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Original Article

Biological screening of extracts from leaf and stem bark of *Croton floribundus* Spreng. (Euphorbiaceae)

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(With 1 figure)

Abstract

This work describes the preliminary evaluation of cytotoxic, antimicrobial, molluscicidal, antioxidant and anticholinesterase activities from leaf (LECF) and stem bark alcoholic extracts (BECF) of the species *Croton floribundus* Spreng. (Euphorbiaceae), popularly known as capixingui or tapixingui. BECF presented significant toxicity (LC_{50} = 89.6 µg/ml) in the *Artemia salina* Leach, 1819 (Crustacea: Branchiopoda) bioassay, whereas LECF did not show activity (LC_{50} > 1000 µg/ml). From DPPH method, the values of IC_{50} for the LECF and BECF were 61.2 µg/ml and 62.2 µg/ml, respectively, showing that *C. floribundus* has an expressive antioxidant activity. Antimicrobial susceptibility was evaluated by microdilution technique and only BECF was active against *Staphylococcus aureus* (MIC = 39.6 µg/ml). The extracts did not present molluscicidal activity against snail *Biomphalaria glabrata* Say, 1818 (Gastropoda: Planorbidae). Both extracts revealed the presence of several components with an inhibiting capacity of acetylcholinesterase enzyme on the bioautographic assay. *C. floribundus* showed to be a promising species considering that it exhibited good biological activity in the most assays performed.

Keywords: anticholinesterase activity, antimicrobial activity, antioxidant activity, capixingui, *Croton*.

Triagem biológica de extratos das folhas e caules de *Croton floribundus* Spreng. (Euphorbiaceae)

Resumo

Este trabalho descreve a avaliação preliminar das atividades citotóxica, antimicrobiana, moluscicida, antioxidante e anticolinesterásica de extratos alcoólicos das folhas (LECF) e das cascas do caule (BECF) da espécie *Croton floribundus* Spreng. (Euphorbiaceae), popularmente conhecida como capixingui ou tapixingui. No bioensaio com *Artemia salina* Leach, 1819 (Crustacea: Branchiopoda), BECF apresentou toxicidade significante (LC_{50} = 89,6 µg/ml), enquanto que LECF não apresentou atividade (LC_{50} > 1000 µg/ml). A partir do método de DPPH, os valores de IC_{50} para o LECF e BECF foram 61,2 µg/ml e 62,2 µg/ml, respectivamente, evidenciando que *C. floribundus* tem uma atividade antioxidante expressiva. A susceptibilidade antimicrobiana foi avaliada pela técnica de microdiluição e apenas BECF foi ativo contra *Staphylococcus aureus* (MIC = 39,6 mg/ml). Os extratos não apresentaram atividade moluscicida contra o caramujo *Biomphalaria glabrata* Say, 1818 (Gastropoda: Planorbidae). Ambos os extratos revelaram a presença de componentes com capacidade inibidora da enzima acetilcolinesterase no ensaio bioautográfico. *C. floribundus* mostrou ser uma espécie promissora considerando que exibiu boa atividade biológica na maioria dos ensaios testados.

Palavras-chave: atividade acetilcolinesterase, atividade antimicrobiana, atividade antioxidante, capixingui, *Croton*.

1. Introduction

Croton L., the second largest genus of family Euphorbiaceae, comprises ca. 1,300 species which are composed of shrubs and herbs distributed mainly in tropical and subtropical regions of the World of which nearly 316 species of *Croton* occur in Brazil, and 253 of them are endemic (Salatino et al., 2007). Several species of this genus also are found in Africa, Asia, and South America.

As most Euphorbiaceae, *Croton* species may contain latex, which is red-colored in some species (Dragon's blood), a characteristic which is usually associated with medicinal properties (Gupta et al., 2008; Biscaro et al., 2013; Jura-Morawiec and Tulik, 2016). This latex has been used for centuries by indigenous communities of Amazon as a medicinal plant for several maladies. It is utilized in the regions of Colombia, Ecuador and Peru to treat wounds, ulcers, herpes lesions, cuts, burns, etc. (Gupta et al., 2008; Bailon-Moscoso et al., 2015). Popular uses of *Croton* ssp. include treatment of cancer, constipation, diabetes, digestive problems, dysentery, external wounds, fever, hypercholesterolemia, hypertension, inflammation, intestinal worms, malaria, pain, ulcers and weight loss (Gupta et al., 2008).

This genus is rich in chemical constituents with biological activities, among them diterpenoids such as phorbol esters, clerodane, labdane, kaurane, grayanane, tigliane etc. (Liu et al., 2014; Wang et al., 2015; Zhang et al., 2015; Jang et al., 2016; Qiu et al., 2016). *Croton* species are also rich in active alkaloids (Queiroz et al., 2014; Cordeiro et al., 2016). Furthermore, various species of the genus are aromatic, indicating the presence of volatile oil constituents (Santos et al., 2014; Donati et al., 2015). In recent reviews on the medicinal use, chemistry and pharmacology of this species, it is evident the potential of this genus (Gupta et al., 2008; Bailon-Moscoso et al., 2015).

Croton floribundus Spreng., popularly known as capixingui or tapixingui, is a latescent bush plant with reddish colour latex. In Brazil, it is found in the remains of the Atlantic Forest, mainly in the states of Rio de Janeiro, São Paulo, Mato Grosso do Sul, Minas Gerais, and Paraná, especially in the deciduous broadleaf forest (Lorenzi, 1992). In the folk medicine, the barks of the trunk of this species are used as a tea against syphilis and hemorrhoids. The leaves are used for ulcers, like cathartic ones. The fruits are considered tonic. According Medina et al. (2009), the alcoholic extracts of leaves and barks and the isolated compound, kaurenoic acid, showed significant molluscicidal and cercaricidal activities against adult *Biomphalaria glabrata* snails and *Schistosoma mansoni* cercariae, respectively. In other work, the *ent*-kaur-16-en-6α,19-diol isolated from hexane extract of root bark showed moderate effect against three cancer cell lines: MDA-MB-435 (melanoma), HCT-8 (colorectal adenocarcinoma) and HCT-116 (colorectal adenocarcinoma) (Uchoa et al., 2013).

Considering potential evident chemistry and pharmacology of *Croton* species and due to the fact of root bark *C. floribundus* has moderate inhibitory for cells cancer; (Uchoa et al., 2013), in our continuing efforts to evaluate the therapeutical properties of medicinal plants found in Brazil, this work has as objective to evaluate the cytotoxic, antioxidant, antimicrobial, molluscicidal, and anticholinesterase activity of the leaves and stem barks of species *C. floribundus*.

2. Materials and Methods

2.1. Plant material

The leaves and stem barks of *Croton floribundus* Spreng. were collected in May 2009 from the semi-deciduous Forest (Estação Ecológica do Caiuá) of the municipality of Diamante do Norte, Paraná State, Brazil, at 270 m above sea level (S22°35'17" and W52°53'44"). The plant was identified in the herbarium of the Universidade Estadual de Maringá where a voucher specimen was deposited according to exsiccate HUEM 16285.

2.2. Extracts preparation

The leaves and stem barks were separated, dried (ca. 37 °C) and were exhaustively extracted by maceration with ethanol (300 g of plant to 1000 mL of ethanol) at room temperature. The filtrates were individually concentrated under reduced pressure to give of crude alcoholic extracts of the leaf (LECF) and stem bark (BECF).

2.3. Determination of cytotoxicity activity: brine shrimp lethality assay

Cytotoxicity evaluation of LECF and BECF extracts was carried out using the brine shrimp *Artemia salina* Leach, 1819 (Crustacea: Branchiopoda) as a model. Brine shrimp eggs were placed in seawater for 48 h before use. The eggs were placed in a two-compartment tank. One side was covered to keep the eggs in the dark while the other was illuminated to attract shrimps through perforations on the boundary plate. After 24 h, the phototropic brine shrimps (nauplii), which went to the illuminated compartment, were collected by pipette and incubated under illumination for 24 h at room temperature (Meyer et al., 1982). Shrimps were added in groups of ten nauplii in four vials with final seawater volume of 5 ml per tested concentration. The preliminary bioassay was carried out with 1000, 100, and 10 µg/ml after testing intermediate dosages. In order to verify the *A. salina* susceptibility, controls used only seawater. Potassium dichromate was used as a positive control in the bioassay. Each assay was performed in quadruplicate. The results were expressed as LC_{50} value (concentration able to kill 50% or more of the nauplii). LC_{50} was found by linear regression (square equation) using a significance level of 5% ($p < 0.05$) and reliability index of 95%. For this, the Probitos Statistical Methods/Microcal Origin 6.0 software was used. When found value of $LC_{50} < 1000$ µg/ml the assayed product was regarded as a toxic bioactive compound; on the other hand, when found value of $LC_{50} \geq 1000$ µg/ml the assayed compound was regarded as non-toxic.

2.4. Determination of antioxidant activity: DPPH method

Scavenging activities of the extracts on the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were assayed using a modified Blois method (Molyneux, 2004) by which the bleaching rate of DPPH is monitored at a characteristic wavelength in the presence of the sample. A volume of 0.1 ml of different concentrations of extracts from 1000 to 6.25 µg/ml, with 2.9 ml of a 60 µM DPPH (Sigma-Aldrich) solution in ethanol. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using Femto Spectrophotometer (700 plus model). Quercetin and butylated hydroxyanisole (BHA) solutions of different concentrations were used as positive controls for antioxidant activity while methanol with DPPH solution (60 µM) was the negative control. The percentage decrease of DPPH was calculated applying the following Equation 1:

$$\%\ of\ \ inhibition = \left[1 - \left(A_s / A_0\right)\right] \times 100 \qquad (1)$$

Where A_s is the absorbance of the sample and $\mathbf{A_0}$ is the absorbance of the DPPH solution. The IC_{50} values denoted the concentration of each sample required to give 50% of the optical density shown by the control. The IC_{50} values were calculated from data obtained graphically. The results were expressed as the mean of three determinations made by duplicate.

2.5. Determination of molluscicidal activity

Molluscicidal evaluation of the *C. floribundus* extracts was performed according to Bulletin World Health Organization guidelines (WHO, 1983) and adaptations (Silva et al., 2008) by a rapid screening procedure. The ethanolic extracts were dissolved in 100 µl DMSO at the concentration of 200, 100, 50 e 25 µg/ml and then added to glass beakers containing 100 ml of water from the aquaria. Two adult *Biomphalaria glabrata* Say, 1818 (Gastropoda: Planorbidae) snails were placed in each container and maintained in a well-aerated place at room temperature. After 24 h the snails were placed on a Petri disc and their heartbeats were checked using a stereomicroscope. To confirm mortality the snails were transferred to vessels containing only deionized water and their condition was re-evaluated 24 h later. Control experiments were performed with deionized and dechlorinated water alone (negative control) or with niclosamide (0.5 µg/ml; Atenase®, Uci-farma) (positive control). Molluscicidal test with each plant extract dose was separately repeated five times.

2.6. Determination of antimicrobial activity

2.6.1. Microorganisms used and growth conditions

The test microorganisms included the bacteria *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027 and the yeast *Candida albicans* ATCC 10231. The bacteria were grown in nutrient broth (Difco Laboratories) at 37 °C and maintained on nutrient agar slants at 4 °C. The yeast was grown and maintained on Sabouraud-dextrose agar (Merck).

2.6.2. Antimicrobial susceptibility testing

The minimal inhibitory concentrations (MIC) of all extracts were determined by microdilution techniques in Müeller-Hinton broth for bacteria (NCCLS, 2004) and Sabouraud broth for yeast (NCCLS, 1997). Each extract (10 mg/ml) was aseptically mixed with inoculums prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard [10^8 colony-forming units (CFU)/ml for bacteria and 106 CFU/ml for yeasts], and diluted 1:10 for the broth microdilution procedure. Microtiter plates were incubated at 37 °C and the MICs were recorded after 24 h of incubation. Two susceptibility endpoints were recorded for each isolated. The MIC was defined as the lowest concentration of compounds that the microorganism tested did not demonstrate visible growth compared with control. The antimicrobial activity of samples and reference antibiotics was established in accordance with the MIC values: MIC > 500 µg/ml (inactive), MIC = 250 and 125 µg/ml (moderately active), MIC = 62.5 and 31.2 µg/ml (active), MIC = 15.6 µg/ml (more active), MIC = 7.8 µg/ml (highly active) (Tona et al., 1999).

2.7. Determination of anticholinesterase activity: bioautographic method

Anticholinesterase activity was measured using a bioautographic assay adapted (Silveira et al., 2011) from Yang et al. (2009). Acetylcholinesterase (AChE - EC 3.1.1.7, Sigma-Aldrich) was dissolved in 0.05 M Tris-hydrochloric acid buffer at pH 7.8 with 1 mg/ml bovine serum albumin (BSA, 98%, Sigma-Aldrich). The stock solution was kept at 4 °C. LECF and BECF extracts were weighed, and made up into two stock solutions of 20 mg/ml with methanol. Then different volumes of LECF and BECF stock solutions (equivalent to 600, 400, 200, 150, 100, 50 and 25 µg dry mass) were loaded on two TLC F_{254} plates (10 × 10 cm, 0.2 mm thickness; Merck) and eluted in dichloromethane: methanol (9:1 v/v) for separation of compounds. The dried TLC plates were sprayed with AChE enzyme solution (1U/ml) and incubated at 37 °C for 20 minutes. For detection of the enzymes inhibitors, solutions of 1-naphthyl acetate (150 mg; Sigma-Aldrich) in ethanol 40% solution (100 ml) and Fast Blue B salt (50 mg; 95%, Sigma-Aldrich) in MilliQ® water (100 ml) were prepared immediately before use. After incubation of TLC plates, the 1-naphthyl acetate and Fast Blue B salt solutions were sprayed on the plates to give a purple coloration after 1-2 minutes. The inhibition of AChE was observed from the white spots on the purple colored dye background of the TLC plates.

2.8. Phytochemical screening

Phytochemical screening for major constituents (alkaloids, saponins, tannins, anthraquinones, coumarins, flavonoids, and cardiac glycosides) was undertaken using standard qualitative methods (Harborne, 1998). The test for tannins was carried out by subjecting 0.3 g of each extract in

6 ml of distilled water, filtered and ferric chloride reagents added to the filtrate. For cardiac glycosides, Keller Kiliani test was adopted (0.5 g of extract was added to 2 ml acetic anhydrate plus H_2SO_4). The test for alkaloids was carried out by subjecting 0.5 g extract in 5 ml 1% HCl, boiled, filtered and Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The extract was subjected to frothing test for the identification of saponin. The extract was also tested for free glycoside bound anthraquinones (Borntraeger's method): 0.5 g of extract was added to 10 ml benzene, filtered and ammonia solution added. The presence of flavonoids was determined using the Shinoda reaction: 1% aluminum chloride solution in methanol concentrated HCl, magnesium turnins, and potassium hydroxide solution.

3. Results and Discussion

The cytotoxic activity of LECF and BECF were verified preliminarily by the lethality assay on brine shrimp *A. salina*. This bioassay allows the evaluation preliminary of general toxicity of extracts and compounds. According to Meyer et al. (1982), this bioassay has shown good correlation with cytotoxicity on 9Kb and 9PS cells (leukemia), corroborating its usefulness as a tool for the preliminary determination of antitumor activity. Some studies have sought to correlate *A. salina* toxicity with other biological activities like antimicrobial, parasiticide, virucide, and molluscicidal activities (Arcanjo et al., 2012).

Based on the classification of toxicity (Meyer et al., 1982) in the present study, only BECF presented significant toxicity (LC_{50} = 89.6 µg/ml) whereas the LECF did not show relevant activity (LC_{50} > 1000 µg/ml). As significant toxicity was observed for BECF, it should be considered as an interesting characteristic to utilize this vegetal extract in further studies involving cytotoxicity.

This is the first report of activity against *A. salina* for *C. floribundus*, however, the same activity has already been described for essential oils of other *Croton* species: fresh leaves of *C. hirtus* (Lima et al., 2012), the bark of *C. niveus* and *C. monteverdensis* (Werka et al., 2007). Moreover, several studies have reported a series of *Croton* species and their secondary metabolites with cytotoxicity on cancer cell lines (Liu et al., 2014; Wang et al., 2015; Zhang et al., 2015; Qiu et al., 2016), including *C. floribundus* (Uchoa et al., 2013), here it was observed that a compound (*ent*-kaur-16-en-6α,19-diol) isolated from hexane extract of root bark showed moderate effect against three cancer cell lines: MDA-MB-435 (melanoma), HCT-8 (colorectal adenocarcinoma) and HCT-116 (colorectal adenocarcinoma). Thus, ours results for *C. floribundus* corroborate with those observed by Uchoa et al. (2013) and also contribute with others reported in the literature for this genus.

Several methods are used in order to determine antioxidant activity of extracts and isolated compounds. One of the most used *in vitro* method consists of the evaluation of the scavenging activity of DPPH free radical (Molyneux, 2004). This method is based on the electron transfer from one antioxidant compound to a DPPH free radical which loses the purple color when reduced, a process monitored by a spectrophotometer (Molyneux, 2004).

In the Figure 1 was analyzed the scavenging activity of LECF and BECF extracts on DPPH free radical when compared with the standard substances (quercetin and BHA) at varying concentrations.

Analyzing the obtained results, it was observed that the evaluated extracts presented good capacity to scavenge DPPH free radical, once the necessary concentration to inhibit half of the free radicals in solution (IC_{50}) was lower than 100 µg/ml. The values of IC_{50} for LECF and BECF were 61.3 µg/ml and 62.2 µg/ml, respectively, demonstrating that *C. floribundus* presents compounds with a significant antioxidant activity. The quercetin and BHA standards presented IC_{50} = 1.1 µg/ml and IC_{50} = 1.9 µg/ml, respectively.

Considering the lack of studies concerning the chemical composition of *C. floribundus*, tests for phytochemical characterization of tannins, anthraquinones, cardiotonics, alkaloids, coumarins, saponins, and flavonoids were done. According to the tests carried out, there was only the indication of the presence of flavonoids and hydrolysable tannins. The presence of coumarins, anthraquinones, saponins, alkaloids and cardiotonics were not observed. According to our results, because the tests were positive for tannins and flavonoids, the observed antioxidant properties may be attributed to the presence of phenolic compounds (Takao et al., 2015).

This is the first report of the antioxidant activity of *C. floribundus*. Other species of *Croton* have been related to possessing an antioxidant activity (Aderogba et al., 2011; Donati et al., 2015; Shahwar et al., 2015). The popular use of these *Croton* species is enhanced by the verification of its antioxidant action (Morais et al., 2006).

The antimicrobial activity of *C. floribundus* extracts was also the target of our survey. LECF and BECF

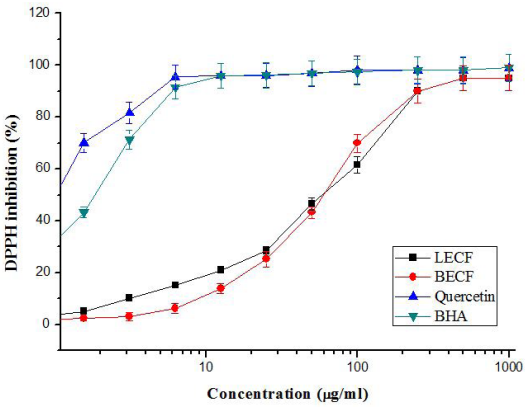

Figure 1. DPPH radical scavenging activity of crude alcoholic extracts of leaves (LECF) and stem barks (BECF) of *C. floribundus* and standard antioxidant compounds: quercetin and BHA.

extracts were evaluated using microorganisms of medical interest, Gram-positive bacterium *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, and the fungus of *Candida albicans*, through the microdilution techniques for determining of minimum inhibitory concentration (MIC). The evaluation results of the antimicrobial activity of *C. floribundus* are found in Table 1.

Although only the crude stem bark extract of *C. floribundus* was active against Gram-positive bacteria *S. aureus*, it was observed only at high concentration (MIC = 39.6 µg/ml). On the other hand, Gram-negative bacteria *E. coli* and *P. aeruginosa* were resistant to all tested concentrations. The walls of Gram-negative and Gram-positive bacteria present distinct differences. The wall of Gram-negative bacteria is composed of several layers, and the periplasmatic gap possesses enzymes that avoid the entrance of strange substances, explaining why Gram-negative bacteria are usually more resistant than Gram-positive ones (Madigan et al., 2014). Therefore, the resistance observed in this study may eventually be related to the structural particularities of cell walls of Gram-negative bacteria, making difficult the penetration of secondary metabolites presents in the *C. floribundus* extract. Although *S. aureus* is susceptible to the action of several active drugs against Gram-positive bacteria, it is also known for its high capacity to develop resistance against several antibiotics. Taking this into considerations, it is important to find new active principles to avoid resistance development.

Based on the historical use of Euphorbiaceae as molluscicidal agent (Al-Zanbagi et al., 2000), it is relevant to investigate this activity for plants of this family. The concern with problems caused by synthetic molluscicide and the search for easily biodegradable compounds that are also inexpensive and locally available has increased the interest for the use of vegetal molluscicide, once some of them have promising activity similar to the synthetics (WHO, 1983). For the extracts to be considered potentially active, they should have activity in concentrations lower than 100 µg/ml (WHO, 1983).

In the present study, a preliminary study of this activity was done with alcoholic extracts of *C. floribundus* leaf and stem bark. The molluscicidal activity was not observed for both extracts tested on *Biomphalaria glabrata* snails in the evaluated concentrations (200, 100 and 50 µg/ml). Medina et al. (2009) reported significant molluscicidal and cercaricidal activities against adult *Biomphalaria glabrata* snails and *Schistosoma mansoni* cercariae for alcoholic extracts of leaves and barks of *C. floribundus* and the isolated compound, kaurenoic acid. The lack of activity observed in our study can be related to the non-production of active metabolites in the studied plant, since the production of secondary metabolites in plants can be influenced by several factors like seasonality, type of soil, weather, rainfall, radiation or any other condition (Al-Zanbagi et al., 2000). Medina et al. (2009) prepared their extracts from especies collected in winter (August) and this work from species collected in the autumn (May) and in locations with different soil characteristics.

Presently, there is a relatively intense effort to find new inhibitors in plant extracts, with a special interest in the discovery and isolation of new inhibitors of acetylcholinesterase enzyme (AChE) (Murray et al., 2013). AChE inhibitors are utilized in the treatment of Alzheimer's disease, a neurodegenerative disorder of the brain, responsible for approximately half of the cases of dementia in sixty-year-olds (Murray et al., 2013). Thus, the increase of acetylcholine produces improvements in cognitive function, one of the signs of the disease (Murray et al., 2013).

Two species of genus *Croton*, *C. heliotropiifolius* (Queiroz et al., 2014) and *C. sparsiflorus* (Shahwar et al., 2015) presented anticholinesterase activity, which led us to evaluate of this activity in *C. floribundus*.

The TLC bioautography-guided strategy was used to separate the anticholinesterase compounds from plant extracts. Further TLC bioautographic analysis showed five active spots for BECF extract and four active spots for LECF extract with different R_f values, as presented in Table 2.

The results showed that BECF presented a higher capacity AChE inhibitory than LECF not only by the amount of active spots (A1-E1), but also because all the components are active in low amount of extract (50 µg), of which two (C1 and E1) were active in the least amount of extract tested (25 µg). The LECF extract showed only one spot (C2) with strong activity, and the other far less active (Table 2). Considering that this is not a quantitative analysis since the concentration of the extracts tested does not reflect the concentration of the active components. Thus, it can be expected that these components if isolated,

Table 1. Antimicrobial activity of crude alcoholic extracts of leaves (LECF) and stem barks (BECF) of *C. floribundus* represented as MIC.

Microorganism	BECF (µg/ml)	LECF (µg/ml)	Levofloxacin (µg/ml)
Escherichia coli ATCC 8739	> 10,000	> 10,000	0.01
Pseudomonas aeruginosa ATCC 9027	> 10,000	> 10,000	0.04
*Staphylococcus aureus*ATCC 6538	39.6	> 10,000	0.11
Candida albicans ATCC 10231	> 10,000	> 10,000	0.24

Table 2. Inhibition of acetylcholinesterase enzyme in the presence of different concentrations (dry mass) of crude alcoholic extracts of leaves (LECF) and stem barks (BECF) of *C. floribundus* by HPTLC bioautographic analyses.

Observed spot	R_f spot	BECF (drymass)						
		600 µg	400 µg	200 µg	150 µg	100 µg	50 µg	25 µg
A1	0.85	+++	+++	+++	+++	++	+	-
B1	0.78	+++	+++	+++	+++	++	+	-
C1	0.41	+++	+++	+++	+++	+++	++	+
D1	0.27	+++	+++	+++	+++	++	+	-
E1	0.11	+++	+++	+++	+++	++	++	+
		LECF (drymass)						
A2	0.85	+++	++	++	+	-	-	-
B2	0.78	++	++	+	-	-	-	-
C2	0.64	+++	+++	+++	+++	++	++	+
D2	0.0	+++	+++	++	++	+	-	-

Legend: (-) no activity; (+) low; (++) moderate; (+++) high activity.

may be active at concentrations well below those tested in this work.

Finally, TLC-bioautography can not only be used for screening of the components with anticholinesterase inhibitor potential but also for the purpose of quality evaluation of interest extracts at the same time. The studied extracts showed significant positive results at the bioautographic tests, revealing a promising source of natural agents with pharmacological potential.

4. Conclusions

The alcoholic extracts of stem bark and leaf of *Croton floribundus* Spreng. showed biological potential observed at different assays performed. The stem bark alcoholic extract of *C. floribundus* showed significant antioxidant, anticholinesterase, cytotoxic and antimicrobial activities. Whereas, the leaf alcoholic extract presented considerable antioxidant and anticholinesterase activities. All these activities have already been observed in other species of genus *Croton* L.; therefore, the present study corroborates information found in the literature for this genus. However, all these observed activities for this native species are being reported for the first time.

Preventive and symptomatic treatment of Alzheimer's disease requires multitarget drug strategy. Therefore, it is suitable to explore a crude extract having both antioxidant and anticholinesterase activities. The obtained results could, therefore, form a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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