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Evaluation of antimicrobial activity of the fixed oil of *Bowdichia Virgilioides* Kunth (fabaceae) seeds

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Abstract – Bacterial resistance to current drugs is a major public health problem worldwide. The search for biologically active compounds that act synergistically with antibiotics for their use at lower concentrations would be of great help in overcoming bacterial resistance. *Bowdichia virgilioides* Kunth, also known as sucupira-preta or sucupira-do-cerrado, is a species of the family Leguminosae-Papilionoidea that occurs in both primary and secondary formations, always in fast-draining areas. The objective of this study was to evaluate of antibiotic modulation through of the fixed oil from the seeds of *B. virgiloides* activity. . The seeds showed a considerable amount of oil, with a yield of approximately 11%. The oil did not inhibit bacterial growth, but its combination with the antibiotics tested produced growth inhibition. Our data indicated that the oil extracted from *B. virgiloides* seeds has no antibacterial activity at clinically relevant concentrations, but when combined with aminoglycoside antibiotics, it showed modulatory activity, lowering the antibiotic resistance of Gram-negative strains

Key Words: *Medicinal plants. Sucupira-preta. Modulation.*

Avaliação da atividade antimicrobiana do óleo fixo das sementes de *Bowdichia Virgilioides* Kunth (fabaceae)

Resumo – A resistência das bactérias aos medicamentos atuais é um importante problema de saúde pública a nível mundial. A busca de compostos biologicamente ativos que agem sinergicamente com antibióticos para a sua utilização em concentrações mais baixas seria de grande ajuda para superar a resistência bacteriana. *Bowdichia virgilioides* Kunth, também conhecida como sucupira-preta ou sucupira-do-cerrado, é uma espécie da família Fabaceae, que ocorre em ambas as formações primárias e secundárias, sempre em áreas de drenagem rápida. O objetivo deste estudo foi avaliar a atividade antimicrobiana e moduladora de antibióticos através do óleo fixo das sementes de *B. virgiloides.* As sementes apresentaram uma quantidade considerável de óleo, com um rendimento de aproximadamente 11%. O óleo não inibiu o crescimento bacteriano, mas a sua combinação com os antibióticos testados inibiram o crescimento. Os nossos dados indicam que o óleo extraído das sementes *B. virgiloides* não tem atividade anti-bacteriana em concentrações clinicamente relevantes, mas quando combinado com os antibióticos aminoglicosídicos, mostrou atividade moduladora, diminuindo a resistência aos antibióticos de estirpes Gram-negativas.

Palavras-Chave: *Plantas medicinais. Sucupira-preta. Modulação*

Evaluación de la actividad antimicrobiana de la aceite fijo de las semillas *Bowdichia Virgilioides* Kunth (fabaceae)

Resumen – La resistencia bacteriana a los fármacos actuales es un problema importante de salud pública en todo el mundo. La búsqueda de compuestos biológicamente activos que actúan sinérgicamente con antibióticos para uso en concentraciones más bajas sería de gran ayuda para superar la resistencia bacteriana. *Bowdichia virgilioides* Kunth, también conocido como Sucupira-negro del cerrado es una especie de la familia Fabaceae, que se produce tanto en la formación primaria y secundaria, siempre en áreas de drenaje rápido. El objetivo de este estudio fue evaluar la actividad antimicrobiana y modulador de antibióticos a través del aceite fijo de las semillas de *B. virgiloides*. Las semillas mostraron una cantidad considerable de aceite, con un rendimiento de aproximadamente 11%. El aceite no inhibió el crecimiento bacteriano, pero su combinación con antibióticos probados crecimiento inhibido. Nuestros datos indican que el aceite extraído de las semillas de *B. virgiloides* no tiene actividad anti-bacteriana a concentraciones clínicamente relevantes, pero cuando se combina con antibióticos aminoglucósidos mostrar actividad moduladora, la disminución de la resistencia a cepas Gram-negativos de los antibióticos.

Palabras Clave: *Las plantas medicinales. Sucupira negro. Modulación.*

Introduction

With the growing incidence of antibiotic resistance, natural plant products can be interesting alternatives against microorganisms (Lu et al. 2007; Mbwambo et al. 2007). Some plant products are known to have antimicrobial properties and can be used in therapeutic treatments (Coutinho et al. 2008a; Coutinho et al. 2008b). Natural products from seeds with pharmacological potential have been studied in recent years as a promising source of biologically active molecules. The use of seeds in popular medicine for the treatment of various diseases has expanded. This knowledge of popular medicine is in agreement with the characterization of these herbal products (Scott et al. 2011a).

In recent years, the effectiveness of these treatments based on natural products has been proven by several research groups (Benoit-Vical et al. 2006; Senatore et al. 2007; Singh et al. 2007). Many plants have been evaluated not only for direct antimicrobial activity, but also as a modifier of antibiotic resistance (Gibbons 2004).

Fixed and essential oils from plants have received enormous attention in the above-mentioned scenario, where they are a promising tool in the treatment of various illnesses. These products extracted from seeds have been studied as sources of substances with the ability to modify antibiotic action (Coutinho 2008).

Studies have shown the antibiotic and antibiotic-modifying activity of fixed and essential oils against a number of pathogens such as *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, *Bacillus subtilis*, *Serratia liquefaciens*, *Lactobacillus carvatus*, *Aspergillus niger*, *Asp. flavus, Asp. ochraceus, Fusariumoxy sporum* and *Penicillium* spp. (Lambert et al. 2001). Such studies have great importance from a clinical point of view with regard to lowering the doses of antibiotics in the treatment of patients.

The family Fabaceae has a large number of representatives used in folk medicine (Borges 2008), and *B. virgilioides* Kunth is one of the species that stand out in the family, since it has some pharmacological activities such as anti-inflammatory (Barroso et al. 2010), wound healing (Agra et al. 2013), antinociceptive (Thomazzi et al. 2010), antimalarial (Deharo et al. 2001), hypoglycemic (Barbosa-Filho et al. 2005), antimicrobial (Almeida et al. 2006) and anti-acetylcholinesterase (Barbosa-Filho et al. 2006).

B. virgilioides, popularly known as sucupira-preta or sucupira-do-cerrado, is a deciduous, heliophytic, selective xerophytic species, characteristic of the Brazilian cerrado, and although having a fairly even distribution, it occurs at a low population density (Lorenzi 1998). Faced with the need to prove the medicinal properties of *B. virgilioides*, the aim of this study was to evaluate the antibiotic activity of *B. virgilioides* fixed oil and its antibiotic-modifying effect with aminoglycoside antibiotics against *Escherichia coli* standard and clinically resistant strains.

Material and Methods

The seeds of *B. virgilioides* were collected from trees in the Chapada do Araripe, Crato, CE and partly provided by the Seed Bank of Chico Mendes Institute, Crato - CE. Initially, the seeds with husk removed were ground in an electric mill and then passed through mesh sieve to obtain the flour. To obtain the fixed oil, 25.0 g flour were transferred to filter paper cartridges for extraction with hexane in a Soxhlet extractor for 6 hours. After extraction, the mixture was decanted, filtered and the solvent removed with a rotary evaporator under reduced pressure equipment and controlled temperature (70 ± 2°C).

The bacterial strains used were the clinical isolates *E. coli* 27 (EC 27) and E. *coli* (ATCC 10536) with the resistance profile described in Table 1. All strains were maintained in slants with heart infusion agar (HIA, Difco Laboratories Ltda.). Prior to assay, cells were grown overnight at 37°C in brain heart infusion (BHI; Difco Laboratories Ltda.).

Table 1 - Bacteria, origin and antibiotic resistance profile

Bacterial strain	Origin	Resistance profile
E. coli 27	Surgical wound	Ast, Ax, Amp, Ami, Amox, Ca, Cfc, Cf, Caz, Cip, Chlo, Im, kan, Szt, Tet, Tob

Ast - aztreonan; Ax - amoxicillin; Amp - ampicillin; Ami - amikacin; Amox - amoxicillin; Ca - cefadroxil; Cfc - cefaclor; Cf - cephalothin; Caz - ceftazidime; Cip - ciprofloxacin; Chlo - chloramphenicol; Im - imipenem; kan - kanamycin; Szt - sulfametrim; Tet - tetracycline; Tob - tobramycin.

The antibiotics gentamicin, amikacin, kanamycin and neomycin were obtained from Sigma Chemical Corp., St. Louis, MO, USA. All these antibiotics were diluted in sterile water prior to testing.

Minimum inhibitory concentration (MIC) was determined by the broth microdilution assay (Javadpour et al. 1996) using 100 µL of each strain suspended in BHI broth at a final concentration of 10^5 CFU/mL in 96-well microtiter plates. The final concentrations of *B. virgilioides* oil were 512 - 8 µg/mL. MIC was the lowest concentration where growth inhibition was observed.

To evaluate modulatory effect of the oil in the presence of aminoglycoside antibiotics, 100 µL oil at a subinhibitory concentration 64 µg/mL were added to each well. Next, 100 µL of bacterial suspension of 10^5 CFU/ml. The drug concentration range was from 2.5 to 0.0012 mg/mL. The MIC was defined as the lowest concentration at which no growth was observed in the presence of antibiotics. Each drug-modifying assay was performed in triplicate. Differences ranging only one point in MIC were not significant.

Results

The highest concentration of fixed oil (512 µg/ml) did not inhibit the growth of *E. coli* 27 (EC 27) and *E. coli* (ATCC 10536). However, fixed oil at a subinhibitory concentration of 64 µg/mL significantly modified the action of aminoglycoside antibiotics in the strains tested.

In the Gram-negative strain *E. coli* 27 (EC 27), amikacin showed no growth inhibition at 2.5 mg/mL (Figure 1). Kanamycin, gentamicin and neomycin inhibited bacterial growth at 0.0049 (Figure 2), 1.25 (Figure 3) and 0.0049 mg/mL (Figure 4), respectively.

In the presence of the fixed oil of *B. virgilioides* seeds (64 µg/mL), MIC values against *E. coli* were 0.078, 0.078, 0.078 and 0.039 µg/mL for amikacin, kanamycin, gentamicin and neomycin, respectively.

Thus, *B. virgilioides* fixed oil had an antagonistic action towards the antibiotics kanamycin and neomycin but enhanced the inhibitory effect of amikacin and gentamicin at least 64 and 32 times respectively.

Figura 1 - Determination of minimum inhibitory concentration of amikacin against E. coli 27 and its modulation by B. virgilioides oil at 64 µg/mL.

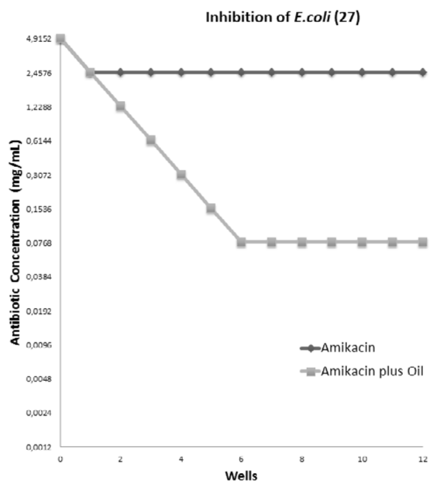


Figure 2 - Determination of minimum inhibitory concentration of kanamycin against E. coli 27 and its modulation by B. virgilioides oil at 64 µg/mL.

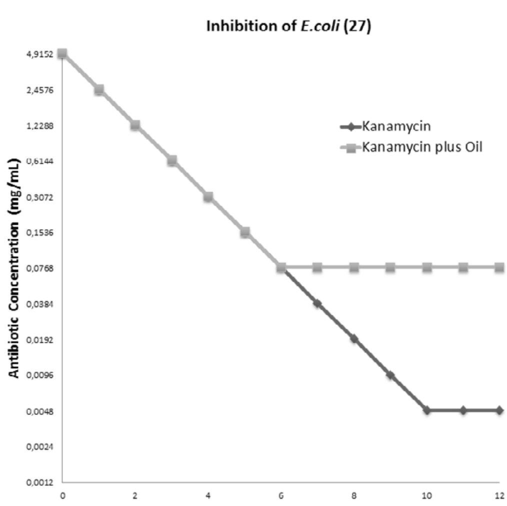


Figure 3 - Determination of minimum inhibitory concentration of gentamcin against E. coli 27 and its modulation by B. virgilioides oil at 64 µg/mL.

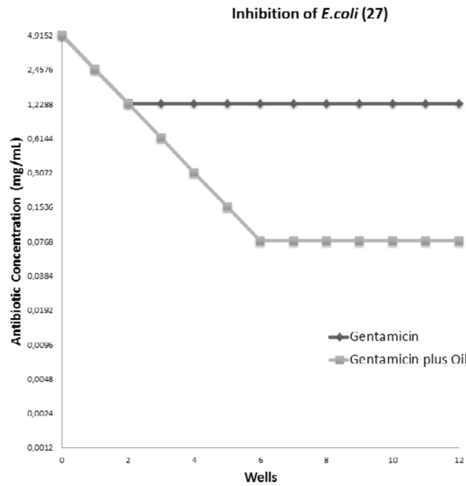
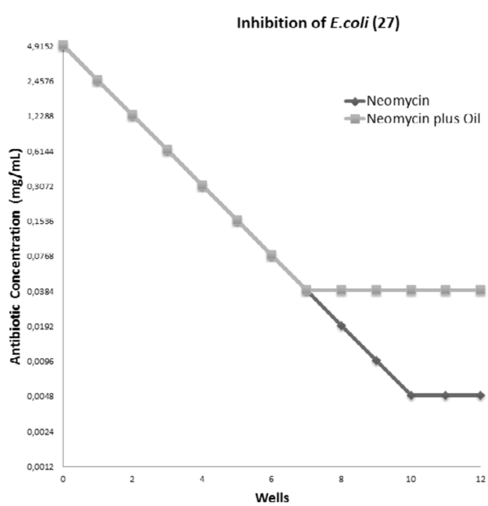


Figure 4 - Determination of minimum inhibitory concentration of neomycin against E. coli 27 and its modulation by B. virgilioides oil at 64 µg/mL.



Discussion

Various synthetic or naturally derived compounds are active against many species of bacteria, such as by increasing the activity of a specific antibiotic, reversing the natural bacterial resistance to specific antibiotics, promoting the elimination of plasmids and inhibiting efflux of antibiotics across the membrane plasma (Coutinho et al. 2009; Coutinho et al. 2010). The potentiation of antibiotic activity or the reversal of antibiotic resistance allows the classification of these compounds as antibiotic activity modifiers (Coutinho et al. 2009; Coutinho et al. 2010; Gunics et al. 2006; Molnar et al. 2004).

Some studies have demonstrated the bioactive potential of fixed oils of plants and animals commonly used in folk medicine in Northeast Brazil, which have, for the most part, antimicrobial and pharmacological activity (Cabral et al. 2014; Saraiva et al. 2011a).

The fixed oil *B. virgiloides* showed no growth inhibitory effect on strains of *E. coli*, indicating that the fixed oil of this plant is ineffective against these bacteria. But it proved to be a strong antibiotic modulator, enhancing the activity of gentamicin and amikacin, although it behaved as an antagonist of neomycin and kanamycin against Gram-negative strains of *E. coli*.

A similar study was reported by Ferreira *et al*. (2009), for the fixed oil the snake *Boa constrictor*, which had no substantial growth inhibitory effect on the same strains of *E. coli*, but showed synergism with aminoglycoside antibiotics, in line with the results obtained in our study.

Some fixed and essential oils have been described as having antimicrobial activity against bacteria at clinically relevant concentrations (Lambert et al. 2001). The mechanisms by which fixed oils can inhibit the growth of microorganisms involve different modes of action, and may be partly due to the hydrophobic characteristics of its components and the presence of polyunsaturated fatty acids, which may have greater interaction with the lipid bilayer of the cell membrane, affecting the respiratory chain and energy production (Nicolson et al. 1999). Singh and colleagues (2007) reported the presence of polyunsaturated fatty acids in fixed oils, which can undergo peroxidation releasing malondialdehyde, a molecule that can bind to DNA and RNA and thus form crosslinks between these molecules resulting in the inactivation of DNA replication and protein synthesis as well. Malondialdehyde can also bind to the amine groups of enzymes of bacteria and thereby inhibit their growth. Although *B. virgiloides* seed oil had no direct effect on bacterial growth, it did show a synergistic effect when combined with some aminoglycoside antibiotics. This effect may be due to the presence Δ^9 octadecenoic fatty acid (oleic acid) as suggested by Scott et al. (2011b).

These demonstrated a synergistic effect of the fixed oil of pequi (*Caryocar coracium*) when combined with aminoglycosides against *E. coli* and *S. aureus,* indicating the bioactive potential of plant fixed oils and thus suggesting that fixed oils rich in oleic acid are potential modifiers of antibiotic resistance in treatments against these pathogens (Saraiva et al. 2011b).

There are various mechanisms for enhancing the action of aminoglycoside antibiotics, particularly the possible role of lipids as antibiotic vehicles because of their lipophilic character that allows good uptake through the cell membrane (Wagner and Wisenauer, 2006). Therefore, further studies are needed for a better understanding of this modulatory action.

The combination of antibiotics with fixed oils of plants is still a new approach in view of the few published studies on this subject, making it necessary to carry out more detailed studies to clarify the mechanism by which these oils modify antibiotic activity for different strains of Gram-positive and Gram-negative bacteria.

Conclusion

Our data indicate that the oil extracted from *B. virgiloides* seeds has no clinically relevant bactericidal activity but shows antibiotic-modifying activity when combined with aminoglycosides, lowering the antibiotic resistance of Gram-negative strains. Thus, this oil can be indicated as a possible modulatory agent in infections caused by these pathogens. Still, studies are needed to determine whether the presence or absence of the bacterial cell wall is involved in the differential response to antibiotics combined with the seed fixed oil.

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