Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Chemical Characterization and Cytoprotective Effect of the Hydroethanol Extract from *Annona coriacea* Mart. (Araticum)

José G. A. S. Júnior, Henrique D. M. Coutinho[1], Ticiana C. C. Boris, Janyketchuly S. Cristo, Nara L. F. Pereira, Fernando G. Figueiredo[2], Francisco A. B. Cunha[1], Pedro E. A. Aquino[3], Polyana A. C. Nascimento[2], Francisco J. C. Mesquita, Paulo H. F. Moreira, Sáskia T. B. Coutinho[2], Ivon T. Souza, Gabriela C. Teixeira[4], Najla M. N. Ferreira, Eleonora O. Farina, Cícero M. G. Torres[5], Vanderlan N. Holanda[5], Vandbergue S. Pereira[3], Maria I. F. Guedes

Department of Biotechnology, Ceará State University, Fortaleza (CE), [1]Department of Biological Chemistry, Regional University of Cariri, Crato (CE), [2]Department of Biomedicine, Leão Sampaio College, Juazeiro do Norte (CE), [3]Department of Medical Microbiology, Federal University of Ceará, Fortaleza (CE), [4]Department of Medicine, Medical School of Juazeiro do Norte - Estacio, Juazeiro do Norte (CE), [5]Department of Health, University of Fortaleza – UNIFOR, Fortaleza (CE) Brazil

ABSTRACT

Introduction: *Annona coriacea* Mart. (araticum) is a widely distributed tree in the cerrado. Its value is attributed principally to the consumption of its fruit which possesses a large nutritive potential. The objective was to identify the chemical profile and evaluate the antimicrobial and cytoprotective activity of the hydroethanol extract of *A. coriacea* Mart. (HEAC) leaves against the toxicity of mercury chloride. **Materials and Methods:** The characterization of components was carried out using high-performance liquid chromatography (HPLC). The minimum inhibitory concentration (MIC) was determined by microdilution method in broth with strains of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. For evaluation of the modulatory and cytoprotective activity of aminoglycoside antibiotics (gentamicin and amikacin) and mercury chloride ($HgCl_2$), the substances were associated with the HEAC at subinhibitory concentrations (MIC/8). **Results and Discussion:** The HPLC analysis revealed the presence of flavonoids such as Luteolin (1.84%) and Quercetin (1.19%) in elevated concentrations. The HEAC presented an MIC ≥512 μg/mL and significant antagonistic action in aminoglycosides modulation, and it also showed cytoprotective activity to *S. aureus* (significance $P < 0.0001$) and *E. coli* (significance $P < 0.05$) bacteria against the mercury chloride heavy metal with significance, this action being attributed to the chelating properties of the flavonoids found in the chemical identification. **Conclusions:** The results acquired in this study show that the HEAC presents cytoprotective activity over the tested strains *in vitro* and can also present antagonistic effect when associated with aminoglycosides, reinforcing the necessity of taking caution when combining natural and pharmaceutical products.

Key words: Aminoglycosides, *Annona coriacea* Mart., antimicrobial, flavonoids, mercury chloride

SUMMARY

- The hydroalcoholic extract of *A. coriacea* Mart. presents *in vitro* cytoprotective activity against the toxic effect of Hg.

Abbreviations Used: HPLC-DAD: High-performance liquid chromatography with a diode array detector; MIC: Minimum inhibitory concentration; DMSO: Dimethyl sulfoxide

Correspondence:
Dr. Henrique D. M. Coutinho,
Departamento de Química Biológica,
Universidade Regional Do Cariri, Av. Cel. Antônio Luiz, 1161, CEP: 63105-000, Crato, CE, Brazil.
E-mail: hdmcoutinho@gmail.com
DOI: 10.4103/0974-8490.188882

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Research for the production of new pharmaceuticals from natural products involve diverse fields of knowledge and various methods of analysis, from the collection and identification of materials, to biological triage and pharmacological tests, determination of the active principle and mechanism of action by which the pharmacological effect is exerted.[1]

Annonaceae is a large family of plants in the cerrado region of Brazil, containing approximately 27 genera and 290 species, and including a great variety of exotic fruits.[2] *Annona coriacea* Mart. popularly known as "araticum" is a widely distributed tree in the cerrado. Its value is attributed principally to the consumption of its fruit which possesses a large nutritive potential and are appreciated for its pulp commonly consumed *in natura* or in the form of sweets, jams, juices, liqueurs and pies, in addition to its pharmacological properties associated with different parts of the plant.[3]

Cite this article as: Junior JG, Coutinho HD, Boris TC, Cristo JS, Pereira NL, Figueiredo FG, *et al*. Chemical characterization and cytoprotective effect of the hydroethanol extract from *Annona coriacea* Mart. (Araticum). Phcog Res 2016;8:253-7.

The necessity of research with natural products motivates the substitution of synthetic chemical substances for natural materials of low cost and of easy access, once the benefits of the research can contribute to the conservation of natural vegetation and sustainability of semi-natural production systems.[4]

The anthropic aggression to the environment has been considered in many forms, with the improper use of mercury normally being shown as one of the more representative examples of what man can do to natural cycles, due to population growth and intensification of human activities involving these elements, the concentration of heavy metals has principally increased in bodies of water, where these levels threaten biotic aquatic and terrestrial organisms, for example, man himself.[5] Mercury is present in the environment in the chemically oxidized form or as metallic mercury, in its elementary state it emerges from volcanoes of the Earth's crust degradation; however, the artificial forms are more diversified than those considered natural.[6]

Metals act as selective agents of resistant bacteria to antibiotics, in contrast to antibiotics, metals suffer a greater difficulty in being naturally degraded; however, microorganisms have developed diverse resistance mechanisms in response to toxic metals, bacterial resistance to toxic metals such as mercury is one of the most studied resistance mechanisms. In Gram-negative bacteria, especially those belonging to the *Escherichia coli* species, the presence of genetic resistance determinants to mercury have been described, which makes a promising alternative to bioremediation processes.[7]

Various genera from the Enterobacteriaceae family, including the *E. coli* (Theodor Escherich) and *Pseudomonas aeruginosa* (Schroeter) species, nonfermenting Gram-negative bacilli have been considered problematic due to the increase in resistance rates to antimicrobials.[8,9] The genus *Staphylococcus aureus* (Rosenbach) is also greatly associated with human infections and are classified as coagulase-positive strains.

This study had its objective to identify the chemical profile and evaluate the antimicrobial and cytoprotective activity of the hydroalcoholic extract from *A. coriacea* Mart. (Araticum) leaves against mercury chloride.

MATERIALS AND METHODS

Plant material *Annona coriacea* Mart.

The botanical material was collected in the Cariri in Crato, CE, Brazil. After collected a voucher, specimen was compared with voucher Herbarium Caririense Dárdano de Andrade-Lima, Regional University of Cariri-URCA and identified as belonging to *A. coriacea* Mart. species.

Concentration of the hydroethanol extract

For preparation of the hydroethanol extract, 500 g of leaves were collected and cut in pieces which were immersed in equal parts of distilled water and ethanol for 72 h at room temperature. After this period, it was filtered and concentrated on rotaevaporator (Q-344B - Quimis model, Brazil), to evaporate the ethanol. Dehydration was performed in Bath Mary (model Q214M2 - Quimis, Brazil), then the material was placed in freezer for freezing and freeze-dried in (Líotop equipment, Model L101). The extract was packed in amber glass and stored at −20°C temperature. The yield of the extract was 3.53%.

Bacterial material

Three microbial standard lineages from the American Type Culture Collection were used: *S. aureus* ATCC 25923; *P. aeruginosa* ATCC 25853; *E. coli* ATCC 9027. To evaluate the modulatory activity of the natural products, the following multiresistant bacterial isolates were used: *S. aureus* SA 358; *P. aeruginosa* PA 03; *E. coli* EC 06 [Table 1]. The strains were maintained using Heart Infusion Agar (HIA, Difco Laboratories Ltda.). Before the tests, the cells were cultivated for 24 h in Brain Heart Infusion (BHI, Difco Laboratories Ltda.).

Quantification of compounds by means of high-performance liquid chromatography-diode array detector

High-performance liquid chromatography-diode array detector (HPLC-DAD) was carried out with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A DAD and LC solution 1.22 SP1 software.

Reverse phase chromatographic analyses were carried out under gradient conditions utilizing a C18 column (4.6 mm × 150 mm) loaded with 5 um diameter particles; the mobile phase was water containing 2% formic acid (A) and acetonitrile (B), and the composition gradient was the following: 17% of B until 10 min and altered to obtain 20%, 30%, 50%, 60%, 70%, 20%, and 10% of B at 20, 30, 40, 50, 60, 70, and 80 min, respectively, following the method described by Kamdem *et al*. (2013) with slight modifications. The Araticum (*A. coriacea* Mart.) hydroalcoholic extract was analyzed to a concentration of 15 mg/mL. The presence of antioxidant compounds was investigated, that is, gallic acid, chlorogenic acid, caffeic acid, coumaric acid, catechin, epicatechin, quercetin, quercitrin, rutin, and luteolin. The identification of these compounds was made by comparison of their retention times and ultraviolet absorbance spectrum with commercial standards.

The rate of flux was 0.6 mL/min, injection volume was 50 µL and wavelength was 271 nm for gallic acid, 276 nm for coumaric acid, 280 nm for catechin and epicatechin, 327 nm for caffeic and chlorogenic acid, and 365 nm for quercetin, quercitrin, rutin, and luteolin. The samples and mobile phases were filtered through the 0.45^ M membrane filter (Millipore) and it was then degassed by ultrasonic bath before use. The standard reference stock solutions were prepared in the mobile phase of the HPLC in a range of concentrations of 0.025–0.300 mg/ml for catechin, epicatechin, quercetin, quercitrin, rutin, and luteolin; 0.030–0.350 mg/ml for caffeic, coumaric, chlorogenic, and gallic acid. The chromatography peaks were confirmed by comparison of the retention time with standard references and by DAD spectrum (200–500 nm). Calibration curve for catechin:

Table 1: Bacterial antibiotic resistance profile

Bacterium	Source	Resistance profile
S. aureus ATCC 25923	-	-
P. aeruginosa ATCC 25853	-	-
E. coli ATCC 9027	-	-
S. aureus SA 358	Surgical wound	Oxa, Gen, Tob, Ami, Neo, Para, But, Sis, Net
P. aeruginosa PA 03	Nasal discharge	Cf, Cfp, Cfd, Caz, Lv, Im Mero, Pip
E. coli EC 06	Urine	Cf, Ca, Clx, Amp, Nor, Lm, Cip, Lv, Of, Ampisul

Amp: Ampicilina; Ampisul: Ampicilina-sulbactam; Ami: Amicacina; Amox: Amoxilina; Ca: Cefadroxil; Cfc: Cefaclor; Cf: Cefalotina; Clx: Cefalexina; Caz: Ceftazidima; Cfp: Cefepimo; Cfd: Ceftaridima; Cip: Ciprofloxacin; Im: Imipenem; Can: Canamicina; Lm: Lomefloxacina; Lv: Levofloxacina; Tob: Tobramicina; Of: Ofloxacin; Oxa: Oxacilina; Gen: Gentamicina; Mero: Meropenen; Nor: Normofloxacina; Neo: Neomicina; Para: Paramomicina; Pip: Piperacilina; But: Butirosina; Sis: Sisomicina; Net: Netilmicina; *S. aureus: Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa; E. coli: Escherichia coli*

Y = 12509x + 1,263.8 (r = 0.9995); epicatechin: Y = 11958x + 1,309.1 (r = 0.9997); gallic acid: Y = 11875x + 1,253.4 (r = 0.9999); caffeic acid: Y =13165x + 1,185.3 (r = 0.9996); chlorogenic acid: Y = 12603x + 1,274.9 (r = 0.9998); rutin: Y = 12657x + 1,378.9 (r = 0.9999); quercetin: Y =13591x + 1,183.7 (r = 0.9995); quercitrin: Y = 11783x + 1,263.8 (r = 0.9999); coumaric acid: Y = 12574x + 1,261.8 (r = 0.9993); and luteolin Y = 13509x + 1,267.5 (r = 0.9997). All the chromatography operations were carried out in room temperature and in triplicates. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope from three independent analysis curves.[10] The LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

Preparation of the original solution and test solutions

In preparing the initial solution, the extract was solubilized in dimethyl sulfoxide (DMSO), BEING as observed following proportions: 10 mg of extract solubilized in 1 ml of DMSO, obtain an initial concentration of 10 mg/ml. Then, this solution was diluted in distilled water reaching extract concentration of 1024 µg/mL (pH 6.76; 2,138 mOsm/kg H_2O) and reducing DMSO concentration paragraph 10%, and from this, effected If serial dilutions 1:1 during the microdilution test, yielding to extract concentrations ranging 512-8 µg/mL DMSO and paragraph 5% concentration.

Determination of the minimum inhibitory concentration and modulation of aminoglycosides

The minimum inhibitory concentrations (MIC) of the *A. coriacea* Mart. hydroalcoholic extract (HEAC) and fractions, were determined by microdilution tests using suspensions of 10^5 CFU/mL of standard strains, and the HEAC in the initial concentration of 1.024 µg/mL.[11,12] For evaluation of aminoglycoside antibiotic modulatory activity (gentamicin and amikacin), the solutions were prepared with the addition of sterile distilled water to obtain a concentration corresponding to 1.024 µg/mL. A volume of 100 µL of each antibiotic solution was serially diluted in wells containing BHI Broth culture medium – BHI at 10% (pH 6,85; 404 mOsm/kg H_2O) and the multiresistant inoculum suspension, with the HEAC at subinhibitory concentration (MIC/8).[13] The culture medium final concentration of antibiotics were of 512-0.5 µg/mL. The plates were incubated for 24 h at 35°C ± 2°C and the activity was evidenced by the use of Resazurin sodium.

Cytoprotective activity against mercury chloride test

For evaluation of the protective effect of HEAC and fractions to mercury chloride ($HgCl_2$), a modulation using sub-inhibitory concentrations of the products were carried out, 10^5 CFU/mL suspensions of *S. aureus* ATCC 25923 and *E. coli* ATCC 9027 in BHI Broth medium – BHI and one concentration of mercury chloride (271.52 g/mol) varying from 10 mM to 0.0048 mM. The microdilution plates were incubated for 24 h at 37°C. The minimum bactericidal concentration was determined using the lowest concentration capable of inhibiting the growth of microorganisms, using petri dishes with BHI Agar – BHI for transferring solutions incubated for 24 h at 37°C in microdilution wells. The natural product was evaluated in relation to the control experiment with only mercury. The petri dishes were incubated in an incubator at approximately 37°C, and the reading was taken after 24 h of incubation.[14]

Statistical analysis

The graphs were produced by the GraphPad Prism 5 software. Statistical analysis was done using a two-way ANOVA followed by the Bonferroni test.[15]

RESULTS AND DISCUSSION

The *A. coriacea* Mart. (Araticum) hydroalcoholic extract in the HPLC analysis revealed the presence of gallic acid (tR = 9.97 min; peak 1), catechin (tR = 15.03 min; peak 2), chlorogenic acid (tR = 21.54 min; peak 3), caffeic acid (tR = 24.98 min; peak 4), coumaric acid (tR = 31.49 min; peak 5), epicatechin (tR = 34.67 min; peak 6), rutin (tR = 42.15 min; peak 7), quercitrin (tR = 49.16 min; peak 8), quercetin (tR = 50.02 min; peak 9), and luteolin (tR = 56.73 min; peak 10) [Figure 1 and Table 2].

Was reported in the phytochemical identification of the genus *Annonaceae* the predominance of the alporphine and oxoalporphine alkaloids, besides these, constituents such as polyphenols, essential oils, terpenes and aromatic substances are also found in the family's representatives.[16]

The alporphine alkaloids possess great representation in the isoquinolines groups and were only found in plants of the *Annonaceae*, *Berberidaceae*, *Lauraceae*, *Magnoliaceae*, *Menispermaceae*, *Monimiaceae*, *Ranunculaceae* (Ranales order), *Papaveraceae* (Rhoedales order), and *Rhamnaceae* (Rhamnales order)[17] families. The presence of the alporphine alkaloids in the *Annonaceae* family is constant in species of the genus *Annona* with

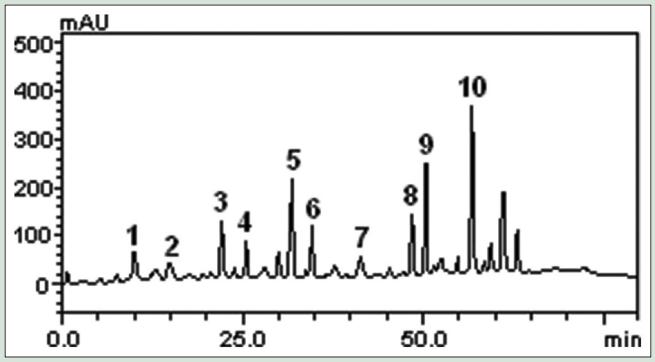

Figure 1: Representative high-performance liquid chromatography profile of Araticum (*Annona coriacea* Mart.) hydroethanolic extract. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), coumarin (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), quercetin (peak 9), and luteolin (peak 10)

Table 2: Components of araticum (*Annona coriacea* Mart.) hydroethanolic extract

Compounds	*A. coriacea* Mart.		µg/mL	
	mg/g	Percentage	LOD	LOQ
Gallic acid	2.97±0.02	0.29	0.009	0.030
Catechin	2.64±0.01	0.26	0.014	0.047
Chlorogenic acid	5.85±0.01	0.58	0.021	0.070
Caffeic acid	3.09±0.03	0.30	0.025	0.083
Coumarin acid	10.72±0.02	1.07	0.017	0.056
Epicatechin	5.81±0.02	0.58	0.008	0.028
Rutin	2.59±0.01	0.25	0.029	0.096
Quercitrin	6.11±0.03	0.61	0.007	0.024
Quercetin	11.93±0.01	1.19	0.011	0.037
Luteolin	18.47±0.03	1.84	0.031	0.102

Results are expressed as mean±SD of three determinations. Averages followed by different letters differ by Tukey test at P<0.05. SD: Standard deviations; LOD: Limit of detection; LOQ: Limit of quantification; *A. coriacea: Annona coriacea*

reports of the isolation and identification in leaves.[18] In this way, the following compounds were found in the chemical identification of the *A. coriacea* Mart. hydroalcoholic extract [Table 2].

Natural products of vegetable and animal origin can alter the effect of antibiotics, be it increasing or decreasing the antibiotic activity, and can be denominated modulators of antibiotic activity.[12,19]

Alkaloids between the already isolated alkaloids of the *Annona* genus, anonaine and estefarina presented antimicrobial activity against *S. aureus* and *Bacillus cereus*, glaziovine presented activity over *E. coli*. Anolobina, nornantenina, and lanuginosina are also found and presented antimicrobial activity over the *Salmonella typhimurium* bacteria.[20]

The presented results for the MIC of the HEAC for all the standard and multiresistant species were ≥512 μg/mL. The graphs below show the evaluation of the modulatory activity of bacterial resistance to aminoglycosides utilizing amikacin and gentamicin combined with the HEAC, determined after the MIC test by microdilution against the multiresistant and standard lineages of *E. coli* [Figure 2], *S. aureus* [Figure 3] and *P. aeruginosa* [Figure 4].

According to the results obtained on the cytoprotective activity [Figure 5], the HEAC presents significant cytoprotection $P < 0.0001$ and $P < 0.05$ in *S. aureus* and *E. coli* strains against mercury chloride ($HgCl_2$). The data obtained indicates the product as a promising source in the combat to heavy metals, presenting itself as protectors of prokaryotic beings.

Mercury has a large cytotoxic effect over various types of cells, principally those of the immune system, being capable of inducing immunosuppression in many animal species.[21] In contrast to antibiotics, metals have a great difficulty in suffering natural degradation, thus increasing the importance of their role as bactericidal agents especially in bacteria that are resistant to antibiotics.

Flavonoid compounds can act with an antioxidant activity, reacting with free radicals and also as metal chelators.[22] The most abundant flavonoid classes in the *Annona* genus are the flavonols and its glycosylated derivatives, being present both the glycosylated O-glycosides and C-glycosides, within these, it is possible to identify the presence of O-glycosides of quercetin, isorhamnetin, kaempferol, and luteolin in leaves of various *Annona* genera.[23]

The presence of these may justify the possible cytoprotective effect observed in the tests since the HPLC analysis demonstrated the presence of flavonoids such as luteolin (1.84%) and quercetin (1.19%) in elevated concentrations ($P < 0.05$).

The antioxidant action of the flavonoids is mainly through the presence of the large number of hydroxyls in the B ring.[24] The cytoprotective property against mercury chloride is directed by the chelation which can maintain the metal in solution, making it unavailable through precipitation or the complex formed.[25]

The extremely rapid combinations of metals with free radicals OH and O_2 through the Fenton or Haber-Weiss reaction, stimulate the presence of hydroxyl radicals.[26,27] The hydroxyl group can inactivate various proteins

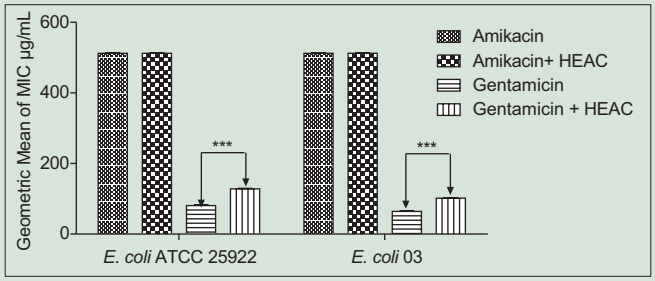

Figure 2: Minimum inhibitory concentration of aminoglycosides in the presence and absence of *Annona coriacea* Mart. extract in a concentration CIM/8, against strains of *Escherichia coli*. HEAC: hydroalcoholic extract of *Annona coriacea* Mart. ***Statistically significant value of $P < 0.0001$

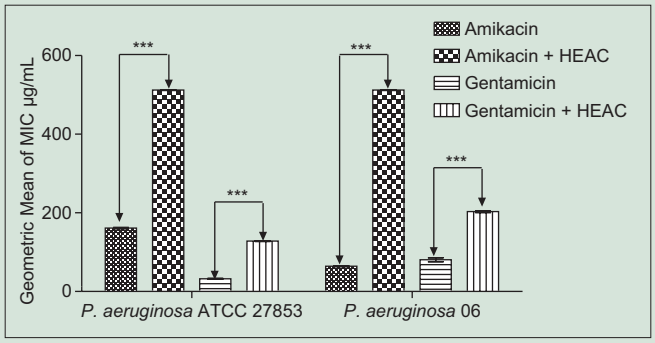

Figure 4: Minimum inhibitory concentration of aminoglycosides in the presence and absence of *Annona coriacea* Mart. extract in a concentration CIM/8, against strains of *Pseudomonas aeruginosa*. HEAC: hydroalcoholic extract of *Annona coriacea* Mart. ***Statistically significant value of $P < 0.0001$

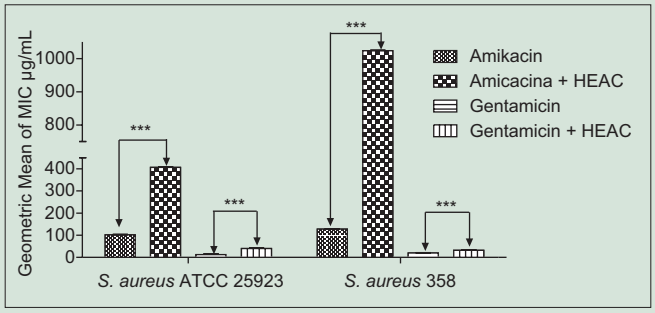

Figure 3: Minimum inhibitory concentration of aminoglycosides in the presence and absence of *Annona coriacea* Mart. extract in a concentration CIM/8, against strains of *Staphylococcus aureus*. HEAC: hydroalcoholic extract of *Annona coriacea* Mart. ***Statistically significant value of $P < 0.0001$

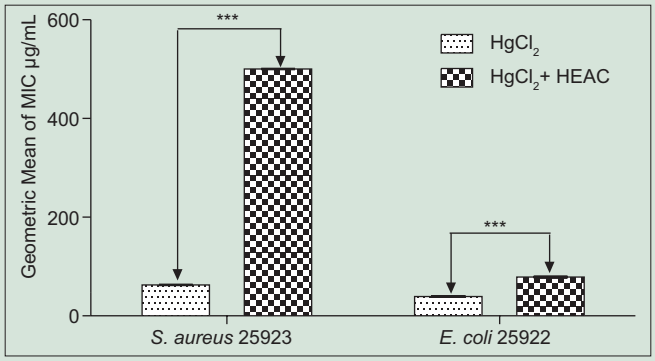

Figure 5: Minimum bactericidal concentration of mercuric chloride in the presence and absence of *Annona coriacea* Mart. extract in a concentration minimum inhibitory concentration/8, against strains of *Staphylococcus aureus* and *Escherichia coli*. HEAC: hydroalcoholic extract of *Annona coriacea* Mart. $HgCl_2$: Mercuric chloride. ***Statistically significant value of $P < 0.0001$

by oxidizing their sulfhydryl (-SH) groups and disulfide bridges (-SS), they can also initiate lipoperoxidation provoking the disruption of the cellular matrix.[28]

Quercetin can inhibit the process of free radical formation through interaction with superoxide ions, the formation of hydroxyl radicals through chelating metal ions and inhibiting lipid peroxidation by reacting with peroxy radicals of lipids, protecting the cell against the cytotoxic action of metals.[29,30]

CONCLUSIONS

The results obtained in this study show that the *A. coriacea* Mart. hydroalcoholic extract can be used as a source of natural products derived from this plant, once cytoprotective activity over tested strains is presented *in vitro*, and can also present an antagonistic effect when associated with aminoglycosides, reinforcing the necessity of caution when combining natural products and pharmaceuticals.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sci 2005;78:431-41.
2. Formagio AS, Vieira MC, Volobuff CR, Silva MS, Matos AI, Cardoso CA, *et al. In vitro* biological screening of the anticholinesterase and antiproliferative activities of medicinal plants belonging to *Annonaceae*. Braz J Med Biol Res 2015;48:308-15.
3. Barbalho SM, Goulart RA, Farinazzi-Machado MV, Souza FS, Bueno MC, Guiguerer PL, *et al. Annona* sp.: Plants with multiple applications as alternative medicine – A review. Curr Bioact Compd 2012;8:277-86.
4. Chou CH, Reigosa MJ, Pedrol N, González L. Allelopathy: A Physiological Process with Ecological Implications. Dordrecht: Springer; 2006. p. 637.
5. Bertolazi AA, Canton GC, Azevedo IG, Cruz ZM, Soares JM, Santos WO, *et al.* The role of ectomycorrhiza in heavy metals bioremediation in soil. Natureza Online 2010;8:24-31.
6. Azevedo FA. Toxicologia do mercúrio. São Carlos/SP: Editora Ri Ma; 2002. p. 272.
7. Zeyaullah M, Nabi G, Malla R, Ali A. Molecular studies of *E. coli* mercuric reductase gene (merA) and its impact on human health. Nepal Med Coll J 2007;9:182-5.
8. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: A clinical update. Clin Microbiol Rev 2005;18:657-86.
9. Slama TG. Gram-negative antibiotic resistance: There is a price to pay. Crit Care 2008;12 Suppl 4:S4.
10. Kamdem JP, Olalekn EO, Hassan W, Kade J, Yetunde O, Boligon AA, *et al. Trichilia catigua* (Catuaba) bark extract exerts neuroprotection against oxidative stress induced by different neurotoxic agents in rat hippocampal slices. Ind Crops Prod 2013;50:625-32.
11. CLSI – Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 5th ed. Villanova, PA: NIH; 2000.
12. Coutinho HD, Costa JG, Lima EO, Falcão-Silva VS, Siqueira-Júnior JP. Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. and chlorpromazine. Chemotherapy 2008;54:328-30.
13. Coutinho HD, Costa JG, Siqueira-Júnior JP, Lima EO. *In vitro* anti-staphylococcal activity of *Hyptis martiusii* Benth against methicillin-resistant *Staphylococcus aureus* – MRSA strains. Rev Bras Farmacog 2008;18:670-5.
14. Lima CN, Valero TF, Leite NF, Alencar LB, Matias EF, Kerntof MR, *et al.* Protective action *Duguetia furfuracea* (A. St.-Hil.) Saff. Against toxicity to mercuric chloride in *Escherichia coli*. Rev Cub Plant Med 2014;19:179-88.
15. Matias EF, Alves EF, Santos BS, Sobral de Souza CE, de Alencar Ferreira JV, Santos de Lavor AK, *et al.* Biological activities and chemical characterization of *Cordia verbenacea* DC. as tool to validate the ethnobiological usage. Evid Based Complement Alternat Med 2013;2013:164215.
16. Leboeuf M, Cavé A, Bhaumik PK, Mukherjee B, Murkherjee R. The phytochemistry of the *Annonaceae*. Phytochemistry 1982;21:2783-813.
17. Guinaudeau H, Lebouef M, Cavé A. Aporphine alkaloids II. J Nat Prod 1979;42:325-60.
18. Debourges D, Roblot F, Hocquemiller R, Cave A. Alkaloids of the *Annonaceae*. Part 77. Alkaloids of *Duguetia spixiana*. J Nat Prod 1987;50:664-73.
19. Rodrigues FF, Costa JG, Coutinho HD. Synergy effects of the antibiotics gentamicin and the essential oil of *Croton zehntneri*. Phytomedicine 2009;16:1052-5.
20. Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. J Ethnopharmacol 1988;23:127-49.
21. Tchounwou PB, Patlolla AK, Centeno JA. Carcinogenic and systemic health effects associated with arsenic exposure – A critical review. Toxicol Pathol 2003;31:575-88.
22. Harbone JB, Williams CA. Advances in flavonoid research since 1992. J Pharmacogn Phytochem 2000;55:481-504.
23. Santos DY, Salatino ML. Foliar flavonoids of *Annonaceae* from Brazil: Taxonomic significance. Phytochemistry 2000;55:567-73.
24. Santos AK, Costa JG, Menezes IR, Cansanção IF, Santos KK, Matias EF, *et al.* Antioxidant activity of five Brazilian plants used as traditional medicines and food in Brazil. Pharmacogn Mag 2010;6:335-8.
25. Stevenson FJ. Humus Chemistry: Genesis, Composition, Reactions. 2nd ed. New York: John Wiley; 1994. p. 496.
26. Middleton E Jr., Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52:673-751.
27. Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000;63:1035-42.
28. Halliwell B, Gutteridge JM. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. Arch Biochem Biophys 1986;246:501-14.
29. Afanas'ev IB, Dorozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem Pharmacol 1989;38:1763-9.
30. Kahraman A, Erkasap N, Serteser M, Köken T. Protective effect of quercetin on renal ischemia/reperfusion injury in rats. J Nephrol 2003;16:219-24.

Henrique D. M. Coutinho

ABOUT AUTHOR

Prof. Henrique D. M. Coutinho, The research theme is about antimicrobial effect of natural products. Recently, this researcher initiated researches about the use of natural products in a environmental perspective, using these products to protect cells against the toxic damages from heavy metals. This researcher is BSc. In Biological Sciences (1996), MSc. In Genetics (2001) and PhD. In Pharmacology (2008). He is full professor of the Regional University of Cariri (Universidade Regional do Cariri – URCA) since 1998 and superisioned several students on their BSc., MSc. and PhD studies. In this moment, is granted by the Brazilian Council of Science and Research (CNPq) as researcher and is the head of the research group of Applied Microbiology, recognized by the CNPq.