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Antioxidant, Cytotoxic, Antiproliferative And Acetylcholinesterase Inhibition Properties Of The Extract From Amburana Cearensis

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***Abstract*— *Amburana cearensis* A.C. Smith is a plant found in Northeastern Brazil that has been shown antioxidant, anti-inflammatory, and immunomodulatory effects. Here, were reported biological effects and some underlying mechanisms of action of *A. cearensis*. Stem powder of *A. cearensis* was used to prepare its hydroalcoholic extract, its elemental analysis, and its antioxidant, antifungal, cytotoxic, antiproliferative, and acetylcholinesterase inhibitory activities. We have found that *A. cearensis* hydroalcoholic extract showed antioxidant and cytotoxic activity, primarily in higher concentration, though reduction of cell viability. The organic compounds indicate the promising potential of this plant as medicine. Interestingly, *A. cearensis* also showed antiproliferative activity against breast carcinoma and demonstrated acetylcholinesterase inhibitory activity, when compared to positive control (Physostigmine®). In conclusion, theses evidences reveal that *A. cearensis* has medicinal proprieties due to its constituents and show that *A. cearensis* presents, at least, antioxidant, cytotoxic, antiproliferative, and acetylcholinesterase inhibitory activities, which could have future therapeutical uses.**

Keywords*— *Amburana cearensis; antioxidant effect; antifungal activities; toxicity; folk medicine.

I. INTRODUCTION

Amburana cearensis A.C. Smith, commonly known as ‘cumaru’ or ‘imburana-de-cheiro’ is a medicinal plant found in Northeastern Brazil widely used in folk medicine. It is a deciduous tree that can grow up to 30 meters tall in the rainforest, but is likely to be no more than 4 - 10 meters tall when growing in the dry forests of northeast Brazil. Moreover, the tree also has several local medicinal uses and is the source of an essential oil. Oily resin obtained from the bark is traditionally used in the treatment of colds, coughs, and bronchitis, asthma and lung ailments. Traditional use includes decoction or cooking barks for pharmacological actions. In addition, among its therapeutical effects, antioxidant (Leal et al. 2008; de Araújo Lopes et al. 2013; Lima Pereira et al. 2017), anti-inflammatory (Leal et al. 2009; de Araújo Lopes et al. 2013; Lima Pereira et al. 2017), antiproliferative (Costa-Lotufo et al. 2003), neuroprotective (Leal et al. 2005), antibacterial (Figueredo et al. 2013; Sá et al. 2014), immunomodulatory (de Araújo Lopes et al. 2013), and bronchodilator (Leal et al. 2003; Leal et al. 2008) are the most reported in the literature.

The first report of *A. cearensis* as anti-inflammatory occurred by Leal et al. (2009) when amburoside A (a phenol glucoside) and isokaempferide (a flavonol) isolated from the trunk bark of *A. cearensis* showed inhibition of inflammatory processes and myeloperoxidase activity in human neutrophils (Leal et al. 2009). In 2013, de Araújo Lopes et al. showed that afrormosin, an isoflavone isolated from *A. cearensis*, was able to modulate intermediary steps of neutrophil reactive oxygen species (ROS) generation process and the result seemed to be directed towards phorbol 12-myristate-13-acetate (PMA)-induced activation, indicating a marked inhibition of protein kinase C activity (de Araújo Lopes et al. 2013). Moreover, neuroprotective effects of *A. cearensis* was initially demonstrated by Leal et al. (2005), which evaluated the glucoside isolated from *A. cearensis* on rat mesencephalic cell cultures exposure to neurotoxin 6-hydroxydopamine. The authors found a significant neuroprotective effect suggesting that glucoside uses as a therapeutic agent in neurodegenerative disease, such as Parkinson's (Leal et al. 2005). Later, Lima Pereira et al. (2017) corroborates the neuroprotective previous findings of the *A. cearensis*. On this study, the seeds extract of *A. cearensis* showed its effect against oxidative stress and toxicity induced by glutamate in a primary cultures of cerebellar cells, which were treated with ethanol, hexane, dichloromethane and ethyl acetate fractions (Lima Pereira et al. 2017). Moreover, Leal et al. (2008) demonstrated that besides the neuroprotection, *A. cearensis* promoted a hepatic protection since it could reverse the oxidative damage induced by carbon

tetrachloride in the liver. The authors suggested that hepatoprotective activity of *A. cearensis* was due to the phenolic nature of this glucoside (Leal et al. 2008).

The antiproliferative effects of several compounds isolated from *A. cearensis* were evaluated by Costa-Lotufo et al. (2003). The cytotoxicity of kaempferol, isokaempferide, but not amburoside A and protocatechuic, inhibited sea urchin egg development, as well as tumor cell lines, but in this assay isokaempferide was more potent than kaempferol. Considering that protocatechuic acid was unique compound capable to induce hemolysis of mouse erythrocytes, it was suggested that cytotoxicity of kaempferol and isokaempeferide was not related to membrane damage (Costa-Lotufo et al. 2003). Furthermore, modulation of antibiotic activity by extracts from *A. cearensis* was reported by Figueredo et al. (2013). A year later, chloroform extract from stem bark of *A. cearensis* showed activity against multiresistant bacteria of different species, as *Pseudomonas aeruginosa* and *Bacillus cereus* (Sá et al. 2014). These findings support that besides anti-inflammatory, neuroprotective and antiproliferative, *A. cearensis* seems to have also antibiotic proprieties.

Last, but not least, since 2003 *A. cearensis* have been described by its role as bronchodilator in rats and guinea-pigs (Leal et al. 2003). Serotonin was able to increase cutaneous vascular permeability and this effect was blocked by hydroalcoholic extract, coumarin and flavonoid fraction isolated from trunk barks of *A. cearensis*. Moreover, when guinea-pig trachea was precontracted with carbachol, histamine or KCl, all three supracited *A. ceasensis* solutions were able to induce a concentration-dependent relaxation in the presence of three agonists. Indeed, hydroalcoholic extract, coumarin and flavonoid fraction were also able to inhibit histamine and serotonin-induced increase of cutaneous vascular permeability in rats (Leal et al. 2003). The same authors showed that a flavonoid isokaempferide (IKPF; 5,7,4'-trihydroxy-3-methoxyflavone) from *A. cearensis* was able to induce relaxation of guinea-pig isolated trachea (Leal et al. 2006). Therefore, considering all *A. cearensis* therapeutical potentials, this study performed its elemental analysis of organic compounds followed by the screening of potential biological activities of hydroalcoholic extract from bark of *A. cearensis* through its antioxidant and antifungal, acetylcholinesterase inhibitory activities, as well as toxic activities against strains of murine fibroblasts (L929), human keratinocytes (HaCaT), human adenocarcinoma mammary gland/breast (MDA-MB-231 and MCF7), and *Artemia salina*.

II. Material and Methods

A. Plant material

The stem powder of *A. cearensis* was purchased at Ver-O-Peso market in Belém, Pará, Brazil in September 2016. The stem powder were dried at 40 °C in an oven and then reduced to powder using a knife mill (Metvisa, Brazil).

B. Preparation of the extract

Ten (10) grams of dried stem powder were macerated and extracted with 500 mL of ethanol-water (70:30, v/v) for 72 hours at room temperature. Then, the residue was removed by filtration, and the extract was evaporated to dryness at a lower temperature (< 40 °C) under reduced pressure in a rotary evaporator (Buchi, Switzerland), followed by lyophilization (Christ, Germany) under 1.8 mbar pressure and −14 °C. The yield of the extract was 8.7% w/w. The material was stored protected from light in air-tight containers with cap at −20 °C until use.

C. Elemental analysis

The elements such as carbon (C), hydrogen (H), nitrogen (N) and sufur (S) were determined through combustion at 925 ºC in elemental analyzer Perkin Elmer CHN/O Analyser 2400 Series II (Perkin Elmer Sdn Bhd.: Selangor, Malaysia). The values were corrected considering dry basis and free of ashes, using information obtained by thermogravimetric analysis. The oxygen (O) level was determined by the difference from the corrected data. The analysis was made in duplicate.

D. Antioxidant activity

The scavenging activity of *A. cearensis* was measured according to 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) method, as described previously by Sreejayan and Rao (1996) (Sreejayan and Rao 1996), with minor modifications. Briefly, sample (50 µL) at different extract concentrations (0.97 – 250 µg/ml) was added to each well of a 96-well microplate and mixed with 150 µL of 50 µM DPPH in ethanol solution. The reaction mixture was then kept for 30 minutes in the dark at room temperature. Then, the absorbance was measured in a spectrophotometer at 510 nm against the negative control (ethanol). Resveratrol (Sigma-Aldrich, USA) was used as positive control at the same concentrations. Inhibition of DPPH radical was calculated using Formula: IC_{50} (%) = 100 x (A0 – As) / Ao, being A0 negative control absorbance and As test-sample absorbance. The IC_{50} value was calculated from the straight-line equation of the linear dispersion graph and represents the extract concentration that inhibits 50% of DPPH radical. All tests were performed in triplicate.

E. Antifungal activity

The standard strains used in this study were as follows: *Candida albicans*, American Type Culture Collection (ATCC) 10231; *C. glabrata* (Taniwaki, M.H.), Collection of Tropical Cultures (CCT) 0728; *C. krusei*, (FTI) CCT 1517; and *C. guilliermondii* (CCT), 1890 from the Foundation André Tosello (Campinas, São Paulo, Brazil). The procedures were performed according to M27-A2 protocol from the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2002). The fungal suspension was prepared in sterile saline

(0.85% NaCl, w/v) and then it was diluted in RPMI 1640 culture medium, buffered with 3-(N-morpholino)-propanesulphonic acid (MOPS) and pH was adjusted to 7.0 ± 0.1, so as to obtain from 5 x 10^2 to 2.5 x 10^3 colony forming units (CFU) per mL. The dried extract was diluted in RPMI 1640 medium buffered with MOPS and 20 µl mL^{-1} Tween-80/dimethyl sulfoxide (DMSO) (1:1, v/v) in a concentration range of 39 to 5,000 µg mL^{-1}. The assay was performed in 96-well sterile microplates to which 100 µL of analogs dilutions and 100 µL RPMI 1640 were added, buffered with MOPS and inoculated with a suitable number of the microorganism's colony forming units. The growth control consisted of 100 µL of the same inoculated culture medium and 20 µl mL^{-1} Tween 80/DMSO (1:1, v/v) and a sufficient quantity of uninoculated medium to make up 200 µL. Negative control was prepared adding 200 µL of uninoculated medium. Amphotericin B (Cristália, Brazil) was used as reference drug at concentrations from 0.0313 to 16.0 µg mL^{-1}. Then, microplates were incubated at 35 °C during 48 hours. The minimum inhibitory concentration (MIC) was established as the lowest concentration at which no turbidity was observed in the culture medium. After checking the MIC, an aliquot of 20 µL was retained from those wells which showed no visible growth and re-incubated with 4 mL of Tryptic Soy Broth (TSB) without addition of antifungal agent, for another 48 hours at 35 °C. The lowest concentration at which no turbidity was noticed after this period was considered to be the Minimum Fungicidal Concentration (MFC).

F. Cytotoxic assay

The two immortalized cell lines, L929 fibroblasts and human keratinocytes (HaCaT), were grown in Dubelcco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg mL^{-1} streptomycin and 10 mM HEPES and maintained at 37 °C in a 5% CO2 humidified atmosphere at pH 7.4. Cell viability was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann 1983). Briefly, L929 or HaCaT cells were seeded in 96-well plates at a density of 5 × 10^4 cells mL^{-1} in 100 µL of medium per well. After 24 hours of incubation, culture medium was replaced by fresh medium with treatments. Quintuplicate wells were treated with *A. cearensis* extract at concentrations ranging from 15.62 to 1,000 µg mL^{-1}. The plates were incubated at 37 °C in 5% CO2. A control experiment was performed under the same conditions without cell treatment. After 48 hours, medium was removed and 100 µl of DMEM with 10% of MTT (5 mg mL^{-1}) dye solution was added, followed by incubation for 3 hours at 37 °C. The precipitated formazan was then dissolved in DMSO, and the absorbance was measured at 540 nm using a microplate reader. The cell viability was determined by equation: cell viability (%) = (A sample / A control) x 100.

G. Brine shrimp lethality assay

The brine shrimp lethality assay was carried out following an already published procedure described by (Meyer et al. 1982) with some modifications. Encysted eggs of the brine shrimp *Artemia salina* Leach were incubated in artificial seawater and after 48 hours of incubation at room temperature, the active nauplii free from egg shells (10 units) were collected and added to each set of wells containing dried extract (10 – 1,000 µg mL^{-1}) dissolved in 0.1 % DMSO and made up to 5 mL total volume using artificial salt water. Thymol and 0.1 % DMSO were used as positive and negative controls, respectively. After 24 hours, the number of survivors was counted using a binocular microscope, and the percentage of death was calculated. The lethal concentration 50% (LC_{50} value) and the standard error were calculated by Probit analysis (Finney 1971).

H. Antiproliferative activity of dried extract of A. cearensis in breast carcinoma

Antiproliferative activity was assessed in human breast adenocarcinoma (MDA-MB-231 and MCF7) and mouse tumor mammary gland (4T1) cells lines by MTT. These cells were cultured in DMEM with 10% FBS and 50 µg mL^{-1} gentamicin and incubated at 37 °C in CO_2 incubator in an atmosphere of humidified 5% CO_2. Exponentially growing MDA-MB-231 cells were seeded into 96-well plates (10^4cells per well in 100 µl of media) and allowed to attach for 24 hours. Test extract was prepared in 1% ethanol (70% v/v) and serially diluted with media to obtain appropriate concentrations (15.62 – 1,000 µg mL^{-1}). Cells were treated with different concentrations of extract and incubated for 48 hours. The test compound containing media was removed and washed with 100 µL of PBS followed by addition of 90 µL of MTT reagent and incubated for 3 hours at 37 °C. The medium was removed and 100 µL DMSO was added and the absorbance measured using a micro plate reader at 570 nm followed by the calculation of percentage viability. Cells from control test were treated with 0.1% (v/v) ethanol 70% in medium. Each treatment was performed in sextuplicate. IC_{50} values were calculated using dose response inhibition curves in GraphPad Prism 5.

I. The multi-well plate AChE inhibition assay

AChE inhibition activity *in vitro* of the dried extract of *A. cearensis* was measured by an adaptation of the method first described by Ellmam et al. (1961). For measuring anti-AChE activity, it was used acetylcholine iodide (AChI, Sigma-Aldrich, USA) as a substrate and 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma-Aldrich, USA) as a reagent. Briefly, to a flat bottom 96-well plate were added 80 µL of Tris-HCl buffer 50 mM, pH 8.0, 120 µL of extract solution in different concentrations ranges, and 25 µL of DTNB 3 mM + AChI 75 mM solution. Then, 25 µL of AChE 0.25 U mL^{-1} solution were joined in order to activate the reaction. Control wells contained Eserine, a parasympathomimetic alkaloid that acts as a reversible cholinesterase inhibitor, in place of the extract. The

concentration of yellow 5-thio-2-nitrobenzoate anion formed by the reaction between DTNB and thiocholine, a resulting product from the hydrolysis of ACh, was measured in a spectrophotometer at a wavelength of 415 nm every 15 s for 41 cycles at 37 °C. IC_{50} for the dried extract of *A. cearensis* was determined by GraphPad Prism 5, and it represents in which extract concentration the hydrolysis of acetylcholine is inhibited in 50%. The experiments were performed in triplicate for each concentration.

J. Statistical analysis

The results were calculated as a mean ± standard deviation (SD). Statistical comparisons were made using the Student t-test, one-way analysis of variance (ANOVA) and Bonferroni's post-hoc test, using the software GraphPad Prism 5. The limit of statistical significance was set at $p < 0.05$.

III. Results and Discussion

The main finding of current study showed that *A. cearensis* hydroalcoholic extract showed: *i)* antioxidant and cytotoxicity activities; *ii)* antiproliferative activity against breast carcinoma, and *iii)* acetylcholinesterase inhibitory activity, whereas *A. cearensis* hydroalcoholic extract have no effect on the fungal growth. The elemental analysis of organic compounds revealed the presence of the essential elements such as C, H and N in the extract sample is depicted in Table I.

TABLE I. Elemental analysis of extract from *A. cearensis* leaves using combustion at 925 °C in elemental analyzer Perkin Elmer CHN/O analyzer 2400 series II

***A. cearensis* stem powder**			
Mean (n=2)	**Weight (mg)**	**Carbon (%)**	**Hidrogen (%)**
	2.3 ± 0.02	47.41 ± 0.06	6.55 ± 0.11
	Nitrogen (%)	**Sulfur (%)**	**Total protein N x 6.25 (%)**
	0.25 ± 0.07	0.97 ± 0.01	1.56

Plants take them from the ground and incorporate them into organic compounds that we consume them by eating either the plants or the animals that are the plants. Interestingly, it was observed the higher percentage of the C in the *A. cearensis* powder (mean: 47.35%), while H, N and S composed 6.67%, 0.18% and 0.99%, respectively.

The antioxidant activity of *A. cearensis* hydroalcoholic extract is depicted in the Table II. Using the method previous described by Sreejayan and Rao (1996), we had found that *A. cearensis* hydroalcoholic extract showed an antioxidant activity when compared to resveratrol (p=0.0132*). In accordance to previous findings from Lima Pereira et al. (2017), seed extracts from *A. cearensis* demonstrated a potential therapeutic agent for neurodegenerative diseases since them showed neuroprotective potential against cytotoxicity induced by glutamate and oxidative stress. Here, the antioxidant propriety was compared to resveratrol, a potent antioxidant compound, which corroborates Lima Pereira et al. (2017) findings.

TABLE II. Antioxidant activity of *A. cearensis* extract and resveratrol

Sample	**Antioxidant activity IC_{50} values (μg mL^{-1}) ± SD**
A. cearensis extract	5.61 ± 0.53*
Resveratrol	7.80 ± 0.72

The superscript (*) indicates a statistically significant difference between resveratrol and A. cearensis extract at $p < 0.05$, as analyzed by Student's t-test (mean ± SD, n=3)

The antifungal activity of *A. cearensis* stem extract is shown in Table III. The results show that only reference drug was active against Candida species with MIC value of 0.0312 to 2 μg mL^{-1}, whereas the antimicrobial activity of *A. cearensis* was >5,000 μg mL^{-1}, which did not demonstrate clinical relevance of the possible use of *A. cearensis* as an antifungal drug. Here, *A. cearensis* stem extract presented weak antifungal activity in accordance to (Webster et al. 2008) who classified plant extracts having MICs of more than 625 μg mL^{-1} as weak antimicrobial activity.

TABLE III. Minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *A. cearensis* extract and reference drug against *Candida* species

Sample	***Candida* species**	**MIC (μg mL^{-1})**	**MFC (μg mL^{-1})**
A. cearensis extract	*	>5,000	>5,000
Amphotericin B	*C. albicans* ATCC 10231	0.12	0.50
	C. glabrata CCT 0728	0.25	0.50
	C. krusei CCT 1517	2	2
	C. guilliermondii CCT 1890	0.031	0.031

**it was inactive for all tested Candida species*

The cytotoxicity activity of *A. cearensis* stem extract is shown in Fig. 1-2 and Table IV. The obtained results demonstrate that almost all extract concentrations displayed significant toxicity activity. The exception to reduce cell viability was the lower concentration (15.62 μg mL^{-1}) of the toxic activities against strains of murine fibroblasts (L929 – Fig. 1), human keratinocytes (HaCaT – Fig. 2), and brine shrimp.

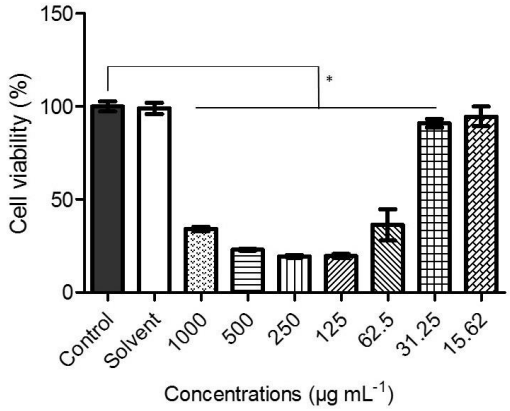

Fig. 1. *Cytotoxicity activity of A. cearensis stems extract on the cell viability using MTT assay. L929 cell were used to test it. The cell viability was determined by Formula: cell viability (%) = (A sample / A control) x 100. All tested concentrations of A. cearensis extract reduced the cell viability, except the lower one. *p<0.05 in comparison to control; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.*

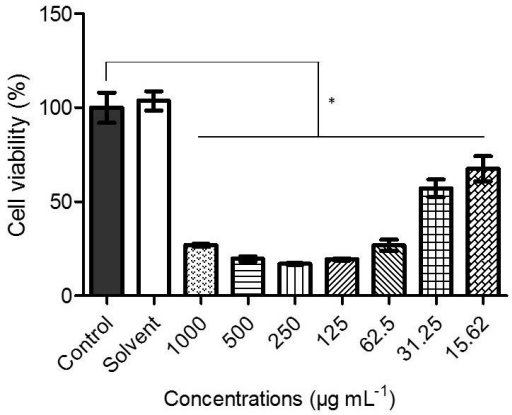

Fig. 2. *Cytotoxicity activity of A. cearensis stems extract on the cell viability using MTT assay. HaCaT cell were used to test it. The cell viability was determined by equation: cell viability (%) = (A sample / A control) x 100. All tested concentrations of A. cearensis extract reduced the cell viability. *p<0.05 in comparison to control; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.*

Costa Lotufo el al. (2003) also showed several compounds isolated from *A. cearensis* with cytotoxic effect. Compared to the thymol, *A. cearensis* also depicted cytotoxic activity (Table IV). It was already observed by Buyukleyla and Rencuzogullari (2009) in human lymphocytes. In agreement with our data, a number of works indicates that thymol showed several biological activities, such as cytotoxicity (Buyukleyla and Rencuzogullari 2009), antifungal, genotoxic (Stammati et al. 1999) and antileishmanial (de Medeiros et al. 2011) effects. Trace element plays a crucial role in the therapeutical value of a natural compound, in health and to treat disease. They play a nutritive, catalytic and balancing function in plants. Moreover, it has been showed that thymol at dose-dependent schedule decreased the nuclear division index (NDI) in human peripheral lymphocytes, and at the lower concentrations (75 and 100 µg mL^{-1}), significantly increased sister chromatid exchange induced both the chromosome aberration (CA) and frequency of micronucleus (MN) (Buyukleyla and Rencuzogullari 2009). Taken together, our data confirms that thymol is an adequate positive control and extract obtained from *A. cearensis* seeds could be cytotoxic to *Artemia salina* probably by modification the structure of DNA. However, further studies are necessary to investigate this hypothesis.

TABLE IV. LC50 VALUE OF THE *A. CEARENSIS* EXTRACT AND POSITIVE CONTROL AGAINST THE BRINE SHRIMP AFTER 24 HOURS

Sample	LC50 (µg mL^{-1})
A. cearensis extract	180.13 ± 5.45*
Thymol	28.0 ± 2.5

The superscript (*) indicates a statistically significant difference between thymol and A. cearensis extract at p < 0.05 as analyzed by Student's t-test (mean ± SD, n=5)

The antiproliferative proprieties of the *A. cearensis* extract is showed in the Fig. 3-5. The results show that almost all extract concentrations showed antiproliferative effect against MDA-MB-231 (Fig. 3), MCF7 (Fig. 4) and 4T1 (Fig. 5). The exception to reduce cell viability was the lower concentration (15.62 µg mL^{-1}) of the human adenocarcinoma mammary gland (MDA-MB-231). Leung et al. (2007), Forgo et al. (2012), and Moghaddam et al. (2012) also showed that isolated compounds from *A. cearensis* showed antiproliferative activity, thus our results extended and corroborated with these previous findings.

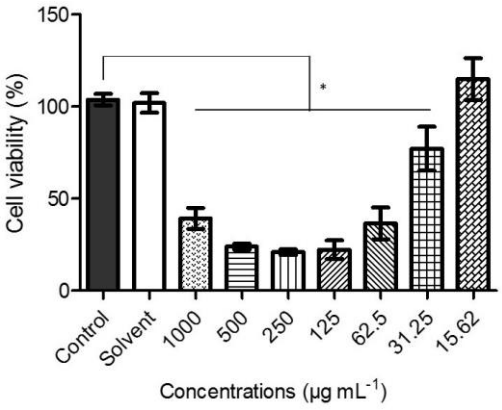

Fig. 3. *Antiproliferantive activity of A. cearensis stems extract on the cell viability using human breast adenocarcinoma (MDA-MB-231) cells lines by MTT. The cell viability was determined by equation: cell viability (%) = (A sample / A control) x 100. All tested concentrations of A. cearensis extract reduced the cell viability, except the lower one. *p<0.05 in comparison to control; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.*

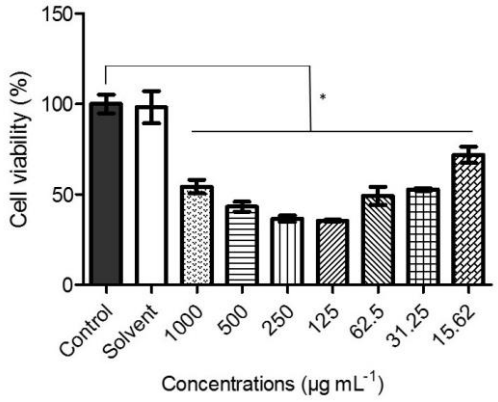

Fig. 4. *Antiproliferantive activity of A. cearensis stems extract on the cell viability using human breast adenocarcinoma (MDA-MCF7) cells lines by MTT. The cell viability was determined by equation: cell viability (%) = (A sample / A control) x 100. All tested concentrations of A. cearensis extract reduced the cell viability. *p<0.05 in comparison to control; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.*

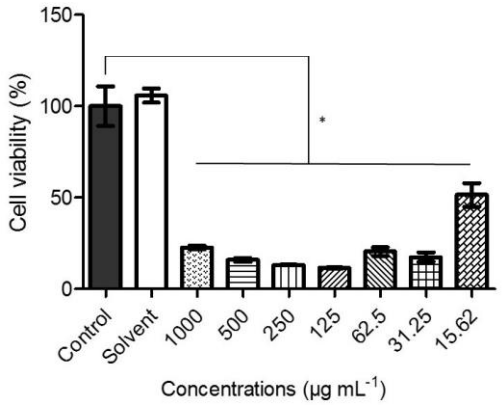

Fig. 5. *Antiproliferantive activity of A. cearensis stems extract on the cell viability using mouse tumor mammary gland (4T1) cells lines by MTT. The cell viability was determined by equation: cell viability (%) = (A sample / A control) x 100. All tested concentrations of A. cearensis extract reduced the cell viability. * p<0.05 in comparison to control; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.*

On the Table V is depicted the acetylcholinesterase inhibitory activity of *A. cearensis*, which corroborates Patel et al. (2018) and Ojo et al. (2018). Both studies point out *A. cearensis* as one of studied medicinal plants with acetylcholinesterase inhibitory activity. A recent review was published focusing medicinal plants with acetylcholinesterase inhibitory activity (Patel et al. 2018). On it, it is possible to observe that many plants exert its effect by their acetylcholinesterase inhibitory profile. Ojo et al. (2018) showed that flavonoids, as kaempferol, were found in phenolic extracts from *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill bark. The authors found antioxidant properties and inhibition of cholinergic enzymes (acetylcholinesterase and butyrylcholinesterase) in the extract. They suggested that a possible mechanism through which the stem bark performs its anti-Alzheimer's disease activity might be inhibition of the cholinesterase activities and by suppressing oxidative-stress-induced neurodegeneration (Ojo et al. 2018).

TABLE V. ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF *A. CEARENSIS* AND REFERENCE STANDARD

Sample	AChE inhibitory activity (%)	IC_{50} values (mg mL^{-1})
A. cearensis extract	83.0	0.3789
Physostigmine®	99.7	0.002

The pharmacological value of plant is due the presence of phyto-compounds which may be useful in defense against persistent disorders due to their physiological actions on the human body. For instance, sesquiterpenes and terpenoids show the antihyperalgesic, alkaloids is effective in headaches associated with hypertension, and others. Thus, Costa-Lotufo et al. (2003) demonstrated that kaempferol, isokaempferide, amburoside A and protocatechuic acid isolated from ethanol extract of trunk bark of *A. cearensis* showed particular profiles of cytotoxicity (Costa-Lotufo et al. 2003). Interestingly, while kaempferol and isokaempferide inhibited sea urchin egg development and tumor cell lines, protocatechuic acid was unique able to induce hemolysis of mouse erythrocytes. Based on the obtained results, the authors suggested that cytotoxicity activity of kaempferol and isokaempeferide was not related to membrane damage (Costa-Lotufo et al. 2003). In addition, there are evidences that flavonol aglycones (as kaempferol) seem to exhibit somewhat stronger cytotoxic activity against blood cancer cells of myeloid lineage compared to lymphocytic leukemia cell lines, whereas flavonol glycosides have no effect on the viability of different blood cancer cells (Sak 2014). Moreover, others cancers type have showed to be sensitive to kaempferol treatment. In ovarian cancer, for example, kaempferol decreased its incidence in individuals with highest quintile of kaempferol consumption compared those in lowest quintile (Li et al. 2009; Luo et al. 2012). Moreover, kaempferol seemed to exhibit higher resistance toward mutant p53 human colon cancer cell lines (Li et al. 2009). Altogether, these evidences support the idea that the compounds of *A. cearensis*, as kaempferol, have strong cytotoxicity/antiproliferative activity, which corroborates with our findings.

Kaempferol has been investigated in relation its effect in lung and leukemia cells. Leung et al. (2007) showed that kaempferol reduced cell viability dose-dependent manner with IC_{50} value of 50 µM and induced apoptosis in human lung non-small carcinoma cells. The apoptosis induced by kaempferol-generated factor (AIF) from mitochondria to nucleus and elicited DNA fragmentation and condensation in H460 cells. After 8 hours kaempferol exposure, the levels of pro-caspase 3 were decreased, and after kaempferol treatment for 24 hours, protein levels of Mn SOD and Cu/Zn SOD were increased (Leung et al. 2007). In regard to pro-myelocytic leukemia cells, they were more sensitive to kaempferol effect since FACS analysis reported that treatment of cells with 10 µM of kaempferol was enough to decrease cell growth. A

mitochondrial potential decrease followed by enhanced expression of active caspase-3 was observed 24 and 72 hours after exposing the cells with kaempferol. Authors suggested that kaempferol growth inhibitory effect on HL-60 leukemia cells was due to heterogeneous response mainly dominated by cell cycle alternation, although some degree of cytotoxicity results from both apoptotic and nonapoptotic process (Leung et al. 2007).

The isokaempferide and afrormosin were also described as components of *A. cearensis* with potential therapeutic effects. It has been described that hydroxyflavones (luteolin, apigenin and isokaempferide) showed comparable antiproliferative/antitumor activities against malignant and normal cells (Forgo et al. 2012; Moghaddam et al. 2012). It could be expected that antiproliferative activity may be useful for treating tumors; however it would exert few side effects in normal tissues. Afrormosin also exhibited key antiproliferative effects. It was demonstrated by Gyémánt et al. (2005) that multidrug-resistant cancer cells, as mouse lymphoma cells and human breast cancer cells, in the presence of afrormosin, amorphigenin, chrysin and rotenone showed a significant antiproliferative effect, which supports the idea that *A. cearensis* prove this therapeutic effect (Gyémánt et al. 2005).

IV. Conclusion and Perspectives

In summary, our study provides evidence that *A. cearensis* extract exerted significant antiproliferative, cytotoxic and acetylcholinesterase inhibitory activities, which could have future therapeutical uses. Moreover, our study provides great implications for ethnopharmacological, ethnobotanical, and clinical research, as well as strongly supports the effectiveness of the *A. cearensis* and its constituents as a new possibility of developing an effective therapy alone, or in combination with existing therapies. Additionally, further investigations are required to clarify other important benefits that could be provided from *A. cearensis*.

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