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Artigo

BIOACTIVE TRITERPENES AND PHENOLICS OF LEAVES OF *Eugenia brasiliensis*

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A chemical investigation of *Eugenia brasiliensis* Lam. (Myrtaceae) leaves led to the isolation of α-amyrin and β-amyrin (in a mixture), betulin, 29-hydroxy-oleanolic acid, quercetin, catechin and gallocatechin. Herein, the identification of 29-hydroxy-oleanolic acid is reported for the first time in the Myrtaceae family. Moreover, in this study, the extract, fractions and six of the seven compounds were monitored for toxicity toward *Artemia salina*, antibacterial and acetylcholinesterase inhibitory activity. The crude ethanol extract of the leaves and fractions were found be active on *A. salina* toxicity bioassay.

Keywords: acetylcholinesterase; triterpenes; *Eugenia brasiliensis*.

INTRODUCTION

Eugenia is one of the 132 genera of the Myrtaceae family, with around 1000 species. It is the largest genus in the Myrtaceae family in Tropical America.[1] *Eugenia* grows from Mexico and the Caribbean to Northern Argentina and it is estimated that 350 species are native from Brazil.[2] The plants of this genus are evergreen trees or shrubs, with spherical fruit which is generally edible,[3] for instance, *Eugenia uniflora* (Surinam cherry; known in Brazil as pitanga) and *Eugenia edulis* (known in Brazil as jaboticaba).

Species of the genus *Eugenia* show a variety of biological activities, such us antimicrobial,[4] antioxidant,[5] antidiabetic[6] and anti-inflammatory activity.[7] The compounds generally associated with this genus are flavonoids and triterpenoids, although chalcones[8] and tannins[9] are also present. Among the flavonoids, such as glycosides or aglycone, there is a predominance of polyhydroxylated flavanols.[10] The majority of compounds isolated from these species[11] are pentacyclic triterpenes with a lupane or oleanane skeleton.

Eugenia brasiliensis Lamarck (synonyms *Eugenia bracteolaris, Eugenia dombeui, Stenocalyx brasiliensis*) is a tree that grows in the Brazilian rainforests commonly known as grumixama.[12] Traditionally, the leaves, fruit and bark of *E. brasiliensis* are used for gastrointestinal disorders, rheumatism, and as a diuretic.[13] Ursolic acid has been isolated from the leaves of *E. brasiliensis*[14] and anthocyanins, ellagic acid, myricetin, quercetin, quercitrin and rutin have also been detected in the fruits.[15] Our studies have shown that the essential oil extracted from the leaves of *E. brasiliensis* has an appreciable antibacterial activity,[16] while the crude hydroalcoholic extract of the leaves and fractions has anti-inflammatory activity.[17]

The use of a simple microorganism such as *Artemia salina* to test *in vivo* lethality can represent a simple tool for guiding the screening and fractionation of physiologically active plant extracts. It has been demonstrated that results on this test correlate reasonably well with cytotoxicity and other biological properties.[18] There have been many reports on the use of *Artemia salina* for the general screening of natural or synthetic bioactive substances.[19]

Microorganisms are frequently the cause of prevailing diseases, presenting a serious public health issue for a significant segment of the population which is not adequately covered by either private or public health care systems. In recent years, multiple resistance in human pathogenic microorganisms has developed due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This fact, together with the undesirable side effects of certain antibiotics, and the emergence of previously uncommon infections, has driven scientists to search for new antimicrobial substances from various sources, including medicinal plants.[20]

The inhibition of acetylcholinesterase, the key enzyme in the breakdown of acetylcholine, is one of the treatment strategies employed against several neurological disorders such as Alzheimer`s disease, senile dementia, ataxia, and myasthenia gravis.[21] Acetylcholine is a compound liberated at the synaptic gap as a neurotransmitter. Neurotransmitter disturbances and insufficient cholinergic functions have been identified as pathological features in central nervous system disorders. The most important changes observed in the brain are a decrease in cortical levels of the neurotransmitter acetylcholine. Inhibition of acetylcholinesterase can therefore restore the level of acetylcholine in the brain. Medicinal plants have been used traditionally to enhance cognitive function and to alleviate other symptoms associated with Alzheimer´s disease.[22] Most of the drugs used in Alzheimer´s therapy contain an enzyme inhibitor, e.g. galantamine.[21]

Few of the 350 native species of *Eugenia* have been studied from at phytochemical and biological point of view. In this context, the present paper reports a phytochemical study on *E. brasiliensis* leaves, describing the isolation and identification of triterpenes α-amyrin (**1**) and β-amyrin (**2**) (in a mixture), betulin (**3**), 29-hydroxy-oleanolic acid (**4**), and the phenolic compounds quercetin (**5**), catechin (**6**) and gallocatechin (**7**). Compound **4** is described for the first time in the Myrtaceae family. The extract, fractions and compounds 1-3 and 5-7 were evaluated based on toxicity toward *Artemia salina*, as well as antibacterial and antiacetylcholinesterase activity.

EXPERIMENTAL

General experimental procedures

1D and 2D ^{1}H, ^{13}C NMR spectra were acquired on a Varian AS-400 spectrometer, operating at 400 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$ or CD_3OD or both solutions, with TMS as the internal standard.

The melting point of the isolated compounds was determined

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using the digital apparatus Micro Chemistry MQA PF-301. A Perkin Elmer FTIR 16 PC spectrometer was used to obtain the spectra in the infrared (IR) region using compressed tablets of KBr or NaCl film and values were recorded in cm^{-1}. Optical rotation measurements were performed on a Schimidt-Haensch Polatronic E polarimeter (Berlin, Gemany).

GC analysis was carried out with a GC-14 B Series instrument (Shimatzu, Japan) equipped with a flame detector (FID) at 290 °C. The chromatographic conditions were as follows: fused silica capillary column (30 m x 0.25 mm) with a DB-1; carrier gas N_2; injector (split/splitless); temperature 300 °C.

Silica gel (70-230 mesh, Merck) was used for the column chromatography (CC) separations and silica gel 60 PF_{254} (Merck) was used for analytical (0.25 mm) thin layer chromatography (TLC).

The solvent used in the liquid-liquid partitioning, column chromatography and thin-layer chromatography, as well as the reagents used for the biological assays, were of analytical grade.

Acetylthiocholine iodide (ATCI), 5,5'-dithiobis-[2-nitrobenzoic acid] (DTNB), acetylcholinesterase (AChE) type VI-S from electric eel, tris-HCl buffer (tris[hydroxymethyl]aminomethane), bovine serum albumin (BSA) and magnesium chloride hexahydrate were supplied by Sigma (USA). The bioassay was performed on a Perkin Elmer Lambda S spectrophotometer.

Plant material

The leaves of *E. brasiliensis* Lam. were collected in Santo Amaro da Imperatriz (SC), Brazil. A voucher specimen was identified by Prof. Dr. D. de B. Falkenberg and deposited in the Universidade Federal de Santa Catarina Herbarium under number FLOR-34675.

Extraction and isolation

The leaves (1.5 kg) were powdered and extracted with 96% ethanol by maceration at room temperature for 7 days. After filtration, the ethanol extract was further evaporated to dryness at 45 °C under reduced pressure, yielding the crude ethanol extract (170 g). This extract was redissolved in a mixture of MeOH:H_2O (3:7 v/v) to yield an insoluble fraction (45.3%). The solution obtained after filtration was partitioned successively with solvents of increasing polarity to give hexane (17.9%) dichloromethane (9.1%) and ethyl acetate (19.6%) fractions.

The non-soluble material (5.0 g) was chromatographed on silica gel using hexane/EtOAc mixtures with increasing polarity yielding 31 fractions (100 mL), which were combined in sub-fractions according to TLC analysis. The sub-fraction 13-15 (hex/AcOEt 90%) yielded a mixture of triterpenes (143 mg), α-amyrin (**1**) and β-amyrin (**2**). Sub-fraction 21-23 (hex/AcOEt 75%) was recrystallized with $CHCl_3$/MeOH 1:1, yielding betulin (**3**) (35 mg).

The dichloromethane fraction (13.0 g) was also chromatographed in a silica gel column applying the same conditions employed for the non-soluble fraction. Sub-fractions 4-9 (hex/AcOEt 80%) and 16 (hex/AcOEt 60%), after observation by TLC, led to the isolation of 510 mg of betulin (**3**) and 15 mg of mesembryanthemoidigenic acid or 29-hydroxy-oleanolic acid (**4**), respectively.

The ethyl acetate fraction (12.0 g) was first chromatographed in a silica gel column with hexane containing increasing amounts of EtOAc. A total of 34 fractions were obtained from this column. The fraction 20-22 (hex/AcOEt 5%) obtained from this column was submitted to CC again by using a mixture of hex/AcOEt 20% and increasing the polarity up to EtOH 100%, giving 34 fractions. The sub-fraction 3-4 (AcOEt/EtOH 20%) yielded compound **5** as an amorphous yellow powder, denominated quercetin (40 mg). The sub-fraction 13-16 (AcOEt/EtOH 10%) afforded 150 mg of pale yellow solid denominated catechin (**6**). The sub-fraction 22-24 (AcOEt/EtOH 5%) was further subjected to silica gel CC eluted with AcOEt 100%, increasing the polarity up to EtOH 100%. Among 26 fractions, the fraction 15-17 (AcOEt/EtOH 1%) afforded 142 mg of a light yellow solid denominated gallocatechin (**7**).

The structural characteristics of the compounds were determined by the spectroscopic methods of infrared spectroscopy, ^1H and ^{13}C nuclear magnetic resonance, with the aid of two dimensional techniques and by comparing the experimental data with those described in the literature.

*Compounds **1** and **2***

α and β-amyrin, white amorphous powder, p.f. 184.9-187.4 °C. IR (KBr) ν_{max} cm^{-1}: 3390 (-OH), 2942 (-CH), 1644 (C=C). These compounds were identified after comparison of the physical chemical data with data available in the literature.[23,24]

*Compound **3***

Betulin or 3β,28-dihydroxy-lup-20(29)-ene, white crystals (recrystallized in $CHCl_3$/MeOH 1:1), p.f. 256-257 °C (lit. 255-257 °C).[25] IR (KBr) ν_{max} cm^{-1}: 3399 (-OH), 2941 (-CH), 1639 (C=C). Identified by co-TLC with an authentic sample and by combination methods (IR, ^1H NMR, ^{13}CNMR spectra) and comparison with literature data.[24,25]

*Compound **4***

Mesembryanthemoidigenic acid or 3β,29-dihydroxy-olean-12-en-28-oic acid, white powder (recrystallized in $CHCl_3$/MeOH 1:1), p.f. 306.4-308.5 °C (lit. 305-309 °C).[26] IR (KBr) ν_{max} cm^{-1}: 3420 (-OH), 2954 (-CH), 1636 (C=C). ^1H NMR δ (400 MHz, $CDCl_3$: 0.77 (*s*, 3H, H-30), 0.81 (*s*, 3H, H-25), 0.93 (*s*, 3H, H-26), 0.94 (*s*, 6H, H-23 and H-24), 1.17 (*s*, 3H, H-27), 3.18 (*s*, 2H, H-29), 3.37 (*m*, 1H, H-3), 5.25 (*t*, 1H, H-12). ^{13}C NMR δ (100 MHz, $CDCl_3$): 180.0 (C-28), 143.9 (C-13), 122.6 (C-12), 78.5 (C-3), 73.2 (C-29), 55.6 (C-5), 46.7 (C-9), 46.4 (C-17), 41.6 (C-14), 40.7 (C-18), 40.2 (C-19), 39.3 (C-8), 38.7 (C-4), 38.6 (C-1), 37.9 (C-22), 36.9 (C-10), 35.6 (C-20), 32.8 (C-7), 29.5 (C-21), 28.1 (C-23), 27.6 (C-15), 27.5 (C-2), 25.2 (C-27), 23.3 (C-11), 22.9 (C-16), 18.3 (C-6 e C-30), 16.5 (C-26), 15.1 (C-24), 14.7 (C-25). Supplementary material.

*Compound **5***

Quercetin or 3,5,7,3',4'-pentahydroxyflavone, light yellow solid, p.f. 304-307 °C (lit. 310-312 °C).[27] IR (KBr) ν_{max} cm^{-1}: 3410 (-OH), 1693 (C=O). Identified by co-TLC with an authentic sample (*Rf* = 0.6, hexane/AcOEt 6:4) and by comparing the spectroscopic data with those available in the literature.[28]

*Compound **6***

Catechin or (+)-(2R,3S)-5,7,3',4'-tetrahydroxyflavan-3-ol, pale yellow solid, p.f. 165.4-172.5 °C (lit. 175-187 °C).[29] $[\alpha]^{25}_D$ = + 13°, 0.4 in 50% aqueous acetone. IR (KBr) ν_{max} cm^{-1}: 3386 cm^{-1} (-OH), 1615 (C=C), 1142 (C-O). ^1H NMR δ (400 MHz, CD_3OD: 2.38 (*dd*, J_1 = 16.0 and J_2 = 7.9 Hz, $1H_{ax}$, H-4), 2.68 (*dd*, J_1 = 16.0 and J_2 = 5.2 Hz, $1H_{eq}$, H-4), 3.86 (*m*, H-3), 4.56 (*d*, J_1 = 7.6 Hz, 1H, H-2), 5.82 (*d*, *J* = 1.6, 1H, H-8), 5.91 (*d*, *J* = 1.6, 1H, H-6), 6.63 (*dd*, J_1 = 8.0 and J_2 = 1.6, 1H, H-6'), 6.66 (*d*, *J* = 8.0, 1H, H-5'), 6.74 (*d*, *J* = 1.6, 1H, H-2').

*Compound **7***

Gallocatechin or (+)-(2R,3S)- 5,7,3',4',5'-pentahydroxyflavan-3-ol, light yellow solid, p.f. 225.0-228.3 °C (lit. 189-191 °C).[30] $[\alpha]^{25}_D$ = + 17°, 0.4 in methanol. IR (KBr) ν_{max} cm^{-1}: 3564 (-OH), 1621 (C=C), 1159 (C-O). ^1H NMR δ (400 MHz, CD_3OD: 2.53 (*dd*, J_1 = 16.0 and J_2 = 7.65 Hz, $1H_{ax}$, H-4), 2.79 (*dd*, J_1 = 16.0 and J_2 = 5.6 Hz, $1H_{eq}$,

H-4), 3.89 (*m*, H-3), 4.52 (*d*, J_1 = 6.8 Hz, 1H, H-2), 5.83 (*d*, *J* = 2.4, 1H, H-8), 5.94 (*d*, *J* = 2.4, 1H, H-6), 6.45 (*s*, H-2' and H-6').

Artemia salina lethality test

The toxic effect of the plant extract and its fractions against *Artemia salina* nauplii was tested according to the method of Sam[31] with minor modifications. Dried *A. salina* eggs were hatched in illuminated artificial seawater at 25 °C. After 48 h incubation, 1000 µL of seawater containing 1% Tween 20 (v/v) and 7-10 free-swimming nauplii was separately transferred to 24-well flat-bottomed tissue culture plates. Toxicities of the extract and fractions were tested at several concentrations (10-1000 µg mL^{-1}). Three replicates were used for each concentration. The culture plates were incubated as described above and the number of dead nauplii were counted after 24 h. Potassium dichromate ($K_2Cr_2O_7$; LC_{50} ~ 20-40 µg mL^{-1}) and seawater solution with 1% Tween 20 v/v were used as positive and negative controls, respectively. The lethal concentrations which led to 50% mortality (LC_{50}) with 95% confidence intervals were determined using the probit method. LC_{50} values were taken as the measure of toxicity of the extract or fractions. LC_{50} values greater than 1000 µg mL^{-1} for the plant extract and the fractions were considered inactive.

Antibacterial assays

Tests were performed on *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) acquired from The American Type Culture Collection (ATCC). All organisms were maintained in brain-heart infusion (BHI) medium containing 30% (v/v) glycerol at 20 °C. Before testing, the suspensions were transferred to trypticase soy agar supplemented with 5% of sheep blood (Difco) and aerobically grown overnight at 35 °C. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 McFarland standard in saline solution (0.9%). The broth microdilution method was used to determine the MIC (minimum inhibitory concentration) of the samples obtained from *Eugenia brasiliensis* against the test organisms as recommended by the National Committee for Clinical Laboratory Standards.[32] This test was performed in sterile 96-well microplates. The samples were properly prepared and transferred to each microplate well in order to obtain a two-fold serial dilution of the original extract (from 1:2 to 1:256 starting from the concentration of 50 mg mL^{-1}). The inocula (100 µL) containing 5 x 10^5 CFU of each microorganism, were added to each well. A number of wells were reserved in each plate for sterility control (no inoculum added), inoculum viability (no extract added), and to assess the inhibitory effect of ethanol. Plates were aerobically incubated at 35 °C. After incubation for 18-24 h, bacterial growth was evaluated by the presence of turbidity and a pellet on the well bottom. MIC was defined as the lowest concentration of samples that had no macroscopically visible growth. Each experiment was repeated at least twice.

Acetylcholinesterase inhibition

The enzymatic activity was measured using an adaptation of the method described by Mata *et al.*.[33] Briefly, 325 µL of 50 mM Tris-HCl buffer, pH 8, 100 µL of a buffer solution of sample (0.1 mg mL^{-1} for the extract and fractions, and 0.01 mg mL^{-1} for isolated compounds, dissolved in EtOH) and 25 µL of acetylcholinesterase (AchE) solution containing 0.28 U/mL (50 mM Tris-HCl, pH 8 buffer, 0.1% BSA) were incubated for 15 min. Subsequently, 75 µL of a solution of ACTI (0.023 mg mL^{-1} in water) and 475 µL of DTNB (3mM in Tris-HCl, pH 8 buffer, 0.1 M NaCl, 0.02 M $MgCl_2$) were added and the final mixture incubated for another 30 min at room temperature. Absorbance of the mixture was measured at 405 nm. A control mixture was prepared, using 100 µL of a solution similar to the sample mixture but with ethanol instead of sample, and was considered as 100% activity of AchE. Inhibition (%) was calculated as follows: I (%) = 100 – (A_{sample} / $A_{control}$) x 100, where A_{sample} is the absorbance of the sample containing the reactant and $A_{control}$ the absorbance of the reaction control. Tests were carried out in triplicate and a blank with Tris-HCl buffer instead of enzyme solution was used. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against the sample solution concentrations. Reminyl containing galantamine was used as the positive control.

RESULTS AND DISCUSSION

The non-soluble, dichloromethane and ethyl acetate fractions obtained from the leaves of *E. brasiliensis* after chromatographic fractionation afforded four triterpenes (**1-4**), one flavonol (**5**), and two catechins (**6-7**).

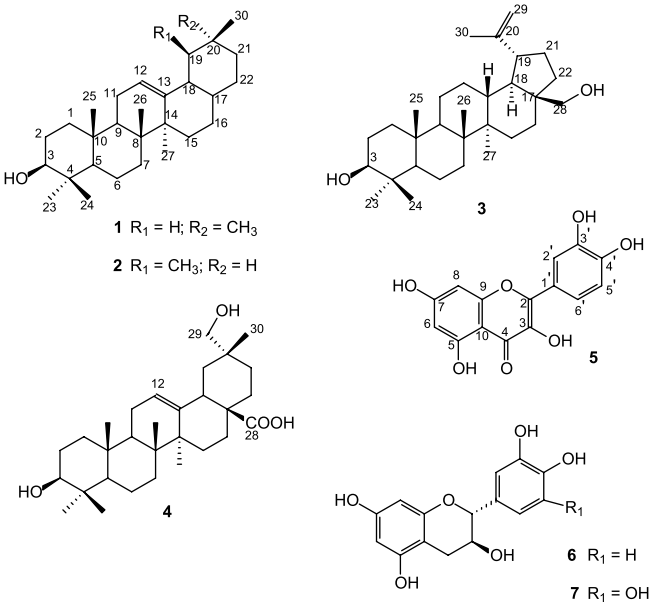

Figure 1. *Structures of compounds isolated from Eugenia brasiliensis*

Compounds **1** and **2** were identified as a mixture of α-amyrin (**1**) and β-amyrin (**2**).[23,24] Comparison of the sample with a standard mixture of α- and β-amyrin by gas chromatography confirmed the presence of these two structures (β-amyrin Tr = 19.1 min and α-amyrin Tr = 20.7 min), and the β-amyrin was found in greater quantity, at a ratio of 3:1. Retention times were similar to those observed when these triterpenes were analyzed in mixture.[34] The ^1H and ^{13}C NMR spectra of compound **3** confirmed the structure of betulin (**3**).[24,25] For triterpene **4**, in addition to six singlets for methyl groups (H-23, H-24, H-25, H-26, H-27 and H-30), signals for oxymethine hydrogen at δ 3.2 (*s*, 2H, H-29) and 3.4 (*m*, 1H, H-3) and olefinic hydrogens at δ 5.2 (*t*, 1H, H-12) were also observed. The ^{13}C NMR spectrum of triterpene **4** showed, in addition, the six signals for C-sp^3, and signals for olefinic carbons, consistent with the presence of triterpene type olean-12-ene at δ 122.6 (C-12) and 143.9 (C-13). Also, signals at δ 78.5 (CH) and 73.2 (CH_2) were observed, characteristic of oxymethine carbon at C-3 and C-29, respectively. Moreover, the signal observed at δ 180.0 corresponded to a carbonyl group (C-28). Comparison of the chemical displacements of the NMR spectra of compound **4** with

data available in the literature[24,35] allowed the unequivocal identification of this structure as 29-hydroxy-oleanolic acid (**4**). There are no previous reports of the isolation or identification of this compound in the Myrtaceae family.

Comparison of the ^{1}H and ^{13}C NMR spectra for compound **5** with data available in the literature allowed the structure to be identified as quercetin.[28] Catechin (**6**) and gallocatechin (**7**) were first identified by co-TLC with an authentic sample (**6**: *Rf* = 0.6, hexane/AcOEt 3:1 and **7**: *Rf* = 0.72, hexane/AcOEt 1:3) and by comparison of their physicochemical data with those of the literature.[29,30] The NMR data were compared with those available in the literature,[29,36] confirming the structure of compound **6** and **7** as catechin and gallocathechin, respectively.

The crude extract, fractions and isolated compounds of *E. brasiliensis* were evaluated for *A. salina* toxicity, as well as antibacterial and antiacetylcholinesterase activities. 29-Hydroxy-oleanolic acid (**4**) was obtained with low yield, and thus this compound was not submitted to the bioassays.

The results for the *A. salina* lethality of the samples are shown in Table 1. The crude extract and all of the fractions studied showed toxicity toward *A. salina* nauplii at concentrations < 1000 μg mL^{-1}, but the ethyl acetate fraction was the most larvicidal sample, with an LC_{50} value of 788.9 μg mL^{-1}.

Table 1 shows the results for the antibacterial activity obtained for the samples tested. For extracts or fractions which had MIC values below 0.1 mg mL^{-1}, antibacterial activity was considered excellent, from 0.1 to 0.5 mg mL^{-1}, moderate, from 0.5 to 1.0 mg mL^{-1} weak, and over 1 mg mL^{-1} inactive.[37] For the isolated compounds, at an MIC value below 0.01 mg mL^{-1} the antibacterial activity was considered excellent, from 0.01 to 0.1 mg mL^{-1} good, and over 0.1 mg mL^{-1} inactive.[38] On the MIC assay, some vegetal samples showed activity against *E. coli* and *P. aeruginosa*. On the other hand, neither the crude extract nor the fractions tested showed activity against *Staphylococcus aureus,* a Gram-positive bacterium. The crude extract and fractions were especially active against *Escherichia coli*, a Gram-negative bacterium. The ethyl acetate fraction was the most active, showing moderate activity, with an MIC value of 0.39 mg mL^{-1}, for this bacterium. The crude extract and dichloromethane fraction exhibited weak activity against *Escherichia coli,* with an MIC value of 0.78 mg mL^{-1}. Another finding was that the ethyl acetate fraction and crude extract showed weak activity against *Pseudomonas aeruginosa,* a Gram-negative bacteria, with an MIC value of 0.78 mg mL^{-1}. This finding is interesting because the great majority of plant extracts are more active against Gram-positive than Gram-negative bacteria. The greater resistance of gram-negative bacteria to plant extracts has been previously reported.[37] This resistance can be explained by the fact that the outer membrane of Gram-negative bacteria is known to present a barrier to many substances, including antibiotics, and the periplasmatic space contains enzymes that are able to breakdown foreign molecules. Moreover, Gram-negative bacteria have efflux pumps that reduce the cellular levels of antibiotics.[39] Among the isolated compounds, only quercetin obtained from the AcOEt fraction showed good activity for all bacteria tested. Thus, quercetin may be responsible for the observed activity of the AcOEt fraction, especially against Gram-negative bacteria.

As summarized in Table 1, all of the vegetal samples exhibited inhibitory activity between 6.7 and 63.0% at a concentration of 0.1 mg mL^{-1} for the AchE. The best inhibitory activity was recorded for the non-soluble fraction (63.9%). Quercetin was the most active compound, with 35.0% inhibition of AchE at a concentration 0.01 mg mL^{-1}. There is a relatively lower number of reports on AChE inhibitory activity of flavonoids, which is the main strategy for the treatment of Alzheimer's Disease. On the other hand, flavonoids as the polyphenolic substances have been known to exhibit strong antioxidant activity, which is an advantage in Alzheimer's disease treatment.[22] Previous studies have shown the value of diverse flavonoid derivatives for their AChE inhibitory effect at a concentration of 1 mg/mL,[40] and amongst these, quercetin was found to be the most active against AChE, having 76.2% inhibition.[41] Docking studies have shown that quercetin binds through strong hydrogen bonds to several important amino acid residues of the enzyme. Hydrophobic interactions could also explain the potency of quercetin for inhibiting AChE.[42] In evaluating the anti AChE activity of extracts and isolated compounds, the extracts and fractions were found to show better inhibition than the isolated substances. This fact is probably due to the presence of other active compounds in the extract, not isolated in the process of fractionation. Another explanation for this finding may be the synergistic effect commonly presented by medicinal plants.[43]

Table 1. Biological activity for the extract, fractions and compounds obtained from *Eugenia brasiliensis*

Sample	*S. aureus* MIC (mg mL^{-1})	*E. coli* MIC (mg mL^{-1})	*P. aeruginosa* MIC (mg mL^{-1})	A. salina LC_{50} (μg mL^{-1})	AchE % inhibition
Crude extract	6.25	0.78	0.78	807.2 738.6-1060.2	40.3 ± 0.1
Non-soluble Fraction	6.25	6.25	6.25	885.1 738.6-1060.2	63.0 ± 0.2
Dichloromethane Fraction	3.12	0.78	6.25	816.6 738.6-1060.2	44.0 ± 0.2
Ethyl acetate Fraction	1.56	0.39	0.78	788.9 738.6-1060.2	50.0 ± 0.1
α,β-amyrin	0.25	0.25	0.12	--	6.7 ± 0.1
Betulin	0.25	0.25	0.25	--	N.O.
Quercetin	0.05	0.10	0.10	--	35.0 ± 0.4
Catechin	0.30	0.30	0.30	--	N.O.
Gallocatechin	0.20	0.40	0.40	--	N.O.
Positive control[a]	0.0001	0.0004	0.0004	25.0	82.7 ± 0.3

[a]gentamycin, for antibacterial assay; $K_2Cr_2O_7$, for brine shrimp lethality; galantamine for acetylcholinesterase assay; -- not tested; N.O. not observed.

CONCLUSIONS

The phenolic and triterpene compounds isolated from *E. brasiliensis* are consistent with biosynthetic routes and with results of phytochemical studies on plants of the genus *Eugenia*, verifying the taxonomic position of this species. The compounds isolated have not been previously reported for the species under study and, of the substances isolated, 29-hydroxy-oleanolic acid (**4**) was found for the first time in the Myrtaceae family.

The results obtained in this study point to the presence of active compounds, especially in the case of the ethyl acetate fraction (the most active fraction) where this may represent a valuable source of novel anti-infectious agents, particularly those active against Gram-negative bacteria.

SUPPLEMENTARY MATERIAL

Available at http://quimicanova.sbq.org.br, in the form of a PDF file with free access.

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