

## 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*.

**Study design:** Copies of plant species *M. lindleyana* were collected in the city of Belém, Pará State. The material analyzed was the leaves and stems of this species and was identified by comparing the Herbarium of the Federal University of Amapá, in the city of Macapa. Laboratory tests were performed at the Laboratory of Pharmacognosy and Phytochemical Pharmacy course at the Federal University of Amapá.

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

**Methodology:** The phytochemical screening was performed according to the method described by Macêdo and by the Brazilian Pharmacopoeia. The bioensaio cytotoxicity on *Artemia salina* was held in ethanol extracts of the sucupijú, where calculations were made for the CL<sub>50</sub> BioEstat ® by PROBIT tool. The microbiological test was performed by the macrodilution method tube in *Escherichia coli* and *Staphylococcus aureus*.

**Results:** The presence of tannins, saponins and alkaloids in the extracts, suggested its popular use, and 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 its popular use, and contributed to the scientific community, opening a new front of research.

**Keywords:** Medicinal plants, secondary metabolites, biological activity.

## 1. INTRODUCTION

Since the beginning, the medicinal plants are used with curative purpose, is essential to health care (BADKE, 2012; CZELUSNIAK, 2012), whose knowledge is based within the family (BADKE, 2012; FIRMO, 2011). Nowadays, the use of plants as medicinal resource was increased, due to the high cost of medicines and the difficult people's access to health care (BADKE, 2012; ARGENTA, 2011).

The Asteraceae family is one of the largest plantae kingdom representing 10% of the world's flora (OLIVEIRA, 2011). According Prudêncio (2012) comprises 1.600-1.700 genera and 24.000-30.000 species, in Brazil, this flowering plant has about 250 genera and 2,000 species. The genres are usually small, species can be shrub, herbaceous, tree, vines and herbs (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). It is found in various locations around the world, such as USA, Brazil, the Mediterranean, Asia and Australia (SILVA, 2011).

The *Mikania* genus has around 450 species, and are distributed in tropical areas of Africa, Asia and America. In Brazil, is described about 200 species of this genus, which concentrates mainly on the north and southeast (SOUZA, 2011). Many species of this genus are of interest to society, being used as medicine, such as *Mikania lindleyana*.

The plant species *Mikania lindleyana* DC, is popularly known as sucurijuzinho, grass-of-snakes, vine-sucuriju among other names. This species only blooms when grown where it will receive direct sunlight. It is a very plant used in folk medicine, in the Amazon region, she is popularly known as anaconda and has the following allegations of popular use: healing, anti-inflammatory, analgesic, diuretic and antihypertensive (VANDERLINDE et al., 2012; SILVA, 2011; SILVA, 2012). This medicinal plant is a climbing shrub, perennial, woody and without tendrils (claws to hold) with voluble stem, fluted cylindrical, green and twiggly, 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), phytochemical is the science that studies the chemical plant. For Silva (2012) 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 protídios, already the secondary metabolism are restricted to a particular taxon, among them are the alkaloids, saponins, tannins, anthocyanin glycosides and several others. The 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 plant material by itself does not guarantee the effectiveness and safety of the final product, because the phytochemical analysis, is governed qualitative testing for metabolites classes, thus the physicochemical analysis associated with the pharmacological, partly ensures the effectiveness and quality of plant material (SIMÕES et al., 2003). Santana (2011) defines the secondary metabolic products as active ingredients, whose function in plants is the defense against pathogens, protection against ultraviolet rays, and also attract pollinators. In humans, the active principles have functions to cause reactions in the organism, may result in the recovery of health (LORENZI; MATOS, 2008). Among the many pharmacological reactions beneficial to the human being that the active ingredients cause, highlights the antibacterial activity.

The first study, in which the antimicrobial activity of plant extracts was evaluated is dated 1881 (SANTANA, 2011). Natural resistance produced by bacteria to antimicrobials is worrying (RANG, 2007; ZUCHINALLI, 2009), with this in recent years, the search for antimicrobial herbal substances had a big boost (SILVA, 2012). Considering that the Brazilian Amazon has a great variety of medicinal plants, in recent years, research was carried out in this region, looking for species that have the presence of secondary metabolites with antimicrobial activity (SARAIVA, 2012).

Nunes (2011) points out that, in the sphere of the study of medicinal plants, 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). 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).

## 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

This study was experimental, exploratory qualitative and quantitative nature. The pharmacognostic analyzes, the phytochemical screening and forward cytotoxicity activity *Artemia salina* were held in Pharmacognosy and Phytochemistry Laboratory of the Federal

University of Amapá (UNIFAP). The microbiological assays were performed in the laboratory Biodiagnostics.

## 2.1 Obtaining Plant Material

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

## 2.2 Obtaining Extracts Crude

The leaves and stem of *a. lindleyana* were initially mechanically fragmented separately. The fragments were subjected to a process of hot ethanol extraction, 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 concentrated on a rotary evaporator, under reduced pressure, thus obtaining, Crude Ethanol Extract of the leaves and stem of the plant species *M. lindleyana* (EBEFM and EBMCM, respectively) (FARMACOPÉIA BRASILEIRA, 2010). 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) and the rules established by the Adolfo Lutz Institute (2008). Were weighed 10 g of plant material in a beaker and diluted with the aid of 100 mL of water. The contents were stirred until the particles were uniformly suspended. The pH was determined using a calibrated pHmeter.

### 2.3.2 Determination of lipids

Were weighed 3g of plant material on filter paper and tied with woolen yarn. The tied filter paper was transferred to the device Soxhlet extractor type, which was engaged in a 250 mL Erlenmeyer flask and heated in a heating mantle to 45 ° C, and he was seated on a refrigerator balls. Was added 150 mL of hexane in the flask heating. After removing the solvent, the flask was placed in an oven at 105 ° C and maintained for one hour, dried for 30 minutes and heavy, this process was repeated until obtaining a constant weight (MACÊDO, 2003; INSTITUTO ADOLFO LUTZ, 2008).

Calculation:

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

Where:

N = weight in grams of lipids

P = weight in grams of the sample

### 2.3.3 Determination residue by incineration - total ash

The total ash include physiological ashes and not physiological ashes. Was weighed about 3 grams of powdered plant material and transferring to a 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, dissected and weighed (MACÊDO, 2005; INSTITUTO ADOLFO LUTZ, 2008; FARMACOPÉIA BRASILEIRA, 2010).

Calculation:

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

Where:

N = weight in grams of ash

P = weight in grams of the sample

### 2.3.4 Moisture determination - gravimetric method

Were weighed Petri dishes 6 washed, dried, weighed and dissected, 3 samples of leaf powder and 3stem powder samples, with about 2 grams each. Then they were

132 subjected to drying in an oven at 105 ° C for 2 hours, dissected and weighed for 30 minutes,  
133 obtaining the sum of the weight plate with desiccated and dry sample. It was then placed  
134 back in the oven at 105 ° C for 30 minutes and in desiccator for 30 minutes and heavy. This  
135 process was repeated until obtaining a constant weight (FARMACOPÉIA BRASILEIRA,  
136 2010).

137 Calculation:

$$138 \quad \frac{P_u - P_s}{P_a} \times 100$$

139 Where:

140 Pa = sample weight in grams.

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

142 Ps = the petri dish containing the sample mass after drying.

## 143 2.4 Screening Phytochemical

144 The phytochemical analysis were performed according to standard methodologies  
145 coloration and precipitation described in Simões (2003).

## 146 2.5 Biological Activity

### 147 2.5.1 Antimicrobial activity

148 To determine the possible antimicrobial activity, was used microdilution method  
149 tube. Two solutions were made, using EBEFM and EBECM, each with 10 mg of the sample  
150 and 10 mL of saline. Then 1 mL were placed in vacuum tubes the concentrate without  
151 anticoagulant, and dilutions 1:2, 1:4, 1:8 and 1:16. A bacterial suspension was prepared  
152 300,000 bacteria per mL Mcfarland of scale, glass tube for *Escherichia coli* and the other for  
153 *Staphylococcus aureus*. Later was inoculated 10 µL of the suspension, corresponding to 300  
154 bacteria per inoculation, at concentrations of plant extracts, then incubated at 36 ° C. After 1  
155 hour sown the strain of *Escherichia coli* amid Eosin Methylene Blue (BEM) and  
156 *Staphylococcus aureus* on blood agar. Concentrations were incubated again and the  
157 procedure repeated for 3 hours (CUNHA, 2012).

### 158 2.5.2 Cytotoxicity activity front of *Artemia salina* L.

#### 159 2.5.2.1 Preparation of artificial marine solution

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

#### 163 2.5.2.2 Obtaining *A. salina* metanauplios

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

#### 170 2.5.2.3 Sample preparation and bioassay

171 The bioassay *A. salina* was based on the technique described by Nascimento  
172 (2008). We used 10 mg of EBEFM and EBECM, to which was added 1 mL of Tween 80 to  
173 5% to help solubilize it. The solution was homogenized, and the volume was made up to 5  
174 mL with artificial sea solution at pH = 9.0. This solution had to be withdrawn aliquots 2500,  
175 1875, 1250, 625, 250 and 125 µL and then were transferred to 5 mL vials and the volume  
176 completed with the same solvent, to give concentrations of 1000, 750, 500, 250, 100 and  
177 50µg/mL of EBEFM and EBECM.

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

#### 2.5.2.4 Statistical analysis

To obtain the Total ash values and Humidity, 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 BioEstat® 5.0 program by PROBIT tool.

### 3. RESULTS AND DISCUSSION

According to the results of the analyzes pharmacognostic (**Table 1**), the pH of the leaves and stems were 5.56 and 5.46 respectively, which may characterize the presence of acidic substances, which 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<sup>+</sup> ions in the reaction medium (SIMÕES; et al., 2003). The result is similar to that reported by Owiti (2011), for him the value found for the anaconda 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 not formed only of lipids, but for all compounds under the conditions of the determination, can be extracted by the solvent, therefore, it is necessary a more rigorous analysis for the identification and qualitative determination result quantification (SIMÕES; et al., 2003).

For moisture, the recommended limit for herbs is 14% (SIMÕES; et al, 2003), with this, it was found that the powder of leaves and stems of *Mikania lindleyana* results presented in percentage of 7.46±0.09 and 7.77±0.06, 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 the values in percentage of total ash for leaves and stem of *Mikania lindleyana*, 17.29±0.99 and 1.41±0.91, compared to 8-14% limit established by the Brazilian Pharmacopoeia (2010), it was observed that the value for the leaves are above recommended levels, suggesting a high concentration of inorganic material (MARQUES, 2012). Owiti (2011) reports the amount of total ash for Sucuriçu sheets 17.067 of ± 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 <sup>a</sup> | 5.46 <sup>a</sup> |
| Lipids (% m/m)              | 3.15 <sup>a</sup> | 0.38 <sup>a</sup> |
| Total ash (% m/m)           | 17.29 ± 0.99      | 1.41 ± 0.91       |
| Humidity (% m/m)            | 7.46 ± 0.09       | 7.77 ± 0.06       |

<sup>a</sup> - 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) who studied the alcoholic extract from sucuriçu leaves found alkaloids, tannins, steroids and triterpenoids, already in the study by Silva (2011) showed the presence of saponins espumílicas the leaves of this species.

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

| Class metabolites | Leaf | Stem |
|-------------------|------|------|
| Alkaloids         | +    | +    |
| Anthraquinones    | -    | -    |

|                            |   |   |
|----------------------------|---|---|
| Steroids and triterpenoids | - | - |
| Anthocyanin glycosides     | + | + |
| Saponins                   | + | + |
| Tannins                    | + | + |

(+) Being (-) Absent.

The presence of these secondary metabolites groups of pharmacological interest highlights its popular use as healing, through the presence of tannins (PEREIRA, 2012), which acts forming a protective layer on injured epithelial tissues, allowing, allowing just below this layer, the tissue repair process occurs naturally (CASTEJON, 2011), anti-inflammatory and antinociceptive, through the alkaloids, which acts biologically to interfere mediators involved in the inflammatory process (SIMÕES, et al., 2003). 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 (SIMÕES; et al., 2003). The anthocyanin glycosides are antioxidants metabolites, acting slowing or preventing oxidative damage, to block the oxidation reactions (SIMÕES; et al., 2003).

For antimicrobial activity, the results demonstrate that the EBEFM and EBECM showed no activity in strains *E. coli* and *S. aureus*, considering that the concentration of 1000 µg/mL is considered toxic, is irrelevant to further testing at higher concentrations. The results were 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*.**

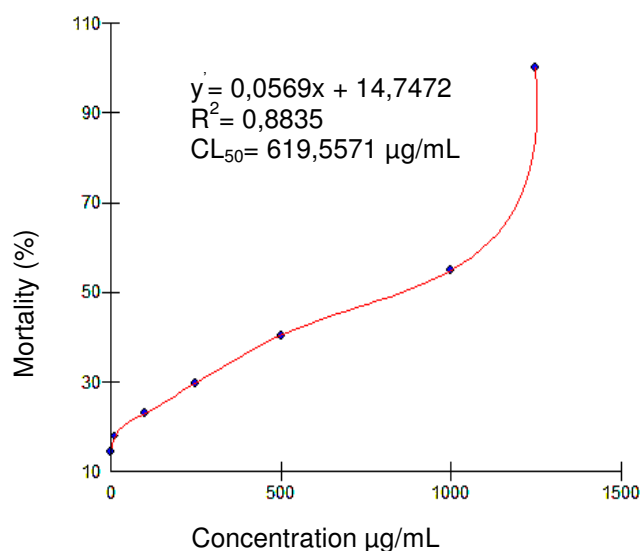
| Sucuriju Extract Dilution | <i>E. coli</i> EBEFM | <i>E. coli</i> EBECM | <i>E. coli</i> EBEFM | <i>E. coli</i> EBECM | <i>E. coli</i> EBEFM | <i>E. coli</i> EBECM |
|---------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|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

|      |   |   |   |   |   |   |
|------|---|---|---|---|---|---|
| 1:8  | + | + | + | + | + | + |
| 1:16 | + | + | + | + | + | + |

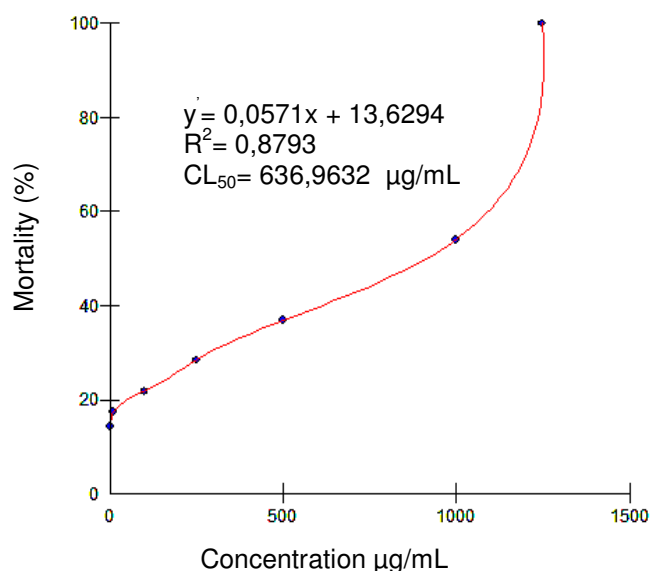
(+) Growth

The cytotoxicity bioassay, on *Artemia salina*, provided the LC50 for EEEFM and EBECM equal to 619.5571 and 636.9632 µg/mL respectively, giving cytotoxic activity both, considering that Nascimento (2008) considers the amounts below 1000 µg/mL as cytotoxic, which can be related to the cytotoxic activity of alkaloids and saponins (SILVA, 2007), in those parts of the plant. The concentration of the solution leaves and stem Sucuriju to kill 50% of microcrustaceans is shown in graphic 1 and 2.

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



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



#### 4. CONCLUSION

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

Also, moisture from the leaves and stem Sucuriçu was consistent with the value set 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 *Mikania lindleyana* was set up by the Brazilian Pharmacopoeia, which possibly corresponds to a high concentration of minerals in its constitution, which did not occur with the stem, what does hurt the little concentration of minerals in this part of the plant.

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

The EBEFM and EBECM not have antimicrobial activity in *Escherichia coli* and *Staphylococcus aureus* which does not exclude the possibility of this kind, have antimicrobial activity in other bacteria. The bioassay on *Artemia salina*, contributed to the population, warning her about the possible toxic concentrations of the species under study, and contribute 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.

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## 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 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.

Bednarczyk 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 sublineata*: phytochemical and active against pernicious *Moniliophthora* fungus associated with cupuaçu (*Theobroma grandiflorum*). *New chemical*, São Paulo, 2012; 35 (11): 2237-2240.

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.

Cunha EL. Evaluation of bacterial contamination and heavy metals in the river shores in the city of Macapa, 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.

- 352 Macêdo JAB. Laboratory Methods of Analysis Physical-Chemical and  
353 Microbiological. 2. ed. Belo Horizonte: CRQ-M.G., 2003.
- 354
- 355 Marques GS, et al. Phytochemical and physicochemical characterization of the  
356 leaves of *Bauhinia forficata* Link collected in two regions. Journal of Pharmaceutical  
357 Sciences Applied Basic, São Paulo, 2012; 33 (1): 57-62.
- 358 B Mendes et al. Information herbal and chemical composition of *Mikania DC*  
359 *lindleyana*. (Asteraceae). Brazilian Journal of Pharmacy, Curitiba, 2002; 83 (1/4): 27-  
360 29.
- 361
- 362 Birth JE, et al. Phytochemical studies and toxicological bioassay against larvae of  
363 *Artemia salina* Leach. three medicinal species of the genus *Phyllanthus*  
364 (*Phyllanthaceae*). Journal of Applied Pharmaceutical Sciences Basic, São Paulo,  
365 2008; 29 (2): 145-150.
- 366 Nunes CR, et al. Inhibition of nitric oxide production and cytotoxic effect of aqueous  
367 extract of *Annona muricata* L. Perspectives online, Rio de Janeiro, 2011; 1 (2): 1-8.
- 368
- 369 Oliveira TS. Anatomy, seed germination and analysis of essential oil *Viguiera*  
370 *arenaria baker* in martius and robust *Viguiera gardner* in hook (*Asteraceae*-  
371 *Heliantheae*) .2011. 95 f. Dissertation (Master of Science) -University of São Paulo  
372 and College of Agriculture "Luiz de Queiroz", Piracicaba, 2011.
- 373 Owiti, AO. Development of a method for characterizing hydroethanolic extract of  
374 leaves of *Mikania lindleyana* DC. by high performance liquid chromatography  
375 (HPLC). 2011. 72 f. Dissertation (Master) -University Federal do Pará, Graduate  
376 Program in Pharmaceutical Sciences, Bethlehem, 2011.
- 377
- 378 Panizza S. Plants that heal: bush smell. IBRASA, São Paulo, 1997; 1 (y / n): 117-  
379 118.
- 380
- 381 Pereira RJ, et al. Plant secondary metabolites and antioxidant benefits. Journal of  
382 Biotechnology and Biodiversity, Tocantins, 2012; 3 (4) 146-152
- 383 Prudencio E. ethnopharmacological *Solidago chilensis* Meyen Survey. "Arnica-  
384 Brazilian" (*Asteraceae*). 2012. 44 f. Work Completion of course (Specialization) -  
385 University of the Extreme South of Santa Catarina, Cricklewood, 2012.
- 386 HP Rang, et al. Pharmacology. 6. ed. Rio de Janeiro: ELSEVIER, 2007.
- 387 Santana RO. Use of plant extracts and essential oils in poultry production. 21 2011.  
388 f. Seminar (Master) -University Federal de Goiás, Veterinary School, Goiânia, 2011.
- 389 Saraiva RMC. Antibacterial activity of medicinal plants front of the multidrug-  
390 resistant bacteria and their interaction with antimicrobial drugs. 2012. 94 f.  
391 Dissertation (Graduate Diploma in Pharmaceutical Sciences) -University Federal do  
392 Pará, Institute of Health Sciences, Bethlehem, 2012.
- 393
- 394 Silva ASB. Evaluation of antinociceptive and anti-inflammatory *Mikania of lindleyana*  
395 *DC* .: validate the use in folk medicine. 2011. 107 f. Dissertation (Master in  
396 Pharmaceutical Sciences) -University Federal do Pará, Institute of Health Sciences,  
397 Graduate Program in Pharmaceutical Sciences, Bethlehem, 2011.

- 398 BSA Silva, et al. Anti-inflammatory and Antinociceptive Activities of the aqueous  
399 extract of mikania lindleyana in rodents. IJPSR, São Paulo, 2012; 3 (6): 1637-1646.  
400
- 401 Smith DB, et al. Isolation and evaluation of the cytotoxic activity of some  
402 oxaporphin alkaloids obtained from Annonaceae. New chemistry, São Paulo,  
403 2007; 30 (8): 1809-1812.
- 404 Silva CG. Ethnobotanical study and antimicrobial activity in vitro of medicinal plants  
405 in Nazaré community, municipality of Miracles, Ceará. 2012. 93 f. Dissertation  
406 (Graduate in Forest Sciences) Federal -University of Campina Grande, Paraíba,  
407 2012.  
408
- 409 Simões CMO, et al. Pharmacognosy: the plant to the drug. 5. ed. Florianópolis  
410 Publisher UFRGS, 2003.
- 411 GS Souza et al. Growth, essential oil content and content of coumarin saplings  
412 guaco (*Mikania glomerata* Spreng.) Cultivated under colored nets. Biotemas,  
413 Florianópolis, 2011; 24 (3): 1-11.  
414
- 415 Vanderlinde FA, et al. Anti-inflammatory and opioid-like Activities in methanol extract  
416 of *Mikania lindleyana*, anaconda. Brazilian Journal of Pharmacognosy, Curitiba,  
417 2012; 22 (1) 150-156
- 418 Zuchinalli A. Study of chemical and biological properties of *Croton urucurana* plant species.  
419 2009. 79 f. Dissertation (Graduate in Chemistry) -University Federal of Santa Catarina,  
420 Center of Physical and Mathematical Sciences, Department of Chemistry, Graduate in  
421 Chemistry Program, Florianópolis, 2009.