IJPSR (2015), Vol. 6, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 March, 2015; received in revised form, 30 April, 2015; accepted, 23 June, 2015; published 01 October, 2015

PHYSICOCHEMICAL CHARACTERIZATION, MICROBIOLOGICAL QUALITY CONTROL AND TOXICITY EVALUATION OF THE HIDROETHANOLIC EXTRACT FROM *CHENOPODIUM AMBROSIOIDES* LINN

Érika da Silva Valério[1], Wagner Luiz Ramos Barbosa[1], Regina Maria Finger[2], Michelle Frazão Muzitano[3], Marlon Heggdorne de Araújo[3], Flavio de Vasconcelos[1] and Francisco Martins Teixeira[1, 2 *]

[1] Programa de Pós-graduação em Ciências Farmacêuticas, Instituto de Ciências da Saúde, Universidade Federal do Pará, Belém, Pará, Brasil; [2] Macaé campus-UFRJ, Universidade Federal do Rio de Janeiro, Macaé, Rio de Janeiro, Brasil; [3] Laboratório de Produtos Naturais, Macaé Campus-UFRJ, Universidade Federal do Rio de Janeiro, Macaé, Rio de Janeiro, Brasil.

Keywords:

Chenopodium ambrosioides, Amaranthaceae, physicochemical characterization, microbiological quality control, acute oral toxicity, cytotoxicity.

Correspondence to Author:

Dr. Francisco Martins Teixeira

Professor Adjunto III da disciplina Controle Biológico e Microbiológico de Qualidade de Produtos Farmacêuticos, Campus UFRJ-Macaé (Coordenação de Farmácia), Avenida Aluizio da Silva Gomes, 50, Granja dos Cavaleiros, Macaé – RJ CEP 27930-560

E-mail: fteixeira@macae.ufrj.br

ABSTRACT: *Chenopodium ambrosioides* Linn, member of the National List of Medicinal Plants of Interest to SUS (RENISUS), is used to treat different diseases and shows good potential to generate products of interest to the Brazilian Unified Health System "Sistema Único de Saúde" (SUS). However, the use of herbal derivatives implies the control of their quality and the evaluation of their toxicity. Pharmacopeial tests were performed aiming at the physicochemical and microbiological characterization of leaves and extract of *C. ambrosioides*. The toxicity of the extract was assessed using MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) and acute toxicity was assayed in mice. The results showed that the leaves and the extract presented acceptable pharmacopeial parameters and the preliminary phytochemical analysis showed the presence mainly of saponins, terpenes, phenols and tannins in the hydroethanolic extract of *C. ambrosioides*. The microbiological evaluation of the extract indicated no apparent growth of pathogenic microorganisms. Although the extract showed cytotoxicity at high concentration, no signs of acute oral toxicity could be observed at the tested doses. These results, besides the partial Pharmacopeial characterization of *C. ambrosioides*, corroborate the literature data, regarding the cytotoxic potential. However, the results reported indicate that the use of this species is safe in appropriate doses

INTRODUCTION: Medicinal plants are used in the treatment of diseases by thousands of people in the world and often represent the main and first source of health care. This form of therapeutic resource is accessible, available and culturally accepted in folk medicine [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(10).4190-97

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(10).4190-97

In Brazil, a great percentage of the population makes use of natural products, mainly from plants, as an alternative source in the cultural context and as phytotherapics [2, 3]. The research interest in using medicinal plants has increased in recent years and among the factors driving this interest is the proven effectiveness of substances derived from plant species. Medicinal plants are important natural sources of biologically active substances, many of which are models for the synthesis of a large number of drugs, those products showing high diversity in structure and physicochemical and biological properties [4-7].

Noting the need of the market and the lack of quality control of many raw materials and extracts, this paper aims to study the hydroethanolic extract of the plant species *Chenopodium ambrosioides* Linn (Amaranthaceae), popularly known as "mastruz" and widely used in folk medicine, being considered one of the plant species with potential for use in the production chain and to generate products of interest to the Brazilian Unified Health System "Sistema Único de Saúde" (SUS), as part of the National List of Medicinal Plants of Interest to SUS (RENISUS) [8, 9].

It is an annual or perennial herbaceous plant with distinctive aroma, upright, that reaches up to 1.5 m high, with dark green and camphorated pubescent leaves. Several therapeutic activities are assigned to the species, such as anti-inflammatory action, antifungal, antitumor, immunomodulatory, analgesic and antibacterial. The species is distinguished by its constitution rich in terpenes, flavonoids, gallic tannins and alkaloids [10-16]. Although there are studies that attribute to *C. ambrosioides* a possible toxic action, allegedly resulting from the presence of the monoterpene ascaridole, constituent abundant in the specie [17-19], there are also reports of no toxicological changes in treating mice with different extracts from *C. ambrosioides* [20, 21]. So, the relevance of research on the toxicity of the species using *in vitro* and *in vivo models* is highlighted.

MATERIALS AND METHODS:

Animals:

10 mice females (*Mus musculus*) were used, divided into two groups of five animals, weighing between 20g - 30g, from the Central Animal Facility of the Federal University of Pará (UFPA). During the adaptation period the animals were kept in the Animal Facility of the Faculty of Pharmacy for a week under standard conditions (temperature 22 ± 3°C, relative humidity 30-70% with control of light/dark cycle), as specified in the literature. The animals had access to food and water *ad libitum*. The methodology was approved by the Ethics Committee on Research with Experimental Animals, under the Protocol BIO098-12, taking into account "*Ethical Principles in Animal Experimentation* of the Brazilian College of Animal Experimentation"[22].

Plant Material:

Leaves of *C. ambrosioides* used in this work were acquired in the Ver-o-Peso Market, coming from the Icoaraci district, municipality of Belém, located around the geographical coordinates of 1º10' and 1º20' south latitude and 48º20' and 48º30' west longitude. The identification of the plant sample was made at the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) and a voucher specimen of the species was deposited under registration number IAN188445 (**Fig. 1**).

FIG.1: VOUCHER SPECIMEN OF THE SPECIES *CHENOPODIUM AMBROSIOIDES* L. REGISTERED UNDER NUMBER IAN 188445

Obtaining the plant drug:

The fresh leaves were dried in an oven with circulating air at 40°C ± 2°C, until stabilizing the weight of an aliquot. After this condition grinding was executed in a Wiley mill **(**NOGUEIRA, Model DM JUNIOR**)**. This process reduces the particle size of the sample and the powder obtained increases the contact surface between plant drug and fluid extractor.

Determination of particle size distribution:

Based on the Brazilian Pharmacopoeia (2010) and to analyze the grain size of the plant drug obtained from dried leaves of *C. ambrosioides* a sample of 25 g of the plant drug was subjected to vibration by

forced passage through sieves with mesh apertures corresponding to 125; 180; 250; 355; 710 and 1700 µm, using shaker sieves (Bertel) at the scale 10 of the apparatus, for thirty minutes. After this process, the plant drug retained in the sieves and collector was weighed. This procedure was performed in triplicate [23].

Determination of loss from drying the plant drug

1 g sample of plant drug were subjected to heating at 110°C by infrared rays for a period of approximately one hour. After this period weighing was done. The test was done in triplicate [23].

Dry Matter Content:

Aliquots of 2 ml of the extract were distributed evenly in an aluminum collector for later subjection to infrared moisture analyzer (Gehaka IV-2000) at a temperature of 105°C for fifteen minutes. The amount of moisture (or dry matter) was given in percentage, accompanied by the arithmetic mean and standard deviation of three determinations[23].

Determination of pH:

In accordance with the Brazilian Pharmacopoeia (2010), the pH of the extract was measured using a potentiometer (HANNA 58210) calibrated previously. Results were expressed as mean of three determinations [23].

Determination of Ash Content

For this test a porcelain crucible was previously calcined in a muffle furnace (ENGRO) for 30 minutes and then cooled in a desiccator for 15 minutes and weighed on an analytical balance. Aliquots of 3 g of plant drugs were distributed uniformly in the crucible and subjected to calcination in a muffle furnace at a temperature of 600°C for 180 minutes. The sample was allowed to cool in a desiccator for 30 min for later weighing. The results were expressed as percentage of the dry weight of drug ash (%, w/w) representing the mean of three determinations [23].

Preliminary Phytochemical Analysis:

The hidroethanolic extract from *Chenopodium ambrosioides* Linn were subjected to preliminary phytochemical analysis aimed to detect different classes of secondary metabolites through chemical reactions, following the methodology described by Camelo and collaborators [24].

Microbiological Quality Control:

The total count of microorganisms and the possible detection of specific pathogens were performed using the methodologies described in the Brazilian Pharmacopoeia 5th Edition, by Pinto and collaborators (2010) and Silva Junior and colleagues (2011) [25, 26]. Standard ATCC strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* were used as positive controls.

Cell Culture Assays for Cytotoxicity: Peritoneal macrophages from murine RAW 264.7 obtained from ATCC (Rockville, MD, USA) were kindly provided by the Laboratory of Natural Products Campus-Macaé, UFRJ (Federal University of Rio de Janeiro), and cultured at 37°C with 5% CO_2 in DMEM/F-12 medium supplemented with 10% fetal bovine serum and gentamicin (50 µg/mL).

Evaluation of cell viability in RAW 264.7 macrophages:

For the cytotoxicity assay the MTT method was used according to the procedure described by Mosmann (1983)[27]. RAW 264.7 macrophages grown in culture bottles were plated at a concentration of 1×10^5 cells/well in a 96 well plate in the presence of the hydroethanolic extract of *Chenopodium ambrosioides* L. in concentrations of 0.8µg/mL, 4 µg/mL, 20 µg/mL and 100 µg/mL in a final volume of 100 µL/well. In the wells used for negative control, macrophages were cultured in DMEM complete only without the extract. In the wells used as positive control, macrophages were treated with 1% Triton X-100. The plates were incubated for 24 hours at 37°C containing 5% CO_2. After this period, 10 µL of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) (Sigma) was added to each well.

After 2 hours of incubation at 37ºC in the dark humid incubator, the supernatant of the plate was removed and the crystals formed were solubilized by 120 µL HCl (4 mM) in 80 mL of isopropanol (1N). Plates were read on a microplate reader using a 570 nm filter. Cytotoxicity was calculated using the following formula (O.D. – optical density):

100 - (O.D. sample - O.D. negative control) * 100/O.D. Triton - O.D. Negative Control)

Acute Oral Toxicity Study (OECD, 2001):

Each group of animals was kept fasting for 12 hours and subsequently received via a stomach tube, by gavage, the dose of 2500 mg/kg of extract *C. ambrosioides* L in 0.9% saline, the dose recommended by OECD for medicinal plants. Systematic behavioral observations were performed to evaluate the Hippocratic screening which provided a general estimate of the toxicity of the substance on the conscious state and general mood, activity and coordination of the motor system, reflexes and activities on the central nervous system and the autonomic nervous system[28]. Parameters such as general activity, vocal tremors, irritability, touch response, response to tail clamping, corneal, tremors, convulsions, hair bristle, hypnosis, anesthesia, lacrimation, ptosis, urination, defecation, piloerection, hypothermia, breathing, cyanosis, hyperemia and death were determined in the 15 min, 30 min, 1 h, 2 h, 4 h and 8 h after administration and thereafter daily until the fourteenth day. The surviving animals were euthanized with a solution of xylazine (Bayer) and ketamine (Laboratory Konig SA) (2:1) at a dose of 2.5mL/kg and the organs were harvested for anatomo-histopathological evaluation.

Statistical Analysis:

Data were analyzed using GraphPad Prism 4 software by analysis of one-way variance (ANOVA) followed by multiple comparison test (Tukey test) those results that showed "*p*" value < 0.05 were considered statistically significant.

RESULTS:

The result of the sieve analysis of vegetable drugs shown in **Fig.2,** shows that the vegetable drug *C. ambrosioides* can be classified as coarse powder, since the entire sample went through the sieve of 1700 µm mesh and about 47% through the 355 µm mesh.

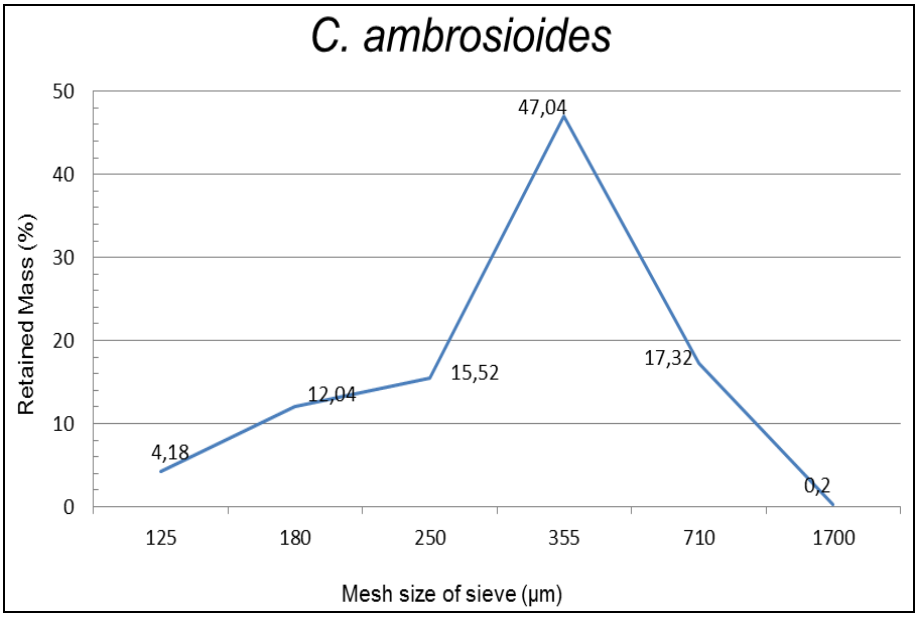


FIG.2: PARTICLE SIZE DISTRIBUTION OF THE PLANT DRUG *CHENOPODIUM AMBROSIOIDES*

TABLE 1: DETERMINATION OF LOSS ON DRYING AND ASH CONTENT OF *C. AMBROSIOIDES* L.

Determinations	Results (%)
Loss from drying	8.9 ± 1
Total ash	10 ± 0.005

Note: All analyzes were performed in triplicate.

The percentage of dry crude extract *C. ambrosioides* L was 3.6% and preliminary data from the phytochemical analysis showed chemical groups that can be employed for the characterization of raw materials. This is the case of phenols, tannins, foam forming saponins, proteins and amino acids, triterpenoids and steroids (**Table 2**).

TABLE 2: DETERMINATION OF pH, DRY MATTER AND PHYTOCHEMICAL OF *C. AMBROSIOIDES* L.

Determinations	Results
pH	6.60 ± 0.03%
Dry matter	3.6 ± 0.3%
Preliminary phytochemical analysis	Foam-forming saponins, proteins, amino acids, phenols, tannins, steroids and terpenoids

Additionally, the microbiological quality control parameters not presented contamination by pathogenic microorganisms (**Table 3**).

TABLE 3: MICROBIOLOGICAL ANALYSIS OF THE HYDROETHANOLIC EXTRACT OF *C. AMBROSIOIDES* L.

Sample	Bacteria (CFU/g)	Fungus (CFU/g)
Hydroethanolic extract of *C. ambrosioides* L.	Absence	Absence
ATCC control strains	> 100 or Uncountable	> 100 or Uncountable

In the evaluation of cytotoxicity and acute oral toxicity of the hydroethanolic extract *C. ambrosioides* it was observed that the extract has a cytotoxic effect at the highest concentration used in this test, 100µg/ml (**Fig.3**). However, in the evaluation of acute oral toxicity, performed in animals treated with the extract at a dose of 2500 mg/kg, no signs or symptoms of alterations, such as compromise of central, autonomic or motor nervous system or behavioral changes were observed, considering the parameters evaluated and recommended by the OECD [28]. Furthermore, no histopathological changes that correlate with toxicological findings in the organs of animals treated with saline (control group) were observed when compared with the group treated with the hydroethanolic extract of *C. ambrosioides* both as regards macroscopic or microscopic aspects, where the brain, lung, heart, kidney and liver were within normal histological limits (**Fig. 4).**

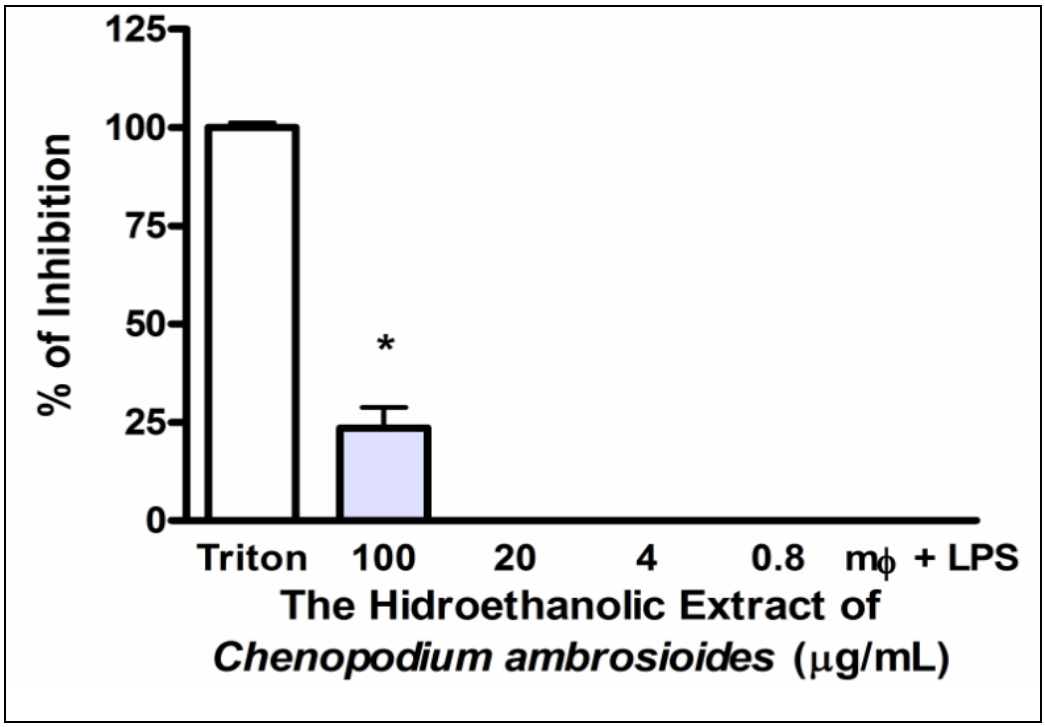
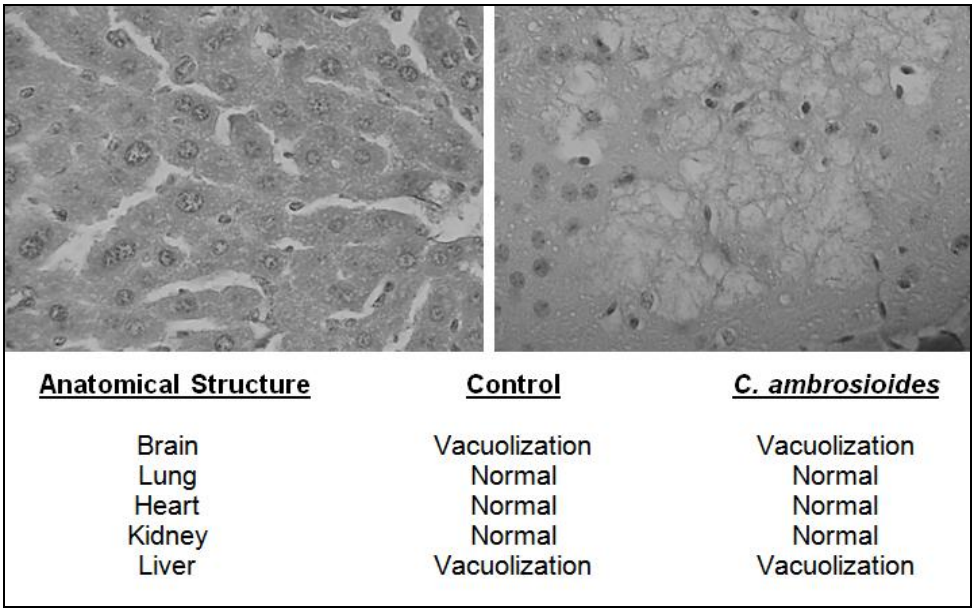


FIG.3: PERCENT INHIBITION OF CELL VIABILITY OF MACROPHAGES STIMULATED WITH LPS AFTER TREATMENT WITH THE HYDROETHANOLIC EXTRACT OF *CHENOPODIUM AMBROSIOIDES* L. TRITON IS USED AS A POSITIVE CONTROL. THE DATA REPRESENT THE MEAN OF TRIPLICATES FROM ONE OF THREE EXPERIMENTS PERFORMED WITH SIMILAR RESULTS.

*** p < 0.001 WHEN COMPARED TO THE NEGATIVE CONTROL (MACROPHAGES STIMULATED WITH LPS AND NOT TREATED WITH EXTRACTS - mØ + LPS).**



Anatomical Structure	Control	*C. ambrosioides*
Brain	Vacuolization	Vacuolization
Lung	Normal	Normal
Heart	Normal	Normal
Kidney	Normal	Normal
Liver	Vacuolization	Vacuolization

FIG. 4: REPRESENTATIVE PHOTOMICROGRAPHS OF LIVER AND BRAIN OF MICE SUBJECTED TO TREATMENT WITH SALINE (CONTROL GROUP) AND THE HYDROETHANOLIC EXTRACT OF *C. AMBROSIOIDES*.

DISCUSSION: Proper pulverization of vegetable raw material is of paramount importance to achieve a good performance in the extraction process of the chemical constituents contained in the plant drug. The particle size of the vegetable raw material is the parameter which determines the contact surface of the drug particles that interact with the solvent in obtaining pharmaceutical ingredients such as dyes and extracts [29-31]. Thus, the particle size of herbal drugs directly affects the efficiency of the extraction process. The loss on drying of plant drug was 8.9% (**Table 1**), these results as well as those of Okhale and colleagues [32], who reported that moisture content of 10% for the drug from plant leaves *C. ambrosioides* are in accordance with the Brazilian Pharmacopoeia 5th Edition, which advocates maximum moisture content of up to 14% for herbal drugs.

The ash content for *C. ambrosioides* was 10%, the result is important for the quality standardization of herbal drugs, since a high ash content might indicate an excess of adherent substances, which may indicate inadequate collection, as well as the drying and transport of materials, or differences in geographic location of the materials analyzed [33-35]. The percentage of dry crude extract *C. ambrosioides* L was 3.6% (**Table 2**). The dry matter is indicative of water content and volatile substances from the extraction of vegetable drugs, these data are important for calculating the yield of extraction, since the drying influences the integrity of cellular components, allowing these components to be exposed to contact with solvents [36, 37], so this percentage corresponds to the concentration of the extract. The value of pH of the crude extract of *C. ambrosioides* is 6.6, which suggests the presence of weakly acidic compounds.

Microbiological quality control is essential to ensure the quality and safety of the product, especially when you have as raw material inputs of vegetable origin [38]. The results presented here indicate that the material used for obtaining the hydroethanolic extract from the leaf of mastruz shows no growth of bacteria and fungus in the culture media used. The evaluation of cytotoxicity and acute oral toxicity of the hydroethanolic extract *C. ambrosioides* data are in agreement with those of literature showing cytotoxicity of the extract, essential oil, and single compounds of this species to eukaryotic cells, and demonstrated antitumor activity [39-42]. However, this cytotoxicity does not implicated in significant toxicological activity in mice at the dose for studying acute oral toxicity.

CONCLUSION: The results presented contribute to the pharmacopeial characterization of derivatives of *C. ambrosioides,* providing inedited data, such as particle size determination and dry matter. Other data confirm the results already published that demonstrate the cytotoxic activity of the species,

moreover, they indicate that the hydroethanolic extract of *C. ambrosioides* presents no significant toxicological activity in mice at the dose recommended by OECD for studies of acute oral toxicity.

ACKNOWLEDGEMENTS: The authors thank CAPES for financial assistance, the UFPA and the UFRJ, for the donation of animals and physical structure for the development of the experiments.

REFERENCES:

1. World Health Organization. WHO Sites. WHO Traditional Medicine Strategy 2014 - 2023. (WHO, 2014) Available in the World Wide Web: http://www.who.int/medicines/publications/traditional/trm_strategy14_23/en/
2. Araújo CRF, Silva AB, Tavares EC, Costa EP, Mariz SR: Perfil e prevalência de uso de plantas medicinais em uma unidade básica de saúde da família em Campina Grande, Paraíba, Brasil. Rev. Ciênc. Farm. Básica Apl. 2014. 35, n.2, 233-238.
3. Stolz ED, Müller LG, Trojan-Rodrigues M, Baumhardt E, Ritter MR, Rates SM: Survey of plants popularly used for pain relief in Rio Grande do Sul, southern Brazil. Rev. Bras. Farmacogn. 2014. 24, n.2, 185-196.
4. Aziz MM, Raza MA, Saleem H, Wajid M, Bashir K, ur Rehman MI: Medicinal values of Herbs and Plants, Importance of Phytochemical evaluation and Ethnopharmacological Screening: An Illustrated review essay. J. Pharm. Cosmet. Sci. 2014. 2, n.1, 6-10.
5. Liu YQ, Li WQ, Morris-Natschke SL, Qian K, Yang L, Zhu GX, Wu XB, Chen AL, Zhang SY, Nan X, Lee KH: Perspectives on biologically active camptothecin derivatives. Med. Res. Rev. 2015. Mar 21. doi:10.1002/med.21342. [Epub ahead of print].
6. Sharma V, Sharma PC, Kumar V: A mini review on pyridoacridines: prospective lead compounds in medicinal chemistry. J. Adv. Res. 2015. 6, n.1, 63-71.
7. Russo P, Frustaci A, Del Bufalo A, Fini M, Cesario A: Multitarget drugs of plants origin acting on Alzheimer's disease. Curr. Med. Chem. 2013. 20, n.13, 1686-1693.
8. Brasil. Ministério da Saúde RENISUS. Relação nacional de plantas medicinais de interesse ao SUS. Espécies vegetais. Available in the World Wide Web: <http://portal.saude.gov.br/portal/arquivos/pdf/RENISUS.pdf>. Accessed on: May, 15th 2013.
9. Sousa ZL, Oliveira FF, Conceição AO, Silva LAM, Rossi MH, Santos JS: Biological activities of extracts from Chenopodium ambrosioides Lineu and Kielmeyera neglecta Saddi. Ann. Clin. Microbiol. Antimicrob. 2012. 11, p.20.
10. Grassi LT, Malheiros A, Meyre-Silva C, Buss ZS, Monguilho ED, Frode TS, Silva KABS, Souza MM: From popular use to pharmacological validation: A study of the anti-inflammatory, anti-nociceptive and healing effects of Chenopodium ambrosioides extract. J. Ethnopharmacol. 2013. 145, 127-138.
11. Jardim CMJ, Jham GM, Dhingrab OD, Freire MM: Chemical Composition and Antifungal Activity of the Hexane Extract of the Brazilian Chenopodium ambrosioides L. J. Braz. Chem. Soc., 2010. 21, n.10, 1814-1818.
12. Nascimento FRF, Cruz GVB, Pereira PVS, Maciel MCG, Silva LA, Azevedo APS, Barroqueiro ESB, Guerra RNM: Ascitic and solid Ehrlich tumor inhibition by Chenopodium ambrosioides L. treatment. Life Sciences. 2006. 78, 2650-2653.
13. Cruz GVB, Pereira PVS, Patricio FJ, Costa GC, Sousa SM, Frazão JB, Aragão-Filho WC, Maciel MCG, Silva LA, Amaral FMM, Barroqueiro ESB, Guerra RNM, Nascimento FRF: Increase of cellular recruitment, phagocytosis ability and nitric oxide production induced by hydroalcoholic extract from Chenopodium ambrosioides leaves. J. Ethnopharmacol. 2007. 111, 148-154.
14. Ibironke JF, Ajiboye KI: Studies on the anti-inflammatory and analgesic properties of Chenopodium ambrosioides leaf extracts in rats. International Journal of Pharmacology. 2007. 3, n.1, 111-115.
15. Lall N, Meyer JJM: In vitro inhibition of drug sensitive strains of Mycobacterium tuberculosis by ethnobotanically selected South African plants. J. Ethnopharmacol. 1999. 66, 347-354.
16. Hallala A, Benalia S, Markouk M, Bekkouchea K, Larhsinia M, Chaitb A, Romanec A, Abbada A, Abdounid MKE: Evaluation of the Analgesic and Antipyretic Activities of Chenopodium ambrosioides L. Asian J. Exp. Biol. Sci. 2010. 1, n.1, 189-192.
17. Dembitsky V, Shkrob I, Hanus LO. Ascaridole and related peroxides from the genus Chenopodium. Biomed. Pap. Med. Fac. Univ. Palacky. Olomouc. Czech. Repub. 2008. 152, 209-215.
18. Pastor J, García M, Steinbauer S, Setzer WN, Scull R, Gille L, Monzote L: Combinations of ascaridole, carvacrol, and caryophyllene oxide against Leishmania. Acta Trop. 2015. 145, 31-38.
19. Monzote L, García M, Pastor J, Gil L, Scull R, Maes L, Cos P, Gille L: Essential oil from Chenopodium ambrosioides and main componentes: activity against Leishmania, their mitochondria and other microorganisms. Exp. Parasitol. 2014. 136, 20-26.
20. Pereira WS, Ribeiro BPR, Sousa AIP, Serra ICPB, Mattar NS, Fortes TS, Reis AS, Silva LA, Barroqueiro ESB, Guerra RNM, Nascimento FRF: Evaluation of the subchronic toxicity of oral treatment with Chenopodium ambrosioides in mice. J. Ethnopharmacol. 2010. 127, 602-605.
21. Silva MGC, Amorim RNL, Câmara CC, Fontenele Neto JD, Soto-Blanco B: Acute and sub-chronic toxicity of aqueous extracts of Chenopodium ambrosioides leaves in rats. J. Med. Food. 2014. 17, n.9, 979-984.
22. COBEA 2003 Principios Éticos na Experimentação Animal do Colégio Brasileiro de Experimentação Animal. Available in: <http://www.cobea.org.br/>. Accessed on: October, 6th 2014.
23. Brazilian Pharmacopoeia, 5ª ed. São Paulo: fiocruz, 2010.
24. Camelo SRP, Costa RS, Ribeiro-Costa RM, Barbosa WLR, Vasconcelos F, Vieira JMS, Silva Júnior JOC: Phytochemical evaluation and antimicrobial activity of ethanolic extract of Vismia guianensis (Aubl.) Choisy. International Journal of Pharmaceutical Sciences and Research, 2011. 2, 3224-3229.
25. Pinto TJA, Kaneko TM, Pinto AFT: Controle biológico de qualidade de produtos farmacêuticos, correlatos e cosméticos. São Paulo, Ateneu, 2010, 7805 p.

26. Silva Júnior JOC, Costa RMR, Teixeira FM, Barbosa WLR Processing and Quality Control of Herbal Drugs and Their Derivatives – Chapter 11 In: Shoyama Y: Quality Control of Herbal Medicines and Related Areas INTECHOPEN 2011. Available in: <http://www.intechopen.com/books/quality-control-of-herbal-medicines-and-related-areas> Accessed on: September, 1st 2014.
27. Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 1983. 16, p.55-63.
28. OECD 2001. Guideline 423: Acute Oral Toxicity – Acute Toxic Class Method. Accessed on: October, 1st 2013 in: www.oecd.org/publications Paris: Head of Publications Service.
29. Govindaraghavan S, Sucher NJ: Quality assessment of medicinal herbs and their extracts: Criteria and prerequisites for consistent safety and efficacy of herbal medicines. Epilepsy & Behav. 2015. Apr 18. doi:10.1016/j.yebeh.2015.03.004 [Epub ahead of print].
30. Alves MSM, Mendes PC, Vieira JGP, Ozela EF, Barbosa WLR, Silva Júnior JOC: Análise farmacognóstica das folhas de Arrabidaea chica (Humb. & Bonpl.) B. Verlt., Bignoniaceae. Rev. Bras. Farmacogn., 2010. 20, 215-221.
31. Kobayashi YTS, Almeida VT, Bandeira T, Alcântara BN, Silva ASB, Barbosa WLR, Silva PB, Monteiro MVB, Almeida MB: Avaliação fitoquímica e potencial cicatrizante do extrato etanólico dos frutos de jucá (Libidibia férrea) em ratos Wistar. Braz. J. Vet. Res. Anim. Sci., 2015, 31, 1-11.
32. Okhale SE, Egharevba HO, Ona EC, Kunle OF: Phytochemical and proximate analyses and thin layer chromatography fingerprinting of the aerial part of Chenopodium ambrosioides Linn. (Chenopodiaceae). J. Med. Plants Res. 2012. 6, n.12, 2289-2294.
33. Sharapin N: Fundamentos de tecnologia de Produtos Fitoterápicos. Santa Fé de Bogotá, D.C., colômbia, Convênio Andrés Bello. 2000. p.145-157.
34. Ayeni MJ, Oyeyemi SD, Kayode J, Peter GP: Phytochemical, proximate and mineral analyses of the leaves of Gossypium hirsutum L. and Momordica charantia L. Journal of Natural Sciences Research. 2015. 5, n.6, 99-107.
35. Hussain J, Rehman NU, Shinwari ZK, Khan AL, Al-Harrasi A, Ali L, Mabood F: Preliminary comparative analysis of four botanicals used in the traditional medicines of Pakistan. Pak. J. Bot. 2014. 46, n.4, 1403-1407.
36. Harborne JB, Mabry TJ, Mabry H The Flavonoids: Advances in Research; Chapman and Hall: London, p.56. 1986.
37. Meneses NG, Martins S, Teixeira JA, Mussatto SI: Influence of extraction solvents on the recovery of antioxidante phenolic compounds from brewer's spent grains. Sep. Purif. Technol. 2013. 108, 152-158.
38. Verdi S, Younes S, Bertol CD: Avaliação da qualidade microbiológica de cápsulas e chás de plantas utilizadas na assistência ao tratamento da obesidade. Rev. bras. plantas med. 2013. v.15, n.4.
39. Jia-Liang W, Dan-Wei M, Ya-Nan W, Hong Z, Bing H, Qun L, Zhi-Yan Z, Jing F: Cytotoxicity of Essential Oil of Chenopodium ambrosioides L against Human Breast Cancer MCF-7 Cells. Trop. J. Pharm. Res. 2013. 12, n.6, 929-933.
40. Monzote L, Stamberg W, Staniek K, Gille L: Toxic effects of carvacrol, caryophyllene oxide, and ascaridole from essential oil of Chenopodium ambrosioides on mitochondria. Toxicol. Appl. Pharmacol. 2009. 240, 337-347.
41. Gadano AB, Gurni AA, Carballo MA: Argentine folk medicine: genotoxic effects of Chenopodiaceae family. J. Ethnopharmacol. 2006. 103, 246-251.
42. Sowemimo AA, Fakoya FA, Awopetu I, Omobuwajo OR, Adesanya AS: Toxicity and mutagenic activity of some selected Nigerian plants. J. Ethnopharmacol. 2007. 113, n.3, 427-432.

How to cite this article:
da Valério É S, Barbosa WLR, Finger RM, Muzitano MF, de Araújo MH, de Vasconcelos F and Teixeira FM: Physicochemical Characterization, Microbiological Quality Control and Toxicity Evaluation of the Hidroethanolic Extract from *Chenopodium Ambrosioides* Linn. Int J Pharm Sci Res 2015; 6(10): 4190-97.doi: 10.13040/IJPSR.0975-8232.6(10).4190-97.