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Original Article

CHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF HYDROETHANOLIC CRUDE EXTRACT OF *EUGENIA FLORIDA* DC (MYRTACEAE) LEAVES

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ABSTRACT

Objective: The present study aimed to characterize and quantify the total phenolics, flavonoids and tannins in a hydroethanolic crude extract (70% (v/v) (EB)) of the leaves of *E. florida* DC, as well as to evaluate the antimicrobial activity of the extract against different species of micro-organisms.

Methods: EB was characterized using a mass spectrometer equipped with a direct insertion device for in-stream injection (FIA). Quantitative analyses of major compounds were carried out by spectrophotometry. In addition, we evaluated the sensitivity profiles of different strains of yeast and bacteria against different concentrations of EB.

Results: The classes found were in agreement with those described in the literature: flavonoids, tannins, phenolic acids and saponins. EB showed levels of phenolic compounds, flavonoids and tannins equal to 25.82 mg gallic acid equivalents per gram of extract (EAG/g), 8.42 mg quercetin equivalents per gram of extract (EQ/g) and 7.30 mg tannic acid equivalents per gram of extract (AT/g), respectively. In the analysis of antimicrobial activity, EB was more active against yeasts but was not effective against the bacteria used in the test.

Conclusion: We can conclude that *E. florida* DC has antimicrobial potential, due to the presence of bioactive secondary metabolites.

Keywords: Phenolics, Micro-organisms, Mass spectrometer, Yeasts, Bacteria, Potential

INTRODUCTION

Medicinal plants are used in some of the oldest strategies employed by man in the treatment of diseases of all kinds, and their use in the prevention or cure of diseases is a habit that has always existed in the history of humanity [1]. In recent decades in Brazil, there has been a growing interest in the use of medicinal plants and their respective extracts for treatments, which can in certain circumstances, be of assistance in primary health care compatible with conventional medicine [2]. The phytotherapy is intended to provide useful information for the development of pharmacological and phytochemicals studies on plant species, as well as providing therapeutic resources, conserving biodiversity, promoting social development, and fostering permanent education [2].

In contrast to synthetic drugs, therapeutic use of herbal products was materialized from the ancient texts and passed on through generations without scientific experimental evidence. For this reason, modern research on the pharmacology of herbal medicine is aimed to validate herbal medicine and to ensure rational use, safety and efficacy [3]. Thus, due to the large diversity of Brazilian plants with therapeutic potential and considering the history and cultures of the population, some species of plants have aroused great interest among researchers [4].

The species *Eugenia florida* DC (Myrtaceae) is a small tree, approximately 3.5 m tall, that blooms in October and fruits at the end of November to mid-December. It is distributed throughout South America, from the Colombia to Bolivia, and from Guyana to the South of Brazil, growing in various ecological landscapes. It is popularly known as black cherry or guamirim cherry. Its fruits have a characteristic odor, and are edible and very tasty [5]. The extensive therapeutic use of the leaves of the species by rural populations has been highlighted. It is used primarily as a hypotensive, hypoglycemic, and antipyretic; for reducing triglycerides and cholesterol levels; and in the treatment of infections, jaundice, heart disease and gastrointestinal disorders [6, 7].

Previous chemical studies of the genus indicate high levels of phenolic compounds such as flavonoids [8, 9], phenolic acids [10] and tannins [11, 12], as well as other classes of compounds, including saponins [13, 14] and terpenoids [15].

However, there are still no detailed chemical and biological studies on the species *E. florida* DC in the literature. Thus, chemical characterization and elucidation of the antimicrobial activity of the plant extract in question, to verify its potential as a medicinal agent, is justified due to the popular use of the plant by rural communities in the treatment of diseases.

MATERIALS AND METHODS

Plant material

Leaves of the species *E. florida* DC were collected in April 2014, the southern state of Minas Gerais, near the "Fazenda Cachoeirinha", W045°56'07.8'', S21°33'51.7'', Alfenas, Minas Gerais, Brazil. The details of the data collection and the coordinates of the site were provided by Dr. Flávio Nunes Ramos (Institute of Natural Sciences, UNIFAL-MG) and the species was identified by Dr. Geraldo Alves da Silva (Faculty of Pharmaceutical Sciences, UNIFAL-MG). A specimen was deposited and recorded in the Herbarium of the UNIFAL-MG under the registration number 2562. The leaves were dried in an oven with air renewal at 45°C for 72 h. After drying, the plant material was sprayed onto a knife mill. The powder obtained was subjected to exhaustive percolation using ethanol (70%) as a liquid extractor. The extraction solution was concentrated in a rotary evaporator under reduced pressure and subsequently lyophilized

and stored, finally obtaining the crude hydroethanolic extract 70% (v/v) (EB).

Chemical characterization by mass spectrometry

For analysis by mass spectrometry, EB of *E. florida* DC (5.0 mg) was dissolved in methanol (3.0 ml), and filtered in a Sepak RP-18® cartridge and then through a Nylon membrane (Flow Supply®) with 22.25 mm diameter and 0.22 μm pore size. The extract (5 ppm) was analyzed online by the LCQ Fleet, Thermo Scientific® mass spectrometer, equipped with a direct sample insertion device for streaming injection analysis (FIA). The sample was ionized by electro spray (ESI) and fragmentations into multiple stages (MS^n) were held in an ion-trap (IT) interface. The negative mode was chosen for the generation and analysis of all the spectra, and the experimental conditions were as follows: capillary voltage-35 V, spray voltage-5000 V, capillary temperature 350°C, drag gas (N_2) and flow rate 60 (arbitrary units). The acquisition track was *m/z* 100-2000, with two or more scan events held simultaneously in the spectrum. The experiment was performed by Laboratory of Bioprospecting of Natural Products (LBPN), UNESP, Coastal Campus-IB-CLP.

Determination of total phenol content

The determination of total phenols was performed according to the colorimetric method based on the formation of complexes with molybdenum and tungsten [16]. Aliquots of the dry extract were diluted in absolute ethanol, to obtain a concentration of 250 μg/ml. 0.5 ml of the sample was added to 2.5 ml of Folin-Ciocalteau reagent 10% (v/v) and 2.0 ml of Na_2CO_3 4% (m/v). The samples were homogenized and kept at rest for 2 h at room temperature and under direct light. After this period, the absorbance at 750 nm was measured in a Shimadzu® TCC 240A UV-Vis spectrophotometer. The same procedure was performed with gallic acid in ethanolic solution (5, 10, 20, 30, 40, 50, 60, and 70 μg/ml), in order to obtain the analytical curve A = 0.0124C+0.0089 (R^2 = 0.9877), where A is the absorbance at 750 nm and C is the concentration of gallic acid. The values obtained for the samples were interpolated on the calibration curve and the concentration values were expressed as milligrams of gallic acid equivalents per gram of extract (mg EAG/g). The experiment was performed in triplicate.

Determination of flavonoid content

Flavonoids were quantified based on the formation of flavonoid-aluminum complexes [17]. Extract aliquots were diluted in absolute ethanol to obtain a concentration of 500 μg/ml. 0.5 ml of sample was added to 0.1 ml of $AlCl_3$. 6 H_2O 10% (m/v), 0.1 ml of CH_3COOK 1.0 mol/l and 2.8 ml of distilled water. The tubes were shaken to homogenization and kept at rest for 30 min. Absorbance at 425 nm were read in a Shimadzu® TCC 240A UV-Vis spectrophotometer. The same procedure was followed using quercetin in ethanolic solution (10, 20, 40, 50, 60, 70, 80, and 100 μg/ml), in order to obtain the analytical curve A = 0.0075C+0.0216 (R^2 = 0.9982), where A is the absorbance at 425 nm and C is the concentration of quercetin. The values obtained for the sample were interpolated on the calibration curve and the concentration values were expressed as milligrams of quercetin equivalents per gram of extract (mg EQ/g). The experiment was performed in triplicate.

Determination of tannin content

Tannins in EB were determined using the method of Hagerman, based on precipitation with albumin and complexation with ferric chloride solution [18]. Extract aliquots were diluted with ethanol at a concentration of 1000 μg/ml. 2.0 ml of bovine albumin 1.0% (m/v) was added to 1.0 ml of extraction solution and, after precipitation, the samples were maintained at room temperature for 15 min and centrifuged at 3000 rpm. The supernatant was discarded and the precipitates were dissolved in 4.0 ml of sodium lauryl sulfate 0.5% (m/v). 1.0 ml of ferric chloride solution 1% (m/v) was added, and the tubes were homogenized and kept at rest for 15 min. Absorbance at 510 nm was measured in a Shimadzu® TCC 240A UV-Vis spectrophotometer. The same procedure was followed with tannic acid in ethanolic solution (10, 20, 40, 50, 60, 70, 80, and 100 μg/ml), in order to obtain the analytical curve A = 0.0059C+0.0014 (R^2 = 0.9846), where A is the absorbance at 510 nm and C is the concentration of tannic acid. The values obtained for the sample were interpolated on the calibration curve and the concentration values were expressed as milligrams of tannic acid equivalents per gram of extract (mg EAT/g). The experiment was performed in triplicate.

Evaluation of antimicrobial activity against yeasts and bacteria

The extract was evaluated *in vitro* for antifungal and antibacterial activities using Mueller-Hinton Broth and the microdilution method following the methodology and interpretative criteria proposed by document M27-S4 for yeasts and document M7A6 for bacteria from the Clinical and Laboratory Standards Institute [19,20], with some modifications. The stock solutions of the extract were prepared in 1% DMSO and tested at the following concentrations (μg/ml): 1000; 500; 250; 125; 62.5; 31.25; 15.63; 7.81; 3.91; 1,95. The standard drugs chloramphenicol and fluconazole were applied as controls of bacteriostatic and fungistatic actions, respectively, at the following concentrations (μg/ml): 64; 32; 16; 8; 4; 2; 1; 0.5; 0.25; 0.125.

The tests were performed with opportunist and pathogenic yeast and bacterial standards from the American Type Culture Collection (ATCC): *Candida albicans* ATCC 10231, *C. krusei* ATCC 6258, *C. glabrata* ATCC 90030, *C. parapsilosis* ATCC 22019, *Staphylococcus aureus* ATCC 6538, and *Escherichia coli* ATCC 25922. The microorganism strains were obtained by Drª. Amanda Latércia Tranches Dias (Laboratory of Research in Microbiology, Institute of Biomedical Sciences, UNIFAL-MG). The microplates were incubated at 37°C for 24 h. Results were visualized and analyzed at 530 nm in an Anthos Zenyth 200rt Microplate Reader®. The concentrations of the compounds inhibitory to microbial growth were determined by identifying the concentrations at which a reduction of 50% (MIC_{50}) and 90% (MIC_{90}) in the absorbance values compared to the absorbance values of maximum growth of the isolates, in μg/ml. The tests were performed in triplicate.

Statistical analysis

Statistical analyses were performed using the Sisvar software Version 5.3/DEX-UFLA (Copyright, 1999-2010). Analysis of variance (ANOVA) was performed followed by Scott & Knott tests with a significance level of 5%. Correlation analyses were performed in the BioEstat software Version 5.0 and the results were presented as Spearman's correlation coefficients (r).

RESULTS

The high percentage of yield observed for EB, equivalent to 23.89%, suggests that the most common compounds present in the extract are polar since the solvent used (ethanol 70%) offers a sufficient degree of polarity to extract such compounds [21].

Chemical characterization by mass spectrometry

The mass spectrum of EB, in *full scan* mode, is shown in fig. 1. The compounds obtained were registered as their respective ions $[M-H]^-$. For these ions, partitions in multiple stages were obtained, which allowed the structures of some molecules to be suggested based on their fragmentation mechanisms, as described in table 1.

Quantitative analysis of main classes of compounds

The results of the quantification of the total phenols, flavonoids and tannins in EB of *E. florida* DC are shown in table 2. EB presented levels of each of these compounds, respectively, equivalent to 25.82 ± 0.85 mg EAG/g, 8.42 ± 1.13 mg EQ/g, and 7.30 ± 1.06 mg EAT/g.

Evaluation of antimicrobial activity

As shown in table 3, EB exhibited MIC_{50} values between 62.5 and 250 μg/ml. EB was more active against the yeast *C. krusei*, with an MIC_{50} calculated as 62.5 μg/ml. The extract did not achieve a growth reduction of 50 % at the concentrations tested for *C. albicans* and *C. parapsilosis*. EB showed a MIC_{90} for *E. coli* of 1000 μg/ml, demonstrating a lower activity against bacteria.

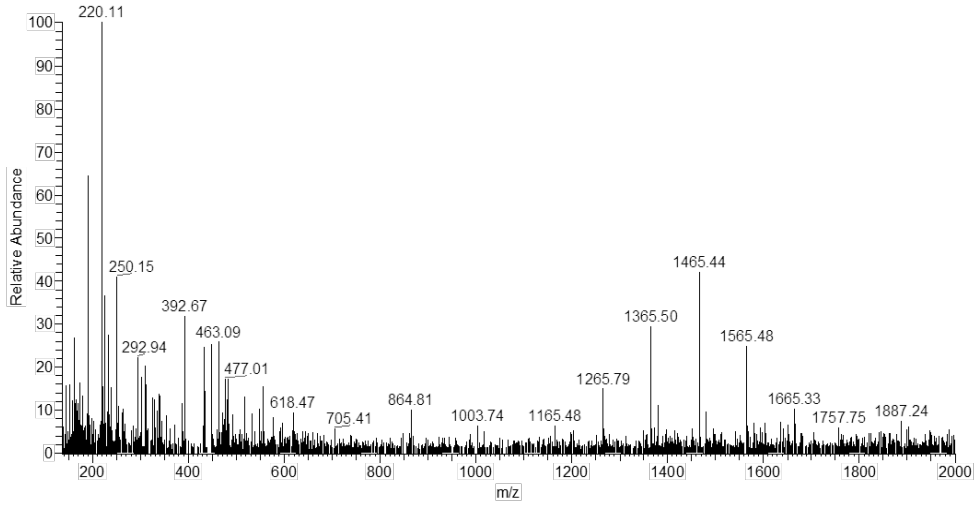

Fig. 1: Mass spectrum in *full scan* mode obtained by direct injection of EB of *E. florida* DC leaves

Table 1: Mass spectrometry by direct injection and electrospray ionization of EB of *E. florida* DC

Compound	Fragmentation by FIA-ESI-IT-MS^n		Reference
	$[M-H]^-$	Major ions produced (MS^n)	
Anhydrous hexose	161	---	[22]
Quinic acid	191	173, 127, 111	[23]
Resveratrol	227	---	[24]
5-hydroxyresveratrol	243	227, 215, 137	[25]
1,2-didehydrotanshinone IIA	293	277, 249, 221, 207	[26]
Quercetin	301	---	[27]
4-allyl-1-hydroxy-2-(2'-allyl-4'-hydroxy-5'-methoxyphenoxy)-benzene	311	283	[28]
4-*O*-*p*-coumaroylquinic acid	337	191, 173, 127, 111	[29]
5-caffeoylquinic acid	353	179	[30]
5-hydroxyeuphene	427	409	[31]
Quercetin-*O*-arabinose	433	301	[32]
Quercetrin	447	301	[18]
Rhododendrin penthoside	459	327, 165	[33]
Quercetin-*O*-glucose	463	301	[18]
Rhamnetin-*O*-glucose	477	315	[34]
Granatunflavanil-*O*-xylose	481	349	[35]
Dystenin	514	482	[36]
O-galloyl desbenzoylpaeoniflorin	527	331, 169	[37]
Procyanidin B_2	577	533, 427, 289	[38]
Hyperin	595	433, 301	[39]
Betulinic acid heteroside	617	455, 411	[40]
Cimicifoetiside A	619	487	[41]
3-*O*-*D*-glucopyranosylserjanic acid	675	499, 455, 397	[42]
3-*O*-caffeoyl-4,5-di-*O*-feruloylquinic acid	705	543, 529, 367, 191, 173	[43]
Kochianoside IV	763	631, 455, 411	[44]
27-*O*-*D*-glucopyranosyl physagulin D	783	621, 459	[45]
Procyanidin C_2	865	823, 715, 577, 533, 425, 289	[46]
Isoastragaloside I	867	705, 489	[47]
Clinopodiside E	994	832, 670, 523	[48]
Aesculuside B	1006	844, 682, 506	[49]
Phytolacagenic acid heteroside	1016	854, 692, 530	[50]
Guaianin D	1026	880, 734, 572, 440	[51]
Tetrameric cathequin	1154	1110, 1002, 865, 823, 715, 577, 533, 425, 289	[46]
Scillasaponin B	1266	1104, 942, 780, 618, 486	[52]

Table 2: Contents of total phenols, flavonoids and tannins in EB of *E. florida* DC.

Compounds	Content
Total phenols (mg EAG/g)	$25.82 \pm 0.85^{c*}$
Flavonoids (mg EQ/g)	$8.42 \pm 1.13^{b*}$
Tannins (mg EAT/g)	$7.30 \pm 1.06^{a*}$

Values expressed as average ± standard deviation (n = 3). The results of each test were analyzed separately. Averages with different letters in the same column are statistically different by Scott and Knott test, *p<0.05.

Table 3: Evaluation of the sensitivity profile of yeasts and bacteria on EB of *E. florida* DC

Microorganism	MIC_{50} FCN (μg/ml)	MIC_{50} CFN (μg/ml)	MIC_{50} EB (μg/ml)	MIC_{90} EB (μg/ml)
Candida albicans	0.5[a*]	---	---	500[b*]
Candida krusei	32[a*]	---	62.5[b*]	500[c*]
Candida glabrata	16[a*]	---	250[b*]	500[c*]
Candida parapsilosis	1[a*]	---	---	500[b*]
Staphylococcus aureus	---	0.975[a*]	250[b*]	500[c*]
Escherichia coli	---	0.975[a*]	500[b*]	1000[c*]

Values expressed as average (n = 3). The results of each test were analyzed separately. Averages with different letters in the same line are statistically different by Scott and Knott test, *p<0.05. FCN, fluconazole; CFN, chloramphenicol.

DISCUSSION

When comparing the full scan spectrum of EB of *E. florida* DC (fig. 1) with the existing data in the literature, it might be said that there was agreement among the classes of secondary metabolites and putatively identified molecules, specially the flavonoids, tannins, phenolic acids, saponins and terpenoids [53].

Phenolic compounds are widely distributed in plants and have multiple biological activities, including prevention and treatment of many diseases, including microbial infections. They are a chemically heterogeneous group, in which most of the compounds are soluble in polar solvents, such as mixtures of water and ethanol [54]. This might account for the high concentrations of phenolic compounds in EB, since the efficiency of their extraction may be relatively higher.

The correlation of the results for total phenols in this study with the type of solvent used in the extraction, and with the chemical characterization by mass spectrometry, suggests the presence of polar phenolic compounds in EB, with an emphasis on those in the heterosidic form. Their relatively high concentration in this extract is likely due to the high solubility in ethanol 70% (v/v).

Among the naturally present compounds in the plants, the significant presence of flavonoids is due to their capacity for protection against ultraviolet radiation. Due to the presence of aromatic rings associated with hydroxyls in their chemical structures, flavonoids have the ability to capture and stabilize radiation avoiding damage to DNA. Flavonoids are relatively important phenolic compounds, especially with regard to their health benefits, which arise from their demonstrated activities [55, 56].

Tannins have been the subject of several studies, most of which discuss the different ecological interactions between vegetable tannins and herbivores. Some research on the biological activity of tannins has shown significant activity against certain microorganisms. In addition, they may act as anti-inflammatories, promote healing and inhibit HIV reverse transcription [57].

When EB obtained from the leaves of *E. florida* DC is compared with rich plant extracts such as green tea (*Camellia sinensis*), which features around 30 to 55 mg/g of phenolic compounds, it can be observed that EB had only slightly lower levels, equivalent to 25.8 mg/g [58].

Total phenols and flavonoids in a hydroethanolic extract of leaves of katukorandi (*Eugenia singampattiana*), a species found in India and used by indigenous tribes in the treatment of rheumatism and respiratory diseases, were determined to be, respectively, 21.58 mg EAG/g and 9.10 mg EQ/g [59]. These total phenol levels are very close to those found in the present study, however the flavonoid content is slightly higher than that found for EB.

Total phenols and flavonoids present in an aqueous extract of "pedra-ume-caá" (*Eugenia punicifolia*) leaves were determined as 21.6 mg EAG/g and 2.62 mg EQ/g, respectively [60]. The total phenol content is quite similar to that in the present study for EB, however the flavonoid content was very low.

However, one must take into consideration that the extract used by the researchers of this study was aqueous, suggesting that the extractor liquid used in the preparation of EB was more efficient in the removal of flavonoid glycosides.

The determination of total tannin content in samples of ethanolic extracts of *Eugenia calycina* revealed concentrations of these compounds equal to 2.43 mg EAT/g [18], lower than that found in EB in this study.

Differences in the levels of total phenols, flavonoids and tannins in this study, with those found in the literature, are due to differences in the various parameters employed in obtaining the extracts. For example, the processes of collecting plant material (date, time, site), the extractive method used, the solvent extractor, the processes of ecological plant interaction with the environment, the age and the stage of plant development can all have major effects. In addition, environmental conditions such as light, temperature, rainfall, radiation, seasonality, availability of water and atmospheric condition interfere directly in the production of secondary metabolites of biological and pharmacological interest. It should also be noted that some differences found in the levels of these metabolites in the literature might be related to different methodologies used for the quantitative determination of the compounds [61].

The extracts which have an MIC_{50} less than 100 μg/ml are considered to have excellent antimicrobial activity, between 100-500 μg/ml is considered moderate, those between 500-1000 μg/ml are slightly active and those with a MIC_{50} greater than 1000 μg/ml are inactive [62]. With respect to *C. krusei*, EB demonstrated excellent antimicrobial activity (MIC_{50} = 62.5 μg/ml). Against *C. glabrata* and *S. aureus* it was moderately active (MIC_{50} = 250 μg/ml), and it was slightly active against *E. coli* (MIC_{50} = 500 μg/ml). It was inactive against *C. albicans* and *C. parapsilosis*. The excellent antimicrobial activity of EB against *C. krusei* is important because this yeast is quite resistant to conventional antifungals, such as fluconazole (MIC_{50} = 32 μg/ml). This result demonstrates that the extract has significant potential as an antimicrobial against *C. krusei*.

The fungal inhibition capacity of phenolic compounds in an extract is due to the stabilization of cellular free radicals, preventing damage to infected cells. By contrast, tannins can inhibit fungal enzymes by complexation with enzyme substrates and metal ions, as well as modifying the microbial cell membrane, altering fungal metabolism [63].

Fungal infections in humans are caused mainly by allergic reactions to fungal proteins or toxins. These infections are responsible for the increased rate of morbidity and mortality in immunocompromised and severely ill patients. *Candida* sp. are yeasts that cause mycosis infections around the world. *C. albicans* and *C. krusei* are major pathogens responsible for the bulk of nosocomial infections. The yeasts *C. glabrata* and *C. parapsilosis* can grow as biofilms in materials used in hospitals such as catheters, needles, and other medical devices. They are considered as additional risk factors in the use of antibiotics [64].

EB of *E. florida* DC showed little activity on *E. coli*, which is a Gram-negative bacterium, compared with the Gram-positive bacterium *S. aureus*. This may be due to different compositions of the bacterial membranes. Gram-negative bacteria have an additional component in their structure: an outer lipid bilayer composed of lipopolysaccharides (LPS) firmly adhered to the peptidoglycan layer of the cell wall. This makes the bacterial cell membrane more lipophilic towards exogenous substances, whose power of penetration and/or action on the membrane determines their

antibiotic ability. Therefore, it is suggested that the flavonoids and tannins have the ability to complex with and/or inactivate enzymes, extracellular proteins, soluble proteins and bacterial cell walls, which determines their likely mechanisms of antimicrobial activity [65].

The antimicrobial activity of a hydroethanolic extract 70% (v/v) of *Eugenia uniflora* leaves on strains of *S. aureus* and *C. albicans* revealed minimum inhibitory concentration values of 90% of the microbial growth (MIC_{90}) of 80 and 500 μg/ml, respectively [66]. These values were demonstrated better antimicrobial activity than that evidenced by the MIC_{90} of EB for *S. aureus* and equal to *C. albicans*, in the present study.

Antimicrobial activity determination of ethanolic extracts of *Eugenia calycina* leaves against *S. aureus*, *E. coli*, *C. albicans* and *C. parapsilosis* demonstrated MIC_{50} values equal to 1000, 2000, 500 and 250 μg/ml, respectively [18]. These MIC_{50} values demonstrated weaker antimicrobial activity than for EB with respect to bacteria. However, they were more effective against the *Candida* yeasts, against which EB did not have significant activity.

It is important to consider the fact that the treatment of microbial infections is not always effective because conventional antimicrobials can cause recurrence of infections, induce resistance in microorganisms and generate toxicity. For these reasons, extracts and natural compounds have been investigated by researchers as new alternatives for the treatment of microbial diseases [66].

CONCLUSION

In this context, studies of chemistry and *in vitro* antifungal and antibacterial activities of EB of *E. florida* DC show relevant importance in phytotherapy. They can assist in improving the screening of chemical compounds in medicinal plants with antimicrobial activity and will aid the identification of potential plant sources for more effective and less toxic therapies.

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CONFLICT OF INTERESTS

All authors have none to declare

REFERENCES

1. Madduluri S, Sitaram B, Balasekharan C. *In vitro* evaluation of anti-inflammatory activity of methanolic and ethanolic leaf extracts of five indigenous plants in South India. Int J PharmTech Res 2014;6:569–74.
2. Firmo WCA, Menezes VJM, Passos CEC, Dias CN, Alves LPL, Dias ICL, *et al.* Historical context, popular use and scientific conception on medicinal plants. Res Notebooks 2011;18:90–5.
3. Chinni S, Dubala A, Kosaraju J, Khatwal RB, Kumar MNS, Kannan E. Effect of crude extract of *Eugenia jambolana* Lam. on human cytochrome P450 enzymes. Phytother Res 2014;28:1731–4.
4. Fouladvand M, Barazesh A, Tahmasebi R. Evaluation of *in vitro* antileishmanial activity of curcumin and its derivatives. Eur Rev Med Pharmacol Sci 2013;17:3306–8.
5. Romagnolo MB, Souza MC. The genus *Eugenia* L. (Myrtaceae) on the upper paraná river floodplain, mato grosso do sul and paraná states, Brazil. Acta Botanica Brasilica 2006;20:529–48.
6. Donato AM, Morretes BL. Foliar anatomy of *Eugenia florida* DC. (Myrtaceae). Braz J Pharmacognosy 2009;19:759–70.
7. Stefanello MEA, Pascoal ACRF, Salvador MJ. Essential oils from neotropical Myrtaceae: chemical diversity and biological properties. Chem Biodivers 2011;8:73–94.
8. Hussein SA, Hashem AN, Seliem MA, Lindequist U, Nawwar MA. Polyoxygenated flavonoids from *Eugenia edulis*. Phytochem 2003;64:883–9.
9. Rattmann YD, Souza LM, Malquevicz-Paiva SM, Dartora N, Sassaki GL, Gorin PA, *et al.* Analysis of flavonoids from *Eugenia uniflora* leaves and its protective effect against murine sepsis. J Evidence-Based Complementary Altern Med 2012;1–12. Doi.org/10.1155/2012/623940. [Article in Press]
10. Haminiuk CW, Plata-Oviedo MS, Mattos G, Carpes ST, Branco IG. Extraction and quantification of phenolic acids and flavonols from *Eugenia pyriformis* using different solvents. J Food Sci Technol 2014;51:2862–6.
11. Yang LL, Lee CY, Yen KY. Induction of apoptosis by hydrolyzable tannins from *Eugenia jambos* L. on human leukemia cells. Cancer Lett 2000;157:65–75.
12. Omar R, Li L, Yuan T, Seeram NP. α-Glucosidase inhibitory hydrolyzable tannins from *Eugenia jambolana* seeds. J Nat Prod 2012;75:1505–9.
13. Jasmine R, Selvakumar BN, Daisy P. Saponins from *Eugenia jambolana* with antibacterial activity against beta-lactamase producing methicillin resistant *Staphylococcus aureus*. J Med Plants Res 2007;1:1–6.
14. Saha S, Subrahmanyam EVS, Chandrashekar K, Shubhash CM, Shashidhara CS. Evaluation of antinociceptive and anti-inflammatory activities of extract and fractions of *Eugenia jambolana* root bark and isolation of phytoconstituents. Braz J Pharmacognosy 2013;23:651–61.
15. Moraes MM, Camara CAG, Santos ML, Fagg CW. Essential oil composition of *Eugenia langsdorffii* O. Berg.: relationships between some terpenoids and toxicity against *Tetranychus urticae*. J Braz Chem Soc 2012;23:1647–56.
16. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods Enzymol 1999;299:152–78.
17. Kalia K, Sharma K, Singh HP, Singh B. Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of *Potentilla astrosanguinea* Lodd. and quantification of its phenolic constituents by RP-HPLC. J Agric Food Chem 2008;56:10129–34.
18. Ferreira FP, Morais SR, Bara MT, Conceição EC, Paula JR, Carvalho TC, *et al. Eugenia calycina* Cambess extracts and their fractions: their antimicrobial activity and the identification of major polar compounds using electrospray ionization FT-ICR mass spectrometry. J Pharm Biomed Anal 2014;99:89–96.
19. CLSI (Clinical and Laboratory Standards Institute): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard; document M7-A6, Wayne, PA, USA; 2003.
20. CLSI (Clinical and Laboratory Standards Institute): Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard; document M27-S4, Wayne, PA, USA; 2012.
21. Okoh-Esene RU, Thomas SJH, Asuquo S. Proximate and phytochemical analysis of leaf, stem and root of *Eugenia uniflora* (Surinam or Pitanga cherry). J Nat Prod Plant Resour 2014;1:1–4.
22. Araújo AS, Rocha LL, Tomazela DM, Sawaya AC, Almeida RR, Catharino RR, *et al.* Electrospray ionization mass spectrometry fingerprinting of beer. Analyst 2005;130:884–9.
23. Bastos DHM, Saldanha LA, Catharino RR, Sawaya ACHF, Cunha IBS, Carvalho PO, *et al.* Phenolic antioxidants identified by ESI-MS from yerba maté (*Ilex paraguariensis*) and green tea (*Camelia sinensis*) extracts. Molecules 2007;12:423–32.
24. La Torre GL, Saitta M, Vilasi F, Pellicanò T, Dugo G. Direct determination of phenolic compounds in Sicilian wines by liquid chromatography with PDA and MS detection. Food Chem 2006;94:640–50.
25. Qiu X, Zhang J, Huang Z, Zhu D, Xu W. Profiling of phenolic constituents in *Polygonum multiflorum* Thunb. by a combination of ultra-high-pressure liquid chromatography with linear ion trap-Orbitrap mass spectrometry. J Chromatogr A 2013;1292:121–31.
26. Zhu Z, Zhang H, Zhao L, Dong X, Li X, Chai Y, *et al.* Rapid separation and identification of phenolic and diterpenoid constituents from radix *Salvia miltiorrhizae* by high-performance liquid chromatography diode-array detection, electrospray ionization time-of-flight mass spectrometry and electrospray ionization quadrupole ion trap mass spectrometry. Rapid Commun Mass Spectrom 2007;21:1855–65.

27. Mullen W, Yokota T, Lean MEJ, Crozier A. Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC-MS^n. Phytochemistry 2003;64:617–24.
28. Kato E, Nakagomi R, Gunawan-Puteri MD, Kawabata J. Identification of hydroxychavicol and its dimers, the lipase inhibitors contained in the Indonesian spice, *Eugenia polyantha*. Food Chem 2013;136:1239–42.
29. Barros L, Dueñas M, Carvalho AM, Ferreira ICFR, Santos-Buelga C. Characterization of phenolic compounds in flowers of wild medicinal plants from Northeastern portugal. Food Chem Toxicol 2012;50:1576–82.
30. Azuma K, Nakayama M, Koshioka M, Ippoushi K, Yamaguchi Y, Kohata K, *et al.* Phenolic antioxidants from the leaves of *Corchorus olitorius* L. J Agric Food Chem 1999;47:3963–6.
31. Mishra M, Shukla YN, Kumar S. Euphane triterpenoid and lipid constituents from *Butea monosperma*. Phytochemistry 2000;54:835–8.
32. Charrouf Z, Hilali M, Jauregui O, Soufiaoui M, Guillaume D. Separation and characterization of phenolic compounds in argan fruit pulp using liquid chromatography-negative electrospray ionization tandem mass spectroscopy. Food Chem 2007;100:1398–401.
33. Mämmelä P. Phenolics in selected European hardwood species by liquid chromatography electrospray ionization mass spectrometry. Analyst 2001;126:1535–8.
34. Engels C, Gräter D, Esquivel P, Jiménez VM, Gänzle MG, Schieber A. Characterization of phenolic compounds in jocote (*Spondias purpurea* L.) peels by ultra high-performance liquid chromatography/electrospray ionization mass spectrometry. Food Res Int 2012;46:557–62.
35. Bagri P, Ali M, Sultana S, Aeri V. New flavonoids from *Punica granatum* flowers. Chem Nat Compd 2010;46:201–4.
36. Hori K, Satake T, Saiki Y, Murakami T, Chen C. Chemical and chemotaxonomical studies of filices. LXXVII. Isolation and structure of novel catechin and proanthocyanidins from *Dennstaedtia distenta* Moore. Chem Pharm Bull 1998;36:4301–6.
37. Cao G, Li Q, Cai H, Tu S, Cai B. Investigation of the chemical changes from crude and processed Paeoniae *Radix alba*-atractylodis macrocephalae rhizoma herbal pair extracts by using Q exactive high-performance benchtop quadrupole-orbitrap LC-MS/MS. J Evidence-Based Complementary Altern Med 2014:1-14. Doi.org/10.1155/2014/170959. [Article in Press].
38. Poupard P, Sanoner P, Baron A, Renard CM, Guyot S. Characterization of procyanidin B2 oxidation products in an apple juice model solution and confirmation of their presence in apple juice by high-performance liquid chromatography coupled to electrospray ion trap mass spectrometry. J Mass Spectrom 2011;46:1186–97.
39. Santos PML, Schripsema J, Kuster RM. Glycosyl flavonoids from *Croton campestris* St. Hill. (Euphorbiaceae). Braz J Pharmacognosy 2005;15:321–5.
40. Vieira TR, Barbosa LCA, Maltha CRA, Paula VF, Nascimento EA. Chemical constituents from *Melaleuca alternifolia* (Myrtaceae). Quím Nova 2004;27:536–9.
41. Sun LR, Qing C, Zhang YL, Jia SY, Li ZR, Pei SJ, *et al.* Cimicifoetisides A and B, two cytotoxic cycloartane triterpenoid glycosides from the rhizomes of *Cimicifuga foetida*, inhibit proliferation of cancer cells. Beilstein J Org Chem 2007;3:1–6.
42. Jayasinghe ULB, Wannigama GP, Macleod JK. Glucuronides of *Diploclisia glaucescens*. J Chem Soc Pak 1998;20:131–7.
43. Jaiswal R, Kuhnert N. Hierarchical scheme for liquid chromatography/multi-stage spectrometric identification of 3,4,5-triacyl chlorogenic acids in green Robusta coffee beans. Rapid Commun Mass Spectrom 2010;24:2283–94.
44. Yoshikawa M, Dai Y, Shimada H, Morikawa T, Matsumura N, Yoshizumi S, *et al.* Studies on Kochiae fructus. II. On the saponin constituents from the fruit of Chinese *Kochia scoparia* (Chenopodiaceae): chemical structures of kochianosides I, II, III, and IV. Chem Pharm Bull 1997;45:1052–5.
45. Jayaprakasam B, Nair MG. Cyclooxygenase-2 enzyme inhibitory withanolides from *Withania somnifera* leaves. Tetrahedron 2003;59:841–9.
46. Hammerstone JF, Lazarus SA, Mitchell AE, Rucker R, Schmitz HH. Identification of procyanidins in cocoa (*Theobroma cacao*) and chocolate using high-performance liquid chromatography/ mass spectrometry. J Agric Food Chem 1999;47:490–6.
47. Zhang X, Xiao HB, Xue XY, Sun YG, Liang XM. Simultaneous characterization of isoflavonoids and astragalosides in two Astragalus species by high-performance liquid chromatography coupled with atmospheric pressure chemical ionization tandem mass spectrometry. J Sep Sci 2007;30:2059–69.
48. Liu Z, Li D, Owen NL, Grant DM, Cates RG, Jia Z. Oleanane triterpene saponins from the Chinese medicinal herb *Clinopodium chinensis*. J Nat Prod 1995;58:1600–4.
49. Zhao J, Yang X, Hattori M. Three new triterpene saponins from the seeds of *Aesculus chinensis*. Chem Pharm Bull 2001;49:626–8.
50. Madl T, Sterk H, Mittelbach M, Rechberger GN. Tandem mass spectrometric analysis of a complex triterpene saponin mixture of *Chenopodium quinoa*. J Am Soc Mass Spectrom 2006;17:795–806.
51. Alves TMA, Nagem TJ, Ribeiro A, Mendes NM, Goméz JD, Zani CL, *et al.* Molluscicidal saponins from *Guaiacum officinale* (Zygophyllaceae). Int J Pharmacogn 1996;34:81–6.
52. Mimaki Y, Ori K, Kubo S, Sashida Y, Nikaido T, Song L. Scillasaponins A, B, and C, new triterpenoid oligosaccharides from the plants of the subfamily Scilloideae. Chem Lett 2002;21:1863–6.
53. Neri-Numa IA, Carvalho-Silva LB, Morales JP, Malta LG, Muramoto MT, Ferreira JEM, *et al.* Evaluation of the antioxidant, antiproliferative and antimutagenic potential of araçá-boi fruit (*Eugenia stipitata* Mc Vaugh–Myrtaceae) of the Brazilian Amazon Forest. Food Res Int 2013;50:70–6.
54. Fang X, Wang J, Hao J, Li X, Guo N. Simultaneous extraction, identification and quantification of phenolic compounds in *Eclipta prostrata* using microwave-assisted extraction combined with HPLC-DAD-ESI-MS/MS. Food Chem 2015;188:527–36.
55. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J 2013;1–16. Doi:10.1155/2013/162750. [Article in Press]
56. Wu P, Ma G, Li N, Deng Q, Yin Y, Huang R. Investigation of *in vitro* and *in vivo* antioxidant activities of flavonoids rich extract from the berries of *Rhodomyrtus tomentosa* (Ait.) Hassk. Food Chem 2015;173:194–202.
57. Monteiro JM, Albuquerque UP, Araújo EL, Amorim ELC. Tannins: from chemistry to ecology. Quím Nova 2005;28:892–6.
58. Pereira VP, Knor FJ, Vellosa JCR, Beltrame FL. Determination of phenolic compounds and antioxidant activity of green, black and white teas of *Cammelia sinensis* (L.) Kuntze, Theaceae. Rev Bras Plantas Med 2014;16:490–8.
59. John KMM, Ayyanar M, Jeeva S, Suresh M, Enkhtaivan G, Kim DH. Metabolic variations, antioxidant potential, and antiviral activity of different extracts of *Eugenia singampattiana* (an endangered medicinal plant used by Kani tribals, Tamil Nadu, India) leaf. BioMed Res Int 2014:1–11. Doi.org/ 10.1155/2014/726145. [Article in Press]
60. Galeno DML, Carvalho RP, Boleti AP, Lima AS, Almeida PDO, Pacheco CC, *et al.* Extract from *Eugenia punicifolia* is an antioxidant and inhibits enzymes related to metabolic syndrome. Appl Biochem Biotechnol 2014;172:311–24.
61. Gobbo-Neto L, Lopes NP. Medicinal plants: factors of influence on the content of secondary metabolites. Quím Nova 2007;30:374–81.
62. Holetz FB, Pessini GL, Sanches NR, Cortez DA, Nakamura CV, Filho BP. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz 2002;97:1027–31.
63. Pandey AK, Kumar S. Perspective on plant products as antimicrobials agents: a review. Pharmacologia 2013;4:469–80.
64. Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. Virulence 2013;4:119–28.
65. Kumar TDK, Murali HS, Batra HV. Simultaneous detection of pathogenic *B. cereus*, *S. aureus* and *L. monocytogenes* by multiplex PCR. Indian J Microbiol 2009;49:283–9.
66. Auricchio MT, Bugno A, Barros SBM, Bacchi EM. Antimicrobial and antioxidant activities and toxicity of *Eugenia uniflora*. Lat Am J Pharm 2007;26:76–81.