# Antimicrobial activity and phytochemical screening of branches, fruits and leaves of *Eugenia brejoensis*

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This study evaluated the antimicrobial activity against Gram-negative and Gram-positive bacteria of Cyclohexane, Ethyl acetate and n-butanol fractions obtained from a liquid-liquid partition of methanolic extract from *E. brejoensis* leaves, fruits and branches. All tested tissue showed antimicrobial action. The leaves showed the best results. Polyphenols, flavanoids and tannin are related as possible active compounds. Our results show at the first time the broad spectrum antimicrobial activity of *E. brejoensis*, an endemic Caatinga plant. The isolation and structural characterization of these antimicrobial compounds is a sustainable application of the natural resource from Caatinga biome.
Keywords: *Eugenia brejoensis;* natural products; antibacterial activity; Caatinga biome

Este trabalho avaliou a atividade antimicrobiana frente à espécies Gram-positivas e Gram-negativas de frações orgânicas obtidas através da partição líquido-líquido de extratos metanólicos de folhas, frutos e ramos de *E. brejoensis.* Todos os tecidos foram capazes de inibir o crescimento bacteriano, tendo as frações de folha maior potencial. Polifenóis, flavanoídes e taninos foram detectados and são relacionados como possíveis agentes ativos. Nossos resultados mostram pela primeira vez a ação antimicrobiana de *E. brejoensis*, uma planta endêmica da Caatinga. O isolamento e caracterização estrutural desses compostos bioativos representa uma aplicação sustentável dos recursos naturaus provenientes da Caatinga.
Palavras-chave: *Eugenia brejoensis;* produtos naturais; atividade antimicrobiana; Caatinga

## 1. INTRODUCTION

The Myrtaceae family comprises a large number of genera (at least 132) and 5,671 species. The economic and therapeutic uses of this family have been report by several authors for the treatment of dysentery, gastroenteritis, stomachaches, indigestion, diabetes mellitus and hypertension [1,2,3,4,5]. In Brazil, Myrtaceae is an ecologically important family, composed of approximately 1000 species distributed in 23 genus [6] and represents the largest number of species in the Brazilian *restinga* [7].

Between the genus of Myrtaceae family, *Eugenia* is the largest with approximately 2000 species. This genus is distributed from the south of Mexico, Cuba and the Antilles to Uruguay and Argentina. *Eugenia* genus is not representative in Africa continent (60 species) [8]. Additionally, some species have edible fruits and have been extremely cultivated in tropical and subtropical regions, for example, in Brazil *Eugenia uniflora* L. and *E. brasiliensis* L.

Recently, a new species of *Eugenia* section *Racemosae* (Myrtaceae) was described in the area of Caatinga biome localized at Pernambuco State, north-eastern Brazil. This species was named as *Eugenia brejoensis* Mazine, due to its localization only from highland humid forests, locally called 'brejo' [9]. In this work we aimed to evaluate the antimicrobial activity of a new Myrtaceae specie *E. brejoensis* and performed a preliminary quantitative phytochemical screening.

## 2. MATERIALS AND METHODS

### 2.1. Preparation and fractionation of the methanolic extract

Samples of branches, fruits and leaves of *E. brejoensis* were collected in *Parque Nacional do Catimbau* (Pernambuco, Brazil, northeastern Brazil, in September 2010) and voucher specimens (IPA 84.033) were deposited in the Instituto de Pesquisa Agronômica de Pernambuco (IPA).

Each tissue was dried and ground into a fine powder (5 g), which were mixed with 50 mL of methanol and submitted to agitation for 15 hours. The supernatants were concentrated at 45°C, dissolved in water and submitted to liquid–liquid partitions successively with cyclohexane, ethyl acetate and n-butanol. The final residues were kept at 20º C until use.

### 2.2. Microbial strains

In this study we used the microorganisms provided by Departamento de Antibióticos, Universidade Federal de Pernambuco: *Bacillus subtilis* (UFPEDA82), *Staphylococcus aureus* (UFPEDA02), *Escherichia coli* (UFPEDA224), *Klebsiella pneumaniae* (UFPEDA396), *Salmonella enteritidis* (UFPEDA414), *Pseudomonas aeruginosa* (UFPEDA416).

### 2.3. Antimicrobial assays

The antibacterial activity was evaluated by the determination of minimum inhibitory concentration (MIC) and the Minimum bactericidal concentration (MBC), through a modified microdilution assay using a Resazurin solution (0.01%) as a growth indicator [10]. Brief, an aliquot of 100 μL of bacterial suspension (approximately 1.5 x $10^8$ CFU/mL) was added to a twofold serial dilution of the crude extract prepared in Mueller Hinton Broth (MHB) and incubated for 24 h at 37°C. The microorganism growth was detected using a Resazurin solution (0.01%): any color changes from purple to pink were recorded as microorganism growth. The lowest concentration at which no color change occurred was taken as the MIC. Afterwards, cultures were seeded in Mueller Hinton Agar and incubated for 24 h at 37°C to determine the MBC which corresponds to the minimum concentration of extract that eliminated the bacteria.

### 2.4. Phytochemical analysis

The phytochemical tests to detect the presence of tannins, flavonoids, anthocyanins, saponins, coumarins, quinones, anthraquinones, tannin, reducers compounds and alkaloids [11].

### 2.5. Statistical analysis

The experiments were performed in triplicate and expressed as the mean ± SD (standard deviation). Statistical analysis was performed by Student's t-test. Differences were considered significant at $p < 0.05$.

## 3. RESULTS AND DISCUSSION

The results of the antimicrobial activities of branches, fruits and leaves of *E. brejoensis* are exhibited in table 1. The concentration of DMSO used did not affect the bacterial growth. The best results were found to crude extract and fractions of *E. brejoensis* leaves ($p< 0.05$), which were able to inhibit both positive and negative microorganisms. For this tissue, the methanolic extract (ELME) showed MIC values of 6.25 mg/mL for *S. aureus*, 12.5 mg/mL for *P. aeruginosa* and 25 mg/mL for *B. subtilis* and *E. coli*. The ethyl acetate fraction (ELEF) showed the best results ($p < 0.05$) with MIC values of 3.12 mg/mL (*B. subtilis* and *P. aeruginosa*), 6.25 mg/mL (*S. aureus* and *S.* enteritidis) and 12.5 mg/mL (*E. coli* and *K. pneumoniae*). It is important to note that despite the ELME, the ELEF was able to inhibit *S. enteritidis* growth. The leaves cyclohexane fraction (ELCF) also showed antimicrobial action against *S. aureus* (MIC of

6.25 mg/mL), *E. coli, K. pneumoniae* (12.5 mg/mL), *B. subtilis* and *S. enteritidis* (25 mg/mL). The butanolic and cloroformic fractions from leaves did not showed antimicrobial activity.

*Table 1: Antimicrobial activity of crude extract and fractions obtained from branches, fruits and leaves of Eugenia brejoensis.*

| TISSUE | SAMPLE | *S. aureus* | | *B. subtilis* | | *E. coli* | | *P.aeruginosa* | | *S. enteritidis* | | *K. pneumoniae* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MIC | CBM | MIC | CBM | MIC | CBM | MIC | CBM | MIC | CBM | MIC | CBM |
| Branches | EBME | > 50 | > 50 | 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 |
| | EBBF | > 50 | > 50 | > 50 | > 50 | 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 |
| | EBChF | 25 | > 50 | 50 | > 50 | 25 | > 50 | 25 | > 50 | 25 | 25 | 50 | > 50 |
| | EBEF | 50 | > 50 | 50 | 50 | 25 | 50 | 25 | 25 | 25 | 50 | 50 | 50 |
| Fruits | EFME | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 |
| | EFBF | > 50 | > 50 | 50 | > 50 | > 50 | > 50 | 25 | > 50 | > 50 | > 50 | 50 | > 50 |
| | EFChF | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 |
| | EFEF | 25 | > 50 | 50 | > 50 | 25 | 50 | 25 | > 50 | 25 | 50 | 25 | 50 |
| Leaves | ELME | 6.25 | > 50 | 25 | > 50 | 25 | > 50 | 12.5 | > 50 | > 50 | > 50 | 25 | > 50 |
| | ELBF | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 |
| | ELCF | 6.25 | > 50 | 50 | > 50 | 12.5 | > 50 | 12.5 | > 50 | 50 | > 50 | 25 | > 50 |
| | ELEF | 6.25 | > 50 | 3.12 | > 50 | 12.5 | > 50 | 3.12 | > 50 | 6.25 | > 50 | 12.5 | 50 |

MIC and MBC are expressed in mg/mL

In other hand, for branches and fruits a weak antimicrobial capacity was observed. The branches cyclohexanic fraction (EBCF) showed MIC values of 25 mg/mL for all tested pathogens, except to *B. subtilis* and *K. pneumoniae* (50 mg/mL). The branches ethyl acetate fraction (EBEF) showed MIC values of 50 mg/mL for *S.aureus* and *B. subtilis*, and MIC of 25 mg/mL for the others bacteria.

In relation to *E. brejoensis* fruits, only ethyl acetate (EFEF) and butanolic (EFBF) fractions showed antibacterial activity. The ethyl acetate fraction showed action against all tested organisms with MIC values of 25 mg/mL and 50 mg/mL (only to *B. subtilis*). The butanolic fraction had MIC values of 25 mg/mL for *P. aeruginosa*, and 50 mg/mL for *B. subtilis* and *K.* pneumoniae.

Our results demonstrated that branches, fruits and leaves of *E. brejoensis* are sources of compounds which have a broad-spectrum bactericidal activity. This action in a same plant material is currently related to the presence of a wide spectrum antimicrobial substance, or by the action of toxins produced by the plant [12].

Additionally, we performed a preliminary quantitative phytochemical screening of the studied samples of *E. brejoensis.* The results showed the presence of polyphenols, flavonoids, tannins (only in the branches). In fact, the antimicrobial activity of these compounds has been documented by diverse researchers [13,14,15]. The presence of carbohydrates also was detected. Finally, the highest antimicrobial potential of *E. brejoensis* leaf can be attributed to the high content of flavonoids in this tissue. Our preliminary studies detected, at least, six possible new types of flavonoids (date not show). Another insight which indicates the importance of flavanoids as active substance in *E. brejoensis* was that the found MBC values were not more than 25 mL. Flavanoids have been characterized as weak bactericidal agents, but they are able to induce the formation of bacterial aggregates, provoking the reduction of the number of viable cells [13].

## 4. CONCLUSION

In conclusion, the results of our research showed that branches, fruits and leaves of *E. brejoensis* have antibacterial potential in a broad-spread way. These tissues were able to inhibit the growth of *B. subtilis*, *E. coli*, *K. pneumoniae*, *S. aureus*, *S. enteritidis*, and *P. aeruginosa*. The preliminary phytochemical analyses suggest that polyphenols, flavonoids and tannins are the main constituintes of the phytocomplex responsible for the biological activity. This is the first report of a biotechnology application of this new Myrtaceae species. The isolation, identification and characterization of bioactive substances of this Caatinga plant are objectives of further studies of our group to verify the antibacterial potential of these compounds.

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