ISOLAMENT OF PHYTOSTEROLS OF *Dalbergia ecastophyllum* (L.) TAUB. (LEGUMINOSAE) AND MODULATION OF ANTIBIOTIC RESISTANCE BY A POSSIBLE MEMBRANE EFFECT.

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INTRODUÇÃO

Improper use of some antimicrobials is causing the drugs lose their effectiveness very quickly, which makes necessary the development of new drugs and effective management techniques. Resistance is a phenomenon characterized not only by evolutionary pressure, but especially by the indiscriminate and irrational use of antimicrobial therapeutics [Coutinho *et al*., 2008]. The species *Pseudomonas aeruginosa* is responsible for a variety of infections, such as those affecting the skin, urinary tract, eye and ear. The wide distribution of Pseudomonas environmental is ensured by its non-fastidious growth requirements, and possesses many structural factors, enzymes and toxins that enhance their virulence, and make them resistant to most common antibiotics [Murray *et al*., 2004]. *Staphylococcus aureus* is often found colonizing the natural microbiota, especially the skin, and with the breakdown of skin barriers or decrease of immunity can become pathogenic. It causes a variety of infections, such as infections of the skin and subcutaneously, post-surgical infections, osteomyelitis, pneumonia, abscesses, endocarditis and bacteremia [Gelatti *et al*., 2009]. *Escherichia coli* is a gram negative bacterium belonging to the family *Enterobacteriaceae* normally found in the intestine of being endothermic, becoming recognized as a pathogen and a harmless commensal versatile [Vogt, 2005]. The rapid evolution of microbial resistance to drugs and alarming slowdown in the development of new drugs, to arouse attention with multidrug treatments (Keith *et al*., 2005) and the combination with the associated natural products of plant origin that can alter the effect of antibiotics, either increasing or decreasing the antibiotic activity [Coutinho *et al*., 2008]. *Dalbergia ecastophyllum* (L.) Taub. (*Leguminosae*), popularly known as rabo-de-bugio, is a kind scandent or semi - prostrate, the *Dalbergia genus* has 41 species distributed in Brazilian ecosystems. [Carvalho, 1997]. In swamps that are the reverse of strings of dunes, which are periodically flooded areas, the *Dalbergia ecastophyllum* has popular use of roots and barks in combating uterine inflammation and anemia. The ecosystem of the dunes presents problems for the survival of plants, due to scarcity of nutrients and various factors that negatively affect the water balance, such as low water holding capacity of the soil, marine strong wind action, high salinity and heat stroke. [Daugsch *et al*., 2007].

OBJETIVOS

The objective of this study was to evaluate the in vitro antimicrobial activity and modulating the ethanol extract and the hexane phase of *Dalbergia ecastophyllum* against bacterial and fungal strains in order to identify this natural product as a new strategy to combat resistance microorganisms to antimicrobials

MATERIAL E MÉTODOS

Bacterial strains The bacterial strains used in the MIC were: E.coli ATCC 10536, S. aureus ATCC 25923 and P.

aeruginosa ATCC 15442; already been used for modulating the multiresistant strains: E.coli 27, S aureus 358 and P. aeruginosa 22. All strains were maintained on slants with Heart Infusion Agar (HIA, Difco laboratorises Ltda.). Plant material The plant *Dalbergia ecastophyllum* was investigated on how to ethanol extract and hexane fraction obtained from its leaves, in order to evaluate the antibacterial and antifungal activity of these samples facing the strains of pathogenic microorganisms. Plant species were collected near the Bessa Mando - Paraíba. The plant material was identified by Evelise Locatelli, curator of the Herbarium Systematics - Federal University of Paraiba, where is deposited exsicata: JPB45738. The preparation of the extract and fractions occurred in UFPB. Processing of chromatographic hexane phase of the ethanol extract An aliquot of 15.0 g of hexane phase (column 1.0) of crude ethanol extract of the aerial parts of *D. ecastophyllum* was subjected to column chromatography using as stationary phase silica gel and 60 were used as the mobile phase hexane, CH2Cl2, EtOAc and MeOH alone or in binary mixtures with increasing polarity gradient, the fractions are concentrated on a rotary evaporator pressure reduced. Preparation of test solution The solution used in the tests were prepared at an initial concentration of 100mg/ml, dissolved in 1ml DMSO, then diluted in distilled water to a concentration of 1024µg/ml. Drugs The antibacterial drugs used at a concentration of 5000µg/ml were: Amikacin, Gentamicin and Neomycin. The drugs used were as antifungals in concentration and were 1024µg/ml: Amphotericin B, Nystatin, Mebendazole and Benzoilmetronidazol. Antibacterial and antifungal activity and modulation of drug The minimum inhibitory concentration (MIC) was determined in a microdilution assay [Javadpour *et al*., 1996]. An amount of 100mL of each strain suspended in brain heart infusion broth (BHI) was dispensed into each well of a microdilution plate. In each cavity was deposited 100µl of solution serial dilution of extract / fraction, with their final concentrations ranging from 1024-2µg/ml. MIC was recorded as the lowest concentration that inhibited growth. The minimum inhibitory concentration was determined for the antibiotic in the BHI broth microdilution assay using suspensions of 105 CFU / ml and a range of drug concentration 5000µg - 2.5 g / ml for antibacterial and 1024 to 0.5 mg / ml for antifungals.

RESULTADOS

Studies involving this species has been reported, according Daugsch [2007] *Dalbergia ecastophyllum* is the botanical origin of propolis (propolis group 13) and that has antimicrobial activity, and that the ethanol extracts of propolis group 13 showed antibacterial activity against *Staphylococcus aureus, Salmonella typhimurium, Streptococcus mutans*. Results The results show a broad spectrum against various microorganisms (fungi, bacteria, viruses, protozoa). Although important biological antioxidant properties, and immunomodulatory acitotóxica been proven. [Bankova *et al*., 2000]. Silva [2008] showed that both the ethanol extract of propolis as the ethanol extract of *Dalbergia ecastophyllum* resin exhibited high antimicrobial activity.

DISCUSSÃO

The verified data point extract and fraction *Dalbergia ecastophyllum* as a potent modulator of resistance of bacteria, since reduced the MIC of the antibiotic assayed. Searches are still to be developed that can support testing in vivo and provide a future therapeutic use.

CONCLUSÃO

The rapid evolution of microbial resistance to drugs and alarming slowdown in the development of new drugs, to arouse attention with multidrug treatments (Keith *et al*., 2005) and the combination with the associated natural products of plant origin that can alter the effect of antibiotics, either increasing or decreasing the antibiotic activity [Coutinho *et al*., 2008]. As a conclusion, we identified that *Dalbergia ecastophyllum* will be a natural product as a new strategy to combat resistance microorganisms to antimicrobials.

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Agradecimento