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Full Length Research Paper

Screening the *in vitro* modulation of antibiotic activity of the extracts and fractions of *Ocimum gratissimum* L.

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***Escherichia coli* is known to produce enterotoxins whose properties and roles in diarrheal disease have been extensively investigated. Some species of *Staphylococcus* are often recognized as etiological agents of many animal and human opportunistic infections. This study is the first test of change in the resistance of antibiotic activity by *Ocimum gratissimum* L. against multi-resistant strains of *E. coli* and *Staphylococcus aureus*. In this study, the hexane and methanol extracts of *O. gratissimum* L. were tested for antibacterial activity alone and in combination with aminoglycosides against bacterial strains. The synergy of the methanolic extracts was verified by micro dilution method. A synergistic effect of both extracts and fractions combined with the aminoglycosides was demonstrated. It is therefore suggested that the extracts from *O. gratissimum* L. could be used as a source of natural products derived from this plant with resistance-modifying antibacterial activity, providing a new weapon against the problem of bacterial resistance to antibiotics.**

Key words: *Ocimum gratissimum* L., methanol extract, fractions, antibacterial activity, modification of resistance, antibiotics, *Staphylococcus aureus*, *Escherichia coli.*

INTRODUCTION

Bacteria of the genus *Staphylococcus* are distributed in nature, as well as being part of the normal microbiota of the skin and of the mucosa of animals including birds. Some specimens of *Staphylococcus* are frequently recognized as etiologic agents of opportunistic infections in many animals and humans (Coutinho et al., 2009a). *S. aureus, S. epidermidis, S. saprophyticus* and *S. haemolyticus* are the most important causative species of human and hospital infections. Besides causing different types of intoxications, *S. aureus* represents the most common etiologic agent of purulent infections (for example, furuncle, carbuncle, abscess, myocarditis, endocarditis, pneumonia, meningitis, bacterial arthritis) (Verhoeff et al., 1999).

E. coli is one of the principal causes of infectious diseases in humans. These bacteria are known to produce enterotoxins whose properties and role in

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Table 1. Bacteria source and antibiotic resistance.

Bacteria	Source	Antibiotic resistance
E. coli ATCC 25922		-
E. coli 27	Surgical would	Ast, Ax, Amp, Ami, Amox, Ca, Cfc, Cf, Caz, Cip, Clo, Im, Can, Szt, Tet, Tob
S. aureus ATCC 12692		-
S. aureus 358	Surgical would	Oxa, Gen, Tob, Ami, Can, Neo, Para, But, Sis, Net

Ast: aztreonam; Ax: amoxacilin; Amp: ampicillin; Ami: amikacin; Amox: amoxicillin; Ca: cefadroxil; Cfc: cefaclor; Cf: cefalotin; Caz: ceftazidime; Cip: ciprofloxacin; Chlo: chloranphenicol; Im: imipenem; Kan: kanamycin; Szt: sulfametim; Tet: tetracyclin; Tob: tobramycin; Oxa: oxacillin; Gen: gentamicin; Neo: neomycin; Para: paramomycin; But: butirosin; Sis: sisomicin; Net: netilmicin.

Table 2. Dry mass and yield of methanol extract and fractions (g).

Specie	Solvent used (Sigla)	Material	Dry mass	Yield
Ocimum gratissimum L.	Methanol (MEOG)	Leaves	332.65	8.98
	Acetate (AFOG)	Extract	8.98	0.44
	Methanol (MFOG)	Extract	8.98	4.37

MEOG – Methanol Extract of *Ocimum gratissimum*; AFOG – Acetate Fraction of Extract Methanol of *Ocimum gratissimum*; MFOG – Methanol Fraction of Methanol Extract of *Ocimum gratissimum*.

diarrheal disease has been widely investigated. The activity of cytotoxins and their role in human infection has been identified (Konowalchuk et al., 1978; Scotland et al., 1980), mainly in infections of the urinary tract (Hughes et al., 1982).

In relation to pathogenic bacteria, a growing and worrisome problem is the increase in bacterial resistance to antibiotics (Nostro et al., 2004). For patients, antimicrobial resistance increases morbidity and mortality, while there is a significant increase in costs for health care institutions (Coutinho et al., 2005). With respect to the growing clinical importance given to the hospital community and bacterial infections and the progressive development of antimicrobial resistance, considerable scientific research has focused on the antibacterial properties of plant products (Aguiar et al., 2008; Silva et al., 2008; Salvagnini et al., 2008; Simões et al., 2008).

In the last years, there has been great scientific interest in chemical and pharmacological investigations of the biological properties of medicinal plants (Barbosa-Filho et al., 2008; Coutinho et al., 2008a, b). Medicinal plants have been the source of many medications that are now applied in clinical practice. The use of extracts as antimicrobial agents shows a low risk of increasing resistance to their action, because they are complex mixtures, making microbial adaptability very difficult (Daferera et al., 2003).

O. gratissimum L. is an aromatic shrub originally from Asia and Africa (Martins and Alvarenga, 2008). Popularly known as alfavaca and manjericão, the leaves are used on traditional medicine. The antimicrobial activity of essential oil from these leaves was observed against several pathogenic microrganisms as *S. aureus*, *Bacillus* spp, *Pseudomonas aeruginosae*, *Klebisiella pneumoniae*, *Proteus mirabilis* and *Leishmania amazonensis* (Matasyoh et al., 2007; Martins and Alvarenga, 2008).

The aim of this study was to do a phytochemical screening of the methanol extracts and fractions of *O. gratissimum L*. and to determine their potentiation of the antibiotic activity of aminoglycosides.

MATERIALS AND METHODS

The bacterial strains utilized were the clinical isolates *E. coli* (EC27), *S. aureus* (SA358) and standard strains of *E. coli* (ATCC25922) and *S. aureus* (ATCC12692) with the resistance profile described in Table 1. All strains were maintained on slants with heart infusion agar (HIA, Difco Laboratories Ltda.). Before the assay, the cells were grown overnight at 37ºC in brain heart infusion broth (BHI, Difco Laboratories Ltda.).

Leaves of *O. gratissimum L.* were collected in the municipality of Crato, Ceará, Brazil. The plant material was identified and dried and pressed specimens were deposited in the Herbarium Dárdano de Andrade Lima of University Regional of Cariri - URCA, as No. 3978.

Preparation of methanol extract e fraction of *O. gratissimum* L.

For the preparation of the extracts, leaves were collected which were kept submersed in methanol separately for 72 h; afterward, the extract was filtered and concentrated using a rotary vacuum evaporator (model Q-344B – Quimis, Brazil) and ultrathermal bath (model Q-214M2 – Quimis, Brazil), obtaining yields of crude extracts, after the extract obtained was performed for obtaining the vacuum fractionation of the crude extract and fractions presented in Table 2. The solution utilized in the tests was prepared at a concentration of 10 mg/ml, dissolved in DMSO and then diluted with distilled water to obtain a concentration of 1024 µg/ml,

Table 3. Phytochemical prospection of methanol extract and fractions of *Ocimum gratissimum* L.

Extracts	Metabolites															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
MEOG	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+
AFOG	-	-	+	-	-	+	+	+	-	-	+	-	-	+	+	+
MFOG	-	-	+	-	-	+	+	+	-	-	+	-	+	+	-	+

1: phenols; 2: Tannin pyrogallates; 3: Tannin phlobaphenes; 4: anthocyanins; 5: anthocyanidins; 6: Flavones; 7: Flavonols; 8: Xanthones; 9: Chalcones; 10: Aurones; 11: Flavononols; 12: leucoanthocyanidins; 13: Catechins; 14: Flavonones; 15: Alkaloids; 16: Terpenes; (+) presence; (-) absence.

Table 4. MIC values (µg/ml) of MEOG, AFOG and MFOG in *Escherichia coli* (ATCC25922 and EC27) and *Staphylococcus aureus* (ATCC12692 and SA358).

Extract/fractions	MIC			
	***E. coli* ATCC 25922**	***E. coli* EC27**	***S. aureus* ATCC 12692**	***S. aureus* SA358**
MEOG	512	512	512	512
AFOG	256	256	128	256
MFOG	512	512	256	512

reducing the concentration of DMSO to 10%, where the microdilution procedure the concentration of DMSO did not reach significant level of toxicity.

Phytochemical prospecting

The phytochemical tests to detect the presence of heterosides, saponins, tannins, flavonoids, steroids, triterpenes, cumarins, quinones, organic acids and alkaloids were performed according to the method described by Matos (1997). The tests were based on the visual observation of a change in color or formation of precipitate after the addition of specific reagents, and the results for the extract and fractions studied show presence of tannins flobatenics, flavonoids (flavones, flavonols, xanthones, chalcones, auron, flavonons, leucoantocianidins, catechins), alkaloids and terpenes.

Drugs

Gentamicin, kanamycin, amikacin and neomycin were obtained from Sigma Chemical Co. All drugs were dissolved in sterile water.

Antibacterial test (MIC) and modulation of antibiotic activity

MIC (minimal inhibitory concentration) was determined in a microdilution assay (Javadpour et al., 1996) utilizing an inoculum of 100 µL of each strain, suspended in brain heart infusion (BHI) broth up to a final concentration of 10^5 CFU/ml in 96-well microtiter plates, using twofold serial dilutions. Each well received 100 µL of each extract solution. The final concentrations of the extracts varied 512 - 8 µg/ml. MICs were recorded as the lowest concentrations required to inhibit growth. The minimal inhibitory concentration for the antibiotics was determined in BHI by the microdilution assay utilizing suspensions of 10^5 CFU/ml and a drug concentration range of 2.500 to 2.4 µg/ml (twofold serial dilutions) (Javadpour et al., 1996). MIC was defined as the lowest concentration at which no growth was observed. For the evaluation of the extracts as modulators of resistance to the antibiotics, MIC of the antibiotics was determined in the presence or absence of MEOG and fractions at sub-inhibitory concentrations (64 – 32 µg/ml) and the plates were incubated for 24 h at 37°C. Each antibacterial assay for MIC determination was carried out in triplicate.

RESULTS

The phytochemical tests show the presence of various compounds indicated in Table 3. Table 4 presents the results of antibacterial activity, indicating that the MIC obtained MEOG and its fractions, when tested on the standards and multidrug-resistant strains of *E. coli* and *S. aureus*. Analyzing the data we observe that the solution of MEOG presented a MIC value of 512 µg/ml, as to the solutions of the fractions of the MIC showed MEOG value ranging from 128 to 512 µg/ml, and the MIC showed higher occurrence of 256 µg/ml.

Table 5 shows the result of the modulator of bacterial resistance to aminoglycosides front, indicating the MIC obtained the aminoglycoside alone and combined with concentrations of subinibitory MEOG and its fractions, when tested against the multidrug-resistant strains of *E. coli* and *S. aureus*. In all tests, the combination of antibiotics and MEOG and its fractions showed considerable reduction in the concentration of antibiotic needed to inhibit bacterial growth. The data revealed that the best MEOG showed synergistic activity with aminoglycosides than fractions when combined with the same antibiotics. The best activity in relation to their MEOG fractions is probably due to combined action of several secondary metabolites present in the extracts and after

Table 5. MIC values (µg/ml) of aminoglycosides in the absence and presence of MEOG, AFOG and MFOG in *Escherichia coli* 27 and *Staphylococcus aureus* 358.

Antibiotics	EC 27				SA 358			
	MIC Alone	MIC Combined			MIC Alone	MIC Combined		
		MEOG (64 µg/ml)	AFOG (16 µg/ml)	MFOG (32 µg/ml)		MEOG (64 µg/ml)	AFOG (16 µg/ml)	MFOG (32 µg/ml)
Gentamicin	78.1	2.4	2.4	2.4	19.5	2.4	2.4	2.4
Kanamicin	625	4.9	9.8	39.1	312.5	2.4	9.8	19.5
Amicacin	625	9.8	4.9	39.1	312.5	2.4	9.8	9.8
Neomicin	625	2.4	9.8	39.1	312.5	2.4	2.4	4.9

fractionation was probably isolated and thus reducing the potential synergistic fraction compared with the MEOG.

DISCUSSION

With the increase in the incidence of resistance to antibiotics, alternative natural products of plants could be of interest (Lu et al., 2007; Mbwambo et al., 2007). Some plant extracts and phytochemicals are known to have antimicrobial properties, which could be of great importance in the therapeutic treatments. In the last years, various studies have been conducted in different countries, demonstrating the efficacy of this type of treatment (Coutinho et al., 2008a, b). Many plants have been evaluated not only for direct antimicrobial activity but also as resistance modifying agents (Gibbons, 2004). Various chemical compounds, synthetic or from natural sources, have direct activity against many species of bacteria, enhancing the activity of a specific antibiotic, reversing the natural resistance of bacteria to specific antibiotics, causing the elimination of plasmids and inhibiting the active efflux of antibiotics through the plasma membrane (Coutinho et al., 2009a, b). The potentiation of antibiotic activity or the reversal of antibiotic resistance allows the classification of these compounds as modifiers of antibiotic activity (Coutinho et al., 2009a, b; Gunics et al., 2006; Molnar et al., 2004).

The phytochemical test shows the presence of various compounds such as of tannins flobatenics, flavonoids, alkaloids and terpenes. Through phytochemical prospecting of the extracts, it was possible to determine the presence of diverse classes of secondary metabolites that show a wide variety of biological activities such as antimicrobial (Djipa et al., 2000; Esquenazi et al., 2002; O'kennedy and Thomes, 1997), antioxidant (Barreiros and David, 2006), antitumor and anti-ophidic (Okuda et al., 1989).

In the tannins, the antimicrobial properties appear to be associated with the hydrolysis of an ester bond with gallic acid, thereby serving as a mechanism of natural defense against microbial infections. The antimicrobial property of tannic acid can also be utilized in food processing to increase shelf life. The tannin components of epicatechin and catechin (*Vaccinium vitisidaea* L.) demonstrated strong anti-microbial activity against bacteria and fungi (Ho et al., 2001).

Flavonoids are synthesized by plants in response to microbial infection (Dixon et al., 1983) and are effective against a broad range of microorganisms. The activity is probably due to their capacity to form complexes with extracellular soluble proteins, which bind to the bacterial cell wall. Some lipophilic flavonoids can also cause rupture of the plasma membrane of microorganisms (Tsuchiya et al., 1996).

Terpenes occur in the form of diterpenes, triterpenes, tetraterpenes as well as hemiterpenes and sesquiterpenes. Terpenenes or terpenoids are active against bacteria (Ahamd et al., 1993). The seeds contain active components; for example, volatile oil and thymoquinone afford protection against nephrotoxicity and hepatotoxicity induced by any disease or chemical product (Ali and Blunden, 2003).

Comparatively, the natural products may have a different antibacterial activity, when we consider the existence of differences in polarity and secondary metabolites, which according to the plant material, solvent fractionation and mechanism of determining the presence of substance and affinities for antimicrobial activity (Matias et al., 2010a, b).

The mechanisms by which extracts can inhibit the growth of microorganisms are varied, and can be due in part to the hydrophobic nature of some components. As a result, they can show greater interaction with the lipid bilayer of the cell membrane, affecting the respiratory chain and the production of energy (Nicolson et al., 1999), or even make the cell more permeable to antibiotics, leading to the interruption of vital cellular activity (Burt, 2004; Juven et al., 1994). Various components of extracts can permeabilize the cell membrane, increasing the penetration of antibiotics (Helander et al., 1998). The interference with bacterial enzyme systems can also be a potential mechanism of action (Wendakoon and Sakaguchi, 1995). These mechanisms of action can be obtained by the combination of antibiotic with extract at a sub-inhibitory concentration applied directly to the

culture medium (Coutinho et al., 2008a, b).

This strategy is called "herbal shotgun" or " synergistic multi-effect targeting" and refers to the utilization of plants and drugs in an approach using mono- or multi-extract combinations, which can affect not only a single target but various targets, where the different therapeutic components collaborate in a synergistic-agonistic manner. This approach is not only for combinations of extracts; combinations between natural products or extracts and synthetic products or antibiotics are also possible (Hemaiswarya et al., 2008; Wagner et al., 2009).

Conclusion

The results obtained indicate that *O. gratissimum L.* could serve as a source of plant-derived natural products that modify antibiotic resistance for use against multidrug-resistant bacteria, such as strains of *E. coli* and *S. aureus* standards and acquired from the hospital and from the community.

REFERENCES

Aguiar JS, Costa MCCD, Nascimento SC, Sena KXFR (2008). Atividade antimicrobiana de Lippia alba (Mill.) N. E. Brown (Verbenaceae). Rev. Bras Farmacogn, 18: 436-440.

Ahamd AA, Mahmoud AA, Williams HJ, Scott AI, Reibebspies JH, Mabry TJ (1993). New sesquiterpene a-methylene lactones from the Egyptian plants *Jasonia candicans*. J. Nat. Prod., 56: 1276–80.

Ali BH, Blunden G (2003). Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res., 17: 299–305.

Barbosa-Filho JM, Alencar AA, Nunes XP, Tomaz ACA, Sena-Filho JG, Athayde-Filho PF. 2008. Sources of alpha-, beta-, gamma-, delta- and epsilon-carotenes: A twentieth century review. Rev. Bras Farmacogn*, 18*: 135-154.

Barreiros ALBS, David JM (2006). Estresse Oxidaditivo: Relação entre geração de espécies reativas e a defesa do organismo. Quim Nova, 29: 113-123.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods – a review. Int. J. Food Microbiol*.,* 94: 223–253.

Coutinho HDM, Cordeiro LN, Bringel KP (2005). Antibiotic resistance of pathogenic bacteria isolated from the population of Juazeiro do Norte - Ceará. Rev. Bras Cienc e Saud, 9: 127-138.

Coutinho HDM, Costa JGM, Lima EO, Falção-Silva VS, Siqueira Jr JP (2008ª). Enhancement of the Antibiotic Activity against a Multiresistant *Escherichia coli* by *Mentha arvensis* L. and Chlorpromazine. *Chemotherapy,* 54: 328–330

Coutinho HDM, Costa JGM, Lima EO, Falção-Silva VS, Siqueira Jr JP (2009ª). Herbal therapy associated with antibiotic therapy: potentiation of the antibiotic activity against methicillin – resistant *Staphylococcus aureus* by *Turnera ulmifolia* L. BMC Complement Altern. Med., 9: 13.

Coutinho HDM, Costa JGM, Lima EO, Siqueira Jr JP (2009b). Effect of *Momordica charantia* L. in the resistance to aminoglycosides in ethicilin-resistant *Staphylococcus aureus*. Comparative immunology, Microbiol. Infect. Dis. (in press) doi:10.1016/j.cimid.2009b.08.001

Coutinho HDM, Costa JGM, Siqueira Jr JP, Lima EO (2008b). In vitro anti- staphylococcal activity of *Hyptis martiusii* Benth against methicillin-resistant *Staphylococcus aureus*-MRSA strains. Rev. Bras Farmacogn, 18(Supl.): 670-675.

Daferera DJ, Ziogas BN, Polissiou MG (2003). The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. michiganensis. Crop Prot., 22: 39-44.

Dixon RA, Dey PM, Lamb CJ (1983). Phytoalexins: enzymology and molecular biology. Adv. Enzymol. Relat. Areas. Mol. Biol., 55: 1–69.

Djipa CD, Delmee M, Quentin-Leclercq J (2000). Antimicrobial Activity of bark extracts of *Syzygium jambos* (Myrtaceae). J. Ethnopharmacol., 71: 307-313.

Esquenazi D, Wigg MD, Miranda MMFS, Rodrigues HM, Tostes JBF, Rozental S, Da Silva AJ, Alviano CS (2002). Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. Res. Microbiol., 53: 647-652.

Gibbons S (2004). Anti-staphylococcal plant natural products. Nat. Prod. Rep., 21: 263-277.

Gunics G, Farkas S, Motohashi N, Shah A, Harsukh G, Kawase M, Molnár J (2006). Interaction between 3,5-diacetyl-1,4-dihydropyridines and ampicillin, and erythromycin on different E. coli strains. *In Vivo, 20*(3): 367-372.

Helander IM, Alakomi HL, Latva-Kala K, Mattila Sandholm T, Pol EJI, Smid EJ, Gorris LGM (1998). Characterization of the action of selected essential oil components on Gram-negative bacteria. J. Agric. Food. Chem., 46: 3590-3595.

Hemaiswarya SH, Kruthiventi AK, Doble M (2008). Synergism between natural products and antibiotics against diseases. Phytomedicine, 15: 639-652.

Ho KY, Tsai CC, Huang JS, Chen CP, Lin TC, Lin CC (2001). Antimicrobial activity of tannin components from *Vaccinium vitisidaea* L. J. Pharm. Pharmacol., *53*: 187–91.

Hughes C, Muller D, Hacher J, Goebel W (1982). Genetics and pathogenic role of *Escherichia coli* haemolysin. Toxicon., 20: 247-252.

Javadpour MM, Juban MM, Lo WC, Bishop SM, Alberty JB, Cowell SM, Becker SL, Mclaughlin ML (1996). *De novo* antimicrobial peptides with low mammalian cell toxicity. J. Med. Chem., 39: 3107-3113.

Juven J, Kanner J, Schved F, Weisslowicz H (1994). Factors that interact with antimicrobial action of thyme essential oil and its active constituents. J Appl. Bacteriol., 76: 626–631.

Konowalchuk J, Dickie NS, Stavri, Speirs JI (1978). Properties of an *Escherichia coli* cytotoxin. Infect. Immun., 20: 575-577.

Lu Y, Zhao YP, Wang ZC, Chen SY, Fu CX (2007). Composition and antimicrobial activity of the essential oil of Actinidia macrosperma from China, Nat. Prod. Res., 21: 227-233.

Martins JR, Alvarenga AA (2008). Leaf Anatomy of alfavaca-cravo plants cultivated under colored nets. Cienc Rural, 39: 82-87

Matasyoh LG, Matasyoh JC, Wachira FN, Kinyua MG, Muigai AWT, Mukiama TK (2007). Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. Afr. J. Biotechnol., 6: 760-765.

Matias EFF, Santos KKA, Almeida TS, Costa JGM, Coutinho HDM. (2010ª). Atividade antibacteriana *In vitro* de *Croton campestris* A., *Ocimum gratissimum* L. e *Cordia verbenacea* DC. Revista Brasileira de Biociências, 8: 294-298

Matias EFF, Santos KKA, Almeida TS, Costa JGM, Coutinho HDM (2010). Enhancement of Antibiotic Activity by *Cordia verbenacea* DC. Acta Farmacéutica Bonaerense, 29: 1049-1052.

Matos FJA (1997). Introdução à Fitoquímica Experimental. 2ª Ed. – Fortaleza: Edições UFC.

Mbwambo ZH, Moshi MJ, Masimba PJ, Kapingu MC, Nondo RS (2007). Antimicrobial activity and brine shrimp toxicity of extracts of *Terminalia brownii* roots and stem. BMC Complement Altern. Med., 7: 9.

Molnar J, Molnar A, Spengler G, Mandi Y (2004). Infectious plasmid resistance and efflux pump mediated resistance. Acta. Microbiol. Immunol. Hung., 51(3): 333-349.

Nicolson K, Evans G, O´Toole PW (1999). Potentiation of methicillin activity against methicillin-resistant Staphylococcus aureus by diterpenes. FEMS Microbiol. Lett., 179: 233–239.

Nostro A, Blanco AR, Cannatelli MA, Enea V, Flamini G, Morelli I (2004). Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. FEMS Microbiol. Let., 230: 191-195.

O'kennedy R, Thomes RD (1997). Coumarins: biology applications and mode of action.New York: John Willey.

Okuda T, Yoshiba T, Hatano T (1989). Ellagitannins as active constituents of medicinal plants. Planta Med., 55: 117-122.

Salvagnini LE, Oliveira JRS, Santos LE, Moreira RRD, Pietro RCLR (2008). Avaliação da atividade antibacteriana de folhas de *Myrtus communis* L. (Myrtaceae). Rev. Bras Farmacogn, 18: 241-244.

Scotland SM, Day NP, Willshaw GA, Rowe B (1980). Cytotoxic enteropathogenic *Escherichia coli*. Lancet, 315: 90.

Silva MAR, Higino JS, Pereira JV, Siqueira-Júnior JP, Pereira MSV (2008). Antibiotic activity of the extract of *Punica granatum* Linn. over bovine strains of *Staphylococcus aureus*. Rev. Bras Farmacogn, 18: 209-212.

Simões CC, Araújo DB, Araújo RPC (2008). Estudo *in vitro* e ex vivo da ação de diferentes concentrações de extratos de própolis frente aos microrganismos presentes na saliva de humanos. Rev. Bras Farmacogn, 18: 84-89.

Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Jinuma M (1996). Comparative study on the antibacterial Activityof phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. J. Ethnopharmacol., 50: 27–34.

Verhoeff J, Beaujean D, Vlok H, Baars A, Meyler A, Werkwn VDC (1999). A dutch approach to methicillin-resistance *Staphylococcus aureus*. Eur. J. Clin. Microbiol. Infect. Dis., 18: 461-466.

Wagner H, Ulrich-Merzenich G (2009). Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine, 16: 97-110.

Wendakoon C, Sakaguchi M (1995). Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. J. Food Prot., 58: 280–283.