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Research Article

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The effect antioxidant aqueous crude extract in *Acmella ciliata (*Kunth.) (*Asteraceae*)

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ABSTRACT

Many species of Asteraceae are used as medicinal plants with different therapeutic indications, preparation and uses. The species Acmella ciliata (Kunth.) Cass belongs to this family. It is a medicinal plant of African origin, brought to Brazil by slaves during the colonial period. The extract from the leaves of A. ciliata (Kunth.) Cass has several uses in Brazilian folk medicine, since it has analgesic and anti-inflammatory properties. Thus, this research aims to identify the chemical compounds present in the aqueous crude extract of leaves of A. ciliata (Kunth.) Cass (Asteraceae) evaluate the antioxidant potential against the DPPH radical and cytotoxic front the Larvae of Artemia Salina Leach. Evaluation of antioxidant activity, with some modifications, was based on the methodology proposed by Sousa and Lopez-Lutz by sequestering capacity of DPPH• (2,2-diphenyl-1-picryl-hidrazila). The cytotoxicity assay front Artemia salina Leach. based on the technique of Araujo and Lôbo with adaptations. In this study, the preliminary phytochemical analysis of EBA A. ciliata (Kunth.) Cass. revealed the presence of saponins, organic acids, reducing sugars, tannins, alkaloids, steroids and triterpenoids. Based on these results it is concluded that the EBA leaves A. ciliata (Kunth.) Cass has in its chemical constitution promising secondary metabolites with antioxidant action. Cytotoxic activity was not significant considered non-toxic in this test. However, it is evident the great therapeutic potential of this species.

Keywords: Antioxidant, Cytotoxic, *Acmella ciliata*.

INTRODUCTION

The Human populations use various plant species to find a cure for various diseases. This procedure is widespread, not only in Brazil but also in other countries, settling-over the time because of the accumulation of knowledge about the plants by diverse ethnic groups [1].

There are about 1.620 genders and 24.000 species of the Asteraceae family, it has a worldwide distribution, and is well represented in temperate and subtropical regions, accounting for the largest family of eudicotyledonous, with more than 1.100 genders and 19.000 species [2]. In Brazil, it is listed up 2.065 species in 288 genders, being considered the center country of the Asteraceae diversity [3].

Many species of the Asteraceae are used as medicinal plants with different therapeutic indications, preparation, and uses. The species *Acmella ciliata (*Kunth.) Cass belongs to this family. It is a medicinal plant of African origin,

brought to Brazil by slaves during the colonial period [4]. In some african-brazilian religions, boldo is considered a sacred plant and widely distributed in the North, Northeast, Midwest and Southeast regions of Brazil [5].

Acmella ciliata (Kunth.) Cass also is known as boldo-baiano, boldo-japonês (Japonese boldo), boldo-chinês (Chinese Boldo), boldo-africano (African boldo), boldo-goiano, boldo-de-Goiás, boldo, acumã, alumã, aloma, aluman, luman, árvore-do-pinguço, fel-de-índio, heparém, assa-peixe, figatil, among other popular names. In fact, It includes the botanical synonyms *Vernonia bahiensis, Vernonanthura condensata Baker, and Vernonia sylvestris* [4. 6].

The extract from the leaves of *A. ciliata* (Kunth.) Cass has several uses in Brazilian folk medicine, because of its analgesic and anti-inflammatory properties [7]. The boldo-da-Bahia is used by medicinal teas or juices. It offers benefits in the treatment of heartburn, gastrointestinal disorders, gastritis control, headache, diarrhea, hypertension, antibacterial, immunomodulatory, anti-histamines [7. 8. 9. 10].

The boldo of Bahia has a glycoside steroid known as vernonioside B, which has been suggested as one of the active ingredients of the analgesic activity (antinociceptive) in the leaves extracts from *A. ciliata* (Kunth.) Cass [11]. It is considered a strong medicine to treat hangover, as well as stimulate appetite and aid digestion. It is believed that the boldo-of-Bahia also helps in the treatment of anorexia, anemia, inflammation and bladder problems [12]. It presents in its constitution phenolic compounds, flavonoids, alkaloids, fructose, carbohydrates, lactose, saponins, sesquiterpene lactones, sucrose, tannin and essential oil [4].

Silva et al. [13] Support the hypothesis that *A. ciliata (Kunth.)* Cass is a promising source of bioactive substances with antioxidant activity because the chemical composition of this species fits the profile of potential antioxidant compounds. Acting generally kidnapping or eliminating free radicals [14. 15]. Therefore, drugs and plant phytochemicals with high concentration of phenolic compounds are substances that can be used to prevent and / or treat oxidative stress.

Given the above, it becomes necessary the scientific validation of the use of medicinal plants in treating diseases arising from the production of free radicals as inflammation, pain, cancer, abortion, and gastrointestinal disorders by experimental studies. Thus, this research aims to identify the chemical compounds present in the aqueous crude extract from the leaves of A. *ciliata (Kunth.)* Cass (Asteraceae), to assess the potential antioxidant against DPPH radical and cytotoxic front of the larvae of *Artemia Salina* Leach.

EXPERIMENTAL SECTION

Plant Material

The samples of *A. ciliata (Kunth.)* Cass, were collected in the district of Fazendinha S 00º00'26.8 "/ W 51 05'04.6 " Macapá- Amapá. The herbarium specimens obtained in the collection were identified and deposited in the Herbarium Amapaense (HAMAB / Institute of Scientific and Technological Research of the State Amapá- IEPA), under the symbol of (IAN): 018879. The selected leaves were dried at 40ºC and then ground in a mill to obtain a fine-grained powder.

Preparation of materials, solutions and reagents (Prospecting Phytochemistry)

All reagents were prepared according to conventional techniques in Phytochemistry [16-19].

Obtaining the aqueous crude extract (ACE) of *A. ciliata* (Kunth.) Cass

The aqueous crude extract was obtained with the dilution of this powder in distilled water in the ratio of approximately 1/20 (w / v) and submitted to hydrodistillation process (100 ° C) in a Clevenger type apparatus for 2 h [20]. Held the extraction, the ACE was evaporated under reduced pressure and further diluted in suitable solvents and concentrations to achieve the phytochemicals and biological assays [19].

Phytochemical Prospection

Phytochemical tests were conducted to verify the presence of saponins, organic acids, reducing sugars, phenols, tannins, alkaloids, Flavonoids, Anthraquinones and Triterpenes [16-21].

Determination of the antioxidant activity by the capture of the DPPH free radical

The evaluation of the antioxidant activity, with some modifications, was based on the methodology proposed by Sousa et al. [24] and Lopez-Lutz et al. [25] through the sequestering capacity of the DPPH (2,2-diphenyl-1-picryl-hidrazila). There was prepared a methanol solution of DPPH at the concentration of 40µg/mL. The ACE *A. ciliata (Kunth.)* Cass. was also diluted in methanol at the following concentrations (5-2,5-1,0-0,75-0,5 e 0,25 mg/mL). To

evaluate the antioxidant activity were made triplicate with volume of 0.3 mL of extract per tube, added to 2.7 mL of DPPH solution. Meanwhile, the white of each concentration was prepared, being it the mixture of 2.7 mL of methanol plus 0.3 mL of the methanolic solution of ACE. After 30 minutes of incubation at room temperature and protected from light, the readings were performed with a spectrophotometer (Biospectro SP-22) at a wavelength of 517nm in quartz cuvette. The antioxidant activity was calculated according to Souza et al. [26].

$$(AA\%) = 100 - \{[(Abs_{sample} - Abs_{white})100]/Abs_{control}\}$$

Cytotoxicity assay with *Artemia salina* Leach.

The cytotoxicity assay of *Artemia salina* Leach. was based on the technique of Araújo et al. [22] and Lôbo et al. [23] with some adaptations. It was prepared a synthetic sea salt solution at 35 g / L, in this were incubated 45 mg of eggs from *A. salina* Leach. The solution was incubated in a dark environment and exposed to a source of artificial heat, within 24 hours to hatch the larvae (nauplii). Then the nauplii were separated and placed in a bright environment at room temperature for 24 hours to achieve the metanauplius stage. The mother solution of ACE from *A. ciliata* (Kunth.) Cass was prepared containing 18 mg of dry extract, solubilized in 1.5 mL of Tween 80 to 5%, facilitating the solubilization of it. Then, it was added to a 7.5mL saline solution. The final volume was 9 mL, the final concentration obtained was 2mg / ml. Subsequently to the end of the light period, the metanauplius were selected and divided into 7 groups of 10 subjects in each test tube. . For group, were added aliquots of 2500, 1900, 1250, 625, 250 and 125µL of ACE, respectively, completing the final volume of 5 mL with a synthetic sea salt solution (35g / L). It was obtained the final solutions with the following concentrations of 1000, 750, 500, 250, 100 and 50 µg/mL, thereby the groups were designated according to their respective concentration and all tests were performed in triplicate. After 24 hours, the number of survivors was counted to determine CL_{50} by the PROBIT analysis from the SPSS® software.

Statistical analysis

The results obtained from the bioassays were expressed by averages of ± Standard error (SEM) organized according to the relevance in tables, charts, tables and figures. The significant differences between treatments were evaluated using the Anova Um Critério test, and followed by Student-Newman-Keuls test. The analysis of correlation and regression were applied to assess the DE_{50} and CL_{50}. The CL_{50} values were determined in PROBIT regression, using the SPSS (*Statical Package for Social Sciences*) program, with probabilistic limit of $p \leq 0.05$.

RESULTS AND DISCURSSION

Phytochemical screening of the ACE from *Acmella ciliata* (Kunth.) Cass.

The result of the phytochemical analysis from the aqueous extract of *Acmella ciliata* (Kunth.) Cass. revealed the presence of saponins, organic acids, reducing sugars, tannins, alkaloids, steroids and triterpenoids (Table 1).

Table 1 Preliminary Results of phytochemical screening of the ACE *Acmella ciliata* (Kunth.)

Secondary Metabolite	*Acmella ciliata* Baker
Saponins	+++
Organic acids	+
Reducing sugars	++
Phenols	-
Alkaloids	+++
Tannins	+
Anthraquinone	-
Flavonoids	-
Steroids	+
Triterpenoids	+

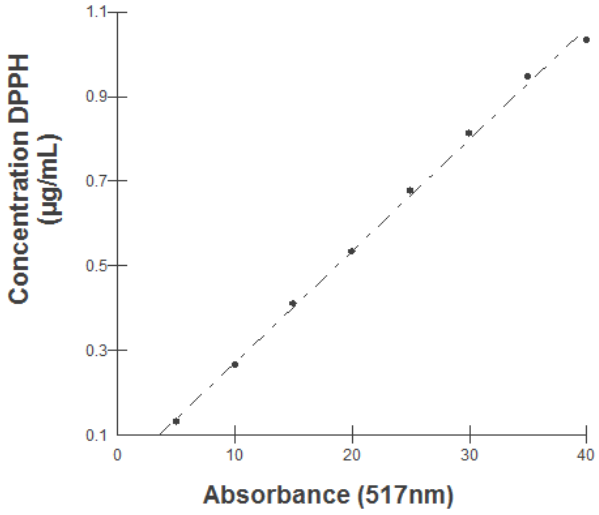
Antioxidant activity of ACE *Acmella ciliata* (Kunth.) Cass. in the elimination of the free radical DPPH.

Table 2 shows the result of the antioxidant activity of ACE *Acmella ciliata* (Kunth.) Cass. on the free radical DPPH. It is observed a strong correlation of the extract to reduce the DPPH radical with ED50 of 0.2137mg / mL, suggesting a strong antioxidant activity as hown in Figure 2. Figure 1 shows the calibration curve of the DPPH radical (40 µg/mL).

Table 2: Antioxidant activity percentual of ACE *Acmella ciliata* on the free radical DPPH•.

Concentration do *Acmella ciliata* Baker	% AA (DPPH•)
5mg/mL	84,066±2,542[a]
2,5mg/mL	78.536±1,221[b]
1,0mg/mL	55.601±1,043[b]
0,75mg/mL	51.785±0,481[b]
0,5mg/mL	49.194±0,622[b]
0,25mg/mL	47.863±0,547[b]

Mean ± standard deviation (SD), [a] p <0.05 to 5 mg / mL when compared to other concentrations



Y=0,079X+0,0264
R^2= 0,9975

Figure 1: Analytics of DPPH curve in length from 517 nanometer (nm)

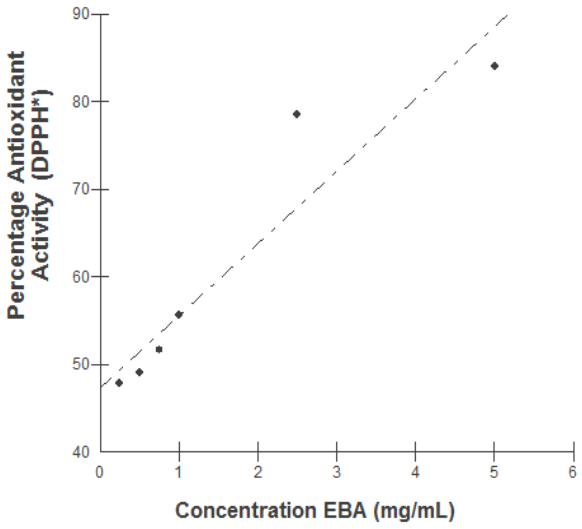


Figure 2: Percentage of EBA antioxidant activity *Acmella ciliata* (Kunth.) on the free radical DPPH
DE_{50}=0,321mg/mL p <0,05

Cytotoxicity in vitro against *Artemia salina* Leach.

The cytotoxic activity of ACE from *Acmella ciliata* (Kunth.) Cass. was evaluated against larvae of the parasite *Artemia Salina* Leach. in different concentrations of the extract. The data show the effective LC_{50} lethal concentration 1557. 55µg / mL of ACE *Acmella ciliata* (Kunth.) Cass. on *A. salina*, showing a non-toxic activity of the extract of *A. salina*, as shown in (Figure 3).

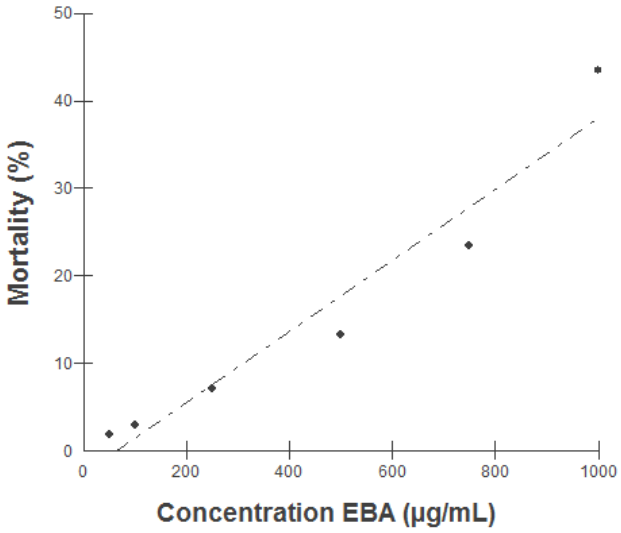


Figure 3: EBA toxicity Test *Acmella ciliata* (Kunth.) at different concentrations on *A. Salina* Leach

The molecule of DPPH • is well known to be characterized as a stable free organic radical and has many advantages such as a good stability in the absence of light, applicability, simplicity, and practicality [27. 28]. According to Moon and Shibamoto [29], the DPPH • method is used in over 90% of the antioxidant evaluation studies of pure substances, mixtures or complex matrices.

Studies show that antioxidants of vegetable origin can have a strong importance as therapeutic agents for various diseases associated with oxidative stress, for these compounds be effective as free radical scavengers and inhibitors of lipid peroxidation, showing to be clinically more effective and less toxic than existing drugs [30. 31].

The species *Acmella ciliata* (Kunth.) Cass. is very consumed by the Brazilian population in the treatment of various diseases as an analgesic, anti-inflammatory, anti-anemic, antibacterial, hepatoprotective and antiulcerogenic [2. 11]. These activities can be attributed to the presence of some metabolites as terpenes, steroids, and flavonoids [10. 32. 33].

In this study, the preliminary phytochemical analysis of ACE from A. *ciliata* (Kunth.) Cass. revealed the presence of saponins, organic acids, reducing sugars, tannins, alkaloids, steroids, and triterpenes, according to previous descriptions of most of these components [4]. Several studies in vitro and in vivo showed that tannins, triterpenoids and other secondary metabolites of plants have analgesic, anti-inflammatory and antioxidant action [12. 34. 35. 36]. The results obtained demonstrate strong antioxidant correlation of the aqueous crude extract of *A. ciliata* (Kunth.) Cass. Leaves, against the free radical DPPH as straight line equation Y=6,9747X+48,5089, R^2 (adjusted) of 0.8290 and IC_{50} (inhibitory concentration) of 0,2137mg / mL, suggesting excellent antioxidant activity (Figure 2). The presence of organic acids (Table 1) reinforces its antioxidant application, widely used in food industry as an additive and preservative [21. 37]. Several plants have the ability to accumulate organic acids found in citrus juices, due to the presence of citric acid, but these acids are not only present in fruit but also in the leaves of some plant species [38. 39]

It is known the large citric acid antioxidant potential, which shows that the species A. *ciliata* (Kunth.) Cass are a promising source of this substance. The same functions are assigned to the phenolic compounds, abundant in many plant species, that regardless of the class, these compounds all have antioxidant potential [40]. Phenolic compounds are abundant in fruits, vegetables and foods derived from plants, which are consistently associated with reduced risk of cardiovascular disease, cancer and other chronic diseases [41].

The ability of these substances to scavenge free radicals and pro-oxidant metals (antioxidant action) explains in part this association. Recent evidence suggests that these compounds may act through other mechanisms besides the antioxidant capacity, as modulation of the activity of different enzymes such as telomerase, lipoxygenase, and cyclooxygenase, interactions with receptors and pathways of signal transduction, cell cycle regulation, among others, essential for the maintenance the homeostasis of living organisms [42].

The reducing sugars present major health benefits to possessing antioxidant and antimutagenic activity. The putative mechanism of antioxidant activity for the reducing sugars is the ability that they have to bind to free radicals, reducing them and promoting the excretion from the body, without the aid of carriers, reducing cell activity without causing oxidative stress and premature aging cells [43. 44].

The steroids are also known for their antioxidant properties, among its benefits to human health there is the reduction of dietary cholesterol absorption, with a consequent reduction in blood levels; reducing the risk of cardiovascular disease; and inhibiting the growth of certain malignant tumors [45].

As to the cytotoxic potential, it is known that certain phenolic compounds are bacteriostatic, fungicidal, and able to inhibit tumor development [46]. Sundarraj et al. [47] demonstrated in vitro the cytotoxic activity of phenolic compounds against lung and breast cancer cells. Boutennoun et al. [48] also confirmed cytotoxic and antioxidant activity of phenolic compounds from the methanol extract of Achillea odorata.

In this study was observed by the adjusted coefficient of determination (R^2) of 0,7894, the correlation between the mortality showed by the larvae of *A. salina* L., facing the different concentrations of ACE *V. condensate*. Whose minimum lethal concentration CL_{50} was 1557,55µg/mL, presenting non-toxic behavior exceeding 1000µg/mL [49, 22].

CONCLUSION

Facing the results attained, it is concluded that the ACE from the leaves of *A. ciliata* (Kunth.) Cass has in its chemical constitution promising secondary metabolites with antioxidant action. The cytotoxic activity was not significant, considered nontoxic for this test. However, it is clear the great therapeutic potential of this species.

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