CHEMICAL ENGINEERING TRANSACTIONS
VOL. 64, 2018
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Enrico Bardone, Antonio Marzocchella, Tajalli Keshavarz

ISBN 978-88-95608-56-3; **ISSN** 2283-9216
DOI: 10.3303/CET1864049

Bioactive Extracts from *Annona hypoglauca*

Ricardo C. dos Santos[a], Edvan A. Chagas[b,e], Antônio A. de Melo Filho[c,e], Jacqueline A. Takahashi[d], Ismael F. Montero[e], Pollyana C. Chagas[e,f], Pedro R. E. Ribeiro[g], Ana Cristina G. R. de Melo[g]

[a]National Postdoctoral Program of CAPES, associated to POSAGRO/UFRR, Campus Cauamé, BR 174, s/n, Km 12, District Monte Cristo, CEP 69310-250, Boa Vista-RR, Brazil
[b]Embrapa, Rodovia 174, Km 8, Industrial District, CEP 69301970, Boa Vista-RR-Brazil;
[c]Post-Graduate Program in Chemistry, Center for Research and Graduate Studies in Science and Technology, UFRR, Campus Paricarana, CEP 69304-000, Boa Vista-RR-Brazil
[d]Department of Chemistry, Institute of Exact Sciences, Universidade Federal de Minas Gerais, UFMG, Av Antonio Carlos, no. 6627, Pampulha, CEP 31270-901, Belo Horizonte-MG-Brazil.
[e]Post Graduate Program in Biodiversity and Biotechnology, Bionorte, State Coordination of Roraima, UFRR, Campus Paricarana, CEP 69304-000, Boa Vista-RR-Brazil
[f]Post Graduate in Agronomy, POSAGRO, UFRR, Campus Paricarana, CEP 69304-000, Boa Vista-RR-Brazil.
[g]Post-Graduate in Natural Resources Program, Federal University of Roraima, Boa Vista-RR-Brazil.
ricardocs.br@gmail.com

The aim was to analyze the bioactive potential of hexane and ethanolic extracts from leaves of *Annona hypoglauca*, Roraima, Brazil, against fungi (*Aspergillus flavus* and *Fusarium proliferatum*) and yeast (*Candida albicans*) and gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus sanguinis*) and gram-negative (*Escherichia coli* and *Salmonella typhimurium*), antioxidant, antiacetylcholinesterase and cytotoxic activities against *Artemia salina*, in addition to verify a phytochemical screening. Antioxidant activity of the leaves ethanolic extract 51% to 50 μg mL^{-1}, 16% at the same concentration for the hexane extract. The antiacetylcholinesterase activity presented high variation of the ethanolic extract and 59.76% in the hexane extract. The cytotoxic activity against *A. salina* was more efficient for the ethanolic extract of the leaves in 27% to 500 μg mL^{-1}. The bioactivity of fungi and yeasts, the ethanolic extract of the leaves of *A. hypoglauca* showed no inhibition on the fungi *F. proliferatum* and *A. flavus*, but the hexane extract inhibited 40.0% and 59.5% on these fungi, respectively. For the inhibition of *C. albicans*, the extracts hexane (94.5-96.9%) and ethanolic (89.2-92.6%) of the leaves of *A. hypoglauca*, at all concentrations. Excellent inhibitions on gram-positive bacteria were given by the hexane extract in 100% to 500 μg mL^{-1}, but low inhibition for the ethanolic extract for S. aureus in 21.6% to 125 μg mL^{-1} and for *S. Sanguinis* 35.5% to 500 μg mL^{-1}. Inhibitions on gram-negative bacteria varied from 26.1% to 42.4% in ethanolic extract and 10.7% to 18.6% in hexanic extract for *E. coli*; as inhibition of *S. typhimurium* occurred from 9.3% to 61.1% in ethanolic extract and from 9.1% to 41.2% in hexane extract. Regarding phytochemical screening, the class of compounds observed qualitatively suggests the presence of saponins, flavonoids, triterpenoids and tannins.

1. Introduction

The species *Annona hypoglauca* belongs to the Annonaceae family, which presents about 112 genera, where almost 37% are in Brazil. One of the most common genera in this family is the genus *Annona*, which has great economic, nutritional and medicinal importance (Rinaldi, 2007). This medicinal use is due to the great diversity of bioactive substances present in species of the genus. The species *A. hypoglauca* is indicated bioactivity in its extracts and substances isolated from wood, as well as inhibition of colon and breast cancer cells and also presented antibacterial potential against the bacteria *Staphylococcus aureus* and *Enterococcus Faecalis* (Rinaldi et al., 2017; Rinaldi, 2007; Suffredini et al., 2007). More recent studies highlight the potential of *A. hypoglauca* seeds oil on gram negative and gram positive bacteria, filamentous and yeast type fungi, and potent inhibitor on the acetylcholinesterase enzyme (Santos et al., 2015). There are no chemical and

Please cite this article as: Carvalho Dos Santos R., Alves Chagas E., Melo Filho A.A., Takahashi J.A., Montero Fernandez I., Cardoso Chagas P., Estevam Ribeiro P.R., Goncalves Reis De Melo A.C., 2018, Bioactive extracts from annona hypoglauca, Chemical Engineering Transactions, 64, 289-294 DOI: 10.3303/CET1864049

bioactivity studies of the leaves of *A. hypoglauca*, for this reason, the aims of the study were to verify the phytochemical profile of *A. hypoglauca* leaf extracts collected in Roraima state, Brazil, as well as to analyze the bioactive potential Of these extracts on antioxidant by DPPH, cytotoxic activities on *Artemia salina*, antiacetylcholinesterase and antimicrobial against yeast (*Candida albicans*), filamentous fungi (*Aspergillus flavus* and *Fusarium proliferatum*) and gram positive (*Staphylococcus aureus* and *Streptococcus sanguinis*) and gram negative (*Escherichia coli* and *Salmonella tiphymurium*) bacteria.

2. Materials and Method

2.1 Collection, preparation of samples and obtaining extracts and phytochemical prospecting of extracts

The leaves of *A. hypoglauca* were collected in Mucajaí city, Roraima state, Brazil, these were taken to the Environmental Chemistry Laboratory, Nucleus of Research and Post-Graduation in Science and Technology of the University Federal of Roraima (NPPGCT/UFRR), sanitized and dried in an air circulating oven at 50 ^{o}C for 48 hours, then ground and sieved between 20-40 mesh (Santos et al., 2015). Phytochemical screening is a screening of the phytochemicals present in the unknown material, whose purpose is to qualitatively present the possible substances present in the extracts. Some specific developers may be used to detect some classes of secondary metabolites. Thus, with chloroform, hexane, ethanolic concentrated crude extracts, phytochemical prospecting was performed to identify chemical constituents, qualitatively, that is, tests for saponins, flavonoids, triterpenoids and tannins (MATOS, 1998).

2.1.1 Test for Tannins

A test tube containing the extract was taken and three drops of $FeCl_3$ alcohol solution was added. It was stirred well and any variation of its color or formation of abundant, dark precipitate was observed. It was compared to a blank test.

2.1.2 Test for Flavonoids

A test tube with the extract was made alkaline at pH 11, which showed a red-orange color indicating the presence of flavonoid constituent (MATOS, 1988).

2.1.3 Test for Saponinas

Using the test tube containing the extract, it was redissolved with 5 to 10 mL of distilled water and filtered in a test tube. The tube was shaken vigorously for 2 to 3 min and foam formation was observed indicating the presence of saponin heterosides (MATOS, 1988).

2.1.4 Test for Triterpenoids

The dried residue was removed from the test tube 2 to 3 times with 1 to 2 mL of chloroform. Then it was filtered with chloroform solution dropwise into a small glass funnel. Immediately after 1 mL of acetic anhydride was added, it was stirred gently and then 3 drops of concentrated H2 SO4 was added, which was gently stirred (MATOS, 1988).

2.2 Antioxidant activity of extracts of *A. hypoglauca*

The antioxidant activity has the purpose to verify the interaction of the extract of *A. hypoglauca* with DPPH (2,2-Diphenyl-1-picrylhydrazyl) and, thus, to reduce its free radicals. The concentration was developed from dilutions of a solution of higher concentration (1 g mL^{-1}), stock solution. The concentrations of the extract were 1000 µg mL^{-1}, 500 µg mL^{-1}, 250 µg mL^{-1}, 125 µg mL^{-1}, 50 µg mL^{-1}, and 10 µg mL^{-1}. In UV-Vis spectrophotometer at 515 nm. This method is described by Molyneux (2004). The standard routine was used as a positive control. The results can also be visualized in percentage of antioxidant activity (%AA).

2.3 Acetylcholinesterase inhibition activity of extracts of *A. hypoglauca*

This test consists of analyzing the bioactivity of the extract of *A. hypoglauca* on the acetylcholinesterase enzyme through the measurement of absorbance at 405 nm, which procedure is described by Frank and Gupta (2005) and Ellman et al. (1961).

2.4 Cytotoxic activity on *Artemia salina* extracts from *A. hypoglauca*

This test aims to analyze the possible antitumor potential of *A. hypoglauca* extract, is low cost and relatively simple. The bioactivity of the extracts at concentrations of 62.5 µg mL^{-1}, 125 µg mL^{-1}, 250 µg mL^{-1}, 500 µg mL^{-1} and 1000 µg mL^{-1}, is verified by counting live saline microcrustaceans and/or mortality, this methodology is described by Meyer et al. (1982).

2.5 Antimicrobial activity of extracts of *A. hypoglauca Filamentous fungi assay*

Filamentous fungi used in this test were *A. flavus* (CCT 4952) and *F. proliferatum* (CML 3287). DMSO was used for sample preparation and the concentration of sample in the assay was 250 mg mL^{-1}. Sabouraud broth was used for fungal growth. A spore suspension at a concentration of $5x10^{-5}$ spores mL^{-1} was used after

spores counting on a Neubauer chamber. The sample incubation time was 48 h after which absorbance was read at 490 nm on a microtitre plate reader. Data were processed using the Outlier method, Grubbs test with 95% significance level (Santos et al., 2015). The results were calculated as percent inhibition using the formula:

$$\% \text{ Inhibition} = 100 - AC1 - AC2 \times 100AH - AM$$

where AC1 = absorbance of the sample; AC2 = absorbance of control sample; AH = absorbance in the control of microorganism and AM = absorbance of the control of the culture medium.

Antibacterial and antifungal assay

E. coli (ATCC 25922), *S. tiphymurium* (ATCC 14028), *S. aureus* (ATCC 25923) and *S. sanguinis (*ATCC 49456*)* and *C. albicans* (ATCC 18804) were used in the assay following the procedures for Minimum Inhibitory Concentration (MIC) described by Zacchino and Gupta (2007). Concentrations assayed were 500, 250, 125, 62.5, 31.25, 15.6, and 3.9 µg mL^{-1}. Microorganism growth was measured in ELISA plate reader (492 nm) immediately after ending the experiment (0 h). They were incubated at 37 °C and read again after 24 h of experiments, ending the test. The results were calculated as percent inhibition using the formula:

$$\% \text{ Inhibition} = 100 - AC1 - AC2 \times 100AH - AM$$

where AC1 = absorbance of the sample; AC2 = absorbance of control sample; AH = absorbance in the control of microorganism and AM = absorbance of the control of the culture medium.

3. Results and Discussion

3.1 Phytochemical prospecting

Quantities of leaf and seed extracts and seed oil were reserved for other activities, such as phytochemical screening, separation, isolation and purification of chemicals, acetylcholinesterase, antimicrobial, antioxidant and cytotoxic inhibition analyzes. A class of compounds observed through phytochemical screening suggests a presence of saponins, flavonoids, triterpenoids and tannins. Note that this identification is only qualitative, not measured concentrations of the aforementioned compounds.

3.2 Determination of the antioxidant activity of extracts

The results of the quantitative evaluation of the percentage of antioxidant activity (%AA) of the extracts and of the positive control, rutin, are presented in Table 1, showing that all analyzed materials have DPPH radical sequestering activity, however the crude hexanic extract was the least active in the five concentrations. The crude ethanolic extract had antioxidant activity higher than 50% in 50 µg mL^{-1}, while the positive control reached 100% in this concentration.

Table 1: Antioxidant activity of crude ethanolic and hexane extracts and routine standard

	Ethanol Extract	Hexane Extract	Rutin
Concentration	**%AA**	**%AA**	**%AA**
1000 µg mL^{-1}	22	14	35
500 µg mL^{-1}	28	14	55
250 µg mL^{-1}	37	14	73
125 µg mL^{-1}	44	15	93
50 µg mL^{-1}	51	16	100

The percentage of antioxidant activity (% AA) corresponds to the amount of DPPH consumed by the antioxidant, and the amount of antioxidant needed to decrease the initial concentration of DPPH by 50% is named the efficient concentration (EC_{50}). The higher the DPPH consumption for a sample, the lower its EC_{50} and the higher its antioxidant activity (Sousa et al., 2007).

According to Melo et al. (2006), plant extracts with inhibition of the DPPH radical higher than 70% are considered to have a high antioxidant action, values of 60 to 70% inhibition are considered as moderate activity, whereas lower values are considered low antioxidant activity.

Rice-Evans et al. (1996) argue that for phenolic compounds to be considered as antioxidants and to exert biological activity at a low concentration they must have the ability to prevent, retard and prevent free radical-mediated oxidation or oxidation and the product formed after the reaction is stable.
This antioxidant capacity is expected and observed, as it is possible to note the presence of phenolic compounds in the ethanolic extract and not in the hexane extract in the phytochemical screening. Thus, this antioxidant activity, according to Saeidnia and Abdollahi (2013), is due to the main phenolic groups present in vegetables, such as flavonoids, anthocyanins, hydroxycinnamic acids and tannins, stilbenes, coumarins, among others. There is no literature as compared with the species under study, but it is observed that there is a relatively high value because it is a crude extract, which is a complex mixture of chemical and potentially antioxidants.

3.3 Cytotoxic activity on *Artemia salina* extracts from *A. hypoglauca*

The verification of the behavior of the crude ethanolic and hexane extracts against *A. salina*, as well as the mortality expressed in percentage are described in Table 2.

Table 2: Mortality (%) of A. salina with ethanol and hexane extracts

Mortality (%)	1000 µg mL^{-1}	500 µg mL^{-1}	250 µg mL^{-1}	125 µg mL^{-1}	62.5 µg mL^{-1}
Ethanol extract	17%	27%	10%	13%	13%
Hexanic extract	30%	0	0	0	0

Thus, according to Meyer et al. (1982) it is possible to verify the biological activity of extracts, fractions and even substances obtained from plants against the microcrustacean *A. salina*, when it presents DL_{50} (lethal dose) less than or equal to 1000 µg mL^{-1}. Thus, the toxicity tests employing this microcrustacean indicate a maximum concentration of 1000 µg mL^{-1}. Following this toxicity criterion Dolabela (1997) presents a classification based on LD_{50} concentrations, where: LD_{50} less than 80 µg mL^{-1} were considered highly toxic, between 80 µg mL^{-1} and 250 µg mL^{-1} moderately toxic and above 250 µg mL^{-1} with low toxicity or non-toxic.
It is observed, therefore, that the ethanolic and hexanic extracts of *A. hypoglauca* leaves do not present toxicity, since they are above the values presented by Meyer et al. (1982) and Dolabela (1997). However, according to Santos Pimenta et al. (2003), the wood of *A. hypoglauca* presents such cytotoxicity against the crustacean.
According to the LD_{50}, 4,843.81 µg mL^{-1} of the crude ethanolic extract is required to cause mortality in 50% of microcrustaceans, this is, LD_{50}> 250 µg mL^{-1}. While in the crude hexane extract it is necessary 1,779.50 µg mL^{-1} to cause mortality in 50% microcrustaceans, that is, LD_{50}> 250 µg mL^{-1}. In this way, crude ethanolic and hexane extracts are non-toxic.

3.4 Acetylcholinesterase inhibition activity

Table 3 shows the percentages of inhibition of AChE by hexane, ethanolic, chloroform extracts from *A. hypoglauca* leaves.

Table 3: Inhibition of acetylcholinesterase by ethanol, hexane and chloroform extracts

Sample	Extract	Inhibition (%)	Inhibition Intensity
AHEE	Ethanol	High variation	-
AHEH	Hexane	59.76	Potent
AHEC	Chloroform	88.47	Potent

OBS: High Variation: the values obtained, after repeating the tests 3 times, were not convergent. This may have occurred because of the staining of the samples or the presence of substances that are affected by the reagents.

It is observed that the highest inhibitory rate was that of the chloroform extract which inhibited 88.47% of this enzyme, followed by the hexane extract with almost 60% inhibition. While *A. hypoglauca* seeds oil showed moderate inhibition. According to Vinutha et al. (2007), they classified their extracts of medicinal plants as potential inhibitors of AChE, such as potent inhibitors (>50% inhibition), moderate inhibitors (30-50% inhibition) and weak inhibitors (<30% inhibition).

3.5 Antimicrobial activity of extracts

The bioactivity of the extracts ethanol, hexane and chloroform is observed in Table 4.

Table 4: MIC bioassay table on Gram positive and Gram negative bacteria

Gram positive bacteria					
S. aureus			***S. sanguinis***		
AHEE	**AHEH**	**AHEC**	**AHEE**	**AHEH**	**AHEC**
21.65% (125 μg mL^{-1})	100.00% (500 μg mL^{-1})	66.83% (500 μg mL^{-1})	35.45% (500 μg mL^{-1})	100.00% (500 μg mL^{-1})	31.00% (250 μg mL^{-1})
Gram negative bacteria					
E. coli			***S. tiphymurium***		
AHEE	**AHEH**	**AHEC**	**AHEE**	**AHEH**	**AHEC**
42.36% (9.375 μg mL^{-1})	18.58% (9.375 μg mL^{-1})	36.54% (500 μg mL^{-1})	61.07% (250 μg mL^{-1})	41.19% (9.375 μg mL^{-1})	58.28% (9.375 μg mL^{-1})

The highest inhibition of *S. aureus* by ethanolic extract in about 22% to 125 μg mL^{-1} and 100% inhibition of *S. aureus* in 500 μg mL^{-1} of hexane extract and about 67% in 500 μg mL^{-1} chloroform extract. Bioactivity on *S. sanguinis* was higher in the hexane extract at 100% at 500 μg mL^{-1}, whereas inhibition at 500 μg mL^{-1} of ethanolic extract was 35.45% and inhibition of this gram positive bacteria at 250 μg mL^{-1} chloroform extract was 31.00%. Regarding the inhibitory potential of gram negative bacteria, inhibition values did not reach 100%, but there was a good inhibition of *E. coli* (42.36%) in 9.375 μg mL^{-1}. The inhibition of *S. tiphymurium* was 41.19% and 58.28% of hexane and chloroform extracts, both at 9.375 μg mL^{-1}. There are only reports of bioactivities of extracts or chemical compounds isolated from the bark (Rinaldi, 2007) and of vegetable oil (Santos et al., 2015), so there are no reports in the literature regarding the bioactivity of leaf extracts of *A. hypoglauca* to Gram positive and Gram negative bacteria, as well as filamentous fungi and yeast, being, therefore, an unpublished work.

3.6 Bioassay of MIC for *C. albicans*

The biological activity on the yeast fungus, *C. albicans*, presented the best results in the concentration of 9.375 μg mL^{-1} for the extracts ethanol (92.56%), hexane (96.95%) and chloroform (97.99%) of the leaves, being superior Miconazole (91.51%) and Nystatin (91.26%) in the same concentration. There was inhibition of yeast at all concentrations, ranging from 89.25% to 97.99%. Inhibition of microorganisms is also observed in the filamentous fungi *A. flavus* and *F. proliferatum* (Table 5).

Table 5: MIC bioassay on filamentous fungi A. flavus and F. proliferatum

% Inhibition	**AHEE**	**AHEH**	**AHEC**
A. flavus	0	59.49%	40.07%
F. proliferatum	0	40.00%	22.66%

The literature reports the potential of *A. hypoglauca* seeds oil on *C. albicas*, *A. flavus* and *F. proliferatum* (Santos et al., 2015). Further study of the bioactive potential on tumor cells can be observed for the wood of this species under study (Rinaldi, 2007). Several studies have shown the antimicrobial potential of the genus *Annona* (Almeida et al., 2004; El-Chaghaby et al., 2004; Padmaja et al., 1995).

4. Conclusions

A. hypoglauca presents antioxidant potential in its ethanolic extract, exceeding 50% reduction of the DPPH radical in 50 μg mL^{-1} concentration of the above-mentioned extract, whereas in the concentrations of hexane extract do not reach 20% reduction of the DPPH radical. Another biological activity was cytotoxic, but the highest value was 27% mortality of *A. salina* in 500 μg mL^{-1} of ethanolic extract and 30% of mortality in 1000 μg mL^{-1} of hexane extract, thus the ethanolic extract was higher than hexane, but both concentrations are considered to be low toxicities or even non-toxic.

As for bioactivity on the acetylcholinesterase enzyme presented 59.76% inhibition using the hexane extract and 88.47% of the chloroform extract, which are considered potent inhibitors. However, it was not possible to quantify the inhibitory potential of the enzyme for the ethanolic extract.
The antimicrobial activity on *A. flavus* and *F. proliferatum* showed that the ethanolic extract had no inhibition on the above mentioned microorganisms, but the greatest inhibitions were on hexane and chloroform extracts. As for yeast *C. albicans* showed excellent inhibition at all concentrations, ranging from 89.25% to 97.99%. The antibacterial activity had its potential in the extracts hexane (100%) and chloroform (66.83%) on the *S. aureus*; and the hexane extract inhibited 100% *S. sanguinis*; The highest inhibitory value was observed by the ethanolic extract (42.36%) on *E. coli*; The extracts ethanol (61.07%), hexane (41.19%) and chloroform (58.28%) presented potential on *S. tiphymurium*.

Acknowledgments

To CAPES for scholarship, to CCT (Sao Paulo) and CML (UFLA, MG) for donation of microorganisms, to CNPq for the financial support and to Research Oleoquímicos Group UFRR.

Reference

Almeida J.R.G.S, Araújo C.S., Pessoa C.Do Ó, Costa M.P., Pacheco A.G.M., 2014, Atividade Antioxidante, Citotóxica e Antimicrobiana de Annona vepretorum Mart. (Annonaceae). Rev. Bras. Frutic. 36, 258-264.

Dolabela M.F., 1997, Triagem in vitro para atividade antitumoral e anti-Tripanossoma cruzi de extratos vegetais, produtos naturais e substancias sintéticas. [Master's thesis] Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, 128p.

El-Chaghaby G.A., Ahmad A.F., Ramis E.S., 2014, Evaluation of the antioxidant and antibacterial properties of various solvents extracts of Annona squamosa L. leaves. Arab. J. Chem. 7, 227-233.

Ellman G.L., Courtney K.D., Jr Andres V., Feather-Stone R.M., 1961, A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-95.

Frank B., Gupta S., 2005, A review of antioxidants and Alzheimer's disease. Ann. Clin. Psychiatry 17, 269-286.

Matos F.J.A., 1988, Introdução à fitoquímica experimental. Fortaleza: UFC.

Melo, E.A., Maciel M.I.S., Lima V.L.A.G., Leal F.L.L., Caetano A.C.S., Nascimento R.J., 2006, Capacidade antioxidante de hortaliças usualmente consumidas. Ciênc. Tecnol. Aliment. 26, 639-644.

Meyer B.N., Ferrigni N.R., Putnan J.E., Jacobsen L.B., Nichols D.E., McLaughlin J.L., 1982, Brine shrimp: A convenient general bioassay for active plant constituents. J. Med. Plant Res. 45, 31-34.

Molyneux P., 2004, The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol. 26, 211–219.

Padmaja V., Thankamany V., Hara N., Fujimoto Y., Hisham A., 1995, Biological activities of Annona glabra. J. Ethnopharmacol. 48, 21-24.

Rice-Evans C.A., Miller N.J., Paganga G., 1996, Structure antioxidant activity relationship of flavonoids and phenolic acid. Free Radic. Biol. Med. 20, 933-956.

Rinaldi, M.V.N., 2007, Avaliação da atividade antibacteriana e citotóxica dos alcaloides isoquinolínicos de Annona hypoglauca Mart. Programa de Pós-graduação em Fármaco e Medicamentos, Área de Insumos Farmacêuticos, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, 125p, São Paulo.

Rinaldi M.V.N., Díaz I.E.C., Suffredini I.B., Moreno P.R.H., 2017, Alkaloids and biological activity of beribá (Annona hypoglauca). Rev. Bras. Farmacogn. 27, 77-83.

Saeidnia S., Abdollahi M., 2013, Toxicological and pharmacological concerns on oxidative stress and related diseases. Toxicol. Appl. Pharmacol. 273, 442-455.

Santos Pimenta L.P.S, Pinto G.B., Takahashi J.A., Silva L.G.F., Boaventura M.A.D., 2003, Biological screening of Annonaceous Brazilian Medicinal Plants using Artemia salina (Brine Shrimp Test), Phytomedicine 10, 209-212.

Santos R.C., Melo Filho A.A., Chagas E.A., Takahashi J.A., Ferraz V.P., Costa A.K.P., Melo A.C.G.R., Montero I.F., Ribeiro P.R.E., 2015, Fatty acid profile and bioactivity from Annona hypoglauca seeds oil. Afr. J. Biotechnol. 14, 2377-2382.

Vinutha B., Prashanth D., Salma K., Sreeja S.L., Pratiti D., Padmaja R., Radhika S., Amit A., Venkateshwarlu K., Deepak M., 2007, Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. J. Ethnopharmacol.109, p.359-363.

Zacchino A.S., Gupta M.P., 2007, Manual de técnicas in vitro para la detección de compuestos antifúngicos. Corpus Editorial y Distribuidora: Rosario, v. 85, 176.