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PHYTOCHEMICAL PROSPECTION AND BIOLOGICAL ACTIVITY OF THE ETHANOLIC EXTRACT OF THE LEAVES OF *CURATELLA AMERICANA L.* (DILLENIACEAE)

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ABSTRACT

The present work describes the phytochemical prospection and biological activity of the leaves of *Curatella americana* L., also known as "Caimbé", belonging to the family Dilleniaceae. The necessary botanical material was obtained in the municipality of Boa Vista, state of Roraima. This species has a wide distributionthroughout the intertropical belt, and can be found throughout the belt from Australia to South America. The extract was obtained from dried and pulverized material. The toxic activity against the microcrustacean Artemia salina was then determined. Tests were performed to determine the antimicrobial activity of *Curatella americana* for gram-positive bacteria (*S. aureus* and *L. monocytogenes*), gram-negative bacteria (*C. freundii* and *P. aeruginosa*) and for yeasts (*C. albicans*). Furthermore, the inhibition of the acetylcholinesterase enzyme was determined by the spectrophotometric method on an Elisa type reader at a wavelength of 600 nm. The phytochemical prospection of *C. Americana* leaf extracts showed the presence of the following secondary metabolites: phenols, tannins, saponins and free steroids. Biological tests of the extract showed low toxicity, antimicrobial activity against some of the microorganisms tested, and inhibition rates of over 50% of the enzyme acetylcholinesterase, therefore classified as a potent inhibitor.

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INTRODUCTION

The origin of man's knowledge about the medicinal properties of plants isintertwined with his own history. For millennia plants have been used by humans in the treatment of diseases, however, only recently have plants become the object of scientific study with regard to their various medicinal properties, including their antibacterial and/or antifungal activity [Novais, 2003]. In Brazil, the use of plants in the treatment of diseases receives fundamental influences from indigenous, African and European cultures. The indigenous people usedphytotherapy within a mystical framework and by observing the plants that animals turned to when sick. A large part of the phytotherapeutic remedies used by the Brazilian population comes from native plants. the national biomes have several plants that can be used for the treatment of diseases. Among this natural wealth is the Brazilian *Cerrado* biome, which comprises approximately 25% of the national territory, and is located largely in the central-western region [Coutinho, 1990]. The *Cerrado* has many medicinal plants. Plants produce a vast and ramified set of organic compounds, classified as primary and secondary metabolites. The primary metabolites include structural, building and energy storage activities [Taiz, 1998]. The secondary metabolites have great value due to their applications as medicines, cosmetics, food and agrochemicals [Phillipson, 1998]. Among the many plant species with pharmacological potential, the Dilleniaceae family stands out. Some studies have proven the existence of important biological activities in plants of the genus Dilleniaceae, such as hypoglycemic, hypolipidemic, antimicrobial, antioxidant, antileukemic and antinociceptive activity of *Dillenia indica* [Kumar et al., 2011]. Among the plants of the Dilleniaceae

family, the species *Curatella americana* L. is highlighted. The plants of this species has healing, antiseptic and hypoglycemic properties, being widely used in folk medicine for the treatment of thrush, headache, intoxication, cancer, arthritis, diabetes and lung problems [Luz, 2001]. In this context, the present work aims at prospecting and evaluating the biological activities of the crude ethanolic extract of *C. americana* leaves, focusing on evaluating the toxic activity thereof against *Artemia salina*, determining antimicrobial activity, and evaluating the inhibition of the acetylcholinesterase enzyme.

MATERIALS AND METHODS

Plant Material: In November 2021, the botanical material (leaves) of *C. americana* L was collected in the municipality of Boa Vista, the capital of the state of Roraima - Brazil. The botanical material went through several steps until the final compound was obtained. The botanical identification of the species was performed by Prof. Dr. Reinaldo Imbrózio Barbosa, a consultant at the INPA herbarium, based on the records 6785, 76079, 80328, 122249, 152159, 175998 and 199505, deposited with the INPA. Herbarium of the National Institute for Amazon Research in the city of Manaus - Brazil.

Extract preparation: 500g of the leaves of *C. americana* were used in the preparation of the extract. The leaves were immersed in 3.0 liters of ethyl alcohol for seven days. Afterwards, the extract obtained was filtered on the analytical filter paper and concentrated under reduced pressure in a roto-evaporator. Then, the extract was stored and preserved until the completion of the tests.

Qualitative classification of secondary metabolites: The phytochemical prospection was performed according to the methodology of Barbosa, et al. (2004) [7]. The ethanolic extract of *C. americana* leaves was subjected to tests for phenols, tannins, flavonols, flavanones, flavanonols, xanthones, flavonoids, sesquiterpenolactones and other lactones, steroids and triterpenoids.

Teste de toxicidade frente a *Artemia salina:* The assay was carried out in partnership with the Natural Products Laboratory of the Federal University of Roraima, by adapting the methodology of Mayer and collaborators (1982) and McLaughlin and collaborators (1993) [Meyer, 1982]. In an *in vivo* system, we placed Artemia salina larvae in various concentrations of the ethanolic extract of *C. american*leaves. After a 24-hour incubation period, the number of dead and live larvae in each test tube was counted. After counting the number of living and dead *Artemia salina* specimens, the mortality and the Lethal Dose 50% (LD_{50}) were calculated.

Determination of Antimicrobial Activity (AAM): The antimicrobial activity test was performed by determining the minimuminhibitory concentration (MIC) for bacteria (gram-negative and gram-positive) and for afungus (yeast). These were carried out in the laboratory of Biotechnology and Bioassays of the Federal University of Minas Gerais. The results containing the rate of inhibition as a function of concentration were submitted and tabulated. The microplates were incubated in an incubator at 37°C and after 24 hours the test was read in an ELISA reader (600 nm). The antibiotic used for quality control was ampicillin.

Determination of Acetylcholinesterase inhibition (AChE)

RESULTS AND DISCUSSION

Extract yield: To obtain the extract, 1 kg of *Curatella americana* dried leaves were used in 5.8L of ethanol, obtaining 98.93g of dry and concentrated extract. The extract had a yield of 9.8%.

Preliminary phytochemical testing of the white ethanolic extract of *C. Americana:* The phytochemical prospection tests were performed with the crude extract of *Curatella americana* and the classes of metabolites found in the ethanolic crude extract of the leaves were: phenols, tannins, saponins, and free steroids (Table 1).

Table 1. Results of the phytochemical prospection of the crude ethanolic extract of *Curatella americana L.* leaves:

Secondary metabolites	Results	Secondarymetabolites	Results
Phenols	+	Chalcones	-
Tannins	+	Isoflavones	-
Phenolic Substances	-	Saponins	+
Flavones	-	Free steroids	+
Flavonols	-	Free pentacyclics	-

(-)Absence; (+) Presence.

In the phytochemical analysis tests performed by Correia *et al.,* (2013) [Correia] with the crude ethanolic extract of the leaves of *C. americana*for the determination of the medicinal character through the leaves; the classes of metabolites found in the extract were: Phenols and tannins, reducing sugars, saponins, steroids and triterpenoids, depsides and depsidones, and alkaloids. The detection of these metabolites confirms the basis for the popular use of *C. americana* as an analgesic and anti-inflammatory, for washing cuts and treatment of ulcers. Metabolites such as saponins, have numerous biological activities and are one ofthe metabolites responsible for the antimicrobial, analgesic, and antioxidant activity of *C. americana* [Santos, 2011].

Toxicity test against *Artemia salina:* The toxicity bioassay against Artemia salina was performed with the ethanolic extract of *Curatella americana*. The test was performed in triplicate for allconcentrations of the samples and for the blank test [control 1 (saline + DMSO) and control 2 (saline)]. Counting of dead or immobilized nauplii was performed after 24 hours with the aid of a magnifying glass. The following results were obtained:

Table 2. Number of dead nauplii in the samples tested.

1000 µg/mL		500 µg µg/mL	250 µg/mL	125 µg/mL	62,5 µg/mL	31,25 µg/mL	15,62 µg µg /mL	C1	C2
Ethanolic extract of the leaves of *C. americana L*									
	M	M	M	M	M	M	M	M	M
S	4	1	0	0	0	0	0	0	0
D	3	0	1	0	0	0	0	0	0
T	2	1	0	0	0	0	0	0	0
X	3	0,6	0,3	0	0	0	0	0	0

S = sample; D = duplicate; T= triplicate; X = arithmetic mean; M= dead; C1= control 1; C2 = control 2.

After counting the number of dead or immobilized Artemia salina specimens, the mortality rate calculation was performed, obtaining the following results:

Table 3. Activity data against *Artemia salina*

Concentrations	mortality rate (%)
0 µg /mL	0
15,62 µg /mL	0
31,25 µg /mL	0
62,5 µg /mL	0
125 µg /mL	0
250 µg /mL	3
500 µg /mL	6
1000 µg /mL	30
Control 1	0
Control 2	0

Using the linear regression formula Y= a +bx , we can calculate the lethal dose (LD_{50}) for the ethanolic extract of *C. americana*..

Y= 50; a= -2,775; b= 0,029
50 = (-2,775) + 0,029x
X is 1792,05 µg /mL

Table 4. Results of the inhibition of microorganisms tested, in percent, of the ethanolicextract of C. americana L. leaves

Microorganisms	% averagesample inhibition	% average inhibition of ampicillin	% average inhibition of miconazole	% average inhibition of nistatina
Staphylococcus aureus	21,56 ± 0,62	94,64 ± 0,62	----	----
Listeria monocytogenes	31,70 ± 3,76	94,51 ± 0,55	----	----
Citrobacter freundii	43,74 ± 1,41	94,65 ± 0,73	----	----
pseudomonas aeruginosa	0 ± 0	94,64 ± 0,74	----	----
Candida albicans	0 ± 0	----	94,80 ± 0,71	92,7 ± 0,50

Table 5. Percentage results of acetylcholinesterase enzyme inhibition for the ethanolicextract of *C. americana L.* leaves

Samples	% of inhibition	Coefficient of Variation
EECA	82,19 ± 1,70	0,02
Eserina (standard)	92,93 ± 0,10	0,00

EECA = Ethanolic extract of *C. americana*

Calculating the arithmetic mean between the values obtained from the extract, a result of 30% mortality was found for the 1000 µg/mL concentration, 6% for the 500 µg/mL concentration, and 3% for the 250 µg/mL concentration. The graph below shows the relationship between extract concentration and % mortality. For data analysis, it can be stated that the ethanolic extract of *C. americana* did not show lethality against *Artemia salina* hence is considered of low toxicity ($LD_{50\%}$ higher than 500 µg /mL) having found the LD_{50} value of 1792.05 µg /mL.

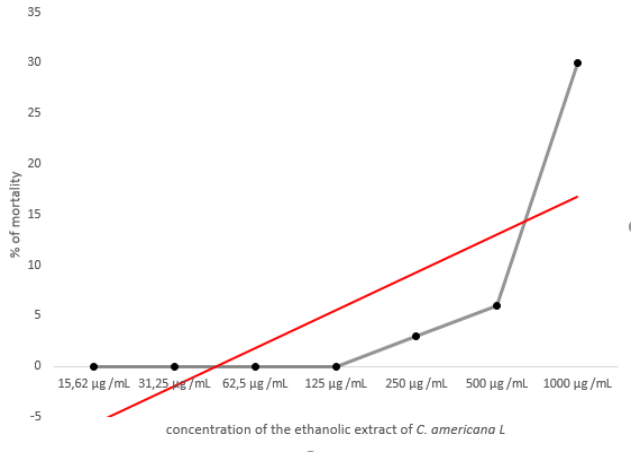

Figure 1. Activity curve against *Artemia salina*

Determination of Antimicrobial Activity (AAM): The bioassays were performed in triplicate for all microorganisms with positive controls. The antibiotic ampicillin for gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and gram-negative (*Citrobacter freundii* and *pseudomon as aeruginosa*) bacteria, and the antifungals miconazole and nystatin for yeast (*C. albicans*) were used as standards (positive control). Through the antimicrobial activity assay, it was possible to verify the activity of the crude ethanolic extract of *C. americana* leaves that showed variations in MIC (minimum inhibitory concentration), ranging from 21.56% to 43.74% for gram-positive bacteria (*S. aureus* and *L. monocytogenes*), MIC ranging from 0% to 31.70% for gram-negative bacteria (*C. freundii* and *P. aeruginosa*) and MIC was 0% for the yeast *Candida albicans*, as shown in Table 4. Although the tested sample has demonstrated antimicrobial activity against some tested microorganisms, it may be concluded that this activity is not satisfactory when compared to the inhibition averages acquired for the positive controls used in the assay.

Determination of Acetylcholinesterase inhibition (AChE): In the test performed to evaluate the inhibition activity of the acetylcholinesterase enzyme, the crude ethanolic extract of C. americana L leavesshowed 82.19% average inhibition of the AChE enzyme, compared to eserine (positive standard) which showed 92.93% inhibition (Table 5). Thus, it may be concluded that the crude ethanolic extract of *C. americana* leaves can be classified as a potent inhibitor to the enzyme (Ache), since it showed an inhibition percentage higher than 50%. This result reinforces the need for further studies, since no data was found in the literature of research on the Acetylcholinesterase enzyme inhibition activity of the crude ethanolic extract of *C. americana*leaves.

CONCLUSION

This work has contributed to biological studies on *C. americana,* known regionally as Caimbé; providing data on the species of the family Dilleniaceae existing in the State of Roraima, Brazil. The main secondary metabolites identified through phytochemical prospection were phenols, tannins, saponins and free steroids. In the toxic evaluation against *Artemia salina*, the extract did not show lethality against microcrustaceans, hence is considered of low toxicity. In the antimicrobial activity analysis, the sample showed activity for gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and gram-negative (*Citrobacter freundii*) bacteria. In the evaluation of the inhibition of the acetylcholinesterase enzyme, the crude ethanolic extract of *C. americana L.* leaves showed a high potential, being considered a potent inhibitor of the enzyme, since its inhibition is higher than 50%. Therefore, the studied species showed low toxicity, besides being a potent inhibitor of the acetylcholinesterase enzyme. Thus, the ethanolic extract of *Curatella americana L* bark can be considered analternative for the treatment of several diseases, besides its high contribution to the search for a cure for Alzheimer's disease.

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