Revista de Ciências Farmacêuticas Básica e Aplicada
Journal of Basic and Applied Pharmaceutical Sciences

Rev. Ciênc. Farm. Básica Apl., v. 29, n.3, p. 245-250, 2008
ISSN 1808-4532

Evaluation of antimicrobial activity of the crude ethanol extract of *Eugenia uniflora* L. leaves

Fiúza, T.S.[1]; Sabóia-Morais, S.M.T.[1]; Paula, J.R.[2]*; Tresvenzol, L.M.F.[2]; Pimenta, F.C.[3]

[1]*Instituto de Ciências Biológicas, Universidade Federal de Goiás, UFG, Goiânia, GO, Brasil.*
[2]*Faculdade de Farmácia, Universidade Federal de Goiás, UFG, Goiânia, GO, Brasil.*
[3]*Instituto de Patologia Tropical, Universidade Federal de Goiás, UFG, Goiânia, GO, Brasil.*

Recebido 06/08/2008 - Aceito 19/01/2009

ABSTRACT

***Eugenia uniflora* L. is a tree whose leaves are used in popular medicine as an antihypertensive, antimicrobial drug, in the treatment of bronchitis, influenza and as an antipyretic. This paper reports the antimicrobial activity of a crude ethanol extract of *E. uniflora* L. leaves. The crude extract was prepared from material collected in Goiânia, Goiás (Brazil), dried, pulverized and subjected to phytochemical screening. The antimicrobial activity was tested against spore-forming and non-sporing Gram-positive and Gram-negative bacteria as well as *Candida albicans*, using the well-diffusion test and the agar dilution method to determine the minimum inhibitory concentration (MIC). The phytochemical screening showed the presence of tannins, steroids, triterpenes, heterosides, anthraquinones, flavonoids and saponins. Antimicrobial activity testing showed that the crude *E. uniflora* L. leaf extract inhibited all the Gram-positive bacteria (MICs from 0.273 to 8.75 mg/mL), among which the spore-formers exhibited MICs from 1.094 to 2.187 mg/mL. The MIC for most Gram-negative bacteria varied from 4.375 to 17.5 mg/mL. *C. albicans* NTC 2010 (MIC of 0.547 mg/mL) inhibition was also noted. The antimicrobial activity found in this *in vitro* study of *E. uniflora* L. may justify its popular use as a medicine.**

Keywords: bacteria; MIC; *Eugenia uniflora* L.

INTRODUCTION

The genus *Eugenia* L. (Myrtaceae) has 14 species, among them *Eugenia uniflora* L., popular in Brazil for the delicious fruit *pitanga* ("Surinam cherry" in English). This plant is a 4 to 10 m tall semi-deciduous tree or bush with a smooth light brown trunk. The leaves are plain, chartaceous, 3 to 7 cm long, with a characteristic odor when crushed. The flowers are white, single or in groups of 2 or 3 on the axils and extremities of the branches. The fruit are drupes, globose and furrowed, bright red, yellow or black in color with a sour-sweet fleshy pulp containing one or two hard stones (Lorenzi & Matos, 2002).

Eugenia uniflora L. is a species found in the subtropical north and north-east of Argentina, in Brazil, Uruguay and Paraguay (Pepato et al*.,* 2001). It became part of empirical medicine thanks to the Guarany Indians in the fifteenth century (Alonso, 1998) and its leaves have been used in popular medicine as an anti-hypertensive and diuretic (Consolini & Sarubbio, 2002); adstringent, for the treatment of digestive disorders (Bandoni et al., 1972); antipyretic and anti-rheumatic (Alice et al., 1991), and antimicrobial drug (Souza et al., 2004). On the island of Madeira, *Eugenia uniflora* L. leaves are used to treat bronchitis, influenza and intestinal problems, and in Nigeria as an antipyretic (Consolini & Sarubbio, 2002). Pharmacological testing of the leaf extract of *E. uniflora* indicated inhibitory activity of the xanthine-oxidase enzyme through flavonoid action (Schmeda–Hirschmann et al., 1987), a reduction in blood pressure measured by direct vasodilation and a slight diuretic effect which could be due to an increase in renal blood flow (Consolini et al., 1999).

Adebajo et al. (1989) demonstrated antimicrobial properties of the *E. uniflora* L. essential oils. The bacterium most susceptible to the essential oils from the leaf and fresh fruit was *Pseudomonas aeruginosa*, whereas *Staphylococcus aureus* and *Serratia marcescens* proved resistant. The most susceptible fungus was *Trichophyton mentagrophytes*, while *Candida albicans* proved the most resistant. Lima et al. (2006) noted that the *E. uniflora* L. essential oil had little inhibitory effect on *C. albicans, Candida guilliermondii, Candida parapsilosis, Candida stellatoidea and Candida tropicalis*, although inhibitory activity was detected on *Candida krusei*.

Holetz et al. (2002) found that a hydroalcoholic leaf extract of *Eugenia uniflora* L. showed moderate activity [minimum inhibitory concentration (MIC) from 100 to 500 μg/mL] for *S. aureus* and *Escherichia coli* and good activity against *C. krusei, C. parapsilosis* and *C. tropicalis* (MIC < 100 μg/mL), but did not inhibit *C. albicans*. Souza

**Autor correspondente:* José Realino de Paula - Faculdade de Farmácia - Universidade Federal de Goiás, UFG - CP 131 - CEP: 74001-970 - Goiânia - GO, Brasil - Telefone: (62) 3209 6044 - Fax: (62) 3209 6037
e-mail: pjrpaula@gmail.com

et al. (2002) showed that *E. uniflora* L. ethanol leaf extract inhibited 19 out of 30 dermatophytes at a 500 µg/mL concentration, including *Microsporum canis, Microsporum gypseum, Trichophyton rubrum,* and *T. mentagrophytes*. On the other hand, Schapoval et al. (1994) did not detect antimicrobial activity in the infusion and decoction of the *E. uniflora* L. leaves against *S. aureus*, *E. coli* or *C. albicans*. Santos et al. (2004) noted antifungal toxicity in the *E. uniflora* L. crude ethanol leaf extract against *Paracoccidioides brasiliensis*, with a MIC of 750 mg/mL, and ether fraction activity with a MIC of 187.5 mg/mL.

Considering the emergence of resistant microorganisms with reduced susceptibility to available antibiotics (Tsuchiya et al., 1996), the aim of this study was to assess the antibacterial activity of *Eugenia uniflora* L. crude ethanol leaf extract against sporing and non-sporing Gram-positive and Gram-negative bacteria, as well as the antifungal activity against *Candida albicans*.

MATERIAL AND METHODS

Plant material

The leaves of *Eugenia uniflora* L. were collected in the city of Goiânia, Goiás, Brazil, which lies at 16º 36´15.1" S and 49º 16´ 0.70" W, at an elevation of 778m above sea level, from February to April 2005, and identified by Dr. José Realino de Paula of the Federal University of Goiás. A voucher specimen was deposited at the herbarium of that university under registration number UFG/29859. The leaves were oven dried in a stream of air at 40ºC and then ground to a powder in a blade mill.

Preparation of the crude ethanol extract

The *E. uniflora* L. leaf powder was soaked in 95% ethanol in a proportion of 1:1 (w/v), at room temperature, undergoing occasional shaking for 72 h, and then filtered. The resulting extract was concentrated by rotary evaporator at 40ºC and the plant residue was extracted similarly twice more, thereby obtaining the crude ethanol extract. The concentrated extract was dissolved in DMSO at a proportion of 1:3 (w/v) and the resulting solution used in the *in vitro* antimicrobial tests.

Phytochemical screening

The *E. uniflora* L. leaf powder was subjected to phytochemical screening, using qualitative analytical techniques for alkaloids, starch, coumarins, anthraquinone heterosides, steroids, triterpenes and digitalis heterosides, flavonoid heterosides, saponin heterosides and tannins. These techniques were adapted from Costa (2001).

Total phenol, tannin and flavonoid contents

Total phenol and tannin contents were determined by the Hagerman & Butler (1978) method, while total flavonoid was assayed by the method described for the *pitanga* tree in *Farmacopéia Brasileira* (2004).

Antimicrobial activity bioassay

Microorganisms

Antimicrobial activity was tested against standard strains of microorganisms obtained from the American Type Culture Collection (ATCC): *Micrococcus roseus ATCC* 1740, *Micrococcus luteus* ATCC 9341, *Bacillus cereus ATCC* 14576, *Bacillus stearothermophylus* ATCC 1262, *Bacillus subtilis* ATCC 6633, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 9027 and *Serratia marcescens* ATCC 14756; and some species of microorganisms obtained from a collection of clinical isolates from the Laboratory of Medical Bacteriology, Tropical Pathology and Public Health Institute, Federal University of Goiás, Brazil: *Staphylococcus aureus* 481, *Enterobacter cloacae* HMA/FTA 502, *E. coli* 8739 and against the fungus *Candida albicans* NTC 2010. To determine the MIC, in addition to the microorganisms above, the following bacteria were used: *S. aureus (*ATCC 6538*,* ATCC 25923*)*, *S. aureus* (897, 912, 915, 934, 937) (from a collection of clinical isolates from the Bacteriology Laboratory of the Department of Microbiology at the Tropical Pathology and Public Health Institute (IPTSP), Federal University of Goiás), *Staphylococcus epidermidis* ATCC 12228, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Antimicrobial activity screening was performed as recommended in NCCLS (2003), with modifications. The inoculum was prepared from cultures on nutrient agar incubated at 37ºC for 24 h, in 2 mL saline solution, at a turbidity of half the McFarland 1.0 scale.

Well diffusion test

The agar plates for the well diffusion test were prepared in two stages. A foundation layer of 20 mL of Mueller Hinton agar was poured into a Petri dish and left to set on a horizontal surface. A second layer was then poured on top, consisting of 100 µL of microbial suspension dispersed in 10.0 mL of molten Mueller Hinton agar, at 50ºC. The plates were kept on the same surface until the agar set.

Equidistant holes, 5.0 mm in diameter, were punched in a circular pattern in the agar and, 10 µL of concentrated ethanol extract, diluted 1:3 (w/v) in DMSO, was inoculated, with a solvent control plate containing only DMSO. This stage was performed in triplicate. A disk

with 10 µg penicillin G (Oxoid®) was placed on the plates containing Gram-positive bacteria as a positive control, while those with Gram- negative bacteria received a disk with 15 µg erythromycin (Oxoid®).

The dishes were preincubated at room temperature for two hours for extract diffusion. Latter, they were incubated for 24 h at 37ºC, after which the inhibition zone was measured with a millimeter ruler. This qualitative screening was performed to detect antimicrobial activity in the extract analyzed.

Determination of minimal inhibitory concentration (MIC)

Concentrated ethanol extract of *E. uniflora* L. leaves was weighed (2,800 mg) and diluted in 2 mL of DMSO in a test tube (E1). A second and third tube (E2 and E3) held 1.0 mL of DMSO and sterile distilled water (1.0mL) was added to a sequence of 7 more test tubes (E4 to E10). A 1.0 mL aliquot was transferred from tube E1 to tube E2 and mixed and the process repeated successively, giving serial dilutions up to E10. Next, 19 mL of molten Mueller Hinton agar, at 50ºC, was poured into each of the test tubes, vortexed and poured rapidly into sterile Petri dishes.

The final concentration of crude ethanol extract in the agar varied from 0.1367 to 70 mg/mL. Control plates containing DMSO were also prepared. A sterility test was performed by incubating all the plates at room temperature for 24 h.

The bacterial suspensions were then transferred to the Steers sampler (Steers et al*.,* 1959) and applied to the Mueller Hinton agar plates containing the various concentrations of plant extract. The plates were incubated at 37ºC for 24 h. The lowest concentration capable of inhibiting microbial development was considered the MIC.

RESULTS

Phytochemical screening

Phytochemical screening of the *Eugenia uniflora* L. leaf powder showed the presence of anthraquinone heterosides, free anthraquinones, steroids and triterpenes, flavonoid heterosides, saponin heterosides and tannins.

Total phenolic, tannin and flavonoid contents

The total phenolic content of the *E. uniflora* L. extract was 9.22%, the tannin content was 5.08% and the total flavonoid content was 0.53%.

Antimicrobial activity in the crude extract

The crude ethanol extract of *E. uniflora* L. leaves inhibited the development of all the Gram-positive bacteria, with MICs varying from 0.273 to 8.75 mg/mL (Table 1). Among these, the three spore-bearing bacteria [*Bacillus cereus* ATCC14576, *Bacillus stearothermophylus* ATCC 1262, *Bacillus subtilis* ATCC 6633] showed MICs of 1.094 mg/mL for *B. cereus* and 2.187 mg/mL for the other two (Table 1).

The minimal inhibitory concentration of crude extract for most of the Gram-negative bacteria was 17.5 mg/mL, except for *P. aerugionosa* ATCC 9027*,* which was inhibited by 4.375mg/mL, while the MIC for *P. aerugionosa* ATCC 27853 was 8.75 mg/mL and that for *Serratia marcescens* ATCC 14756, 35.0 mg/mL. *Candida albicans* NTC 2010 was also inhibited, the MIC being 0.547 mg/mL (Table 1).

DISCUSSION

The phytochemical screening of the *E. uniflora* L. leaf extract showed the presence of tannins, steroids, triterpenes, flavonoid heterosides, saponins, and anthraquinones, as previously reported by Lorenzi & Matos (2002) for this species. While investigating the phenolic constituents of *E. uniflora* L. leaves, Lee et al. (1997) reported the presence of eugeniflorin D1 and eugeniflorin D2, as well as two hydrolysable macrocyclic tannins, in the methanolic extract. Tannins can act as antiseptic and antimicrobial agents and have antihemorrhagic, antidiarrheic and wound-healing properties (Simões et al., 2004). Scalbert (1991) noted that various substrates rich in tannins inhibited bacteria belonging to the genera *Bacillus*, *Clostridium*, *Enterobacter*, *Pseudomonas*, *Nitrobacter*, *Staphylococcus* and *Streptococcus*, as well as fungi belonging to *Aspergillus*, *Botrytis*, *Colletotrichum*, *Penicillium* and *Trichoderma*. On the other hand, many flavonoids presented antioxidant, anti-inflammatory and antibacterial activity (Perruchon et al., 2002).

In the present study, the crude extract of *E. uniflora* L. leaves exhibited a broad spectrum of antimicrobial activity, inhibiting both spore-forming and non-sporing Gram-positive and Gram-negative bacteria, as well as the fungus *C. albicans* NTC 2010. Some authors describe similar antimicrobial activity in *E. uniflora* L. against *S. aureus*, *Bacillus subtilis*, *Micrococcus luteus* (Souza et al., 2004), *Providencia* spp. (Gonçalves et al., 2006) and *Bacillus cereus* (Ogunwande et al., 2005).

The antimicrobial activity of the crude ethanolic leaf extract of *E. uniflora* L. against *P. aeruginosa* should be highlighted. This bacterium is the most important pathogen within the genus *Pseudomonas* and is usually associated with humid environments such as water, soil and sewage. It is opportunistic and frequently infects patients with metabolic and hematological diseases, as well as others, and is an important cause of hospital infection (Thomas et al., 2005). *P. aeruginosa* is intrinsically resistant to many antimicrobial agents including many *β*-lactams, quinolones, chloramphenicol, tetracycline, macrolides,

Table 1 - Mean inhibition zone diameters (mm) in the agar diffusion test and minimal inhibitory concentrations (mg/mL) of crude ethanol extract of *E. uniflora* L. leaves and mean inhibition zones (mm) of controls (penicillin G and erythromycin disks).

MICROORGANISMS	Crude ethanol extract of *E. uniflora* L. leaves		Control Inhibition Zone (mm)
Gram-positive Bacteria	Inhibition Zone (mm)	MIC (mg/mL)	Penicillin G
Staphylococcus aureus ATCC 6538	-	2.187	-
S. aureus ATCC 25923	-	2.187	-
S. aureus 481	25	8.75	44
S. aureus 897	-	2.187	-
S. aureus 912	-	2.187	-
S. aureus 915	-	2.187	-
S. aureus 934	-	2.187	-
S. aureus 937	-	2.187	-
S. epidermidis ATCC 12228	-	0.273	-
Micrococcus roseus ATCC 1740	21	2.187	20
M. luteus ATCC 9341	23	0.273	77
Sporing Gram-positive Bacteria			
Bacillus cereus ATCC 14576	15	1.094	12
B. stearothermophylus ATCC 1262	16	2.187	53
B. subtilis ATCC 6633	15	2.187	37
Gram-negative Bacteria			Erythromycin
Enterobacter cloacae HMA/FTA 502	17	17.5	N
E. aerogenes ATCC 13048	17	17.5	N
Escherichia coli 8739	16	17.5	11
E. coli ATCC 11229	14	17.5	18
E. coli ATCC 25922	-	17.5	-
Pseudomonas aerugionosa ATCC 9027	25	4.375	10
P. aerugionosa ATCC 27853	-	8.75	-
Serratia marcescens ATCC 14756	15	35.0	15
Fungus			
Candida albicans NTC 2010	25	0.547	-

N : No inhibition zone formed; **- :** not tested; MIC **:** minimum inhibitory concentration

trimethoprim-sulfamethoxazole and rifampicin (Rossolini & Mantegoli, 2005). Resistance may be induced during antimicrobial chemotherapy and result in therapeutic failure. This phenomenon is correlated with an increase in morbidity and mortality, length of hospitalization and total hospital costs (Carmeli et al., 1999). There is, therefore, an increasing need for new antimicrobial agents and *E. uniflora* L. extract has proved to be a promising alternative source. More research is necessary to verify exactly which of the chemical compounds in the extract are responsible for its antimicrobial activity, degree of toxicity and *in vivo* pharmacological properties.

In conclusion, the antimicrobial activities found in this *in vitro* study may justify the popular use of *E. uniflora* L. as an antimicrobial agent (Souza et al., 2004), to treat bronchitis and intestinal problems (Consolini & Sarubbio, 2002), among other infections. These activities may be due to the presence of flavonoids and tannins in its chemical constitution.

ACKNOWLEDGEMENTS

The authors thank the Fundação de Apoio à Pesquisa (FUNAPE/UFG), the Faculdade de Farmácia/UFG, the Instituto de Ciências Biológicas/UFG and would like to thank Sharon Lois Vinaud for the translation into English.

RESUMO

Avaliação da atividade antimicrobiana do extrato etanólico bruto das folhas de Eugenia uniflora *L.*

A *Eugenia uniflora* L. é uma planta utilizada como fármaco anti-hipertensivo, antimicrobiano, no tratamento de bronquites, gripes e como antipirético. Este estudo avalia a atividade antimicrobiana do extrato etanólico bruto das folhas de *E. uniflora* L. O extrato etanólico bruto foi obtido do material coletado em Goiânia, Goiás (Brasil), dessecado, pulverizado e submetido à triagem fitoquímica. A atividade antimicrobiana foi avaliada com bactérias Gram-positivas esporuladas e não esporuladas, Gram-negativas, bem como *Candida albicans*, usando o teste de difusão em poço e o método de diluição em ágar para determinação da concentração inibitória mínima (CIM). A triagem fitoquímica evidenciou a presença de taninos, esteróides, triterpenos, heterosídeos antraquinônicos, flavonóides e saponínicos. A avaliação da atividade antimicrobiana mostrou que o extrato bruto das folhas de *E. uniflora* L. inibiu o desenvolvimento das bactérias Gram-positivas (CIM de 0,273 mg/mL a 8,75 mg/mL) e Gram-positivas esporuladas (CIM de 1,094 mg/mL a 2,187 mg/mL). A CIM para a maioria das bactérias Gram-negativas variou de 4,375 mg/mL a 17,5 mg/mL. A inibição da *C. albicans* NTC 2010 (CIM de 0,547 mg/mL) também foi notada. As atividades antimicrobianas encontradas neste estudo *in vitro* podem justificar o uso popular da *E. uniflora* L.

Palavras-chave: bactérias; CIM; *Eugenia uniflora* L.

REFERENCES

Adebajo AC, Oloke KJ, Aladesanmi AJ. Antimicrobial activities and microbial transformation of volatile oils of *Eugenia uniflora*. *Fitoterapia* 1989; 60:451-5.

Alice CB, Vargas VM, Silva GA, de Siqueira NC, Schapoval EE, Gleye J, Henriques JA, Henriques AT. Screening of plants used in south Brazilian folk medicine. *J Ethnopharmacol* 1991; **35:**165-71.

Alonso JR. *Tratado de fitomedicina:* bases clínicas y farmacológicas. Buenos Aires: Isis Ediciones S.R.L.; 1998. p.808-10.

Bandoni AL, Mendiondo ME, Rondina RV, Coussio JD. Survey of Argentine medicinal plants. I. Folklore and phytochemical screening. *Lloydia* 1972; **35:**69–80.

Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant *Pseudomonas aeruginosa*: comparison of risks associated with different antipseudomonal agents. *Antimicrob Agents Chemother* 1999; 43:1379-82.

Consolini AE, Baldini OA, Amat AG. Pharmacological basis for the empirical use of *Eugenia uniflora* L. (Myrtaceae) as antihypertensive. *J Ethnopharmacol* 1999; 66:33-9.

Consolini AE, Sarubbio MG. Pharmacological effects of *Eugenia uniflora* (Myrtaceae) aqueous crude extract on rat's heart. *J Ethnopharmacol* 2002; 81:57-63.

Costa AF. *Farmacognosia*. 3.ed. Lisboa: Fundação Calouste Gulbenkian; 2001.

Farmacopéia Brasileira. 4. ed. São Paulo: Atheneu; 2004. pt. 2. p.119-23.

Gonçalves AL, Alves Filho A, Menezes H. Atividade antimicrobiana de algumas plantas medicinais nativas contra bactérias encontradas em úlceras de decúbito. *Biológico* 2006; 68:1-5.

Hagerman AE, Butler LG. Protein precipitation method for the quantitative determination of tannins. *J Agric Food Chem* 1978; 26:809-12.

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(7):1027-31.

Lee MH, Nishimoto S, Yang LL, Yen KY, Hatano T, Yoshida T, Okuda Y. Two macrocyclic hydrolysable tannin dimers from *Eugenia uniflora*. *Phytochemistry* 1997; 44:1343-9.

Lima IO, Oliveira RAG, Lima EO, Farias NMP, Souza EL. Atividade antifúngica de óleos essenciais sobre espécies de *Candida*. *Rev Bras Farmacogn* 2006; 16(2):197-201.

Lorenzi H, Matos FJA. *Plantas medicinais no Brasil:* nativas e exóticas. Nova Odessa, SP: Instituto Plantarum; 2002. p.350-1.

NCCLS. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. NCCLS document M7-A6. 6th.ed. Wayne PA; 2003. p.1-47.

Ogunwande IA, Olawore NO, Ekundayo O, Walker TM, Schmidt JM, Setzer WN. Studies on the essential oils composition, antibacterial and cytotoxicity of *Eugenia uniflora* L. *Inter J Aromather* 2005; 15:147-52.

Pepato MT, Folgado VBB, Kettelhut IC, Brunetti IL. Lack of antidiabetic effect of a *Eugenia jambolana* leaf decoction on rat streptozotocin diabetes. *Braz J Med Biol Res* 2001; 34:389-95.

Perruchon S, Carola C, Buchholz H, Moinet C, Fressner WD. Estudo das propriedades dos flavonóides para cosméticos através do relacionamento função-estrutura. *Cosmet Toilet* (Ed. Português) 2002; 14(6): 74.

Rossolini GM, Mantengoli E. Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. *Clin Microbiol Infect* 2005; 11:17-32.

Santos SC, Ribeiro JP, Guimarães DO, Silva MO, Ferri PH, Garcia ACF, Pires JS, Castro ACM, Silva MRR, Paula JR. Antifungical activity of *Eugenia uniflora* L. fractions against *Paracoccidioides brasiliensis* (splendore) Almeida. *Rev Bras Plantas Med* 2004; 7(1):30-3.

Scalbert A. Antimicrobial properties of tannins. *Phytochemistry* 1991; 30(12):3875-83.

Schapoval EE, Silveira SM, Miranda ML, Alice CB, Henriques AT. Evaluation of some pharmacological activities of *Eugenia uniflora* L. *J Ethnopharmacol* 1994; 44:137-42.

Schmeda-Hirschmann G, Theoduloz C, Franco L, Ferro E, de Arias AR. Preliminary pharmacological studies on *Eugenia uniflora* leaves: xanthine oxidade inhibitory activity. *J Ethnopharmacol* 1987; 21:183-6.

Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR. *Farmacognosia:* da planta ao medicamento. 5.ed. Florianópolis: UFSC; 2004. p.615-56.

Souza LKH, Oliveira CMA, Ferri PH, Santos SC, Oliveira Júnior JG, Miranda ATB, Lião LM, Silva MRR. Antifungical properties of Brazilian cerrado plants. *Braz J Microbiol* 2002; 33:247-9.

Souza GC, Haas AP, von Poser GL, Schapoval EE, Elisabetsky E. Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. *J Ethnopharmacol* 2004; 90:135-43.

Steers E, Foltz EL, Graves BS. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot Chemother* 1959; 9:307-11.

Thomas L, Russell AD, Maillard JY. Antimicrobial activity of chlorhexidine diacetate and benzalkonium chloride against *Pseudomonas aeruginosa* and its response to biocide residues. *J Appl Microbiol* 2005; 98:533-43.

Tsuchiya H, Sato M, Miyazaki T, Fugiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant Staphylococcus aureus. *J Ethnopharmacol* 1996; 50:27-34.