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Antioxidant and cytotoxic potential of aqueous crude extract of *Acmella oleracea* (L.) R. K. Jansen

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ABSTRACT

Acmella oleracea (L.) R.K. Jansen (Asteraceae), popularly known in Brazil as Jambu is a vegetable distributed in tropical and subtropical regions, with important chemical properties. Its leaves and flowers are used in traditional medicine with antioxidant and cytotoxic. The present study aims to evaluate the chemical composition of crude aqueous from the extract of the leaves of A. oleracea its antioxidant potential against the radical DPPH and cytotoxic potential front of the larvae of Artemia Salina. The aqueous crude extract was obtained from this dilution in distilled water and submitted to hydrodistillation .The evaluation of antioxidant activity, was based on the methodology through the sequestering capacity of DPPH. There was prepared a methanol solution of DPPH at the concentration of 40µg/mL. The EBA Acmella oleracea was diluted with methanol at the following concentrations (5/2.5 /1.0/0.75/0.5 and 0.25 mg/mL). The cytotoxicity assay with A. salina was based on the technique of Araújo et al. (2010) and Lôbo et al. (2010) with adaptations. The preliminary phytochemical analysis of crude aqueous extract of the leaves of A. oleracea showed the presence of saponins, organic acids, reducing sugar, phenols, alkaloids, steroids and triterpenoids. A. oleracea showed reduction activity on the DPPH directly proportional to extract concentration using the (DE50%=2.76mg). The data show the effective and lethal concentration CL50 of 730.85µg/mL from EBA A.oleracea on A. salina. Based on these results it is concluded that the EBA of A.oleracea leaves presents in its chemical composition secondary metabolites with antioxidant and / or cytotoxic action.

Keywords: Antioxidant, Cytotoxic, Jambu**,** *A. oleracea.*

INTRODUCTION

Medicinal plants are rich in secondary metabolites such as flavonoids, anthocyanins, polyphenols, anthraquinones, alkaloids, tannins, catechins, terpenes and others. The majority of these metabolites have anti-inflammatory, antibacterial, cytotoxic, antifungal, anti-hypertensive, and anti-oxidant action [1].

Among the various vegetal species used for herbal purposes, are the Asteraceae family species that stands out for being a highly diverse family, where the species *Acmella oleracea* (L.) R.K. Jansen possesses numerous applications in the field of popular medicine in the Amazon, and its pharmacological activities have been the subject of many studies [2,3].

Acmella oleracea (L.) R.K. Jansen, popularly known in Brazil as Jambu is a vegetable distributed in tropical and subtropical regions, with important chemical properties. It is well appreciated in the northern region of Brazil, which is part of the local cuisine and folk medicine. It has many popular synonyms such as agrião from Pará, agrião from Brazil, agrião from North, jabuaçú, crazy plant, jaburama, buttercup, and toothache plant [4-7].

The synonyms of *Acmella oleracea* (L.) R.K. Jansen are: *Spilanthes oleracea* L., *Cotula pyretharia* L., *Pyrethrum spilanthus* Medik., *Spilanthes acmella* var *oleracea* (L.), *Spilanthes fusca* MART [8], *Bidens fervida* Lam., *Bidens fusca* Lam., *Isocarpa pyrethraria* (L.) Cass, *Spilanthes radicans* Schrad., *Spilanthes oleracea* b fusca (Lam.) D. C. [9].

Acmella oleracea (L.) R.K. Jansen is characterized by being a herbaceous plant, from 30 to 60 cm tall, semi-upright or nearly tripped, with a cylindrical stem, with fleshy and decumbent branches [10]. Its leaves are simple, with broadly ovate blade, sparse hair on both surfaces [9]. The flowers are small and yellow, arranged in globose chapters that measure about 1 cm in diameter Figure 1 [11,12].

Figure 1: Leaves and flowers of *Acmella oleracea* (L.) RK Jansen
Source: Author

Its leaves and flowers are used in traditional medicine in the form of infusion and macerated. It helps the treatment of diseases such as dyspepsia, malaria, infections of the mouth and throat [13-16], sexual deficiencies, especially due to the aging, [17], it is diuretic [11], anti-inflammatory [18], larvicides, insecticides [19], local anesthetic [20], analgesic [21], antioxidant and cytotoxic [22,23]. According to Borges (2012) [24], the pharmacological effect is due to their chemical substances, of which the trans-caryophyllene, germacrene D, L-dodecene and spathulenol and espilantol.

The espilantol, a substance found in species *Acmella oleracea* (L.) R.K. Jansen, is an aliphatic amide (N-isobutylamides) of molecular formula $C_{14}H_{23}NO$, described as a yellow burning viscous oil, which produces an anesthetic effect and tingling on the tongue, because of that, it is used in diseases of the mouth and throat and as a treatment of dental pain [25]. Its chemical composition has potential for industrial use as an additive for food and beverages; cleaning agent in preparations for the body and hair; product used as an insecticide for the control of insects and microorganisms in plants, and it is also referred to as target for various diseases [26].

Addition of the espilantol, *Acmella oleracea* (L.) R.K. Jansen is also rich in bioactive phenolic compounds such as coumarin (scopoletin) and triterpenoids, which can be attributed to these a possible antioxidant activity [23]. Among *Asteraceae* family species, is cited *Achillea odorata* [27] and *Eriocephalus africanus* [28] that not only were confirmed the presence of phenolic compounds in the extracts of the leaves but also cytotoxic and antioxidant activity.

Scientific research intensifies efforts in the search for antioxidants less harmful to health, with the product source of antioxidants plant replacing synthetic origin. Phenolic compounds found in fruits, vegetables and herbs have received increasing attention for their potential in preventing degenerative diseases. It is known the importance of the antioxidant in scavenging free radicals helping to prevent and treat many degenerative chronic diseases, inflammations, allergies, hypertension, tumors as well as the better preservation of the food thereby increasing shelf-life [1,29].

Scientific studies have tried to correlate the toxicity of *Artemia salina* Leach with activities such as antifungal, virucidal, antimicrobial, parasiticide, trypanocidal, and in the preliminary evaluation of antitumor plant extracts. *Artemia salina* is a saltwater microcrustacean, which is used as live food for fish, and their eggs are easily found in stores of aquarists. The simplicity of the bioassay favors its routine use, and it may be developed in the laboratory [30].

Studies for cytotoxic activity using the bioassay with *A. salina* Leach, which is characterized by being low-cost, fast and does not require aseptic techniques. Numerous bioactive constituents of plant extracts have been obtained using this test in the monitoring phytochemical studies [31].

Given the above, the present study aims to evaluate the chemical composition of crude aqueous from the extract of the leaves of *Acmella oleracea* (L.) R.K. Jansen (*Asteraceae*) its antioxidant potential against the radical DPPH and cytotoxic potential front of the larvae of *Artemia Salina* Leach.

EXPERIMENTAL SECTION

Plant Material

Samples of *Acmella oleracea* (L.) R.K. Jansen were collected in the district of Fazendinha (S 0 ° 02'30.40 " / W 51 06'37.5 "), Macapá-Amapá, one of the largest suppliers of Jambu for various street markets in the city of Macapá. 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 of Amapá- IEPA), under the code (IAN): 188088. The selected leaves were dried at 40ºC and then crushed in a mill to obtain a fine-grained powder.

Obtaining the aqueous crude extract (EAB)

The aqueous crude extract was obtained from this dilution in distilled water in an approximate proportion of 1/20 (w / v) and submitted to hydrodistillation process (100 °C) in a Clevenger-type apparatus for 2h [32]. Held the extraction, the EBA evaporated under reduced pressure and further diluted in suitable solvents and concentrations to achieve the phytochemicals and biological assays [33].

Prospecting Phytochemistry

Phytochemical tests were conducted to verify the presence of Saponins, organic Acids, reducing Sugars, Phenols, Tannins, Alkaloids, Flavonoids, Anthraquinones and Triterpenes [33-37].

Determination of antioxidant activity by DPPH free radical capture

The evaluation of antioxidant activity, with some modifications, was based on the methodology proposed by Sousa et al. (2007) [38] and Lopez-Lutz *et al*. (2008) [39], through the sequestering capacity of DPPH (2,2-diphenyl-1-picryl-hidrazila). There was prepared a methanol solution of DPPH at the concentration of 40µg/mL. The EBA *Acmella oleracea* (L.) R.K. Jansen was diluted with methanol at the following concentrations (5-2.5-1.0-0.75-0.5 and 0.25 mg / mL). To evaluate the antioxidant activity were made triplicate with the 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, and it is the mixture of 2.7 mL of methanol plus 0.3 mL of the methanolic solution of EBA. 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 a quartz cuvette. The antioxidant activity was calculated according to Souza et al. (2009) [40].

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

Cytotoxicity assay with *Artemia salina* Leach

The cytotoxicity assay with *Artemia salina* Leach. was based on the technique of Araujo *et al*. (2010) [41] and Lobo *et al.* (2010) [42] with adaptations. It was prepared as synthetic sea salt solution at 35 g/L and were incubated 45 mg of eggs from *A. salina* Leach. The solution was incubated in a dark container and exposed to a source of artificial heat, within 24 hours to hatch 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 EBA from *Acmella oleracea* (L.) R.K. Jansen was prepared containing 18 mg of dry extract, solubilized in 1.5 mL of Tween 80 to 5% to facilitate the solubilization of it. 7,5mL of saline solution were added to amounting the final volume of 9 mL, the final concentration obtained was 2mg/mL. Subsequently, the end of the light period, the metanauplius were selected and divided into 7 groups with 10 individuals in each test tube. For group, were added aliquots of 2500, 1900, 1250, 625, 250 and 125µL of EBA, 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 ± standard deviation (SD) organized, according to the relevance, in tables, charts, tables and figures. Data were subjected to one way analysis of variance (ANOVA). The CL_{50} values were determined in PROBIT regression, using SPSS (Statical Package for Social Sciences) with probabilistic limit $p \leq 0.05$.

RESULTS

Phytochemical analysis of the EBA *Acmella oleracea* **(L.) R.K. Jansen**

The preliminary phytochemical analysis of crude aqueous extract of the leaves of *Acmella oleracea* (L.) R.K. Jansen showed the presence of saponins, organic acids, reducing sugar, phenols, alkaloids, steroids and triterpenoids as shown in Table1.

Table 1: Preliminary result of phytochemical screening of the leaves of EBA *Acmella oleracea*

Secondary metabolite	EBA *Acmella oleracea* (L.).
Saponines	+
Organic acids	+
Reducing sugars	++
Phenols	++
Alkaloids	+++
Tannins	-
Anthraquinones	-
Flavonoids	-
Steroids	-
Triterpenoids	-

Antioxidant activity of EBA Acmella oleracea (L.) R.K. Jansen in eliminating free radical DPPH

Figure 2 shows the radical DPPH calibration curve (40µg/mL). For assessing the percentage of antioxidant activity of the EBA *Acmella oleracea* (L.) R.K. Jansen showed reduction activity on the DPPH directly proportional to extract concentration using the following equation of the line (Y=2,8947X+42,0089, R^2= 0,9856 e DE50%=2,76mg) as shown in **Figure 3.**

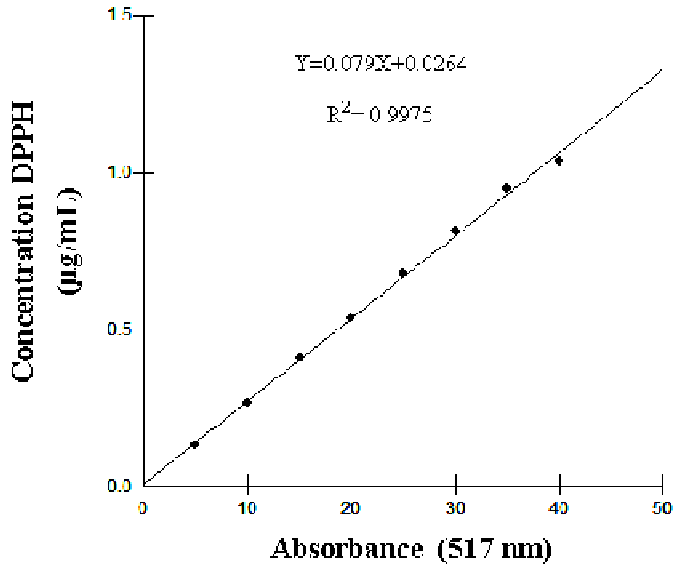

Figure 2: Analytics of DPPH curve in length from 517 nanometer (nm)

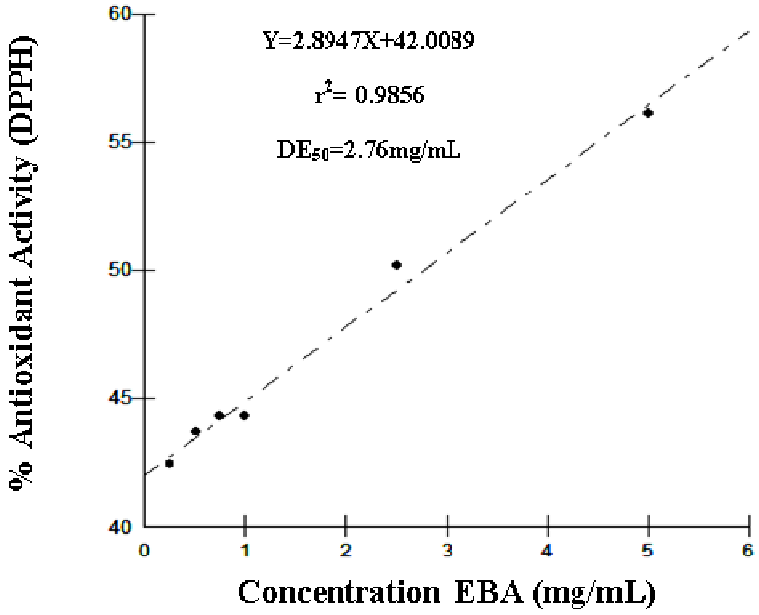

Figure 3: Percentage of EBA antioxidant activity *Acmella oleracea* (L.) RK Jansen on the free radical DPPH

Cytotoxicity in vitro against *Artemia salina* Leach.

The cytotoxic activity of EBA from *A. oleracea* (L.) R.K. Jansen was evaluated against larvae of the parasite *Artemia Salina* Leach. in different concentrations of the extract. The data show the effective and lethal concentration CL_{50} of 730.85µg/mL from EBA *A. oleracea* on *A. salina* as shown in **Figure 4.**

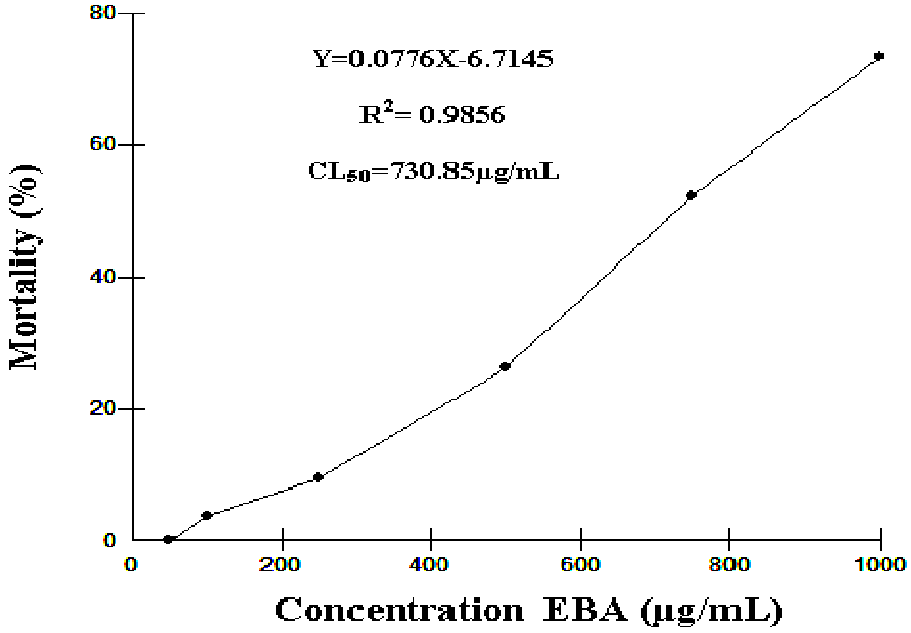

Figure 4: EBA toxicity Test *Acmella oleracea* (L.) RK Jansen at different concentrations on *A. Salina* Leach

DISCUSSION

Free radicals and other oxidants have been considered in recent years as a major cause of various diseases such as cancer, cardiovascular disease, cataracts, immune system decline, brain dysfunction and diabetes mellitus type I [40]. This evidence has suggested that diseases caused by oxidative reactions can be reduced by the intake of natural antioxidants found in the diet [43].

The *A.oleracea* species (L.) R.K. Jansen has been well documented for its popular use as antibacterial, antifungal, antimalarial, larvicide, insecticidal, cytotoxic and antioxidant [18-23].

Before the phytochemical analysis of the EBA *A.oleracea*, there is a great antioxidant and cytotoxic potential of the species (Table 2). According to Alves et al. (2007) the higher the consumption of DPPH by the sample, the higher its antioxidant activity (AA%) [44]. In (Figure 3) it is observed that the consumption of DPPH was directly proportional to the concentration of EBA *A.oleracea* as the equation of Y=2,8947X+42,0089 e DE50 de 2.76mg (effective dose) below the largest 5mg of EBA *A.oleracea* corroborating Wongsawatkul et al. (2008) [22].

Table 2: Mean ± standard deviation (SD), p <0.001 to 5 mg / mL when compared to other concentrations

Concentration EBA *Acmella oleracea* (L.).	% Antioxidant Activity (DPPH)
5mg/mL	56.128±0,538
2.5mg/mL	50.245±0,998[a]
1.0mg/mL	44.328±1,211[a]
0.75mg/mL	44.398±0,641[a]
0.5mg/mL	43.663±0,615[a]
0.25mg/mL	42.367±0,893[a]

The presence of organic acids (Table 1) reinforces their antifungal, antimicrobial and antioxidant application. It is widely used in food industry as an additive and preservative [43,45]. Several plants have the ability to accumulate organic acids found in citrus juices, due to the presence of citric acid, however, these acids are not only present in fruit, but also in the leaves of some plant species [46,47].

It is known the large antioxidant potential of the citric acid, which shows that *A.oleracea* species is a promising source of this substance. The same functions are attributed to phenolic compounds, abundant in many plant species, which regardless of the compounds class [48]. Phenolic compounds are abundant in fruits, vegetables, and foods derived of the plant, that are consistently associated with reduced risk of cardiovascular disease, cancer and other chronic diseases [49].

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 signal transduction pathways, cell cycle regulation, among others, essential for the maintenance of homeostasis of living organisms [50].

About 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 it has to bind to free radicals reducing them and promoting excretion from the body, without the aid of carriers, reducing cell activity without causing oxidative stress and premature aging cells [51,52].

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 [53].

As to the cytotoxic potential, it is known that certain phenolic compounds are bacteriostatic, fungicidal and able to inhibit tumor development [54]. Sundarraj et al. (2012) [55] demonstrated in vitro cytotoxic activity of phenolic compounds against lung and breast cancer cells . Boutennoun et al. (2014) [27] also confirmed cytotoxic and antioxidant activity of phenolic compounds from the methanol extract of *Achillea odorata*.

In this study, it is observed by the adjusted coefficient of determination (R^2) of 0.9856. The relation between the mortality of the larvae of *A. salina* L facing the EBA *A.oleracea* concentrations, whose minimum lethal concentration CL_{50} was 730.85µg/mL, shows the significant toxicity below the highest dose of 1000µg/mL (Figure 4) according to Born et al. (2008) [41] and Araújo et al. (2010) [56].

This activity can also be assigned to saponins, which have anthelmintic activity, antiviral, spermicidal, haemolytic and molusquicida [43,45]. Due to the amphipathic behavior of saponins and the ability to form complexes with steroids, proteins, and phospholipids membrane. It is suggested that some saponins have the ability to disrupt the cell membrane of microorganisms, resulting in leakage of cellular contents and eventually death [57].

Another metabolite that may be involved in cytotoxic activity are the alkaloids, which due to its toxicity and bitterness, act as amebicide, antiviral and antitumor [43].

CONCLUSION

Based on these results it is concluded that the EBA of *A.oleracea* (L.) R.K. Jansen leaves presents in its chemical composition secondary metabolites with antioxidant and / or cytotoxic action. Proving to be a promising source of natural resources, used in the pharmaceutical industry.

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