academicJournals

Vol. 9(12), pp. 395-399, 25 March, 2015
DOI: 10.5897/JMPR2013.5290
Article Number: A7612E551952
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Evaluation of antibacterial activity and modulatory effect of the hexane fraction from methanol extract of *Cordia verbenacea* DC leaves

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Received November 1, 2013; Accepted 16 March, 2015

Medicinal plants have been used since the beginning of civilization, and even today, it is still widely used mainly due to the lack of necessary information on their physical and chemical components. *Cordia verbenacea* DC is a typical plant of the Brazilian coast, popularly known as "grass whaling" and is mainly used as antimicrobial agents, anti- inflammatory and analgesic. This study was conducted in order to identify the phytochemical analysis of the main secondary metabolites, and also to evaluate the antibacterial effect and modulate bacterial resistance to aminoglycoside, the hexane fraction of the methanol extract of *C. verbenacea* (HFMECV) against the standards and resistant strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The phytochemical screening showed the presence of compounds such as tannins flobatenics, flavones, flavonols, xanthones, flavononls, leucoanthocyanidins, catechins, flavones and terpenes. Regarding antibacterial activity, the fraction showed minimum inhibitory concentration (MIC) ≥ 1024 µg/ml, being a value not clinically relevant, but when combined with the sub-MIC, the aminoglycosides antibiotics showed synergism. With these results, we conclude that the HFMECV presented itself as an excellent modulator of bacterial resistance to aminoglycosides, thereby indicating a possibility that through further research there could arise a new herbal that work with modulating function and antibacterial.

Key words: Aminoglycosides, phytochemical analysis, antibacterial activity, minimum inhibitory concentration (MIC), modulation.

INTRODUCTION

Since the beginning of civilization, there is a great use of medicinal plants primarily as a therapeutic or for the prevention and cure of certain diseases. This practice is widely used today because the general population does not have the necessary information on chemical, physical and constituents of many existing plants, as it is in the case of some exotic plants (Viegas et al., 2006; Veiga Junior, 2008).

A family of medicinal plant largely researched is the Boraginaceae, which have been found with about 100

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genera and over 2000 species distributed throughout the planet. A species well known today is *Cordia verbenacea* DC. it is a perennial and shrub located largely around the Brazilian coast and popularly known as "erva baleeira" (Sertié et al., 2005). *C. verbenacea* is widely used as anti-inflammatory and analgesic, and confirmed its anti-inflammatory effects through pharmacological tests (Rosa et al., 2008; Matias et al., 2010). Currently, medicinal plants are of great importance especially to the pharmaceutical industries, as these extracts have produced active compounds that may be part of the new formulations of synthetic drugs (Simões and Schenkel, 2002). This search for new components is often due to dissatisfaction with allopathic medicine for side effects through the inappropriate use of drugs (Rates, 2001; Ribeiro et al., 2005) or due to the growth and spread of microorganisms resistant to existing drugs (Penha et al., 2011).

Pseudomonas aeruginosa are a group of bacteria responsible for nosocomial infection in immune-compromised patients and they can be found in water, soil, plants and animals as well as in the normal intestinal flora of the human body (Brooks et al., 2009). *Escherichia coli* type bacteria are gram-negative participants in the human gastrointestinal flora, and they are the major cause of diarrheal and urinary tract diseases (Cunha et al., 2012). *Staphylococos aureus* bacterial strains are also responsible for hospital infections, but also the leading cause of purulent infections of carbuncle and furuncle (Matias et al., 2010).

This study was conducted in order to identify the main classes of secondary metabolites by phytochemical, and also to evaluate the antibacterial effect of modulating bacterial resistance to aminoglycoside and the hexane fraction of the methanol extract of *Cordia verbenacea* (HFMECV) against bacterial strains and patterns of multiresistant.

MATERIALS AND METHODS

Bacterial material

The following bacterial strains used were: *S. aureus* (ATCC25923 and SA358), *E. coli* (ATCC10536 and EC27) and *P. aeruginosa* (ATCC15442 and PA03). All strains were maintained in Heart Infusion Agar (HIA) at 37°C.

Plant material

Leaves of *C. verbenacea* DC. were collected in the city of Crato, Ceará, Brazil. The plant material was identified and a voucher specimen was deposited in the Herbarium Prisco Bezerra of Federal University of Ceará - UFC, under No. 044 171.

Preparation of the extract, fraction and antibacterial test solution

For preparation of extracts, leaves were collected and then passed through the grinding process which increased its contact surface, the grinded leaves were wrapped in a container with solvent (methanol) volume enough to submerge the whole plant material, remaining there for 72h. After this period, the eluent was filtered through filter paper to separate the solid concentrated in rotary vacuum condenser (model Q-344B - Quimis, Brazil) (model Q-214M2 - Quimis, Brazil). The extracts mixed with silica where then subjected to vacuum filtration process with solvents of different polarity. For microdilution tests, solutions prepared from the fraction in a concentration of 10 mg/ml, dissolved in DMSO (dimethyl sulfoxide) then diluted with distilled water to a concentration of 1024 µg/ml were used. Preliminary evaluation were performed with DMSO and showed no toxicity and interference with concentration below 5%. DMSO used at concentration of 9%, during the tests ranged 4.5 to 0.03%, and is considered non-toxic concentration.

Phytochemical screening

Phytochemicals tests were performed to detect the presence of glycosides, saponins, tannins, flavonoids, steroids, triterpenes, coumarins, quinones, organic acids and alkaloids using the method described by Matos (1997). The tests are based on visual observation of color change or precipitate formation after addition of specific reagents.

Antibacterial activity and modulating resistance to aminoglycosides

The MIC was determined from microdilution broth, using an inoculum of each strain of 100 µL/ml suspended in Brain Heart Infusion (BHI liquid) to give a final concentration of 10^5 CFU/ml. In microdilution, plates with 96 wells were distributed into each well, promoting 1:1 dilution series of the test solution, promoting concentrations ranging from 512 to 8µl. To obtain the controls, antibiotics: amikacin , gentamicin and neomycin were used. The plates were all incubated for 16 to 24h at 37°C. The MIC was determined as the lowest concentration able to inhibit bacterial growth which was performed by triplicate. To evaluate the modulating action of bacterial resistance towards antibiotics, microdilution methodology described earlier was used. The modulatory activity determined by the MIC of aminoglycosides, where all the plates were incubated for 16 to 24h at 37°C. Results were obtained by triplicate, tabulated from the average geometrical data, statistically analyzed by two-way ANOVA and subjected to Bonferroni post-test in GraphPad Prism 5.0 (Matias et al., 2013).

RESULTS

The phytochemical screening was performed to identify the classes of secondary metabolites. Table 1 presented the presence of several bioactive compounds in the extract fraction rated as: tannins flobatenics, flavones, flavonols, xanthones, flavononls, leucoanthocyanidins, catechins, flavanones and terpenes. In trials to test the antimicrobial activity of the hexane fraction of the methanol extract of *C. verbenacea* (HFMECV), for all bacterial strains tested (ATCC and multiresistant Gram positive and negative) was observed MIC value ≥ 1024 µg/ml. Being the amount considered clinically significant when the MIC is MIC ≤ 256 µg/ml. Figure 1 shows the interference of HFMECV on the activity of

Table 1. Phytochemistry screening of hexane fraction of the methanol extract *C. verbenacea* DC. leaves.

Natural product	Phenols	Tannins pirogalics	Tanins flobatenics	Anthoc yanins	Anthocy anidins	Flavones	Flavonols	Xanthones	Chalcones	Aurons	Flavononols	Leuco-anthocyanidins	Catechins	Flavonones	Alkaloids	Terpenes
HFMECV	-	-	+	-	-	+	+	+	-	-	+	+	+	+	-	+

(+) Presence, (-) absence. HFMECV (hexane fraction of the methanol extract of *C. verbenacea* DC.

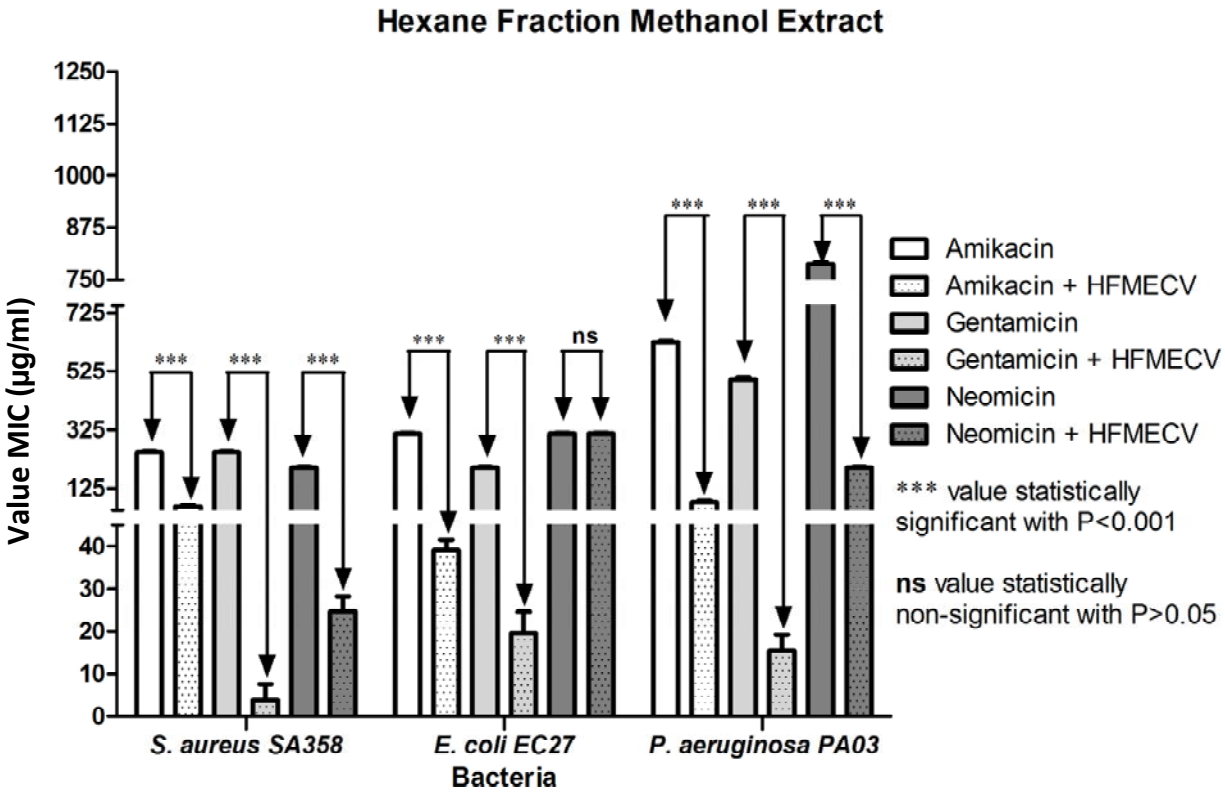

Figure 1. Activity modulating bacterial resistance against the aminoglycosides by hexane fraction of the methanol extract of *Cordia verbenacea* DC. leaves (HFMECV).

aminoglycosides (gentamicin, amikacin and neomycin) showing that there was a reduction in antibiotic concentration when combined with HFMECV sub-inhibitory concentration in bacterial strains tested. All other antibiotics tested against resistant strains were significantly relevant except

opposite strain of E. coli which showed no statistical significance in testing the modulation with the aminoglycoside neomycin.

DISCUSSION

Plants tend to synthesize some products or sub-products that have various applications such as attractive to pollinators and protective function. One of such product that serves as protection against fungi and bacteria are the flavonoids, where currently there are over 6000 types that have been described in the literature, among them are: flavonols, flavones, flavanones and catechins which are constituents of *C. verbenacea*. Flavonoids have certain capabilities, among them are antimicrobial and antiviral which are the most important (Machado et al., 2008) and so may be an explanation for the tests modulation minimum concentrations observed when considering only the use of antibiotics used.

Many medicinal plants used by the general population in their compositions have a class of metabolite known as tannins which have antibacterial properties and they mainly cause a higher sensitivity in *S. aureus* strains, but also may sensitize strains of *Streptococcus pneumoniae*, *Bacillus anthracis*, and *Shigella dysentery* (Monteiro et al., 2005). Combinations of antibiotics of the aminoglycoside type with hexane fraction of methanol extract have synergistic actions when tested with the bacterial strains multiresistant patterns and promoting the reduction of MICs, thus leading to a decreased concentration of antibiotics in order to obtain a therapeutic effect (Figueredo et al., 2013).

This strategy is known as "herbal shotgun" or "the synergistic multi-target effect" because the components obtained from these compounds are medicinal plants, most often by multi combined extracts to be responsible for acting not only on one site action, but also on a variety of targets that may have a synergistic or antagonistic action depending on its composition. This can occur not only with combinations of more extracts but also with the combination of antibiotics with natural synthetic (Matias et al., 2013).

The most favorable results were opposite lineage *S. aureus* possibly because it is a bacterium that has gram positive cell wall. The other strains tested which include *E. coli* and *P. aeruginosa* results were not as relevant as *S. aureus*, but this may have occurred because these bacteria have a greater chemical complexity (Rodrigues et al., 2012).

CONCLUSION

Conclusively, it was found that the hexane fraction of the methanol extract has no clinical significance since it has relevant MIC ≥ 1024 µg/ml, but has a higher efficiency when used together to aminoglycoside antibiotics. Therefore, new research is suggested to have more information on the mechanisms of action and possible development of herbal medicines with antibacterial activity, and in modulating the products obtained from the leaves of *C. verbenacea*.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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