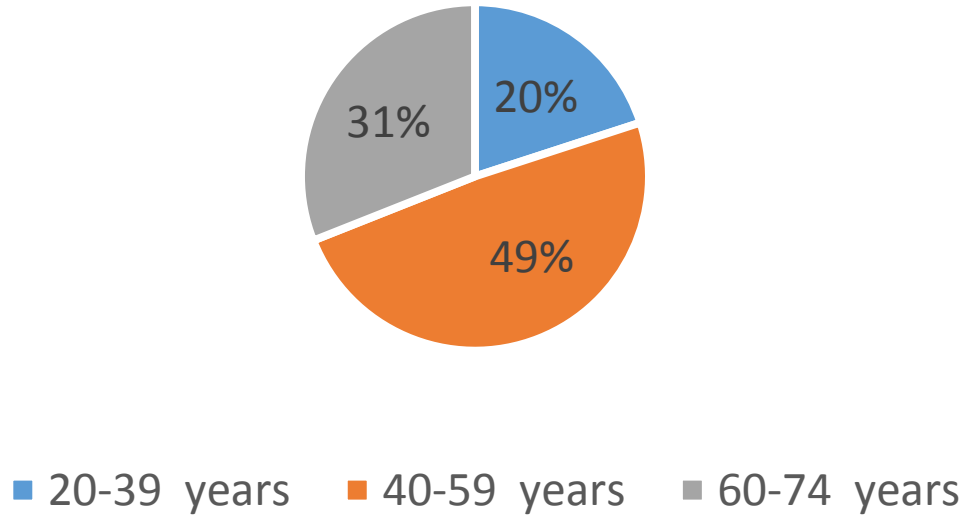

Figure 2. Percentage referring to the age categories of the research participants who are residents of the neighborhoods Nova Cidade and Raiar do Sol.

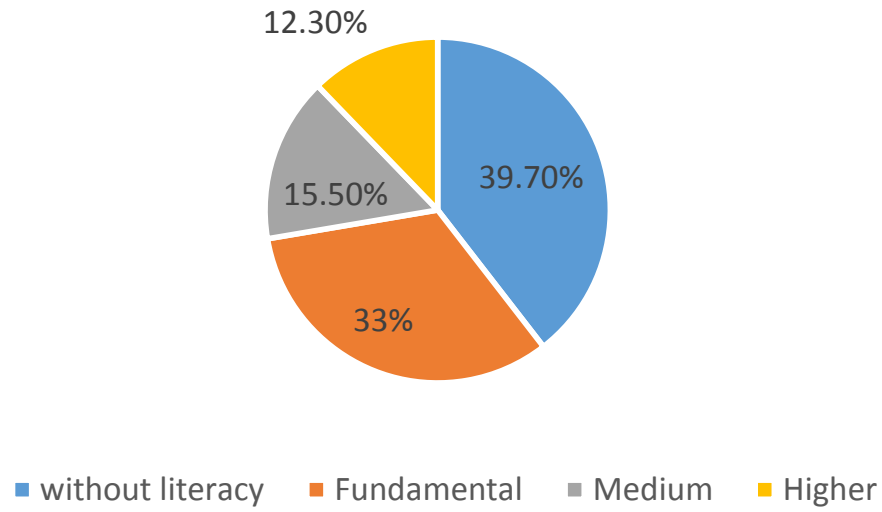

Figure 3. Percentage of educational level of traditional medical professional.

medicine practitioners and practitioners tends to decrease with the level of schooling. Since educational level is associated with economic conditions, the lower educational level associated with greater familiarity with the medicinal power of plant species may reflect the need, due to low purchasing power to find ways of treating diseases other than purchasing expensive drugs. It may also be inferred that increased levels of schooling involve a certain massification of customs, related to globalization; leading to a gradual loss of ancestral habits related to phytotherapy. These findings support the findings of other studies, such as Santos et al. (2008) and Souza et al. (2017) (Figure 3 and 4).

Family income

Figure 5 shows the average income groups of study participants' families: up to one family member earning minimum wage (30%); from 01 to 03 family members earning minimum wage (67%); and above 03 family members earning minimum wage (03%). Figure 5 shows that an inverse relationship exists between the minimum wage and the percentage of people receiving it, highlighting the low economic conditions of most of the respondents. These conditions may drive them to use other health strategies, such as herbal medicine, to improve health. This explains the use of the form either in

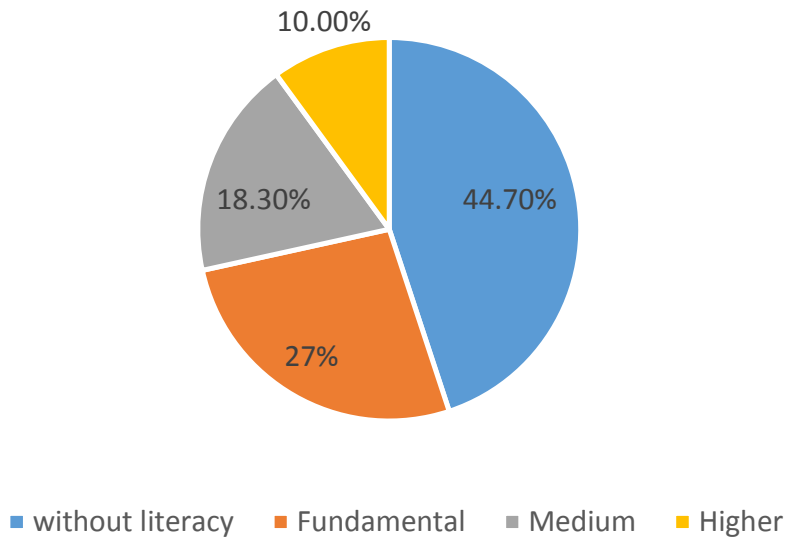

Figure 4. Percentage Level of schooling of traditional medicine practitioners

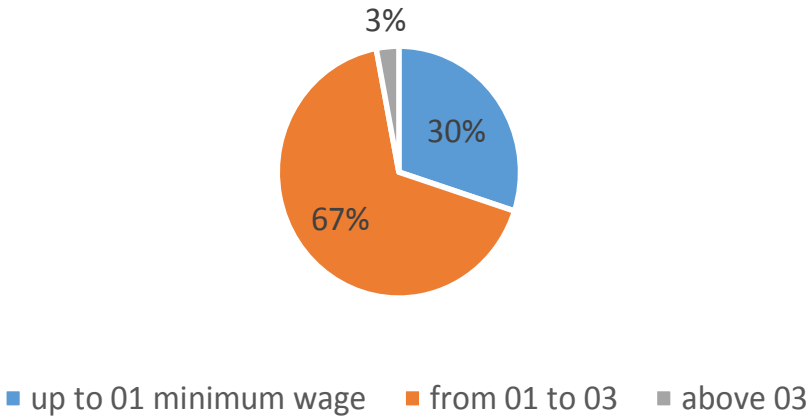

Figure 5. Percentage referring to the average family income of the research participants who are residents of the neighborhoods of Cidade Nova and Raiar do Sol.

addition to or instead of the services offered by SUS. Strategies are invented and reinvented every day according to health needs and problems, utilizing local knowledge and available resources, be they financial, agricultural or even cultural. Among the participants, 58% receive benefits and 42% do not. These data reinforce the idea that they are low-income people, that is, they have low purchasing power. Again, low income levels can influence therapeutic decisions, since people need to use strategies within economic reach for health services. Using herbal and phytotherapeutic plants can be an important strategy for coping with existing health inequalities in Boa Vista. Plant-based medicine offers lower-cost options for healthcare among populations with poor social and health indicators. According to the analysis made by Sundquist (1995), socioeconomic factors influence health in its various dimensions—to a certain point. Among higher income classes, however, differences in health practices depend, above all, on individual choices conditioned by cultural factors.

Lifting of medical plants in the two neighborhoods, New City Center and Raiar of the Sun

The National List of Medicinal Plants of Interest to SUS (RESINUS), elaborated by the Ministry of Health (MS) in 2009, lists 71 species of medicinal plants that are used by the Brazilian population for which there is some scientific evidence about their uses in basic healthcare.

Nevertheless, the need for studies that broaden and deepen the knowledge about these and other species, as well as how to disseminate knowledge about uses, safety and efficacy, are still urgent (Brazil, 2009). This study contributes to that knowledge base, which can contribute to expand the available healthcare options. The dissemination of the results to more people is one of the contributions of this research carried out in two districts in Boa Vista/RR by the present author.

From the verification of the applied questionnaires, the names of the vegetable species most cited by the residents of the neighborhoods Nova Cidade and Raiar do Sol, shown in Table 1, were obtained, in a total of 77 species within 46 families. The chi-square test showed a significant difference between the number of plant citations made by the residents of the two studied districts ($\chi^2 = 29.26$, gl = 9, $p < 0.001$). Interviewees said that many of the medicinal plants on this list can be found in their backyards and that most are available at fairs and other places where herbal medicines are sold in Boa Vista.

Table 1 lists the most frequently cited medicinal plants by the research participants. Through the analyzed data, it was verified that, in cases of less complex diseases in the primary health treatment, the use of medicinal plants, through home remedies or phytotherapeutics, is recommended by relatives, neighbors, religious leaders or health post staff and are the most immediate measures.

The number of species recorded in the present study is lower than the results found in other similar studies of ethnobotanicals and medicinal plants (Vasquez et al., 2014; Oliveira et al., 2010; Brito and Senna-Valle, 2011). This finding can be explained with the fact that, in the present study, only species mentioned by the sample of participants and which were related to their knowledge and uses were included.

The number of plant species cited by respondents varied by the age categories. The average was 26 species (20%) from the 20-39-year group; 56 species (49%) from the 40-59 year olds; and 32 species (39%) from the 60-74-year group. The most cited species were cows (56%), lemongrass (42%), stone breakage (48%) and cow's foot (32%). The paw plant (*Bauhinia forficata*), which has proven hypoglycemic activity and is indicated for use by the Ministry of Health in Brazil, was the most prominent among the mentioned species.

As for the number of citations per informant, the mean was 16 (± 09) for each species. Female informants cited more than males. Despite numerical differences between the number of citations for male and female informants in the range of 40-59 years, there were few significant differences between genders. The categories in 40-59 years of age did not present significant differences among themselves; however, a tendency was observed for the older participants to know more uses for the species, in both genders.

Information acquired in communities that use medicinal flora should be combined with chemical and pharmacological studies conducted in specialized laboratories to test hypotheses about the pharmacological activities and the active substances responsible for the therapeutic actions. This integration of traditional and scientific approaches will optimize local knowledge and benefit the population by generating safety and effectiveness data. This will facilitate, at least for the population studied, the implementation of Portaria no. 971/2006 of the Ministry of Health of Brazil.

Parts of medicinal plants cited by research participants

Figure 6 shows which parts of plants were used and how frequently in the preparation of remedies: flower, leaf, stem, bark, root, sap, seed and fruit. Leaves, bark, and roots or other parts were most frequently used (60, 30 and 10%, respectively). The chi-square test showed that the difference between the number of citations of plant parts is significant ($\chi^2 = 484.54$, gl = 10, $p < 0.001$).

The study by Lacerda et al. (2013) reported the following citation results: the leaves (440 citations), the peels (130 citations), the fruits (72 citations)), flowers (68 citations), latex (44 citations), seeds (27 citations), bulbs (17 citations), intercuts (14 citations) and roots (5 citations). Thus, disease treatments based on phytotherapy can be derived from various parts of plants, such as roots, barks, leaves, fruits and seeds, according to the herbs in question.

This study in Boa Vista/Roraima corroborates other works, such as that of Vasquez et al. (2014) and Alves and Povh (2013). The most frequently cited vegetative part was the leaf, which presented the highest total number (290) of citations and a higher percentage among women than men. This was followed by bark (150 citations) and roots/other parts (50 citations). Women presented a higher percentage of citations referring to leaves, fruit, seeds, and bark, while men presented a higher percentage of roots and stems uses.

"Ethnobotany of medicinal plants in riverside communities of the Municipality of Manacapuru, Amazonas, Brazil" by Vasquez et al. (2014) found that the vegetable part mostly used in the preparations of the home remedies was the leaves (68%). Other parts used were stem bark (12%), fruit (7.4%), stem (5.5%), root (3%), seed (2%), and flower (1%).

"The Use of Medicinal Plants with Antimicrobial Activity by Users of the Public Health Service in Campina Grande - Paraíba" by Souza et al. (2013) reached similar conclusions to the present study in Roraima regarding the way that residents of Raiar do Sol and New Town districts use plants and other products in their traditional medicine; it is subordinated not only looking for efficacy enshrined in the experience of use but also how they perceive the etiology of diseases and the therapeutic actions of the remedies. This highlights the importance of

Table 1. List of medicinal species indicated by the informants in the neighborhoods of Nova Cidade and Raiar do Sol, in the municipality of Boa Vista, Roraima, Brazil.

Family	Scientific name	Popular name	Used part	Therapeutic indication
Malvaceae	*Abelmoschus esculentum L.*	Okra	Seed	Asthma
	Hibiscus sabdariffa	Vinegar	Leaves	Antifungal
Meliaceae	*Carapa guianensis aubl*	Andiroba	Seed	Healing, the flu
Musaceae	*Musa paradisiaca L.*	Banana	Fruit	Ulcer, toothache, wart,
Malpighiaceae	*Malpighia emarginata*	Acerola	Fruit	The flu
Lamiaceae	*Rosmarinus officinalis*	Rosemary	Leaf	The flu
	Mentha spicata	Mint	Leaf	The flu / analgesic
Monimiaceae	*Plectranthus barbatus*	Boldo	Leaf	Intestine function
Anacardiaceae	*Anacardium occidentale*	Cashew	Stalk	Anti-inflammatory / dysentery
	Myracrodruon urundeuva	Aroeira	Stalk	Anti-inflammatory
Asteraceae	*Matricaria chamomilla*	Chamomile	Leaf	Soothing
Myrtaceae	*Eucalyptus globulus*	Eucalyptus	Leaf, fruit	Hypoglycaemic, antipyretic / antibiotic
	Psidium guajava	Guava	Leaf	Disinterest
Rutaceae	*Citrus sinensis Osbeck*	Orange	Leaf	Soothing / the flu
	Citrus aurantifolia	Lemon	Fruit	The flu, sore throat
	Citrus reticulata B.	Tangerine	Fruit	High cholesterol, labyrinthitis
	Ruta graveolens L.	Rue	Leaf	Menstrual cramps, ear pain, general aches, cramps
Lythraceae	*Punica granatum*	Pomegranate	Fruit	Anti-inflammatory
Cucurbitaceae	*Momordica charantia L.*	Melon de-são-caetano	Leaf/ seed	Verminose, antidiarrheal, hemostatic, burns
	Cucumis sativus L.	Cucumber	Fruit	High pressure
	Sechium edule SW	Chuchu	Fruit	High pressure
Fabaceae	*Libidibia ferrea (Mart. ex Tul.) L.P.Queiroz*	Jucá	Stalk/ fruit	The flu, inflammation of the kidneys, soothing
	Mimosa arenosa (Willd.) Poir.	Cat nail	Root	The flu
	Bauhinia foficata	Cow's foot	Leaf/ stalk	Antidiabetic / diuretic
	Stryphnodendron barbatimam Mart	Barbatimão	Stalk	Pain / Astringent and Antiseptic Infections
Acanthaceae	*Ocimum sp.*	Alfavaca	Root	The flu
Passifloraceae	*Passiflora foetida L*	Passion fruit	Root, leaf and fruit	Inflammation of the throat, insomnia, depression, antirheumatic
Phyllanthaceae	*Phyllanthus niruri L.*	Smasher	Root and leaf	Kidney stones liver
Acanthaceae	*Justicia pectoralis Jacq*	Noun	Leaf	Dor de cabeça
Anacardiaceae	*Mangifera indica L*	Mango	Leaf	The flu
	Spondia purpurea L	Stick it	Leaf	The flu
Annonaceae	*Annona muricata L*	Graviola	Fruit and leaf	Lose weight

Table 1. Contd.

Apiaceae	*Petroselinum crispum L*	Parsley	Leaf	Ear infection, hepatitis
	Pimpinella anisum L	Fennel	Leaf	Bellyache (child), soothing
Asteraceae	*Matricaria chamomilla L.*	Chamomile	Leaf	Clean infant's intestine, soothing
	Bidens pilosa L	Picão	Leaf	Hepatitis
Bignoniaceae	*Tabebuia caraiba*	Yellow ipê	Leaf	Wound / uterus
	Tabebuia hepthaphyla	Purple ipê	Leaf	Lung / cough / wound in uterus
Caricaceae	*Carica papaia L*	Papaya	Fruit	Clean skin, verminoses, bad digestion
Euphorbiaceae	*Manihot esculenta L*	Manioc	Stalk	Malnutrition
	Phyllanthus orbiculatus L L	Breaking stone	Leaf	Kidney stones
Lamiaceae	*Mentha piperita L*	Mint	Leaf	Verminose,
	Rosmarium offcinale L	Rosemary	Leaf	Brokenness in children, infection, heart
	Salvia officinalis L	Sage	Leaf	The Flu, infection
Lauraceae	*Cinamomum zeylannicum Breyn*	Cinnamon	Stalk	Internal infection, hypoglycemic
Liliaceae	*Allium sativum L*	Garlic	Seed	Cough, verminose, antioxidant
Arecaceae	*Cocos nucifera L.*	Coconut	Fruit	Urinary tract infection, kidney pain
Asteraceae	*Baccharis trimera (Less.)*	Carqueja	Leaf	Pain in the liver, hypoglycemic
Brassicaceae	*Brassica integrifólia (H. West) Rupr*	Mustard	Seed	Thrombosis, dizziness, stroke, headache, girth
	Nastrutium officinale W. t.	Cress	Leaf	Hoarseness, child colic, earache
Bromeliáceas	*Ananas comosus (L.) Merril*	Pineapple	Fruit	Cough
Caricaceae	*Carica papaya L.*	Papaya	Leaf and fruit	Flu, poor digestion, child cramps, bowel
Amaranthaceae	*Chenopodium ambrosioides L.*	Mastruz	Leaf	Rheumatism, ulcer, strokes, healing, inflammation, pain in the stomach, fractures
Convolvulaceae	*Ipomoea batatas (L.) Lam*	Sweet potato	Root	Inflamed tooth
Cucurbitaceae	*Citrullus vulgaris Schrad*	Watermelon	Seed	Stomach ache
Fabaceae	*Stryphnodendron coriaceum Benth.*	Barbatimão	Leaf	Inflammation
Lamiaceae	*Pogostemon cablin (Blanco) Benth*	Patchuli	Leaf	Heart disease
Lauraceae	*Cinnamomum zeylanicum Blume*	Cinnamon	Stalk	Soothing, high blood pressure
	Laurus nobilis L.	Blond	Leaf	Menstrual cramps
	Persea americana Mill.	Avocado	Semente	Inflammation of the mouth, kidney stones, urinary tract infection, kidney pain
Leguminosae	*Allium cepa L.*	White onion	Leaf	Flu, bronchitis, sinusitis
	Allium sativum L.	Garlic	Leaf	Flu and sore throat, high blood pressure, cramps, weight loss, headache, fever, cancer
	Aloe vera (L.) Burm. f.	Slug	Leaf	Inflammation, flu, blood thinner, hair tonic, cancer, hemorrhoids, general pain

Table 1. Contd.

Monimiaceae	*Peumus boldus Mol*	Boldo	Leaf	Labirinitis, stomach pain, general pain, flu, poor digestion, stomach pain, intestinal infection, liver pain
	Eugenia caryophyllus Spreg.	Clove of india	Leaf	Menstrual cramps
Myrtaceae	*Eugenia Malaccensis Linn.*	Jambo	Stalk	Toothache
	Psidium guajava L.	Guava	Fruit	Diarrhea
Pedaliaceae	*Sesamum indicum L.*	Sesame	Stalk	Fever, tones blood, inflammation, hair tonic, bruising
Piperaceae	*Piper nigrum L.*	Black pepper	Fruit	Depurative
	Cymbopogon citratus (D.C.) Stapf.	Holy grass	Fruit	Nervousness, flu, soothing, high blood pressure, lack of appetite
Poaceae	*Saccharum officinarum L.*	Purple sugar cane	Fruit	High blood pressure, swelling, ophthalmic problems
	Zea mays L.	Corn	Stalk	Stomach pain, Vomiting, Poor digestion
Rubiaceae	*Coffea arabica L.*	Coffee	Seed	Sore throat
	Genipa americana L.	Genipap	Fruit	Fractures
Solanaceae	*Solanum tuberosum L.*	English potato	Root	Gastritis, migraine
	Solanum lycopersicum L.	Tomato	Fruit/ stalk	Ophthalmologic problems
	Solanum melongena L.	Eggplant	Leaf	High cholesterol
	Waltheria indica L.	Kingdom mallow	Leaf	Inflamed throat, flu, headache, cough, inflammation, stomach pain,

Source: Data from the survey conducted in the neighborhoods Nova Cidade and Raiar do Sol, 2018.

discussing perceptions and concepts of health and disease in the construction of public health policies focused on health promotion.

The most used parts according to studies by Alves and Povh (2013) and Soares et al. (2009) were leaves (73%), flower (17%), fruit (7%) and seed (3%). Validating the data of Alves and Povh (2013), Soares et al. (2009) also demonstrate the predominance of leaf use in the preparation of home remedies. The data of Alves and Povh (2013) affirm that, after the leaf, the vegetal parts most used by their interviewees were the root, the fruit and the bark.

Similar results were also reported by Birhanu and Ayalew (2018), in the study entitled, "Indigenous knowledge on medicinal plants used in and around the town of Robe, in the Bale region, in the Oromia region of southeastern Ethiopia"; they found that the leaf is the most harvested plant part (52.7%) in the study area for medicinal use, followed by seed (9.4%), root (8.14.5%), stem (4.3%), root leaf (2.6%), fruit (2.6.6%) and bulb (1.8%).

Finally, Barbara Bäckström (2011) presented similar results in her study on "Health Behaviors and Disease in a Cape Verdian Community in Lisbon". The author states that flora plays a fundamental role as a medical resource and is explained by its isolated geographical situation. The plants are mainly used in the form of infusions or syrups, prepared using leaves and peels.

Therapeutic procedures

Methods of preparation and administration

The oral route was mentioned most as the route for administering therapeutic treatment of many diseases (219 citations, 44.7%), followed by the topical route (90 citations, 18.4%); this was a significant result (χ^2 = 30.69, gl = 1, p <0.001). These data corroborate the findings of Ribeiro et al. (2014), in which the external route represented 79% of the household income; while the topical route, only 21%. It also corroborates the studies of Vasquez et al. (2014), in which the oral route was the most frequently mentioned as an administrative route for the therapeutic treatment of many diseases (244 citations, 64%), followed by the topical route (136 citations, 34%) (χ^2 = 30.69, gl = 1, p <0.001).

There are also different forms of elaboration, with tea being the most used in decoction or infusion preparations, similar to results found in studies by Lacerda et al. (2013), Silva et al. (2014) and Ribeiro et al. (2014).

Vasquez et al. (2014) recorded various practical procedures for the treatment of diseases: the use

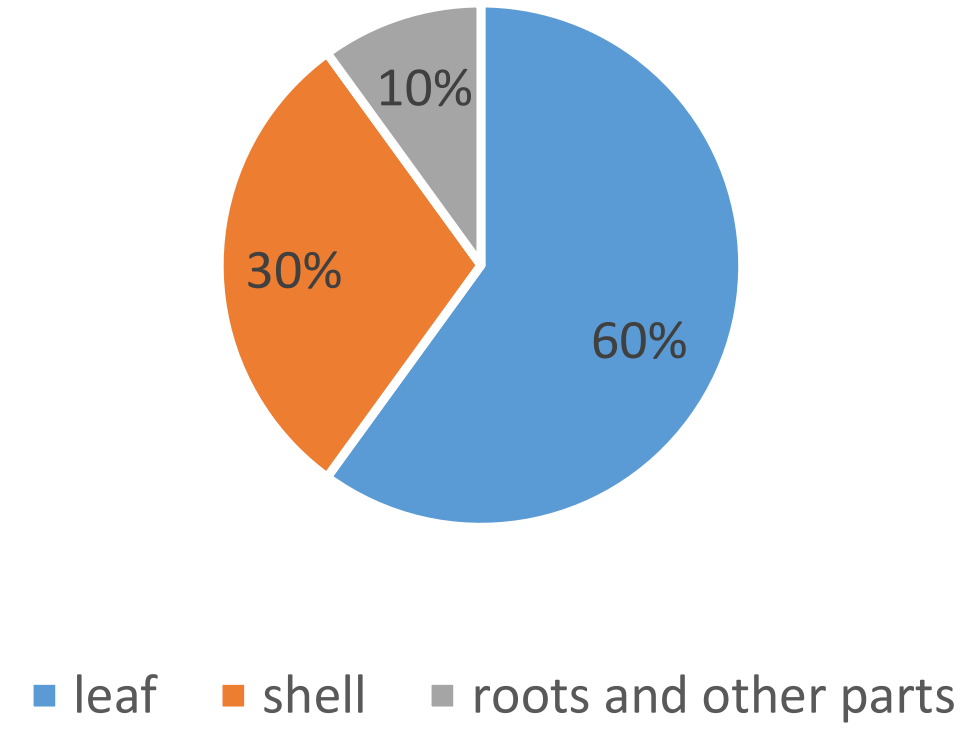

Figure 6. Percentage referring to the main parts of the plants mentioned by the research participants who are residents of the neighborhoods Nova Cidade and Raiar do Sol.

of friction, application of patches, use of oral tea, and application of baths and toiletries. In the Boa Vista/Roraima research, the preparation of the remedies used in the therapeutic procedures also showed the combined uses of plants in these treatments, being used several times for several medicinal plants or several parts of the same plant species, mainly in bottles or baths.

Birhanu and Ayalew (2018) researched the indigenous knowledge of medicinal plants used in and around the town of Robe in the Bale region in the Oromia region of southeastern Ethiopia (26.47, 3%) who were the most prominent, followed by the dermal (19.34, 5%).

In the study by Vasquez et al. (2014), the method of preparation of the medicinal plants was diverse, the main one being tea (62.2%) used in the preparation of several species, followed by juice (11.8%), plaster (7.1%), maceration (7%), bath (6.1%), syrup (4.8%) and juice (2.2%). According to the informants, teas can be prepared by infusion or by decoction depending on the part of the plant being used.

Transmission of knowledge

The study methodology was designed to ensure that all the participants knew and used the plants they cited as medicine. Their local knowledge of plants has been acquired over the years, both by trial and error and by the transmission of knowledge from one generation to the next. The chi-square test showed that there was a significant difference ($\chi^2 = 14.02$, gl = 5, $p < 0.01$) among the different individuals responsible for knowledge transmission in the study districts. Knowledge about the use of plants as medicines was transmitted mainly through the family (including parents, grandparents, siblings, aunts and uncles) (48%) (Figure 8).

Figure 8 shows how knowledge is transmitted or learned by residents of the New City and Sun Ratio neighborhoods. It is often passed on from generation to generation (48%); other times, it is taught by neighbors (13%), which demonstrate the existence of a network of relationships. At other times, people use plant-based medicines under the guidance of healers, raitarians and spiritual counselors (10%) in their religious or faith network. Others learned by reading books or magazines (20%). Some also mentioned having been guided by community health agents (ACS) (7%) working in the formal healthcare network. Additionally, 2% reported having acquired knowledge through the internet. When referring to the term network, it means the set of relations constructed with a common goal.

Most research subjects said that they usually pass on knowledge to their children, neighbors, friends and anyone who needs guidance on home remedies. In the Raiar do Sol neighborhood (Boa Vista/RR) there was a practice of selling 'bottled' medicinal preparations, which are an alternative source of income for some residents who are traditional medicine professionals.

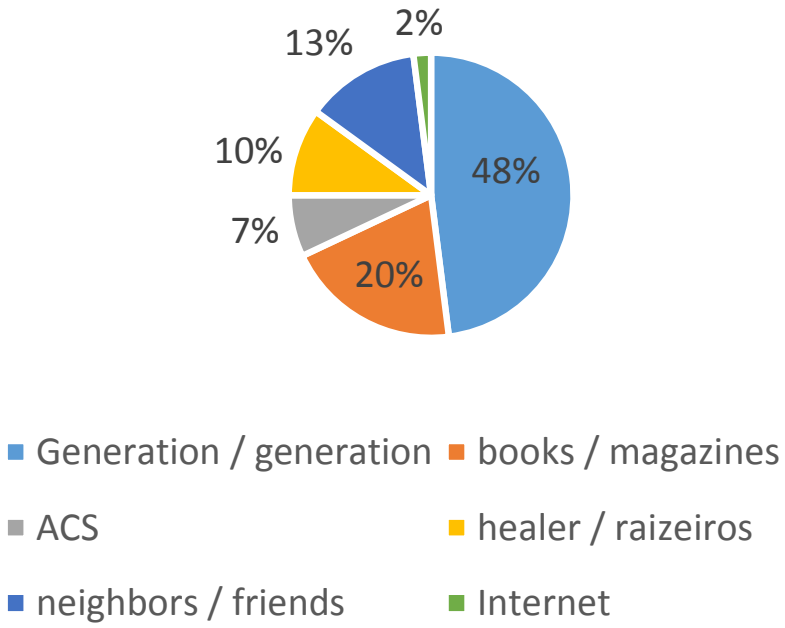

Figure 7. Percentage referring to the main forms of transmission of local knowledge cited by the research participants who are residents of the neighborhoods, Nova Cidade and Raiar do Sol.

Contrary to what happens in the Raiar do Sol neighborhood, according to reports from the residents of the Nova Cidade neighborhood, there is no practice in this area of selling of medicinal preparations by traditional medicine professionals. Instead, phytotherapeutic products are easily found in pharmacies in the neighborhood and in many pharmacies of manipulation and networks of existent franks in the municipality of Boa Vista. In this neighborhood, a religious institution and the basic health unit had long been offering phytotherapy to assist the treatment of the needy population. However, for a number of reasons, it is suggested that some of them do not do it anymore (because of lack of interest of people involved in the process, work overload, lack of skilled people, and lack of financial resources, among others).

Since these are neighborhoods in which there are large populations of indigenous, Afrodescendant and Northeastern (migrant) peoples, the inhabitants have brought in other local knowledge, which is then passed on to their families, neighbors and friends. These form a network of relationships and transmission of knowledge. Thus, migration is important to the dissemination and transfer of knowledge.

It can be emphasized that the family network of knowledge transmission is highly relevant in these neighborhoods and reinforces the transfer of local knowledge among the people who settle there. The community's existing therapeutic system is basically syncretic, with the presence of indigenous, African, northeastern and northern elements. New knowledge, once achieved, beyond purely academic interest, can bring practical results by providing resources to lay on the foundation of health systems more adapted to the culture and conditions of the region. Flor and Barbosa (2014) reported similar findings in a study on popular wisdom in the use of medicinal plants by the residents of the quiet district in the district of Marudá – PA (Figure 7).

Conclusion

Although modern medicine is continually growing in the two neighborhoods where the research was conducted, traditional medicine still plays a large role in treating different diseases. It has been proven that this biodiversity is being used in addressing a variety of diseases. The results show the richness in the knowledge and use of medicinal flora present in the Northern Brazilian Amazon. The main diseases mentioned in the research as responding to plant-based medicine were diabetes mellitus, hypertension, cardiorespiratory and digestive disorders, parasitoses, skin diseases and others. This study will allow for the expansion of therapeutic options for users through access to medicinal plants.

The ethnobotanical study with emphasis on collective health showed remarkable knowledge and use of therapeutic properties of a range of species. Although the number of exotic medicinal plants was high, species such

as *Bauhinia forficata* stood out and is a promising subject for bioprospecting studies. The present work is the first to document the vegetal species Bauhina forficata in Roraima, and knowledge of its traditional use as hypoglycemic in the concomitant treatment to the biomedical model of the diabetes mellitus type II metabolic syndrome in the Nova Cidade and Raiar do Sol neighborhoods, Good View/RR. Of all the plants cited, most show broad medical potential, although there are just little scientific proofs on their biological activities.

Various species—*Chenopodium ambrosioides* (mastruz), *Myracrodruon urundeuva* (aroeira), *Plectranthus barbatus* (boldo), *Mentha spicata* (peppermint), *Citrus sinensis* (orange), *Cymbopogon citratus* and the culturally important *Ruta graveolens*—deserve special attention for future pharmacological and phytochemical studies to confirm their therapeutic properties and to test their toxicity or safety for humans. Popular knowledge that is backed by scientific knowledge will contribute to the rational use of medicinal plants and awareness of the importance of conserving them.

From this perspective, this study can contribute to further work related to the practices, using medicinal plants. The information provided is essential for guiding and consolidating knowledge of the therapeutic properties present in key species and also contributes to the formulation of public policies in the state of Roraima/Brazil.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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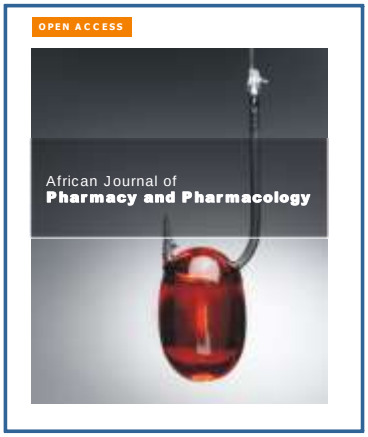

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