

Original Research Article

Phytochemical Study, Microbiological Activity and Cytotoxicity of Leaves and Stem from Plant Species *Mikania lindleyana* DC.

ABSTRACT

Aims: The objective of this research was to study phytochemical, antimicrobial activity and cytotoxicity of crude ethanol extract ~~of the leaves and stems of~~ *Mikania lindleyana* leaves and stems.

Study design: ~~Copies-Samples~~ of plant species *M. lindleyana* were collected in the city of Belém, Pará State, Brazil. The ~~material analyzed was the~~ leaves and stems of this species were analyzed and ~~was the plant was~~ identified by comparing with the voucher specimen at the Herbarium of the Federal University of Amapá, in the city of Macapá. Laboratory Experimental tests were performed at the Laboratory of Pharmacognosy and Phytochemical of the Pharmacy Undergraduate Course at the Federal University of Amapá.

Place and Duration of Study: The samples collection occurred in the city of Belém-Pará-Brazil ~~occurred~~ in December, 2012. The survey study was conducted between January and September, 2013 ~~in the laboratory of pharmacological and phytochemical gnosia~~ at the Federal University of Amapá.

Methodology: The phytochemical screening was performed according to the method described by Macêdo and by the Brazilian Pharmacopoeia. The ~~bioassaio~~ bioassay cytotoxicity on *Artemia salina* was performed using the ~~held in~~ ethanol extracts of the *Mikania lindleyana* sucuriú, where calculations were made for the CL₅₀ employing BioEstat ® ~~by and~~ PROBIT tool. The microbiological test was performed by the tube ~~microdilution~~ method ~~tube~~ in *Escherichia coli* and *Staphylococcus aureus*.

Results: The Screening Phytochemical analysis of the leaf and stem ethanol extract from the Mikania lindleyana DC indicated the presence of tannins, saponins and ~~alkaloids in the extracts, suggested its popular use, and~~ The extracts showed no antimicrobial activity on the bacteria tested. The cytotoxicity of the extracts, might be related to the alkaloids and saponins present in its constitution.

Conclusion: The study helped the community, suggesting that despite its popular use is not recommended as antimicrobial agent, and contributed to the scientific community, opening a new front of research.

Keywords: Medicinal plants, secondary metabolites, biological activity, Mikania lindleyana.

1. INTRODUCTION

~~Since the beginning, the medicinal plants are~~ Medicinal plants are widely used ~~as with~~ curative purpose, ~~is and they are~~ essential to health care ~~(BADKE, 2012; CZELUSNIAK, 2012)[1,2]~~, whose knowledge is based ~~within the family on traditional use~~ ~~(BADKE, 2012; FIRMO, 2011)[1,3]~~. Nowadays, the use-employment of plants as medicinal resource ~~was has~~ increased, due to the high cost of medicines-drugs and medications and the difficulties for the people's to access ~~to~~ health care ~~(BADKE, 2012; ARGENTA, 2011)[1,4]~~.

The Asteraceae family is one of the largest plantae kingdom families representing 10% of the world's flora (OLIVEIRA, 2011)[5]. According to Prudêncio (2012)[6], it comprises 1,600 to 1,700 genera and 24,000 to 30,000 species. In Brazil, this flowering plant has about 250 genera and 2,000 species. The genres are usually small, species can be presented as shrub, herbaceous, tree, vines and herbs [6](PRUDÊNCIO, 2012). The Asteraceae family is of much interest to health, since there is scientific evidence regarding drug stock of plants of the family (SILVA, 2011)[7]. Worldwide, it is found in various locations around the world, such as USA, Brazil, the Mediterranean, Asia and Australia [7](SILVA, 2011).

The *Mikania* genus has around 450 species, and they are distributed over tropical areas in Africa, Asia and America. In Brazil, there is a description of about 200 species of this genus, which concentrates mainly on the north and southeast region (SOUZA, 2011)[7]. Many species of this genus are of interest to society, being used as medicinal plantse, such as *Mikania lindleyana*.

The plant species *Mikania lindleyana* DC, is popularly known in Brazil as sucurijuzinho, grass-of-snakes, vine-sucuriju among other names. This plant species only blooms under when grown where it will receive direct sunlight conditions. It is a very plant commonly used as a popular folk medicine, in the Amazon region, she is popularly known as anaconda and has the following allegations of popular use applications: healing^[u1], anti-inflammatory, analgesic, diuretic and antihypertensive (VANDERLINDE et al., 2012[7-9]; SILVA, 2011; SILVA, 2012). Many people use this plant to prepare tea with fresh leaves for therapeutic purposes, without knowing the quality of the plant material, which becomes relevant to study this species (OWITI, 2011).

This medicinal plant is a climbing shrub, perennial, woody and without tendrils (claws to hold) with voluble stem, fluted cylindrical, green and twiggy, belongs to the Asteraceae family and gender *Mikania*, originally from South America and Brazil. Presents opposite leaves, of bright green color, petiolate, cordate, smooth consistency, almost leathery and triangular, entire board, with three ribs on the base. Many people use this plant as tea fresh leaves for therapeutic purposes, without knowing the quality of plant material, which becomes relevant studies about this species (OWITI, 2011).

According to Carvalho (2012)[11], phytochemical is the science that studies the chemistry of the plants. For Silva (2012)[9] these chemicals vegetables are coming from their own metabolism, which is divided into primary and secondary. Originated from the primary metabolism metabolites are essential for the growth and development of all vegetables, and are classified as carbohydrates, lipids and proteins^[u2] in, already the secondary metabolism products are restricted to a particular taxon, among them are the and may occur as alkaloids, saponins, tannins, anthocyanin glycosides and several others. They secondary metabolites comprise a rich and diverse class of compounds intended to give properties and functional activities of a given plant. However, the quality of the raw plant material by itself does not guarantee the effectiveness and safety of the final product, because the phytochemical analysis is governed used to qualitative testing for metabolites classes, thus and the physicochemical analysis associated with the pharmacological, partly ensures the effectiveness and quality of plant material (SIMÕES et al., 2003)[12]. Santana (2011)[13] defines the secondary metabolic products as active ingredients, whose function in plants is the can be: defense against pathogens, protection against ultraviolet rays, and also attraction of pollinators. In humans, the active principles have functions to cause reactions in the organism, which may result in the recovery of health (LORENZI; MATOS, 2008)[14]. Among the many pharmacological reactions beneficial to the human being that the active ingredients cause, highlights the antibacterial activity can be emphasized.

The first antimicrobial activity of plant extracts study, in which of the antimicrobial activity of plant extracts was evaluated is dated of 1881 [13](SANTANA, 2011). Natural resistance produced developed by bacteria to antimicrobials is worrying troublesome

(RANG, 2007; ZUCHINALLI, 2009)[15,16]; with this and in recent years, the interest and search for antimicrobial herbal substances ~~has had a big boost~~ increased (SILVA, 2012)[9]. Considering that the Brazilian Amazon has a great variety of medicinal plants, ~~in recent years~~, research ~~was carried out in this region~~, efforts are done looking for species that have the presence of secondary metabolites with antimicrobial activity (SARAIVA, 2012)[17].

Nunes (2011)[18] points out that, in the sphere of the study of medicinal plants, it is necessary to perform cytotoxicity studies since the use of medicinal plants can trigger in humans adverse reactions by their own chemical constituents (BARBOSA, 2012)[19]. A cytotoxicity evaluation model that is emerging among researchers is the bioassay on *Artemia salina*. This method is widely used, due to its simplicity, speed and low cost, which favors its use in several studies of plant species (BEDNARCZUK, 2010)[20].

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

This study ~~was involved~~ experimental, exploratory, qualitative and quantitative ~~nature aspects~~. The pharmacognostic analyzes, the phytochemical screening and forward cytotoxicity activity on *Artemia salina* were held ~~in at the~~ Pharmacognosy and Phytochemistry Laboratory of the Federal University of Amapá (UNIFAP). The microbiological assays were performed in the ~~laboratory Biodiagnostics at the same University~~ private clinical laboratory Biodiagnostics in the city of Macapá.

2.1 Obtaining Plant Material

~~Copies of p~~Plant species of *M. lindleyana* were collected in the city of Belém, State of Pará, Brazil. The ~~material analyzed~~ material was included the leaves and stems of this species and ~~was the plant was~~ identified by comparing with the specimens at the Herbarium of the Federal University of Amapá, in the city of Macapá, Amapá, Brazil.

2.2 Obtaining Extracts Crude

The leaves and stems of *M. lindleyana* were initially mechanically ~~fragmented~~ chopped, separately. ~~The Portions of fragments~~ 250 grams were subjected to a process of hot ethanol extraction, employing a reflux at 45 °C for 45 minutes, using 700 mL for each extraction, and this process was performed in triplicate. Then, the materials were filtered and the ethanol extracts ~~obtained were~~ concentrated on a rotary evaporator, under reduced pressure, thus obtaining, Crude Ethanol Extracts of the leaves and stems of the plant species *M. lindleyana* (EBEFM and EBMCM, respectively) (FARMACOPÉIA BRASILEIRA, 2010)[21]. The extracts were used for phytochemical screening, microbiological analysis and cytotoxicity.

2.3 Pharmacognostic Analysis

2.3.1 Determination of pH

The pH determination was made according to Macêdo (2003)[22] and the rules established by the Adolfo Lutz Institute (2008)[23]. ~~Ten grams of plant material w~~ere weighed ~~10 g of plant material~~ in a beaker and diluted with ~~the aid of~~ 100 mL of distilled water. The contents were stirred until the particles were uniformly suspended. The pH was determined using a calibrated pH meter.

2.3.2 Determination of lipids

~~Three grams of plant material w~~ere weighed ~~3g of plant material~~ on a filter paper and tied with woolen yarn. The tied filter paper was transferred to the Soxhlet extractor type device ~~Soxhlet extractor type~~, which was ~~engaged connected to~~ a 250 mL Erlenmeyer flask and heated in a heating mantle to 45 °C, and he was seated on a refrigerator balls. Hexane (150 mL) ~~w~~as added ~~150 mL of hexane in to~~ the ~~heating flask heating~~. After

removing the solvent, the flask was placed in an oven at 105 °C and maintained for one hour. After cooling in a dessicator for dried for 30 minutes and heavy the Erlenmeyer was weighed and, this process was repeated until obtaining a constant weight [22,23](MACÊDO, 2003; INSTITUTO ADOLFO LUTZ, 2008).

The calculation of percentage of lipids was done using the following equation Calculation:

$$\frac{100 \times N}{P} = \% \text{ lipids, m/m}$$

Where:

N = weight of lipids in grams of lipids

P = weight of the sample in grams of the sample

2.3.3 Determination of the residue by incineration - total ash

The total ash includes physiological ashes and not non-physiological ashes. Three grams of powdered plant material were weighed about 3 grams of powdered plant material and transferred to a porcelain crucible hairy and horny. The sample was evenly distributed in the crucible and incinerated at a temperature gradually increased from 200 °C to 600 °C ± 25 °C, until the total elimination of coal. The Total ash was cooled inside a dessicator, dissected and weighed (MACÊDO, 2005; INSTITUTO ADOLFO LUTZ, 2008; FARMACOPÉIA BRASILEIRA, 2010)[21-23].

The Calculation total ash percentage was calculated by the equation:

$$\frac{100 \times N}{P} = \% \text{ ASH, m/m}$$

Where:

N = weight of ash in grams of ash

P = weight of the sample in grams of the sample

2.3.4 Moisture determination - gravimetric method

Were weighed Petri dishes (6 units) were washed, dried, weighed and dissected left in a dessicator. Three samples of leaf powder and three stem powder samples, with about 2 grams each were distributed on each Petri dish. Then, they were subjected to drying dried in an oven at 105 °C for 2 hours, dissected cooled inside a dessicator for 30 minutes and weighed for 30 minutes, obtaining the sum of the weight plate with desiccated and dry sample. It was then placed back in the oven at 105 °C for 30 minutes and in desiccator for 30 minutes and heavy. This process was repeated until obtaining a constant weight (FARMACOPÉIA BRASILEIRA, 2010)[21].

The equation used for the Calculation of the moisture was as follows:

$$\frac{P_u - P_s}{P_a} \times 100$$

Where:

Pa = sample-weight of the sample in grams.

Pu = weight of the petri dish containing the sample weight before drying.

Ps = weight of the petri dish containing the sample mass after drying.

2.4 Screening Phytochemical

The phytochemical analysis were performed according to standard methodologies coloration and precipitation described in-by Simões (2003)[12].

2.5 Biological Activity

2.5.1 Antimicrobial activity

To determine the possible antimicrobial activity, ~~was used the~~ microdilution tube method ~~tube was used~~. Two solutions were ~~made prepared~~, using EBEFM and EBECM, each with 10 mg of the sample and 10 mL of ~~saline solution~~^{saline}_[u4]. Then 1 mL ~~of the concentrates without anticoagulant~~ were placed in vacuum tubes ~~the concentrate without anticoagulant~~, and dilutions 1:2, 1:4, 1:8 and 1:16 ~~were made~~. ~~A b~~Bacterial suspensions ~~were as~~ prepared ~~in glass tubes with~~ 300,000 bacteria per mL (Mcfarland ~~of~~ scale), ~~glass tube~~ for *Escherichia coli* and ~~the other~~ for *Staphylococcus aureus*. ~~Later After that 10 µL of the suspension was were~~ inoculated ~~10 µL of the suspension~~, corresponding to 300 bacteria per inoculation, ~~at on concentrations of the concentrated~~ plant extracts, ~~then and~~ incubated at 36 °C. After 1 hour ~~sown~~ the strains of *Escherichia coli* ~~amid were inoculated on~~ Eosin Methylene Blue (BEM) and ~~the Staphylococcus aureus~~ on blood agar. ~~Concentrations; they~~ were incubated again and ~~the procedure repeated~~ for 3 hours ~~at 36 °C (CUNHA, 2012)[24]~~.

2.5.2 Cytotoxicity activity ~~front of~~ *Artemia salina* L.

2.5.2.1 Preparation of artificial marine solution

The saline solution was prepared with 34.2 g of sodium chloride; 1.425 g of ~~m~~Magnesium ~~s~~Sulfate; 4.75 g ~~s~~Sodium ~~b~~Bicarbonate and 951 mL of distilled water. After homogenized, the pH was adjusted to 9.0, using a solution of 2 mol/L ~~of~~ sodium hydroxide.

2.5.2.2 Obtaining *A. salina* metanauplios

To obtain the metanauplios, *A. salina* cysts were incubated in artificial marine solution (pH 9.0 and 28 °C) under artificial lighting (~~a~~ 40W bulb ~~light~~ for 24 hours). After hatching, the metanauplios migrated ~~u~~ through a perforated plate to another compartment with free incidence of light, due to its phototropism, thus separating ~~ing~~ the larvae of cysts which have not hatched. Later, the metanauplios were separated into petri dish and allowed to ~~stay under~~ artificial light for 24 hours to obtain the nauplio.

2.5.2.3 Sample preparation and bioassay

The bioassay ~~employing~~ *A. salina* was based on the technique described by Nascimento (2008)^[25]. ~~We It was~~ used 10 mg of EBEFM and EBECM, to which was added 1 mL of Tween 80 to 5% to help solubilize it. The solution was homogenized, and the volume was ~~made up completed~~ to 5 mL with artificial sea solution at pH = 9.0. ~~From t~~This solution, ~~had to be it was~~ withdrawn aliquots ~~of~~ 2500, 1875, 1250, 625, 250 and 125 µL and ~~then were~~ transferred ~~separately~~ to 5 mL vials. ~~and T~~he volumes ~~were~~ completed with the same solvent, to give concentrations of 1000, 750, 500, 250, 100 and 50 µg/mL of ~~each~~ EBEFM and EBECM ~~extracts~~.

The nauplio were divided into seven groups, each containing ten individuals. The first group received the control solution (marine solution) and ~~received the following six~~ *M. lindleyana* solution of extract in different concentrations. The samples were submitted to artificial light for 24 hours; ~~after this period were it was~~ recorded living and dead larvae. Dead larvae were considered those that showed no active movement in about twenty seconds of observation. The experiment was performed in triplicate for each extract.

2.5.2.4 Statistical analysis

To obtain the ~~t~~Total ash values and ~~Humidity~~moisture content, Microsoft Excel 2007 was used ~~at the tool~~. The calculations to determine the mortality rate of *Artemia salina* versus concentration of EBEFM and EBECM were ~~made in executed using~~ BioEstat® 5.0 program by PROBIT ~~tool~~.

3. RESULTS AND DISCUSSION

According to the results of the pharmacognostic analyzes ~~pharmacognostic~~ (Table 1), the pH of the leaves and stems suspensions were 5.56 and 5.46 respectively, ~~which that~~ may characterize the presence of acidic substances, ~~which-This~~ is probably related to the presence of saponins and tannins, because they tend to lower the pH ~~at which the species are present~~, due to the release of H⁺ ions in the reaction medium (SIMÕES; et al., 2003)[12]. The result is similar to that reported by Owiti (2011)[10], ~~for him~~ which the value found for the anaconda-sucuriju [u5] leaves was 5.0.

The values obtained for total lipids were 3.15% and 0.38% for leaves and stem respectively, but the residues obtained in the extraction ~~are-were~~ not ~~formed-only~~ composed by lipids, but for all compounds ~~under the conditions of the determination, that~~ can be extracted by the solvent, ~~T~~therefore, it is necessary a more rigorous analysis for the identification and qualitative determination for the adequate result quantification (SIMÕES; et al., 2003)[12].

For moisture, the recommended maximum limit for herbs is 14% (SIMÕES; et al., 2003)[12], ~~with this, it~~ was found that the powder of leaves and stems of *Mikania lindleyana* ~~results-presented~~ moisture content in percentage of 7.46 ± 0.09 and 7.77 ± 0.06 in percentage, which allows to predict that the plant material is protected from attacks by microorganisms and enzymatic actions that may modify its natural properties.

~~As for t~~The values in percentage of total ash for leaves and stem of *Mikania lindleyana*, ~~were~~ 17.29 ± 0.99 and 1.41 ± 0.91 , respectively, compared to 8-14% limit established by the Brazilian Pharmacopoeia (2010)[21], ~~it~~ was observed that the value for the leaves are above recommended levels, suggesting a high concentration of inorganic material (MARQUES, 2012)[26]. Owiti (2011)[10] ~~reporteds~~ the amount of total ash for sSucuriju sheets-leaves of $17.067 \pm 0.5\%$, which is similar to that found in this study.

Table 1. Analysis pharmacognostic leaves and stem of the plant specie *Mikania lindleyana* DC.

Physical Chemistry Analysis	Leaves	Stem
pH	5.56 ^a	5.46 ^a
Lipids (% m/m)	3.15 ^a	0.38 ^a
Total ash (% m/m)	17.29 ± 0.99	1.41 ± 0.91
Humidity (% m/m)	7.46 ± 0.09	7.77 ± 0.06

^a - Standard deviation null

The phytochemical research provided the detection of secondary metabolites, which are: alkaloids, glycosides anthocyanins, tannins and saponins (Table 2). These results are similar to those reported by Mendes (2002)[27] who studied the alcoholic extract from sucuriju leaves and found alkaloids, tannins, steroids and triterpenoids, ~~already-i~~ in the study by Silva (2011)[7] this author showed the presence of saponins ~~espumílicas~~ in the leaves of this species.

Table 2. Screening pPhytochemical leaf and stem of the plant species *Mikania lindleyana* DC.

Class metabolites	Leaf	Stem
Alkaloids	+	+
Anthraquinones	-	-
Steroids and triterpenoids	-	-
Anthocyanin glycosides	+	+
Saponins	+	+
Tannins	+	+

(+) Being-Presence (-) Absencent.

The presence of these secondary metabolites groups ~~of have~~ pharmacological interest ~~highlights and justifies its the~~ popular use ~~of the plant as healing, through the~~ presence of tannins [28](PEREIRA, 2012), ~~which acts forming helps to form~~ a protective layer on injured epithelial tissues, allowing, ~~allowing~~ just below this layer, the ~~occurrence~~ tissue repair process ~~occurs naturally (CASTEJON, 2011)[29].~~ A anti-inflammatory and antinociceptive ~~functions~~, through the alkaloids, ~~which may acts~~ biologically to interfere mediators involved in the inflammatory process (SIMÕES, et al., 2003)[12]. Saponins are able to lower cholesterol levels, by increasing its excretion, which occurs due to its ability to bind to sterol, therefore, a hypolipidemic agent [12](SIMÕES; et al., 2003). The anthocyanin glycosides are antioxidants metabolites, ~~acting slowing or preventing oxidative damage, by~~ blocking the oxidation reactions [12](SIMÕES; et al., 2003).

For antimicrobial activity, the results demonstrated that the EBEFM and EBECM showed no activity ~~in over the strains of~~ *E. coli* and *S. aureus*, considering that the concentration of 1000 µg/mL is ~~considered toxic. Thus, it,~~ is irrelevant to further testing at higher concentrations. The results ~~were are~~ shown in Tables 3 and 4.

Table 3. Growth of *E. coli* strains in EBEFM and EBECM concentrated and diluted *Mikania lindleyana*.

Sucuriju Extract Dilution	<i>E. coli</i> EBEFM	<i>E. coli</i> EBECM	<i>E. coli</i> EBEFM	<i>E. coli</i> EBECM	<i>E. coli</i> EBEFM	<i>E. coli</i> EBECM
	1 hour	1 hour	2 hours	2 hours	3 hours	3 hours
1000 µg/mL	+	+	+	+	+	+
1:2	+	+	+	+	+	+
1:4	+	+	+	+	+	+
1:8	+	+	+	+	+	+
1:16	+	+	+	+	+	+

(+) Growth

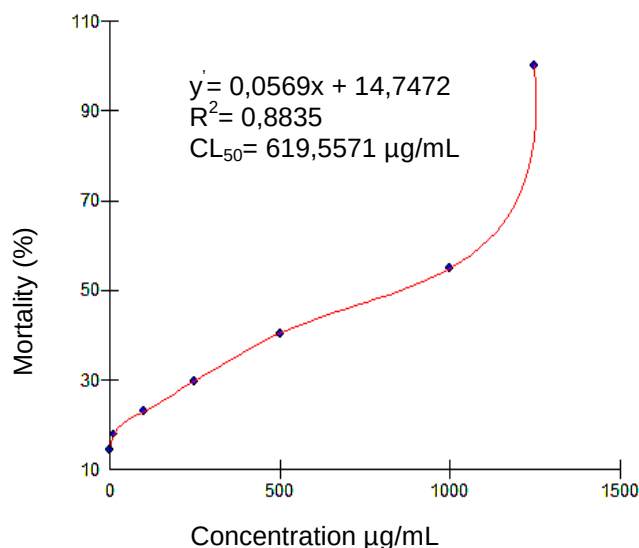
Table 4. Growth of *S. aureus* strains in EBEFM and EBECM concentrated and diluted *Mikania lindleyana*.

Sucuriju Extract Dilution	<i>E. coli</i> EBEFM	<i>E. coli</i> EBECM	<i>E. coli</i> EBEFM	<i>E. coli</i> EBECM	<i>E. coli</i> EBEFM	<i>E. coli</i> EBECM
	1 hour	1 hour	2 hours	2 hours	3 hours	3 hours
1000 µg/mL	+	+	+	+	+	+
1:2	+	+	+	+	+	+
1:4	+	+	+	+	+	+
1:8	+	+	+	+	+	+
1:16	+	+	+	+	+	+

(+) Growth

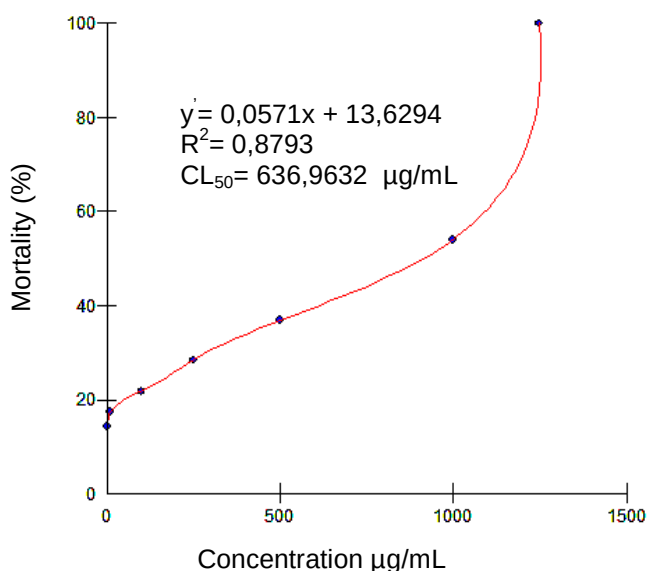
The cytotoxicity bioassay, on *Artemia salina*, provided the LC50 for EBEFM and EBECM equal to 619.65571 and 6376.09632 µg/mL respectively, ~~giving assigning~~ cytotoxic activity ~~to both extracts, considering that~~ Nascimento (2008)[25] considers ~~that the amounts~~ quantity below 1000 µg/mL as cytotoxic, which can ~~is be~~ related to the cytotoxic activity of alkaloids and saponins [30](SILVA, 2007), ~~in those parts of the plant.~~ The ~~concentration-response curves of the Artemia salina mortality after 24 hours submitted to EBEFM and EBECM extracts.concentration of the solution leaves and stem~~ Sucuriju to kill 50% of microcrustaceans ~~is are~~ shown in Graphics 1 and 2, respectively.

Graphic 1. Concentration-response curve of the *Artemia salina* EBEFM-mortality after 24 hours submitted to EBEFM extracts.



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Graphic 2. Concentration-response curve of the *Artemia salina* EBECM-mortality after 24 hours submitted to EBECM extracts.



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4. CONCLUSION

Through pharmacognostic analysis, it was possible to obtain quality control parameters consistent with the Brazilian Pharmacopoeia. The pH of the leaves and stems of *Mikania lindleyana* had showed acid characteristics, because of the potential ability of tannins and saponins in-to releasing ions H^+ in the reaction-aqueous medium. In the lipid analysis, residues obtained in the extraction are-were not formed only of-by lipids, but for all compounds under the conditions of-the-determination-that could be extracted by the solvent. I thus, becomes necessary a more rigorous analysis for the qualitative detection and therefore the identification and quantification are necessary.

Also, moisture from the leaves and stem *Sucuriçu* of the *Mikania lindleyana* was consistent with the values suggested by the Brazilian Pharmacopoeia, which allows to predict that the plant material is protected from attacks by microorganisms and enzyme action that may modify its natural properties. The value of total ash to leaves was high compared to *Mikania lindleyana* was set up by the Brazilian Pharmacopoeia standards, which possibly corresponds to a high concentration of minerals in its constitution, which did not occur with the stem; what does hurt this part of the plant showed little concentration of minerals in this part of the plant.

The phytochemical screening suggests justified its the popular use of the studied species as healing, anti-inflammatory and analgesic, through the biological action of tannins and alkaloids, plus add other possible activities. Also it may act, as lipid-lowering and antioxidant by the presence of saponins and glycosides anthocyanin, respectively, what makes this study relevant for society and collaborating in to the chemosystematics of the species.

The EBEFM and EBECM did not have antimicrobial activity in over *Escherichia coli* and *Staphylococcus aureus* which does not exclude the possibility of this kind, have developing antimicrobial activity in related to other bacterias.

The bioassay on *Artemia salina*, contributed to the population, warning her about the possible toxic concentrations of the species under study, and contributed to the scientific community, opening a new front of research, whereas the saponins have cytotoxic action against anti-tumor cells and that many cytotoxic drugs of plant origin are used in cancer treatment.

REFERENCES

SC Argenta et al. Medicinal plants: popular culture versus ciência. *Vivências, Erechim*, 2011; 7 (12): 51-60.

Badke MR, et al. Knowledge and popular health care practices using medicinais. *Texto context nursing plants, Florianópolis*, 2012; 21 (2): 363-70.

Barbosa LGRS. Analysis of the importance of rational use of medicinal plants and their microbiological control. 2012. 24 f. Monograph (Undergraduate Pharmacy) – Faculdade of Pindamonhangaba, Pindamonhangaba 2012.

Bednarczuk VO, et al. In vitro and in vivo tests used in toxicological screening. *Academic Vision, Curitiba*, 2010; 11 (2): 43-50.

Belcavello L, et al. Cytotoxicity and DNA damage induced by *Zorniadiphylla* extract, a medicinal plant. *ESFA, Holy Spirit*, 2012; 10 (3): 140-145.

Oak JR, et al. Phytochemical screening of *Nasturtium officinale* r. br.: quality control. UFPR, Paraná, November 2008.

LE Carvalho, et al. Study on root and leaf rachis of *Spathelia sublimis*: phytochemical and active against pernicious *Moniliophthora* fungus associated with cupuaçu (*Theobroma grandiflorum*). *New chemical, São Paulo*, 2012; 35 (11): 2237-2240.

Gastejon FV. Tannins and saponins. 2011. 26 f. Seminar (Master) – University Federal de Goiás, School of Veterinary and Animal Science, Graduate Program in Animal Science, Goiânia, 2011.

Cunha EL. Evaluation of bacterial contamination and heavy metals in the river shores in the city of Macapá, Amapá. 2012. 150 f. dissertation (Master of Tropical Biodiversity)

Federal University of Amapá, Graduate Diploma in Tropical Biodiversity Program, Macapa, 2012.

Czelusniak KE, et al. Pharmacobotany, phytochemical and pharmacological the guaco: *Mikania glomerata* Sprengel considering revision and *Mikania laevigata* Schulyz Bip. ex Baker. Magazine Brazilian Medicinal Plants, Botucatu, 2012; 14 (2): 400-409.

Brazilian Pharmacopoeia. 5th. ed. London: Atheneu; 2010.

WCA aight, et al. Historical, popular use and scientific conception of medicinal plants. Notebook Research, St. Louis, 2011; 18 (special).

Instituto Adolfo Lutz 2008. Chemical and physical methods for food analysis. London: IMESP.

Lorenzi M, Matos FJA. Medicinal Plants in Brazil: Native and Exotic. 2. ed. London: Institute plantarum, 2008.

Macêdo JAB. Laboratory Methods of Analysis Physical-Chemical and Microbiological. 2. ed. Belo Horizonte: CRQ-M.G., 2003.

Marques GS, et al. Phytochemical and physicochemical characterization of the leaves of *Bauhinia forficata* Link collected in two regions. Journal of Pharmaceutical Sciences Applied Basic, São Paulo, 2012; 33 (1): 57-62.

B Mendes et al. Information herbal and chemical composition of *Mikania DC lindleyana*. (Asteraceae). Brazilian Journal of Pharmacy, Curitiba, 2002; 83 (1/4): 27-29.

Birth JE, et al. Phytochemical studies and toxicological bioassay against larvae of *Artemia salina* Leach. three medicinal species of the genus *Phyllanthus* (Phyllanthaceae). Journal of Applied Pharmaceutical Sciences Basic, São Paulo, 2008; 29 (2): 145-150.

Nunes GR, et al. Inhibition of nitric oxide production and cytotoxic effect of aqueous extract of *Annona muricata* L. Perspectives online, Rio de Janeiro, 2011; 1 (2): 1-8.

Oliveira TS. Anatomy, seed germination and analysis of essential oil *Viguiera arenaria* baker in martius and robust *Viguiera gardner* in hook (Asteraceae-Heliantheae) .2011. 95 f. Dissertation (Master of Science) University of São Paulo and College of Agriculture "Luiz de Queiroz", Piracicaba, 2011.

Owiti, AO. Development of a method for characterizing hydroethanolic extract of leaves of *Mikania lindleyana* DC. by high performance liquid chromatography (HPLC). 2011. 72 f. Dissertation (Master) University Federal do Pará, Graduate Program in Pharmaceutical Sciences, Bethlehem, 2011.

Panizza S. Plants that heal: bush smell. IBRASA, São Paulo, 1997; 1 (y / n): 117-118.

Pereira RJ, et al. Plant secondary metabolites and antioxidant benefits. Journal of Biotechnology and Biodiversity, Tocantins, 2012; 3 (4) 146-152

Prudencio E. ethnopharmacological *Solidago chilensis* Meyen Survey. "Arnica Brazilian" (Asteraceae). 2012. 44 f. Work Completion of course (Specialization) University of the Extreme South of Santa Catarina, Cricklewood, 2012.

HP Rang, et al. Pharmacology. 6. ed. Rio de Janeiro: ELSEVIER, 2007.

Santana RO. Use of plant extracts and essential oils in poultry production. 21 2011. f. Seminar (Master) University Federal de Goiás, Veterinary School, Goiânia, 2011.

Saraiva RMC. Antibacterial activity of medicinal plants front of the multidrug-resistant bacteria and their interaction with antimicrobial drugs. 2012. 94 f. Dissertation (Graduate Diploma in Pharmaceutical Sciences) -University Federal do Pará, Institute of Health Sciences, Bethlehem, 2012.

Silva ASB. Evaluation of antinociceptive and anti-inflammatory *Mikania lindleyana* DC. validate the use in folk medicine. 2011. 107 f. Dissertation (Master in Pharmaceutical Sciences) -University Federal do Pará, Institute of Health Sciences, Graduate Program in Pharmaceutical Sciences, Bethlehem, 2011.

BSA Silva, et al. Anti-inflammatory and Antinociceptive Activities of the aqueous extract of *mikania lindleyana* in rodents. IJPSR, São Paulo, 2012; 3 (6): 1637-1646.

Smith DB, et al. Isolation and evaluation of the cytotoxic activity of some oxaporfínicos alkaloids obtained from Annonaceae. New chemistry, São Paulo, 2007; 30 (8): 1809-1812.

Silva CG. Ethnobotanical study and antimicrobial activity in vitro of medicinal plants in Nazaré community, municipality of Miracoles, Ceará. 2012. 93 f. Dissertation (Graduate in Forest Sciences) Federal -University of Campina Grande, Paraíba, 2012.

Simões CMO, et al. Pharmacognosy: the plant to the drug. 5. ed. Florianopolis Publisher UFRGS, 2003.

GS Souza et al. Growth, essential oil content and content of coumarin saplings guaco (*Mikania glomerata* Spreng.) Cultivated under colored nets. Biotemas, Florianópolis, 2011; 24 (3): 1-11.

Vanderlinde FA, et al. Anti-inflammatory and opioid-like Activities in methanol extract of *Mikania lindleyana*, anaconda. Brazilian Journal of Pharmacognosy, Curitiba, 2012; 22 (1) 150-156

Zuchinalli A. Study of chemical and biological properties of *Crotonurucurana* plant species. 2009.79 f. Dissertation (Graduate in Chemistry) -University Federal of Santa Catarina, Center of Physical and Mathematical Sciences, Department of Chemistry, Graduate in Chemistry Program, Florianópolis, 2009.[1] Badke MR et al. Knowledge and popular health care practices using medicinals. Texto context nursing plants, Florianópolis, 2012;21(2):363-70.

[2] Czelusniak KE, et al. Pharmacobotany, phytochemical and pharmacological the guaco: *Mikania glomerata* Sprengel considering revision and *Mikania laevigata* Schulyz Bip. ex Baker. Magazine Brazilian Medicinal Plants, Botucatu, 2012;14(2):400-409.

[3] Firmo WCA et al. Historical Context, Popular Use and Scientific Design on Medicinal Plants. Research notebooks (UFMA) São Luís, 2011;18(special).

[4] Argenta SC et al. Medicinal plants: popular culture versus science. Vivências, Erechim, 2011;7 (12):51-60.

[5] Oliveira TS. Anatomy, seed germination and analysis of essential oil *Viguiera arenaria* baker in martius and robust *Viguiera gardner* in hook (Asteraceae- Heliantheae) .2011. 95 f. Dissertation (Master of Science) -University of São Paulo and College of Agriculture "Luiz de Queiroz", Piracicaba, 2011.

[6] Prudêncio E. ethnopharmacological *Solidago chilensis* Meyen Survey. "Arnica-Brazilian" (Asteraceae). 2012. 44 f. Work Completion of course (Specialization) - University of the Extreme South of Santa Catarina, Cricklewood, 2012.

- [7] Silva ASB. Evaluation of antinociceptive and anti-inflammatory of *Mikania lindleyana* DC.: validate the use in folk medicine. 2011. 107 f. Dissertation (Master in Pharmaceutical Sciences) -University Federal do Pará, Institute of Health Sciences, Graduate Program in Pharmaceutical Sciences, Bethlehem, 2011.
- [8] Vanderlinde FA et al. Anti-inflammatory and opioid-like Activities in methanol extract of *Mikania lindleyana*, sucuriú. Brazilian Journal of Pharmacognosy, Curitiba, 2012;22(1)150-156.
- [9] Silva CG. Ethnobotanical study and antimicrobial activity in vitro of medicinal plants in Nazaré community, municipality of Miracles, Ceará. 2012. 93 f. Dissertation (Graduate in Forest Sciences) Federal -University of Campina Grande, Paraíba, 2012.
- [10] Owiti, AO. Development of a method for characterizing hydroethanolic extract of leaves of *Mikania lindleyana* DC. by high performance liquid chromatography (HPLC). 2011. 72 f. Dissertation (Master) -University Federal do Pará, Graduate Program in Pharmaceutical Sciences, Bethlehem, 2011.
- [11] Carvalho LE et al. Study on root and leaf rachis of *Spathelia sublime*: phytochemical and active against pernicious *Moniliophthora* fungus associated with cupuaçu (*Theobroma grandiflorum*). New chemical, São Paulo, 2012;35(11):2237-2240.
- [12] Simões CMO, et al. Pharmacognosy: the plant to the drug. 5. ed. Florianopolis Publisher UFRGS, 2003.
- [13] Santana RO. Use of plant extracts and essential oils in poultry production. 21 2011. f. Seminar (Master) -University Federal de Goiás, Veterinary School, Goiânia, 2011.
- [14] Lorenzi M, Matos FJA. Medicinal Plants in Brazil: Native and Exotic. 2. ed. London: Institute plantarum, 2008.
- [15] Rang HP et al. Pharmacology. 6. ed. Rio de Janeiro: ELSEVIER, 2007.
- [16] Zuchinalli A. Study of chemical and biological properties of *Croton urucurana* plant species. 2009.79 f. Dissertation (Graduate in Chemistry) -University Federal of Santa Catarina, Center of Physical and Mathematical Sciences, Department of Chemistry, Graduate in Chemistry Program, Florianópolis, 2009.
- [17] Saraiva RMC. Antibacterial activity of medicinal plants front of the multidrug-resistant bacteria and their interaction with antimicrobial drugs. 2012. 94 f. Dissertation (Graduate Diploma in Pharmaceutical Sciences) -University Federal do Pará, Institute of Health Sciences, Bethlehem, 2012.
- [18] Nunes CR et al. Inhibition of nitric oxide production and cytotoxic effect of aqueous extract of *Annona muricata* L. Perspectives online, Rio de Janeiro, 2011;1(2):1-8.
- [19] Barbosa LCRS. Analysis of the importance of rational use of medicinal plants and their microbiological control. 2012. 24 f. Monograph (Undergraduate Pharmacy) - Faculdade of Pindamonhangaba, Pindamonhangaba 2012.

- [20] Bednarczuk VO et al. In vitro and in vivo tests used in toxicological screening. Academic Vision, Curitiba, 2010;11(2):43-50.
- [21] Brazilian Pharmacopoeia. 5th. ed. London: Atheneu; 2010.
- [22] Macêdo JAB. Laboratory Methods of Analysis Physical-Chemical and Microbiological. 2. ed. Belo Horizonte: CRQ-M.G., 2003.
- [23] Instituto Adolfo Lutz 2008. Chemical and physical methods for food analysis. London: IMESP.
- [24] Cunha EL. Evaluation of bacterial contamination and heavy metals in the river shores in the city of Macapá, Amapá. 2012. 150 f. dissertation (Master of Tropical Biodiversity) Federal -University of Amapá, Graduate Diploma in Tropical Biodiversity Program, Macapá, 2012.
- [25] Nascimento JE et al. Phytochemical studies and toxicology bioassay against larvae of *Artemia salina* Leach. three medicinal species of the genus *Phyllanthus* (phyllanthaceae). Journal of Basic and Applied Pharmaceutical Sciences, 2008;29(1):145-150. Portuguese
- [26] Marques GS et al. Phytochemical and physicochemical characterization of the leaves of *Bauhinia forficata* Link collected in two regions. Journal of Pharmaceutical Sciences Applied Basic, São Paulo, 2012;33(1):57-62.
- [27] Mendes B et al. Information herbal and chemical composition of *Mikania lindleyana* DC. (Asteraceae). Brazilian Journal of Pharmacy, Curitiba, 2002;83(1/4):27-29.
- [28] Pereira RJ, et al. Plant secondary metabolites and antioxidant benefits. Journal of Biotechnology and Biodiversity, Tocantins, 2012;3(4)146-152.
- [29] Castejon FV. Tannins and saponins. 2011. 26 f. Seminar (Master) -University Federal de Goiás, School of Veterinary and Animal Science, Graduate Program in Animal Science, Goiânia, 2011.
- [30] Silva JG et al. Atividade antimicrobiana do extrato de *Anacardium occidentale* L. em amostras multiresistentes de *Staphylococcus aureus*. Revista Brasileira de Farmacognosia, v.17, n.4, p. 572-577, 2007.