Vol. 14(3), pp. 88-97, March, 2020
DOI: 10.5897/JMPR2019.6790
Article Number: 2805D9D63145
ISSN 1996-0875

http://www.academicjournals.org/JMPR

ACADEMIC JOURNALS
Journal of Medicinal Plants Research

Full Length Research Paper

Antimicrobial activity and acetilcolinesterase inhibition of oils and Amazon fruit extracts

Ismael Montero Fernández*[1], Edvan Alves Chagas[1,2,3], Selvin Antonio Saravia Maldonado[4] Jacqueline Aparecida Takahashi[5], Ricardo Santos Alemán[6], Antonio Alves de Melo Filho[1], Ricardo Carvalho dos Santos[7], Pedro Rômulo Estevam Ribeiro[1], Jhunior Abrahan Marcia Fuentes[8], Pollyana Cardoso Chagas[1,2] and Ana Cristina Gonçalves Reis de Melo[9]

[1]Post-graduate Program in Biodiversity and Biotecnology, State Coordination of Roraima, Federal University of Roraima, Campus Paricarana, Brazil
[2]Post-graduate Program in Agronomy, Federal University of Roraima, Campus Cauamé, Boa Vista, Brazil
[3]Brazilian Agricultural Research Corporation (Embrapa), Boa Vista, Brazil; Productivity Research Scholarship – CNPq
[4]Faculty of Earth Sciences and Conservation, National University of Agriculture, Catacamas, Olancho, Honduras
[5]Departament of Chemistry. Federal University of Minas Gerais, Brazil.
[6]Departament of Food Science. Louisiana State University, United States.
[7]Instituto Insikiran de Formação Superior Indígena, Federal University of Roraima, Campus Paricarana, Brazil
[8]Faculty of Technological Sciences, National University of Agriculture, Catacamas, Olancho, Honduras
[9].Research and Postgraduate Nucleus in Science and Technology, Federal University of Roraima, Brazil.

Received 26 February, 2018; Accepted 26 March, 2018

The present work consists of the evaluation of antimicrobial activity and inhibition of the enzyme acetylcholinesterase (AChE) of fixed oils and hexane extracts of nine fruits with the following native names: *abiu* (*Pouteria caimito*), *acerola* (*Malpighia emarginata*), *araçá* (*Psidium cattleianum*), *bacuparí* (*Rheedia gardneriana*), *biribá* (*Rollinia mucosa*), *camu-camu* (*Myrciaria dubia*), *fruta-do-conde* (*Annona squamosa*), *graviola* (*Annona muricata*) and *taperebá* (*Spondias mombin* L.). Different evaluations were carried out with different parts of the fruits, pulp, seed and barks. The antimicrobial assay was carried out with the following microorganisms: *Candida albicans* ATCC 18804, *Staphylococcus aureus* ATCC 29212, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028. Of these microorganisms, the best inhibition results were obtained for yeast *C. albicans* with percent inhibition of 94.46% by *taperebá* barks extracts, *acerola* barks (87.12%), *araçá* seed (85.23%) and *taperebá* pulp (85.22%). Against the bacteria tested, percent inhibition was low, showing that the extracts have good antifungal selectivity. Some extracts were able to inhibit the enzyme AChE and high percentage of inhibition was observed for the oils, especially from *biribá* barks, with 86.39% inhibition, *taperebá* seeds with 62.17% and *acerola* pulp with 52.18%. Methods of Multivariate Analysis were applied through Principal Component Analysis (PCA) and Hierarchical component analysis (HCA), to establish correlations and groupings between the data obtained, justifying 82.3% of cases for pulps, 73.2% for the barks and 65.7% for the seeds according to the PCA.

Key words: Bacteria, yeasts, Alzheimer, principal component analysis (PCA), hierarchical component analysis (HCA).

INTRODUCTION

In Brazil there are ten thousand plants considered medicinal, aromatic and useful, but around 99.6% of these

plants are little known about the chemical composition (Silva et al., 2002). Many of these plant species have in their chemical composition secondary metabolites with a defensive function when they are attacked by certain microorganisms such as bacteria, fungi, parasites or virus among others. The compounds with antibacterial action usually are terpenoids, phenolic compounds, alkaloids, polypeptics, coumarins and camphors, being extremely numerous and at the same time, their chemical structures present high selectivity and specificity (Simões, 2003; Reschke et al., 2007; Chen et al., 2015).

Fungi and bacteria present in plants environment make the latter to act in the fight against these phytopathogens, as well as against insect pests and herbivores (Peixoto et al., 2009). Since natural products have high biological activity, it is increasingly common to use extracts as an alternative, for example against certain diseases such as candidiasis treatment (Reis et al., 2011). Infectious diseases represent an important cause of morbidity and mortality in humans, especially in developing countries, and pharmaceutical industries have been motivated in recent years for the development of new antimicrobial drugs, especially due to the occurrence of microbial resistance to such diseases as the bacteria possess genetic ability and acquire resistance to drugs used as therapeutic agents (Nascimento et al., 2000).

On the other hand, there are compounds of natural origin that have the property of inhibiting acetylcholinesterase (AChE), a key enzyme in Alzheimer's disease. These compounds can be either isolated from plants or from microorganisms (dos Santos et al., 2017). Inhibition of AChE *in vitro* is attributed to several reasons, including the structure of phenolic compounds, considering the metabolism suffered by phenolic compounds after their ingestion at gastrointestinal tract and liver level (Roseiro et al., 2012).

In this context, the objective of this work was to perform a bioassay to evaluate inhibition of Gram-positive and Gram-negative bacteria, yeasts and the inhibition of the acetylcholinesterase enzyme by different oils and fruit extracts (*abiu, acerola, camu-camu, bacupari, graviola, araçá, biribá* and *taperebá*) of Northern Amazon and to correlate the different results through multivariate analysis techniques (PCA and HCA), aiming to collaborate to future pharmaceutical applications.

MATERIALS AND METHODS

Sample preparation

Samples of the different fruits studied were collected in randomized points of the State of Roraima (Brazil) to guarantee the representativeness of the sample. Each of the fruits was collected in the corresponding production period and were collected in the ripening stage suitable for consumption. From all the samples collected at the different sampling points, a single composite sample was prepared for each of the fruits where they were taken to the Environmental Chemistry Laboratory of the Federal University of Roraima, where those that presented an optimum conservation status were selected. washed with 1% sodium hypochlorite solution and again with distilled water.

Subsequently, a representative sample of each fruit was selected according to the following criteria: acerola, camu-camu and taperebá was selected 1 kg of fresh fruit, abiu, araçá and bacuparí was selected 2 kg of fruit and for biribá, fruta-do-conde and graviola were selected 10 units according with NTON 17002-02 (2002). All of them were separated into pulp, barks and seed and were placed in Ultrafreezer at -80ºC and then lyophilized in lyophilizer LIOTOP model L 101 for 48 hours until complete drying of the material and subsequently ground in LABOR model SP31 punch mill and stored material in airtight bags in the absence of light until the moment of performing the different analyzes.

Preparation of the extracts to realize the bioassays

The oil and extracts were obtained by extraction from hexane solvent in a Soxhlet apparatus for 6 h. The hexane was evaporated on a rotaevaporator and the oil and extracts were properly packaged in amber vials under nitrogen atmosphere and stored in a freezer (Jorge and Luzia, 2012).

Bioassays of fungi and yeasts

The extracts of different parts of the fruit studied were tested against the following microorganisms: yeast *Candida albicans* (ATCC 18804), Gram-positive bacteria *Staphylococcus aureus* (ATCC 29212) and *Bacillus cereus* (ATCC 11778), Gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028).

A pre-inoculum was prepared in which the microorganisms were transferred from the culture medium where they were stored into test tubes containing 3.0 mL of culture medium (BHI for bacteria and Sabouraud Broth for yeast). The tubes were then incubated in an oven at 37.5°C for 24 to 48 h. With the aid of a micropipette, 500 µL of this pre-inoculum were transferred to test tubes containing sterile distilled water. The tubes were homogenized and the concentration adjusted to 600 nm (bacteria) and 530 nm (yeast), until obtaining transmittance between 74 and 75% (bacteria) and 75 and 76% (yeast), corresponding to the 0.5 McFarland standard turbidity, thus obtaining the suspensions of the inocula used in the bioassay. To prepare the working solution, the samples were previously solubilized in 12.5 $mg.mL^{-1}$ dimethylsulfoxide (DMSO). From this solution, an aliquot of 40 µL was added to 960 µL of the culture medium used in the bioassay, obtaining a solution with concentration of 500 $\mu g.mL^{-1}$. The bioassays were run in 96-well plates in triplicate, adding 100 µL of the working solution at the concentration of 500 $\mu g.mL^{-1}$ in three wells. Then, 100 µL of standardized microorganism inoculum was added to each well. Four controls were performed: growth control of the microorganism (to verify cell viability); the blank, which consists of the sample solution at the same concentrations evaluated, replacing the inoculum with sterile distilled water; positive control (the working

*Corresponding author. E-mail: ismonterof@unex.es.

Table 1. Names and families of the Northern Amazon fruits utilized in this work.

Scientific name	Family	Common name in Brazil
Pouteria caimito	Sapotaceae	*Abiu*
Malpighia emarginata	Malpighiaceae	*Acerola*
Psidium cattleianum	Myrtaceae	*Araçá*
Rheedia gardneriana Planch & Triana	Clusiaceae	*Bacuparí*
Rollinia mucosa	Annonaceae	*Biribá*
Myrciaria dúbia (Krunth) Mc Vaugh	Myrtaceae	*Camu-camu*
Annona squamosa	Annonaceae	*Fruta-do-conde*
Annona muricata	Annonaceae	*Graviola*
Spondias mombin L.	Anacardiaceae	*Taperebá*

Table 2. Inhibitory potential of oils and extracts from pulps against yeast, bacteria and AChE.

Sample	*C. albicans* ATCC 18804	*S. aureus* ATCC 29212	*B. cereus* ATCC 11778	*E. coli* ATCC 25922	*S. typhimurium* ATCC 14028	AChE
	% Inhibition					
Abiu	76.71± 5.62	25.85 ± 2.67	16.75 ± 1.80	25.33 ± 2.29	2.28 ± 1.18	29.28 ± 3.03
Acerola	0.00	17.98± 2.96	6.68 ± 2.70	21.65± 10.35	8.64 ± 3.99	52.18 ± 6.30
Araçá	64.28 ± 11.74	25.04 ± 1.55	15.97 ± 2.51	24.09 ± 2.17	13.20 ± 1.45	27.40 ± 4.38
Bacupari	N.D.	N.D.	N.D.	N.D.	N.D.	20.08 ± 4.41
Biribá	N.D.	8.32 ± 0.15	8.22 ± 0.14	15.61 ± 1.27	5.04 ± 0.79	43.42 ± 2.15
Camu-camu	20.52 ± 14.81	28.80 ± 2.49	N.D.	30.68 ± 2.48	28.22 ± 4.48	N.D.
Fruta-do-conde	16.67 ± 4.27	23.20 ± 3.03	N.D.	14.41 ± 1.12	13.56 ± 2.69	25.71 ± 1.19
Graviola	N.D.	15.35 ± 4.85	N.D.	16.28 ± 0.07	12.79 ± 1.04	44.10 ± 0.62
Taperebá	85.22 ± 19.60	17.36 ± 3.43	13.06 ± 10.87	15.57 ± 3.54	2.73 ± 1.04	40.83 ± 3.60
Standard	**Miconazole**			**Ampicillin**		**Eserine**
	82.82 ±13.65	98.8 ± 1.90	96.69 ± 4.94	96.03 ± 0.59	96.00 ± 0.84	80.04 ± 0.18

N.D.: Not detected.

solution is replaced by a commercial antibiotic) and the sterility control of the culture medium containing 100 µL of culture medium and 100 µL of sterile distilled water. The microplates were incubated in an oven at 37.5°C and after 24 h the plate reader was read at 490 nm. The antibiotics used for the quality control of the assays were: ampicillin, for bacteria and miconazole, for yeast, previously prepared as described for the samples tested (CLSI, 2012).

Inhibition bioassay of the enzyme acetylcholinesterase (AChE)

The extracts of the different parts of Amazonian fruits were tested for inhibition of AChE enzyme using UV-visible molecular spectrophotometry method in 96-well microplates. A negative control of the assay was performed without the presence of inhibitors. The tests were performed in quintuplicate. An aliquot of 25 µL of acetylcholine iodide (15 mM) was pipetted into each well followed by 125 µL of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB); 50 µL of 0.1% w/v tris-HCl pH 8 bovine serum albumin and 25 µL of the samples (10 $mg.mL^{-1}$) solubilized in DMSO with 10% v/v tween. The plates were read at 405 nm for 9 times over a period of 8 min. Immediately after the first reading, 25 µL of the enzyme acetylcholinesterase (*Electrophorus electricus*, Sigma Aldrich) (0.222 $U.mL^{-1}$) was added and 9 readings were performed over a period of 8 min at 405 nm, the percentage inhibition of the enzyme shown in Equation 1. Eserine (10 mg mL^{-1}) was used as positive standard control (Ellman et al., 1961; Rhee et al., 2001).

$$\% \text{ Inhibition} = ((C-A) \times 100) / C \quad (1)$$

where C = Control containing enzyme and substrate and A = Assay containing sample, enzyme and substrate.

Statistical analysis

Principal component analysis (PSA) and component hierarchy analysis (HSA) for total phenolic compounds and antioxidant activity by the two methods for the different parts of the fruit were evaluated using InfoStat software version 2016 (Rienzo et al., 2016).

RESULTS AND DISCUSSION

Results of the activity of pulps, barks and seeds of the different fruits studied in the microbiological inhibition and

Table 3. Inhibitory potential of oils and extracts from barks against yeasts, bacteria and AChE.

Sample	*C. albicans* ATCC 18804	*S. aureus* ATCC 29212	*B. cereus* ATCC 11778	*E. coli* ATCC 25922	*S. typhimurium* ATCC 14028	AChE
	% inhibition					
Abiu	48.13 ± 16.22	20.53 ± 1.17	12.69 ± 1.90	21.53 ± 2.67	8.62 ± 1.10	35.31 ± 4.22
Acerola	87.12 ± 25.70	12.91 ± 2.20	5.88 ± 2.13	24.65 ± 3.84	19.01 ± 0.11	N.D.
Araçá	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bacupari	31.85 ± 11.79	11.84 ± 2.71	38.70 ± 3.35	17.7 ± 5.21	8.12 ± 2.45	N.D.
Biribá	39.14 ± 9.56	18.77 ± 2.88	N.D.	13.42 ± 1.77	15.09 ± 2.55	86.39 ± 8.76
Camu-camu	71.35 ± 16.35	35.72 ± 1.95	14.06 ± 5.17	13.12 ± 5.67	28.04 ± 4.40	N.D.
Fruta-do-conde	N.D.	15.28 ± 2.05	N.D.	11.70 ± 1.90	N.D.	43.22 ± 3.06
Graviola	30.75 ± 1.96	14.6 ± 4.90	N.D.	16.28 ± 0.07	11.53 ± 2.28	29.45 ± 3.18
Taperebá	94.46 ± 7.82	23.26 ± 1.39	19.60 ± 5.93	18.91 ± 4.55	5.85 ± 2.00	56.88 ± 2.32
Standard	**Miconazole**			**Ampicillin**		**Eserine**
	82.82 ±13.65	98.8 ± 1.90	96.69 ± 4.94	96.03 ± 0.59	96.00 ± 0.84	80.04 ± 0.18

N.D.: Not detected.

Table 4. Inhibitory potential of oils and extracts prepared from seeds against yeasts, bacterium and AChE.

Sample	*C. albicans* ATCC 18804	*S. aureus* ATCC 29212	*B. cereus* ATCC 11778	*E. coli* ATCC 25922	*S. typhimurium* ATCC 14028	AChE
	% inhibition					
Abiu	59.87 ± 10.33	22.13 ± 2.19	12.07 ± 3.71	22.76 ± 2.04	2.96 ± 0.94	N.D.
Acerola	N.D.	26.78 ± 2.39	17.28 ± 1.72	27.99 ± 2.28	13.21 ± 0.31	30.19 ± 6.04
Araçá	85.23 ± 13.11	25.30 ± 3.43	13.07 ± 1.85	25.55 ± 3.40	16.94 ± 2.46	22.71 ± 4.97
Bacupari	N.D.	16.47 ± 1.37	26.73 ± 3.79	17.51 ± 3.05	14.29 ± 1.93	N.D.
Biribá	N.D.	7.95 ± 4.08	7.85 ± 2.27	13.04 ± 2.90	13.82 ± 0.24	59.34 ± 7.48
Camu-camu	39.26 ± 14.61	27.11 ± 3.74	N.D.	18.76 ± 3.86	19.13 ± 1.57	33.10 ± 6.10
Fruta-do-conde	N.D.	24.89 ± 4.34	N.D.	12.71 ± 3.39	17.50 ± 2.80	54.49 ± 4.93
Graviola	N.D.	15.18 ± 0.90	N.D.	13.50 ± 4.05	10.00 ± 0.95	48.88 ± 3.29
Taperebá	7.44 ± 0.32	17.07 ± 3.21	N.D.	13.63 ± 4.36	15.35 ± 2.31	62.17 ± 5.14
Standard	**Miconazole**			**Ampicillin**		**Eserine**
	82.82 ±13.65	98.8 ± 1.90	96.69 ± 4.94	96.03 ± 0.59	96.00 ± 0.84	80.04 ± 0.18

N.D.: Not detected.

AChE inhibition tests, respectively are shown in Tables 2 to 4.

The inhibitory potential of pulp oils and extracts against *S. aureus* was relatively low for the oils tested, reaching only 28.80% for *camu-camu* pulp oil, and 25% inhibition for *abiu* and *araçá*, compared to amipicillin, the standard utilized, which presented 98.8% inhibition. The other Gram-positive bacterium tested, *B. cereus*, was less inhibited than *S. aureus* bacterium by the oils and pulps extracts from the Amazonian fruits. The highest percentages of inhibition were observed for *abiu* oil with 16.75% inhibition and *araçá* (15.97%), being very low values in relation to the standard ampicillin that showed 96.69% inhibition. According to Cordeiro (2011) *S. aureus* is present in both skin and mucous membranes, being considered as a causal agent of 7.7% of outbreaks of food poisoning in Brazil (Brazil, 2015). Morais et al., (2018) developed a study of biological activity for peppers from species *Capsium* species from the Amazonian region finding percent inhibition of *S. aureus* bacteria (13.71%) in line with the values found in this work.

B. cereus is present in food since it is resistant to the process of pasteurization. This bacterium reaches the environment easily contaminating food when the appropriate processing conditions are not used. It produces different toxins that shave human health when

consumed in foods contaminated by this microorganism in concentrations of 105 to 108 CFU per gram of food (Granum and Lund, 1997).

For the two Gram-negative bacteria that were tested for the oils, *E. coli* was more inhibited, however in low percentages in relation to the tested standard ampicillin (inhibition = 96.03%). *E. coli* causes different types of diarrheogenic diseases (Kuhnert et al., 2000). The highest inhibition values were presented for the oil obtained from *camu-camu* pulp with 30.68% and *abiu* pulp with 25.33% inhibition. The other Gram-negative bacterium utilized in this screening was *S. typhimurium* which was also low inhibited by the oils tested. This bacterium is implicated in food poisoning problems causing gastrointestinal problems (Morpeth et al., 2009). Pulp of *camu-camu* was the best inhibitor of *S. typhimurium* (28.22% inhibition).

From the microorganisms tested in this study, the yeast *C. albicans* was the most susceptible microorganism to the oils tested. For instance, *taperebá* oil presented 85.22% inhibition, a percentage higher than that obtained for miconazole, the standard tested (82.82%), followed by *abiu* oil (76.71%) and *aracá* oil (64.28%). From the health point of view, it is interesting to look for new natural compounds with the capacity to inhibit this yeast since it causes candidiasis in the human body, an infection that can manifest in both oral and vaginal mucosa. *C. albicans* is the yeast from *Candida* genus predominant in the infection of candidiasis with 50%. *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis* are the minor yeats present in that infection. The worldwide mortality rate due to diseases due to yeasts from *Candida* genus is between 15 and 25% in adults and 10 and 15% in children (Alangaden, 2011).

As for the potential inhibition of acetylcholinesterase enzyme (AChE) by the oils and fats of the nine pulps, *acerola* pulp was the one with the most potent inhibition of AChE. *Biribá*, *graviola* and *taperebá* presented moderate inhibition potential and the other samples presented weak AChE inhibition potential (Vinutha et al., 2007) since it was considered that, for crude vegetable extracts, values above 50% mean potent inhibitors; between 30 and 50% are the moderate inhibitors and below 30% are weak inhibitors on AChE.

The inhibitory potential of the oils and extracts of the barks against *S. aureus* was lower than for the oils extracted from the corresponding pulps, since the samples tested did not reach 25% inhibition. *Taperebá* presented the highest percentage of inhibition against *S. aureus* (23.26%) while the tested standard amipicillin presented 98.8%. For the other Gram-positive bacterium tested (*B. cereus*), the oils from barks also presented low inhibition, the major percentage of inhibition found for the oil extracted from *bacuparí* barks with 38.70% of inhibition.

Form the two Gram-negative bacteria tested, *E. coli* presented the highest percentage of inhibition, but the values found are still low in relation to the tested standard ampicillin. The better inhibition was presented by *acerola* extract with 24.65% of inhibition followed by *abiu* barks oil with 21.53% inhibition. For *S. typhimurium*, the highest percentage of inhibition was for the oil from *camu-camu* barks (28.04%) followed by oil from *acerola* barks oil (19.01% inhibition). Among the microbiological inhibition tests for fruits barks, the best results were obtained towards the yeast *C. albicans.* The oil from barks of *taperebá* presented a potent antimicrobial effect with 94.46% inhibition. This value is even higher than that obtained for miconazole, the standard tested that showed 82.82% inhibition. The oil from barks of acerola inhibited 87.12% of *C. albicans* growth, which was also superior to the inhibition presented by the standard tested. Another extract very active against this yeast was that from *camu-camu* barks with 71.35% inhibition.

Concerning the barks of the studied fruits, the oil extracted from *biribá* barks presented good inhibition of AChE (86.39%), even higher than the value found for the positive standard serine, in the conditions utilized in this test. The oil of *taperebá* barks also presented potent inhibition while *abiu* barks and *fruta-do-conde* presented moderate inhibition. Mota et al. (2012) tested different ethanolic extracts of medicinal plants in Brazil for the inhibitory capacity towards the AChE enzyme. In that study, the potent inhibition of AChE by the aqueous extract of *Vitex agnus-castus* L. was highlighted (74%), a value slightly lower than that found in this work for *biribá* barks oil.

The inhibitory potential of the oils and extracts prepared from the seeds (Table 4) was low. The oil of *camu-camu* seeds presented the best behavior but inhibition of *S. aureus* was only 27.11% while ampicillin achieved 98.8% inhibition, while for *B. cereus, bacuparí* seeds inhibited 26.73% followed by the *acerola* seed with 17.28% (ampicillin with 96.69% inhibition).

The Gram-negative *bacteria* were again, only slighted inhibited by the extracts. *E. coli* was more susceptible to the oil of *acerola* seeds (27.99% inhibition) and *aracá* seeds (25.55%), values very low compared to positive control (96.63%). For S. *typhmurium*, inhibition by seeds extracts and oils were still lower (19.13% inhibition for *camu-camu* oil). Only four of the nine oils and extracts from seeds inhibited *C. albicans. Aracá* oil was slightly more active (85.23% inhibition) than miconazole (82.82% inhibition).

Biribá, *taperebá* and *fruta-do-conde* oils presented potent inhibitory potential towards AChE according to the classification proposed by Vinutha et al. (2007). *Acerola*, *graviola* and *camu-camu* seeds presented moderate potential and the remaining seed oils presented weak inhibitory potential or did not present any inhibition of enzyme AChE.

Dos Santos et al. (2015) studied the bioactive potential of *Annona hypoglauca* seeds, another species of *Annona*, finding 79.55% of AChE inhibition, which is

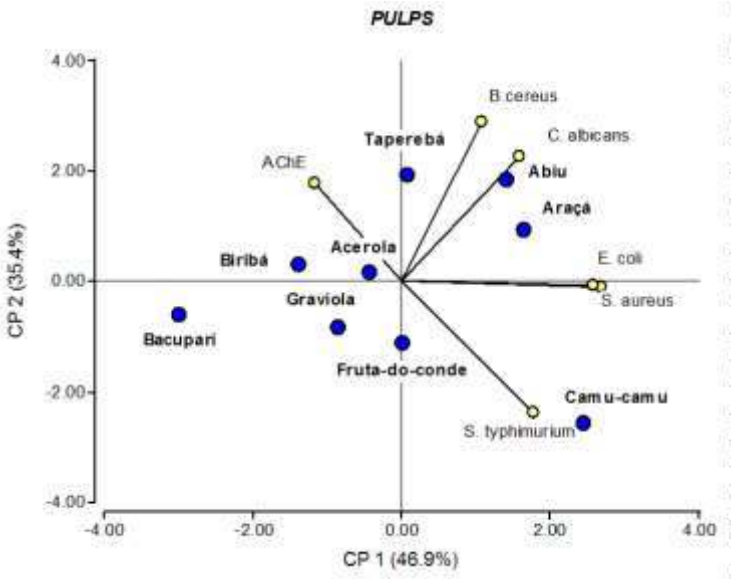

Figure 1. Distribution of the original variables among the different fruits for the pulp on the first and second main component (CP1 and CP2).

higher than the results found for the Annonaceae seeds studied in the present work. The percentage of *C. albicans* inhibition reported for *A. hypoglauca* (90.11%) while for the bacteria *E. coli* and *S. aureus*, literature results agree with those presented in this work.

Statistical analysis

Firstly, the coefficient was calculated to evaluate the consistency of the hierarchical groupings, obtaining a value of 0.973, and values close to the unit indicate a better representation (Ferreira et al., 2002; Cruz and Carneriro 2003; Moura et al., 2006).

Principal component analysis (PCA)

The main components analyses were carried out jointly for the evaluated systems (*abiu*, *bacuparí*, *acerola*, *graviola*, *camu-camu*, *fruta-do-conde, araçá, biribá* and *taperebá*) independently for each part of the fruits for *C. albicans*, *S. aureus, B. cereus. E. coli, S. typhimurium* and AChE in the different parts of the fruit in order to find variables (main components) which are not correlated to explain the structure of the variation. The weight of each variable analyzed in each component (axes) is represented. The main components blipot for the different parts of the evaluated fruit are as shown in Figures 1 to 3.

In blipot (Figure 1), the results of the principal component analysis (PCA) for the microbiological inhibition of the extracts of the different pulps studied are represented and 82.3% of the original variability of the data retained in these components is explained.

The arrangement of the sequence in Figure 1 shows that the systems can be grouped into two sets, the first major component (CP1) contributed 46.9% of the total variance explained, however most of the variables that were strongly affected contributed from all bacteria and yeasts (*B. cereus*, *C. albicans*, *E. coli*, *S. aureus,* and *S. typhimurium*) were positive for CP1; the other test was inhibition of the AChE enzyme.

The second main component (CP2) explained 35.4% of the total data appearing in this case, the percentage of AChE inhibition. Analysis of this component also showed that this attribute negatively projects on the bacteria and yeast tested being the extracts of the *acerola, biribá, graviola* and *bacuparí* pulps have been associated.

In blipot (Figure 2), the results of the principal components analysis (PCA) for the microbiological inhibition of the extracts of the different pulps studied are represented and 65.7% of the original variability of the data retained in these components.

The arrangement of the sequence in Figure 2 shows that the systems can be grouped into two sets. The firstmajor component (CP1) contributed 38.3% of the total variance explained. However, most of the variables that were strongly affected that contributed to all bacteria and yeasts (*B. cereus, C. albicans, E. coli*, *S. aureus* and *S. typhimurium*) were positive and the other test was the inhibition of the AChE enzyme. These results indicate that CP1 allowed to distinguish the oils from fruits that are strongly associated, being *taperebá*, *abiu*, *bacuparí* and *acerola* barks. The second main component (CP2) explained 27.4% of the total data, appearing in this case, the percentage of inhibition of AChE. The analysis of this component also showed that this attribute projects

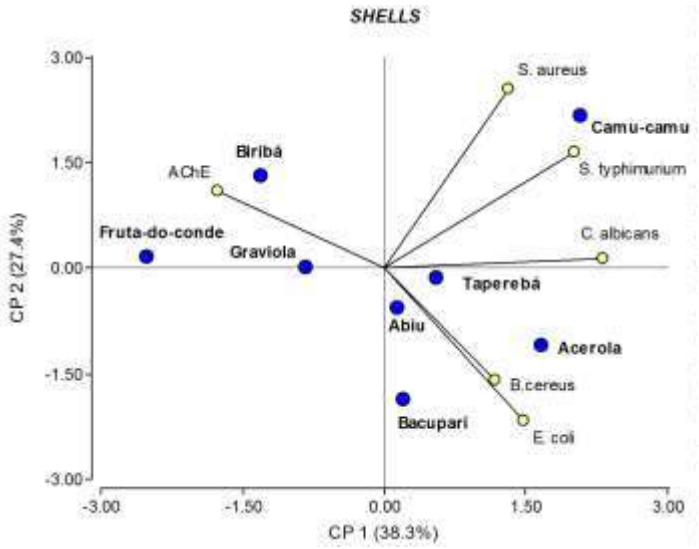

Figure 2. Distribution of the original variables between the different fruits for the barks on the first and second main components (CP1 and CP2).

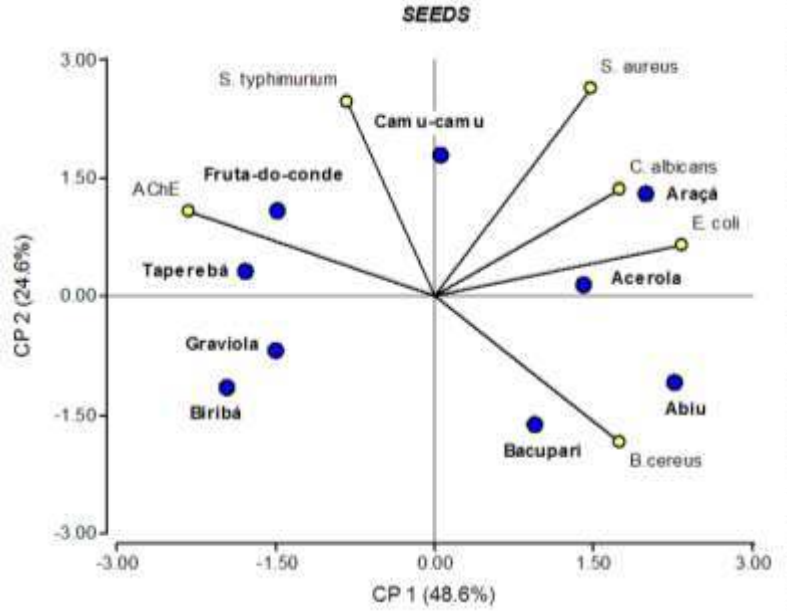

Figure 3. Distribution of the original variables among the different fruits for the seeds on the first and second main component (CP1 and CP2).

negatively on the tested bacteria and yeast, being the extracts of the *biribá*, *fruta-do-conde* and *graviola* barks, who have been associated.

In blipot (Figure 3), the results of the principal component analysis (PCA) for the microbiological inhibition of the extracts of the different pulps studied are presented explaining the 73.2% of the original variability of the data retained in these components.

The arrangement of the sequence in Figure 3 shows that the systems can be grouped into two sets, the first major component (CP1) contributed 48.6% of the total variance explained. However most of the variables that were strongly affected contributed from positive form to CP1 the bacteria and yeasts (*S. aureus, C. albicans, E. coli* and *B. cereus*) and inverse with the other test that was the inhibition of the enzyme AChE and *S.*

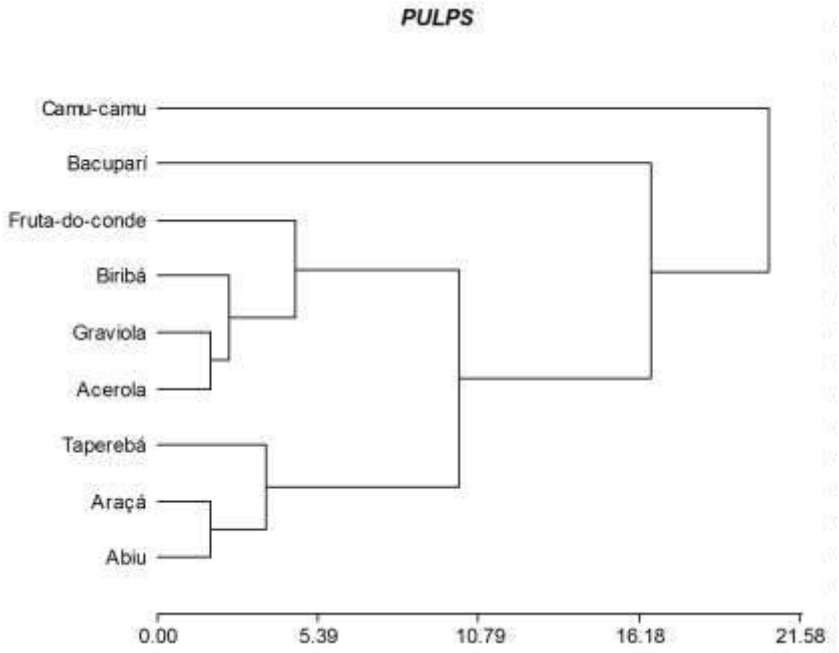

Figure 4. Dendogram by HCA. Euclidean distance and incremental connection technique for the percentage of inhibition present in fruit pulp extracts studied.

typhimurium. These results indicate that CP1 allowed distinguishing oils from fruits that are strongly associated with them being *camu-camu* with *araçá* and *acerola* and *abiu* with *bacuparí*. The second main component (CP2) explained 24.6% of the total data, appearing in this case, the percentage of inhibition of AChE and *S. typhimurium*. The analysis of this component also showed that this attribute projects negatively on the other bacteria and yeast tested being the oils and extracts of the seeds of the *fruta-do-conde* and *taperebá* were associated.

Analysis of hierarchical groupings (HCA)

Through HCA, data can be displayed in a two-dimensional space in order to emphasize their natural groupings and patterns, relating the samples so that the most similar are related to each other presenting the samples in dendogram, grouping the samples and variables according to their similarity. Figures 4 to 6 show the dendrograms for HCA analysis of inhibition of the different strata studied.

For the percentages of inhibition of oils and extracts of the fruits studied, the trends observed through the analysis of PCA main components were observed through the HCA, mainly observing two large groups: one of them formed by the association of *araçá* with *abiu* that present a major contribution, smaller Euclidean distance and they are grouped together with *taperebá* for a major Euclidean distance, but are still associated. On the other hand, the other existing grouping just as happens in the HCA it is for *acerola* and *graviola* that they are strongly associated and increasing the Euclidean distance, they are associated with the *fruta-do-conde*. Finally, the two fruits whose extracts have opposing antimicrobial properties which are the *bacuparí* pulp with the *camu-camu* pulp which are not related in the HCA joining, the elevated Euclidean distance of 21.58%.

For the percentages of inhibition of fruits oils and extracts studied, the trends observed through the analysis of PCA main components of the barks were observed by HCA where there is great dispersion between the grouping of the studied fruits where the main grouping to be highlighted with smaller Euclidean distance is for *graviola* with *abiu*.

For the seeds two large clusters are observed being in agreement with the results presented by PCA. The first association is that between *taperebá* and *graviola* with smaller Euclidean distance that are later associated with *biribá*. The other association with Euclidean distance of 4.09 is for the strata of the *fruta-do-conde* and *camu-camu*. All these fruits are grouped together and the HCA shows that they do not have a relation with or another group of fruits that contribute in an opposite way (*bacuparí*, *araçá*, *acerola* and *abiu*) whose connection happens with elevated Euclidean distance of 16.38.

Conclusion

Given the potent results of the oils and extracts tested in this article against the potential inhibition of *C. albicans*

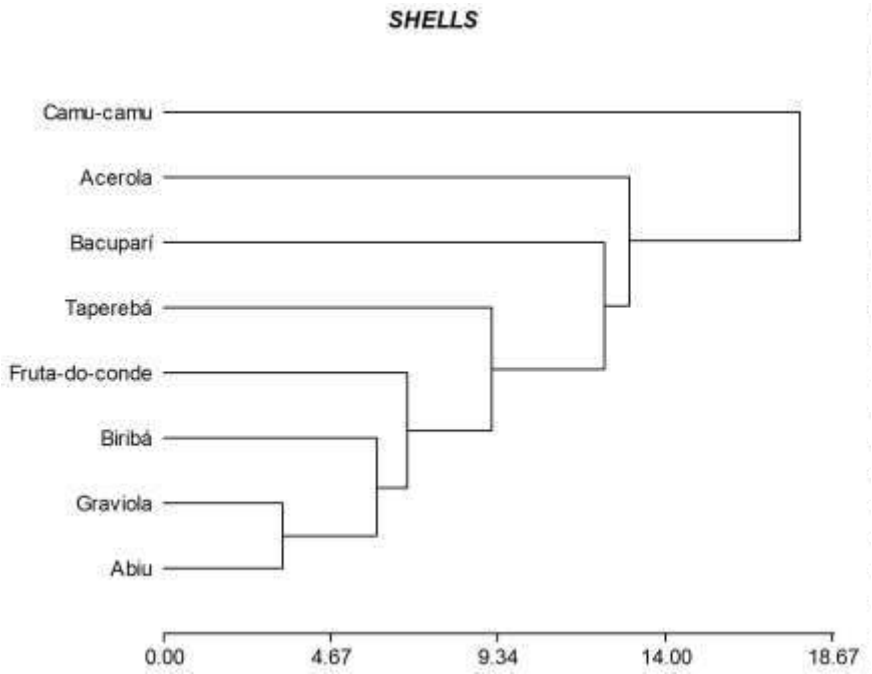

Figure 5. Dendogram by HCA. Euclidean distance and incremental connection technique for the percentage of inhibition present in extracts of fruit barks studied.

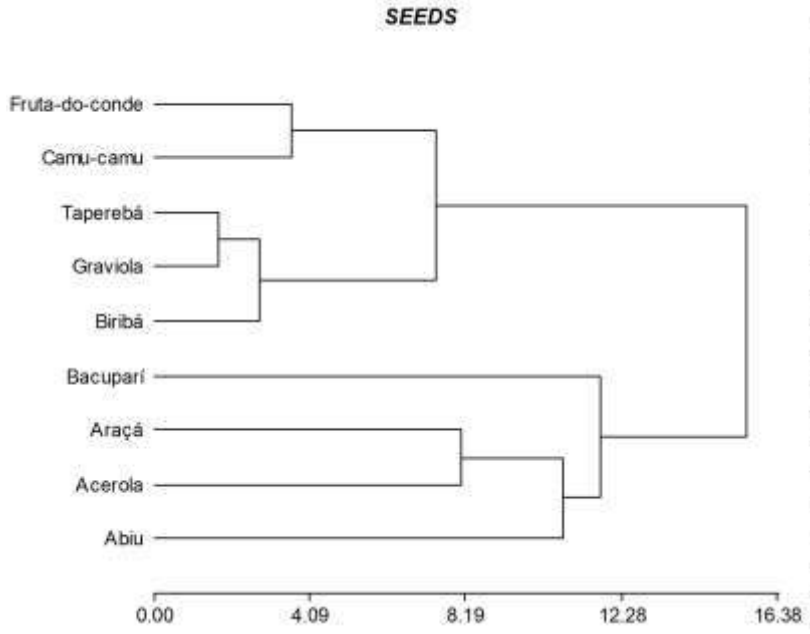

Figure 6. Dendogram by HCA. Euclidean distance and incremental connection technique for the percentage of inhibition present in fruit extract extracts studied.

yeasts such as the barks and pulps of *taperebá*, *acerola* barks or *araçá* seed, this can be a starting point for the development of new drugs with specific action to minimize virulent factors making difficult the development of the infectious process of candidiasis caused by the yeast *C. albicans*.

On the other hand, the results found for the antibacterial action of the oils and extracts did not present high inhibition percentage to inhibit the bacteria of pathogenic action.

Finally, in relation to inhibition of AChE, several extracts demonstrate a strong inhibitory capacity of the enzyme such as *biribá* barks, *taperebá* seed or *acerola* pulp, but in most of the studies, the isolated compounds responsible for the AChE inhibitory activity have not been identified or characterized.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alangaden SC (2011). Nosocomial fungal infections: epidemiology, infection control and prevention. Infectious Disease Clinics of North America 25(1):201-225.

BRASIL (2015). Ministério da Saúde. Secretaria de Vigilância em Saúde. Doenças Trasnmitidas por alimentos. Avaliable at: http://u.saude. gov.br/images/pdf/2015/novembro/09/ApresentaodadosgeraisDTA2015.pdf.

Chen J, Li W, Yao H, Xu J (2015). Insights into drug Discovery from natural products through structural modification. Fitoterapia 1(1):231-241.

Clinical and Laboratory Standards Institute (CLSI) (2012). Métodos de diluição testes de susceptibilidade antimicrobiana para bactérias que crescem aerobicamente. Aprovado Padrão - 9ª Edição - M7 - A9. CLSI. 32(2). Tradução pela ANVISA com permissão do CLSI.

Cordeiro MM (2011). Caracterização molecular de cepas de Staphylococcus aureus isolados no Hospital Municipal de Ipatinga/MG. Disertação (Mestrado). Universidade Federal do Ouro Preto. Ouro Preto.

Cruz CD, Carneriro PCS (2003). Modelos biométricos aplicados ao melhoramento genético. Viçosa: UFV. 585 p.

Dos Santos GF, Pereira RG, Boaventura MAD, Macias FA, Lima GS, Coelho ACS, Molinillo JMG, Cala A, Takahashi JÁ (2017). Structure-activity relationship study of diterpenes for treatment of Alzheimer's Disease. Química Nova 40(9):1045-1050.

Dos Santos RC, de Melo Filho AA, Chagas EA, Takahashi JA, Ferraz VP, Costa AKP, de Melo AGCR, Montero IF, Ribeiro PRE (2015). Fatty acid profile and bioactivity from Annona hypoglauca sedes oil. African Journal of Biotechnology 14(30):2377-2382.

Ellman GL, Courtiney KD, Andres VJ, Feather-Stone RM (1961). A new rapid colorimetric determination of acetylcholinesterase activity. Biochemistry and Pharmacology 7(1):88-95.

Ferreira EC, Rodrigues SHBG, Ferreira MMC, Nobrega JÁ, Nogueira ARA (2002). Application of the exploratory analysis of data in the geographical discrimination of okra from Rio Grande do Norte and Pernambuco. Eclética Química 27(1):77-91.

Granum PE, Lund T (1997). *Bacillus cereus* and its food poisoning toxins. FEMS Micobiology Letters 157(2):223-228.

Jorge N, Luzia DMM (2012). Caracterização do óleo das sementes de Pachira aquatica Aublet para aproveitamento alimentar. Acta Amazonica 42(1):149-156.

Kuhnert P, Boerlin P, Frey J (2000). Target genes for virulence assessment of *Escherichia coli* isolates from water, food and the environment. FEMS Microbiology Reviews 24(1):107-117.

Morais KS, Melo Filho AA, Vilarinho LBO, Morais BS, Cardoso PC, dos Santos RC, de Melo ACGR, Takahashi JÁ.(2018). Biological Activity of Hexane Extracts of the Northern Amazon Species Capsicum spp. Chemical Engineering Transactions 64(1):277-282.

Morpeth SC, Ramadhani HO, Crump JA (2009). Invasive non-typhi Salmonella disease in Africa. Clinical Infectious Diseases 49(4):606-611.

Moura MCS, Lopes ANC, Moita GC, Neto JMM (2006). Estudo multivariado de solos urbanos da cidade de Teresina. Química Nova 29(1):429-435.

Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian Journal of Microbiology 31(4):247-256.

Norma de Procedimientos para muestreo de produtos vegetales. NTON 17002-02 (2002). Comision Nacional de Normalización Técnica y Calidad del Ministerio de Fometo, industria y comercio. Norma técnica Nicaraguense (NTN).

Peixoto NPAS, Azevedo J,. Araújo WL (2009). Microrganismos endofíticos: interação com as plantas e potencial biotecnológico. Biotecnologia Ciência & Desenvolvimento 29(1):517-525.

Reis NTP, Lelis TC, Mendonça AT, Chavsco JK (2011). Avaliação da ação de extratos vegetais sobre a formação de biofilmes por Candida albicans. Revista da Universidade Vale do Rio Verde 9(2):337-343.

Reschke A, Marques LM, Mayworm MAS (2007). Atividade antibacteriana de *Fícus benjamina* L. (Moraceae). Brazilian Journal of Medicinal Plants 9(2):67-70.

Rhee IK, Meent M, Ingkaninan K, Verporte R (2001). Screening for acetylcholinesterase inhibitors from Amaryllidaceae using gel thin-layer chromatography in combination with bioactivity staining. Journal of Chromatograpghy A 915(1-2):217-223.

Rienzo JA, Casanoves F, Balzarini MG, Gonzales L, Tablada M, Robledo CW (2016). InfoStat Release 2016. InfoStat Group FCA, Universidad Nacional de Córdoba, Argentina. Disponível em: http://www.infoestar.com.ar.

Roseiro L, Rauter A, Serralheiro M (2012). Polyphenols as acetylcholinesterase inhibitors: Structural specificity and impact on human disease. Nutrition and Aging 1(1):99-111.

Silva RL, de Melo GB, Antoniolli AR, Lima SO, de Melo VA, Ramalho LNZ, Zucoloto S, Júnior OC (2002). Effect of the aqueous extract of Hyptis Pectinata on hepatocyte proliferation after partial hepatectomy. Acta Cirurgica Brasileira 17(3):101-105.

Simões OMC (2003). Farmacognosia: da planta ao medicamento. 5 Ed. Porto Alegre: Florianópolis pp. 14-15.

Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratiti D, Padmaia R, Radhika S, Amit A, Venkateshwarlu K, Deepak M (2007). Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. Journal of Ethnopharmacology 109(1):359-363.