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Bioactive Extracts of *Capsicum chinense* in the Northern Amazon

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The objective of this work was to analyze the bioactive potential of hexane and methanol extracts of fruits of *Capsicum* spp., a kind of pepper collected in Roraima, Brazil, against the yeast *Candida albicans*, as well as antioxidant activity and antiacetylcholinesterase inhibition. The antioxidant activity of the methanol extract of the fruits was 82%. The antiacetylcholinesterase activity was high in the hexane extract reaching 71%. Hexane extract of the fruits did not present inhibition for *Candida albicans*. The present study demonstrated the efficacy of *Capsicum* spp. as a natural antioxidant and the presence of active chemical constituents that can aid in the treatment of neurodegenerative diseases such as Alzheimer's. However, further studies are still needed to consolidate its use and application in the pharmaceutical field.

1. Introduction

Peppers of *Capsicum* genus are important ingredients used as food and for seasoning because of their typical color, pungency, distinct taste and aroma (Junior et al., 2015). Epidemiological studies demonstrate the benefits of spicy food in reducing mortality and improving quality of health (LV et al., 2015). There are many nutritional benefits associated with consumption of peppers, such as anti-inflammatory, analgesic, glycemic regulation and antioxidant activities. Functional properties are often attributed peppers mainly because they are sources of carotenoids, vitamin C, vitamin E, alkaloids, flavonoids and capsaicinoids, which are their predominant phenolic constituents (Bogusz Jr et al., 2018).

Pepper cultivars are generally found in tropical and subtropical regions. They require humid climate and can be grown in a variety of soils. Besides, peppers have the reputation of having medicinal properties. Today, although synthetic drugs are readily available in most countries and highly effective in curing various diseases, there is still people who opt for traditional folk medicines because they usually have fewer harmful side effects (Reddy et al., 2011). There is a great diversity of compounds, especially secondary metabolites found and isolated from plants. More recently, a number of biological properties and potential health benefits of consuming peppers have been reinforced, as for antioxidant property (Sricharoen et al., 2017). Some studies have shown beneficial therapeutic effects of compounds from peppers as anticancer and antibacterial agents (Junior et al., 2015).

In Roraima, one of the first studies on the chemical composition of fruits of *Capsicum* genus was conducted by Marangon et al. (2014), where they described the characterization of minerals in the fruits of two species of this genus. The following year, Borges et al. (2015) conducted another research with six varieties of this group, being identified some morpho-anatomical and physico-chemical properties of the fruits, as well as the fatty acids contents. Recently Morais et al. (2018) reported the antibacterial extract of a species of *Capsicum* spp. against bacteria pathogenic to humans.

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Due to the abundance of various types of peppers in Roraima state that are part of the natives diet, the objective of this work was to carry out *in vitro* biological assay with the species *Capsicum chinense*. This plant has been identified at the species level for the present work as it was previously referred as *Capsicum* sp. (Morais et al., 2018). In this context, the objective of this work was to analyze the antifungal and antioxidant activities and acetylcholinesterase inhibition of *C. chinense* hexane extracts.

2. Material and methods

The experiment was conducted at the Federal University of Roraima - UFRR, which a pepper species of the genus *C. chinense* purchased at the rural producer's fair in the city of Boa Vista (RR, Brazil) and then planted in a greenhouse for botanical identification species. After full development, the species was identified and exsiccates were deposited at the UFRR herbarium under the number UFRR / 8989. Figure 1 shows the macroscopic aspect of *C. chinense* fruits utilized in the present work.

Figure 1: Macroscopic aspect of Capsicum chinense fruits.

For the preparation of the hexane extracts, the chilies were previously sanitized and weighed to obtain the initial mass, after which the fruits were subjected to forced ventilation at 65 ºC for 72 h until obtaining constant biomass for stabilization of the compounds and fruit picking. moisture. The extracts of the nuts were then prepared by continuous extraction in Soxhlet apparatus at the Laboratory of Environmental Chemistry of the Federal University of Roraima-UFRR. Material 33g was subjected to continuous extraction for six hours using 500 ml of hexane as the extractive solvent. The extraction was done in triplicate and after the extractions were finished, the diluted extracts were subjected to a rotaevaporation process to remove the solvent. After the concentration, the extracts were transferred to labeled and weighed glass vials. This procedure was performed according to the methodology described by Silva and Queiroz (2002).

2.1 Microorganism

The microorganism tested was the fungus *Candida albicans* (ATCC 10231) and the antifungal assay was carried out at the Laboratory of Biotechnology and Bioassays Laboratory of the Departament of Chemistry of the Federal University of Minas Gerais (Brazil).

2.2 Bioassays of anti-*Candida albicans* activity

The analyzes for evaluation of the antifungal activity were carried out at the Biotechnology and Bioassays Laboratory of the Federal University of Minas Gerais - UFMG, following the Minimal Inhibitory Concentration (MIC) method described below. The concentrations tested were 250, 125, 62.5, 31.25, 15.6 and 3.9 µg mL^{-1} (Zacchino and Gupta, 2007). Nystatin (standard antifungico) was used as a control in the concentration of 12.5 mg mL^{-1}. Samples were weighed and dissolved in DMSO at 50 mg mL^{-1}. A volume (40 µL) of this solution was added to a flask containing 960 µL BHI (Brain Heart Infusion) Broth to prepare the working solution. A pre-inoculum was prepared in which *C. albicans*, stored under refrigeration, was transferred with a platinum loop to test tubes containing 3 mL of freshly prepared BHI broth. The tubes were incubated at 37 °C for 18 h. Then the pre-inoculum (500 µL) was transferred to tubes containing 4.5 mL of sterile distilled water. The tubes were homogenized and the concentration was adjusted to 0.5 to reach McFarland turbidity standard (10^8 CFU mL^{-1}), thereby obtaining the inoculum used in the bioassays. The assays were performed in duplicate 96-well

plates. BHI broth (100 µL) was added to each well. In the first well, 100 µL of working solution was also added. The resulting solution was homogenized and 100 µL of the mixture was transferred to the next well and so on until the last well, from which 100 µL of the material were discarded. Then, 100 µL of microorganism inoculum was added to the wells. Six different concentrations of the sample were tested. A positive control lacking of the working solution allowed examining the positive growth of the microorganism. A negative control, lacking the inoculum, was performed to discount the color present in the extracts present in the working solution. A control plate containing 100 µL of BHI culture medium and 100 µL of sterile distilled water was added to the experiment to control BHI broth sterility. Growth of the microorganism was measured on an ELISA plate reader (492 nm) immediately after the start of the experiment (0 h). They were incubated at 37 ^{0}C and read again after 24 h of incubation, terminating the test.

2.3 Inhibition of acetylcholinesterase (AChE)

Aliquots of a working solution (25 µL) (DMSO sample 10 mg mL^{-1}) were added to the wells of the microplate and positive and negative controls were also prepared. To the first five wells of a column (positive control) were added 25 µL of a solution of eserin and galantamine prepared at 10 mg mL^{-1} (31 mM, 2.7 mM in the entire reaction mixture 275 µL) in Tris / HCl at pH 8.0). Then, 25 µL of 15 mM acetylthiocholine iodide (ATChI, Sigma A5751); (DTNB, Sigma D8130) (3 mM) and 50 µL of Tris / HCl (50 mM, pH 8) containing 0.1% (m / v) bovine serum albumin was added to each well. The absorbance was measured at 405 nm every 1 min for 8 times. Then, 25 µl (0.226 U mL^{-1}) of eel AChE (type VI-S) provided by Sigma (C3389-500UN) in Tris / HCl was added to each well. The absorbance was measured at 405 nm for 10-fold (Dominguete & Takahashi, 2018).

2.4 Determination of total phenolic compounds

Methanol extracts (4.0 g) prepared utilizing previously lyophilized peppers was mixed with 35 mL of methanol 80% (v / v) acidified with 0.5% (v / v) of hydrochloric acid in falcon tubes and, afterwards, this mixture was placed in a boiling water bath for 30 minutes. The supernatant was separated and remaining material was and treated again with the same conditions as above. The methanol fractions were then collected and centrifuged at 6000 rpm for 30 minutes. The resulting material was placed in amber glass vials and stored in the refrigerator at 2 °C until analysis. According to Singleton et al. (1999), to make the readings, gallic acid (GA) was used as the reference standard, using the Shimadzu UV-1800 spectrophotometer. The method involves the reduction of the Folin Ciocalteau reagent when phenolic compounds are present in the sample with formation of a complex with blue color. An aliquot of the methanol extracts (0.1 mL) was transferred to a 10 mL test tube and 3 mL of distilled water was added followed by 0.25 mL Folin Ciocalteau reagent. The mixture was allowed to stand for 3 minutes and finally 2 mL of a sodium carbonate 7.5% (w / v) solution was added. A blank test was also used under the same conditions, in which 0.1 mL of distilled water was used instead of the samples. They were incubated in a water bath at 37 °C for half an hour and the readings were made in a spectrophotometer at 765 nm. Quantification of total phenols in the extracts was expressed as mg GA /100 g sample.

2.5 Determination of antioxidant activity

Determination of the antioxidant activity in the different extracts was evaluated by an absorption method that monitor the extinction of the 1,1-diphenyl-2-picryl hydrazyl (DDPH) radical. The DDPH method was developed using visible ultraviolet molecular absorption spectrophotometry measured at 515 nm (Miranda and Fraga, 2006) on the Shimadzu model UV-1800 Spectrometer. The technique consists in preparing a 300 µL incubation of the methanolic extract with 2.7 mL of the 0.06 mM DPPH solution, leaving for 60 minutes in incubation and obscuring to be read later at 515 nm. The calibration curve was made by preparing diluted standards from the 60 mM stock concentration in the range of 10-50 mM and, at the same time, a blank was made with methanol.

3. Results and Discussion

3.1 Evaluation of anti-*Candida* potential

Table 1 shows the inhibition results of the hexanic extract of *Capsicum chinese* fruit against *C. albicans* and having positive control of Nystatin.

Santos (2010) in his experiments with extracts of *Capsicum annuum* also identified low biological activity against the fungus *Candida albicans*. However, the antifungal capacity of C. chinense species has been reported for aqueous extracts of these peppers with significant inhibition rates against phytopathogenic fungi, such as *Rhizoctonia solani* (Matos et al., 2011). The control of all these challenging phytopathogens growth shows that this extract has bioactive compounds that can control phytopathogenic filamentous fungi.

Table1: The biological activity of C. chinense hexane extract against the pathogenic microorganism C. albicans.

C. chinense extract Concentration (µg mL^{-1})	*C. albicans* (%)	Nystatin (%)
250	21.31	98.89
125	8.06	94.92
62.5	2.02	86.95
31.85	-	81.44
15.625	-	57.24
3.90625	-	-

Subtitle: (-) there was no inhibition.

According to Oliveira (2011), the aqueous extract of finger pepper inhibited *Cladosporium cladosporioides* in 49.44% of its growth, being considered as an alternative to the substitution of chemical pesticides.

Pereira (2015), when investigating antimicrobial peptides in ethanolic extracts of *C. annuum* L., observed the inhibition in the growth of phytopathogenic fungi *C. lindemuthianum*, *C. gloeosporioides* that cause the anthracnose and of the bacterium *Xanthomonas euvesicatoria* that causes the bacterial spot in plants .

3.2 Acetylcholinesterase inhibitory activity

The hexanic extract of *C. chinense* inhibited the enzyme AChE at the concentration of 10 mg mL^{-1} in 71.00%, while the standard inhibitors, such as eserin and galantamine, inhibited 91.93% and 94.36%, respectively, in the concentration of the extract. Vinutha et al. (2007) classify AChE inhibition as potent when a sample exhibits more than 50% inhibition, whereas AChE inhibitors are considered moderate (30-50% inhibition) or weak (<30% inhibition) according to degree of activity. Therefore, according to the classification the hexane extract of *C. chinense* can be considered a potent inhibitor of AChE. Moreover, according to Trevisan and Macedo (2003), extracts with inhibition greater than or equal to 50% may be candidates for fractionation and isolation of bioactive metabolites.

3.3 Determination of antioxidant activity by DPPH

In order to evaluate the ability of *C. chinense* extract methanol constituents to capture free radicals, the behavior of solutions of this extract in the presence of DPPH solution was analyzed. The results were expressed as percentage of oxidation inhibition, that is, the percentage of antioxidant activity corresponding to the amount of DPPH consumed by the antioxidant. According to Alves et al. (2007), the consumption of DPPH by the sample is directly proportional to its antioxidant activity. From the result obtained with the methanol extract of *C. chinense*, the linear regression analysis was determined, with the equation of the line y = 0.0099x + 0.0077 with the correlation coefficient *r* equal to 0.9966. The concentration may range from 0 to 60 mg L^{-1}. The result obtained in the determination of the antioxidant activity of *C. chinense* extract was 81.97% of antioxidant activity. According to the DPPH calibration curve, the corresponding concentration is between 10-20 mg L^{-1}, that is a high antioxidant capacity. Sricharoen et al. (2017) in their experiment with several species of peppers also proved a high level of antioxidant potential among the studied accessions of this plant.

3.4 Determination of total phenolic compounds

The phenol content is used extensively in the determination of various extracts and evaluated by the Folin-Ciocalteu reagent. From the reaction between gallic acid and Folin-Ciocalteu reagent (Da Silva et al., 2013) the linear regression analysis was determined with the line equation y = 0.00193x + 0.0459 with correlation coefficient *r* equal to 0.9943. The concentration range was 2 to 16 mg L^{-1}. The total phenol content of the extract methanol was expressed in mg equivalents of gallic acid per 100 g of the extract (mg EAG 100 g^{-1}). The total phenol content found in the crude methanolic extract was 241.89 mg EAG 100 g-1. For the interpretation of the result Rufino et al. (2006) report that values up to 100 mg EAG 100 g^{-1} show low concentration of total phenols, while values between 100-500 mg EAG 100 g^{-1} indicate mean concentration of phenols. Values higher than 500 mg EAG 100 g^{-1} indicate, therefore, a high concentration of total phenols. Thus, *C. chinense* can be considered as an average source of total phenol compounds, since the result was greater at 100 mg EAG 100 g^{-1}. Farhoudi et al. (2017) corroborates that phenolic compounds have attracted the interest of researchers because they present antioxidant activity and can protect the human body from free radicals, and play a role in the prevention of some diseases, including cancer, cardiovascular diseases and neurodegenerative diseases disorders.

4. Conclusions

The hexanic extract of this species showed low inhibition for the fungus C. albicans (21.31%), in the concentration of 250 µg mL-1. The study of inhibition of AChE was considered potent (71.00%), which corroborates for a greater phytochemical investigation in the plant.
For the phenolic compounds the crude methanolic extract of C. chinense presented 241.89 mg EAG 100, whose result represents a mean potential of total phenol content. In the antioxidant activity of C. chinense, the result obtained was 81.97% and shows a positive correlation with the antioxidant activity. This potential may have positive effects on various diseases derived from oxidative stress, such as cancer, for example.
The research demonstrated the effectiveness of Capsicum chinense as a natural antioxidant and the presence of active substances whose bioprospecting may result in molecules capable of interfering positively with diseases that compromise the human nervous system. However, further studies are still needed to consolidate its use and application in the pharmaceutical field.

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References

Alves C.Q., Brandão H.N., David J.M., David J.P., Lima L.S., 2007, Evaluation of the antioxidant activity of flavonoids, Diário Ciênc. 5, 7-8.

Bogusz Jr, S., Libardi, S. H., Dias, F. F., Coutinho, J. P., Bochi, V. C., Rodrigues, D., Godoy, H. T., 2018, Brazilian *Capsicum* peppers: capsaicinoid content and antioxidant activity. Journal of the Science of Food and Agriculture, *98*(1), 217-224.

Borges K.M., Vilarinho L.B.O., Melo Filho A.A., Morais B.S., Rodrigues R.N.S., 2015, Morpho-agronomic and physicochemical characterisation of the pepper for the State of Roraima, Agro@mbiente On-Line. 9, 292-299.

Da Silva E.C.C., Muniz M.P., Nunomura R.C.S., Nunomura S.M., Zilse G.A.C., 2013, Phenolic constituents and antioxidant activity of geopropolis from two species of stingless Amazonian bees, Quim. Nova 36, 628-633.

Dominguete, L. C. B. ; Takahashi, J.A., 2018, Filamentous Fungi as Source of Biotechnologically Useful Metabolites and Natural Supplements for Neurodegenerative Diseases Treatment. Chemical Engineering Transactions, v. 64, p. 295-300.

Farhoudi, R., Mehrnia, M. A., & Lee, D. J., 2017, Antioxidant activities and bioactive compounds of five Jalopeno peppers (*Capsicum annuum*) cultivars. Natural Product Research, 1-4.

Junior, S. B., Março, P. H., Valderrama, P., Damasceno, F. C., Aranda, M. S., Zini, C. A., Godoy, H. T., 2015, Analysis of volatile compounds in *Capsicum* spp. by headspace solid-phase microextraction and GC× GC-TOFMS. *Analytical Methods*, *7*(2), 521-529.

Lv, J., Qi, L., Yu, C., Yang, L., Guo, Y., Chen, Y., Tang, Z., 2015, Consumption of spicy foods and total and cause specific mortality: population based cohort study. BMJ (Clinical research ed.) v. 351, p. h3942-h3942.

Marangon C., Rizzatti I.M., Costa H.N.R., 2014, Determination of macro and micronutrients in four pepper species Capsicum ssp. cultivated in Roraima. In: I North Chemistry Meeting, SBQNorte 2018. <http://files.sbqnorte.webnode.com/2000000215ab995bb38/SBQNORTE_AMB02.pdf>

Matos, S., Vieira Junior, J. R., Fernandes, C. D. F., Almeida, U., Santana, L., Minosso, C.,Lima, R., 2017, (March). Pepper extracts of the genus Capsicum on the inhibition of mycelial growth of Rhizoctonia solani Kuhn, fungus that causes mela disease in common bean. In: BRAZILIAN PHYTOPATHOLOGY CONGRESS, 44., 2011, Bento Gonçalves. Anais... Tropical Plant Pathology, Brasília, DF, v. 36, 2011.

Miranda A.L.P., Fraga C.A.M., 2006, Free radical scavenger activity: determination of the antioxidant profile of bioactive substances. In: Monge A., Ganellin C.R. (Ed.), Practical Studies for Medicinal Chemistry, Genebra: IUPAC.

Morais, K. S., Melo Filho, A. A., Vilarinho, L. B. O., Morais, B. S., Chagas, P. C., Dos Santos, R. C. & Takahashi, J. A., 2018, Biological Activity of Hexane Extracts of the Northern Amazon Species *Capsicum* spp. Chemical Engineering Transactions, *64*, 277-282.

Oliveira, C. M., Regasini, L. O., Silva, G. H., Pfenning, L. H., Young, M. C., Berlinck, R. G., ... & Araujo, A. R. (2011). Dihydroisocoumarins produced by Xylaria sp. and Penicillium sp., endophytic fungi associated with Piper aduncum and Alibertia macrophylla. Phytochemistry letters, 4(2), 93-96.

Pereira, L. S. Peptídeos antimicrobianos de folhas e raízes de *Capsicum annuum* L.; 2015: caracterização atividade inibitória sobre microrganismos fitopatogênicos. Tese (Mestrado em Genética e Melhoramento de Plantas). Centro de Ciências e Tecnologias Agropecuárias da Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes. 96 p.

Reddy, K. K., Ravinder, T., Prasad, R. B., & Kanjilal, S., 2010, Evaluation of the antioxidant activity of capsiate analogues in polar, nonpolar, and micellar media. Journal of Agricultural and Food Chemistry, 59(2), 564-569.

Santos, M. M. P., 2010, In vitro antimicrobial activity of plant extracts of Mangifera species indicates Eugenia jambolana, Schinus terebinthifolius, Capsicum annuum, and capsaicin analogues, against microorganisms of the oral cavity. Field of the Goytacazes: State University of the North Fluminense Darcy Ribeiro.

Silva D.J., Queiroz A.C., 2002, Food Analysis (Chemical and Biological Methods). Ed. 3a. Vicosa, MG: State University of the Viçosa, 235p.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M., 1999, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology (Vol. 299, pp. 152-178). Academic press.

Sricharoen, P., Lamaiphan, N., Patthawaro, P., Limchoowong, N., Techawongstien, S., & Chanthai, S., 2017, Phytochemicals in Capsicum oleoresin from different varieties of hot chilli peppers with their antidiabetic and antioxidant activities due to some phenolic compounds. Ultrasonics sonochemistry, *38*, 629-639.

Trevisan M.T.S., Macedo F.V.V., 2003, Screening for acetylcholinesterase inhibitors from plants to treat Alzheimer's disease, Química Nova, 26, 301-304.

Vinutha B., Prashanth D., Salma K., Sreeja S.L., Pratiti D., Padmaja R., Radhika S., Amita A., Venkateshwarlu K., Deepak M., 2007, Screening of selected Indian medicinal plants for acetylcolinesterase inhibitory activity, Jounal Ethnopharmacol*y.* 109, 359-363.

Zacchino A.S., Gupta M.P., 2007, Manual de técnicas *in vitro* para la detección de compuestos antifúngicos, vol. 85, Corpus Editorial y Distribuidora: Rosario.