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Evaluation of the modulatory and antibacterial activity of the ethanolic extract and fractions of *Duguetia furfuracea* A. St.-Hil.

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Medicinal plants have been the subject of research in several countries such as Brazil. The *Duguetia furfuracea* A. St.-Hil., popularly known as araticum-bravo, ata-brava and ata de lobo, has been used in folk medicine as anti-rheumatic drugs, for the treatment of renal dysfunction, spinal pain and stomach, and against pediculosis. This work aimed to analyze the antibacterial effect of the crude extract and fractions obtained from the leaves of *D. furfuracea*. The characterization of secondary metabolites was carried out through phytochemical prospection, being checked for the presence of tannins, flavonoids and alkaloids. The minimum inhibitory concentration (MIC) was determined by using broth microdilution method and its modulating activity of antibiotic activity in sub-inhibitory (MIC/8) concentration. When standard bacterial strain is used for the MIC and multidrug-resistant strains of modulation, all samples had a MIC ≥ 1024 µg/ml. The samples when combined with aminoglycosides demonstrated synergistic activity against the *Escherichia coli* 27 and *Staphylococcus aureus* 358. The results of this study indicate the species *D. furfuracea* as a promising source in combating bacterial multidrug resistance, increasing the potential of antibiotics.

Key words: *Duguetia furfuracea* A. St.-Hil., modulation, antibacterial activity, aminoglycosides.

INTRODUCTION

The use of medicinal plants for the treatment of diseases is one of the oldest practices of mankind, and the information for their use comes through popular culture (Funke and Melzig, 2006; Biavatti et al., 2007; Oliveira et al., 2007; Agra et al., 2007). The use of these plants, especially in South America contributes significantly to the basic health care. For the treatment of common infections, many plants are used in Brazil in the form of crude extract, infusions or plasters, without any scientific evidence of its effectiveness (Nakamura et al., 1999).

With respect to pathogenic bacteria, bacterial resistance to antibiotics is a growing problem and concern (Georgopapadakou, 2005; Nostro et al., 2004). The constant use of antibiotics has caused a lot of problems among which we can highlight the imbalance of human ecology and microbial resistance. This context shows that

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there is a need to search for new antibiotics that are effective, opening paths for the development of research, because the development of any new antimicrobial comes with resistance of microorganisms; the emergence of resistant pathogens is a threat to these advances (Moellering, 2000).

Research on natural products with antimicrobial activity has increased significantly in recent years. Medicinal plants have been the subject of research in several countries such as Brazil. This country holds a rich biodiversity and possessor of a diverse flora. In this way, the diversity of molecules found in plants makes them promising sources of new antimicrobials (Di Stasi et al., 2002; Estevam et al., 2009). The Annonaceae family has approximately 135 genera and 2,500 species of tropical and subtropical trees and shrubs, fruit trees (Fechine et al., 2002; Chatrou et al., 2004), and in Brazil 33 genera and 250 species were identified (Souza and Lorenzi, 2005). Within this family, the genre *Duguetia* stands by the various structural classes found: alkaloids, amides, diterpenes, steroids, flavonoids and roundness (Pontes et al., 2004; Carrolo et al., 2006).

The species *Duguetia furfuracea* St.-Hil., occurs in several regions in Brazil, the state of Mato Grosso do Sul; this species often becomes an invading plant when the cerrado is turned into pastures. Popularly known as araticum-bravo, ata-brava and ata de lobo, has been used in folk medicine as anti-rheumatic drugs, for the treatment of renal dysfunction, spinal pain and stomach, and against pediculosis (Rodrigues and Carvalho, 2001; Carolo et al., 2006; Pott and Pott, 1994; Gottsberge, 1987; Gavilanes and Brandão, 1998).

This work aimed to analyze the antibacterial effect of the crude extract and fractions obtained from the leaves of *D. furfuracea*.

MATERIALS AND METHODS

Bacterial

The bacterial strains used in this study were four standard strains of bacteria (Gram-positive and Gram-negative): *E. coli* (ATCC10536) and clinical isolated (EC27), *Staphylococcus aureus* (ATCC25923) and clinical isolated (SA358), *Pseudomonas aeruginosa* (ATCC15442) and *Klebsiella pneumoniae* (ATCC4362) with resistance profile shown in Table 1. The microorganisms used in the tests were obtained from Instituto Nacional de Controle de Qualidade em Saúde (INCQS) da Fundação Oswaldo Cruz, Ministério da Saúde. All strains were maintained on Heart Infusion Agar (HIA; Difco laboratories Ltda), being subsequently cultivated by 24 h in Brain-Heart Infusion (BHI – Difco, Laboratories Ltda).

Plant

The leaves of *D. furfuracea* were collected in June 2010, at Sítio Barreiro Grande, in the municipality of Crato, Ceará, Brazil. A plant was prepared and sent for identification in the Herbarium Dárdano Andrade Lima of Regional University of Cariri (URCA) and registered under number # 5508.

Preparation of crude extract and fractions

The ethanolic extract was prepared from the fresh leaves of *D. furfuracea* (637 g) by cold extraction method. The leaves of the species were previously washed in running water, crushed and macerated and then subjected to solvent extraction in ethanol P.A (Dinâmica, Brasil) for 72 h. After this period the ethanolic solvent has been distilled using a Rotary evaporator unit at 60°C under reduced pressure, yield getting of 1.57%. After this process, it was made the fractionation of ethanolic extract (10 g) under vacuum filtration, using three solvents (according to polarity scale): hexane, ethyl acetate and methanol.

Drugs

Neomycin, Kanamycin, Gentamicin and Amikacin were obtained from Sigma Chemical Laboratory Corp., St. Louis, MO, USA. All drugs were dissolved in sterile water.

Phytochemical prospecting

The phytochemical prospecting of ethanolic extract of *D. furfuracea* leaves was done through the methodology of Matos (1997); where to identify the classes of secondary metabolites it was observed that the color changed the formation of precipitates after addition of specific reagent. The results obtained after the tests are described as shown in Table 2.

Test of modulatory and antibacterial activity of aminoglycosides

The solutions of the crude ethanolic extract and fractions (hexane, ethyl acetate and methanol) were prepared using 10 mg samples dissolved in 1 ml of dimethyl sulfoxide (DMSO), obtaining an initial concentration of 10 mg/ml, if required, diluted in distilled water reaching concentration of 1024 µg/ml.

The minimum inhibitory concentration (MIC) was determined through microdilution method in 10% BHI with suspension of 10^5 UFC/ml, from the inoculum of 100 µl of each standard lineage of bacteria and then added 100 µl of each natural product, being diluted in serial manner with final concentrations of samples ranging from 512 to 8 µg/ml (Javadpour et al., 1996). For the evaluation of the samples as modulator of antibiotic activity, the MIC of antibiotics were determined in the presence and absence of natural products in sub-inhibitory concentrations (MIC/8) (Coutinho et al., 2008). Aminoglycosides have been assessed in concentrations that varied from 2,500 to 1.22 µg/ml. The plates were incubated at 35°C for 24 h.

RESULTS AND DISCUSSION

The results of the MIC to all samples were ≥1024 µg/ml, this value does not demonstrate clinical relevance. However, in modulating activity of aminoglycosides was checked, synergistic activity with some of the antibiotics tested.

Table 1. Bacterial origin and profile of antibiotic resistance.

Bacteria	Origin	Resistance profile
E. coli 27	Surgical wound	Ast, Ax, Amp, Ami, Amox, Ca, Cfc, Cf, Caz, Cip, Clo, Im, Can, Szt, Tet, Tob
E. coli ATCC 10536	-	-
K. pneumoniae ATCC 4362	-	-
P. aeruginosa ATCC 15442	-	-
S. aureus 358	Surgical wound	Oxa, Gen, Tob, Ami, Can, Neo, Para, But, Sis, Net
S. aureus ATCC 25923	-	-

Ast: Aztreonan; Ax: Amoxicillin; Amp: Ampicilin; Ami: Amikacin; Amox: Amoxilina; Ca: Cefadroxil; Cfc: Cefaclor; Cf : Ceftazidime; Cip: Ciprofloxacin; Clo: Chloramphenicol; Im: Imipenem; Can: kanamycin; Szt: Sulfametrin; Tet: Tetracycline; Tob: Tombramicina; Oxa: Oxacillin; Gen: Gentamicin; Neo: Neomycin; Para: Paromomycin; But: Butirosin; Sis: Sisomycin; Net: Netilmicin.

Table 2. Classes of secondary metabolites found in the crude ethanolic leaves of *Duguetia furfuracea*.

Classes of metabolites	(+) Presence(-) Absence
Phenols	-
Pyrogallic tannins	-
Condensed tannins	+
Anthocyanins	-
Anthocyanidins	-
Flavones	-
Flavonols	-
Xanthones	-
Chalcones	+
Aurones	+
Flavanonol	-
Leucoanthocyanidin	-
Catechin	+
Flavanone	+
Alkaloids	+

Tables 3 and 4 show synergistic or antagonistic modulating activity to the antibiotics kanamycin, amikacin, neomycin or gentamicin in association to the crude ethanolic extract and fractions. Against multi-drug resistant bacteria *E. coli* (27), the ethyl acetate and methanol fractions demonstrated synergism when associated to the antibiotic kanamycin, showing a reduction of MIC to 625 µg/ml. In the association of amikacin with hexane and ethyl acetate fractions showed a reduction of the MIC to 78, 12 and 312.5 µg/ml, respectively. To neomycin, both the crude extract and the hexane and methanol fractions promoted reduction of MIC in 156.25 µg/ml. In relation to multiresistant bacteria *S. aureus* (358), the crude ethanolic extract when associated with the antibiotic kanamycin, obtained a MIC reduction of 78.12 µg/ml. However, the mikacin when combined with the crude ethanolic extract and with hexane and methanol fractions promoted a reduction of MIC in 156.25 µg/ml. The crude ethanolic extract association with gentamicin, presented the synergism reducing the MIC from the antibiotic to 2.44 µg/ml.

Natural compounds, either from plant and animal origin can cause alteration of the effect of antibiotics, either increasing or antagonizing the antibiotic activity (Coutinho et al., 2008; Rodrigues et al., 2009; Tintino et al., 2013). We note that both extract and fractions acted synergistically when associated with aminoglycosides, showing a catalyzing effect, favoring an antibacterial activity to them. This was also observed as an antagonistic effect of natural products in association with aminoglycoside gentamicin against *E. coli* (27). This same effect has also been observed in studies by Veras et al. (2011), which reported a significant increase in MIC among natural products and aminoglycosides. According to Granowitz and Brown (2008), the antagonistic effects of combined use of antibiotics can be assigned to mutual chelation. According to Behling et al. (2004), the antagonistic effects of combined use of antibiotics can be

Table 3. Modulatory activity of *Duguetia furfuracea* against bacteria *E. coli* (27) with: crude ethanolic extract, hexane fraction, ethyl acetate fraction and methanolic fraction.

E. coli 27	+EEDF	+FH	+FAE	+FM	Control
Kanamycin	2.500	2.500	625	625	2.500
Amikacin	1.250	78,12	312,5	2.500	>2.500
Neomycin	156.25	156.25	625	156.25	625
Gentamicin	1.250	625	312.5	1.250	19.53

EEDF: Ethanolic extract of *Duguetia furfuracea*; FH: hexane fraction; FAE: ethyl acetate fraction; FM: methanolic fraction.

Table 4. Modulatory activity of *Duguetia furfuracea* against bacteria *S. aureus* (358) with: crude ethanolic extract, hexane fraction, ethyl acetate fraction and methanolic fraction.

S. aureus 358	+EEDF	+FH	+FAE	+FM	Control
Kanamycin	78.12	312.5	312.5	312.5	312.5
Amikacin	156.25	156.25	625	156.25	625
Neomycin	312.5	312.5	312.5	312.5	312.5
Gentamicin	2.44	19.53	19.53	19.53	19.53

EEDF: Ethanolic extract of *Duguetia furfuracea*; FH: hexane fraction; FAE: ethyl acetate fraction; FM: methanolic fraction.

assigned to mutual chelation. This possibly explains the reduction in the activity of aminoglycoside antibiotics in the presence of ethanolic extract of leaves of *D. furfuracea*, which demonstrated the presence of flavonoids by phytochemical survey carried out.

The results observed in species confirm with those found by Bento (2010), where the leaf extract of *Annona muricata* demonstrated a catalyzing effect against the same strains and species belonging to the same family. It is suggested that the synergistic effect brought by the extract and fractions can be due to the presence of compounds that exhibit antibacterial activity such as tannins and flavonoids. In tannins, antibacterial properties can be associated with the hydrolysis of an ester linkage between gallic acid, thereby serving as a natural defense mechanism against infections (Ho et al., 2001). However, the flavonoids have activity that probably is due to its ability to form complexes with soluble extra-cel proteins that bind to the bacterial cell wall (Tsuchiya et al., 1996).

The data obtained indicate the species *D. furfuracea* as a promising source in combating bacterial multidrug resistance, presenting itself as potentiating antibiotic.

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