

SUPPLEMENTARY MATERIAL

Enhancement of antibiotic activity by phytochemicals of *Turnera subulata*

Cicero L.A. Freitas, Francisca F.P. Santos, Orlando M. Dantas-Junior, Vital V. Inácio, Edinardo F.F. Matias, José J.S. Aguiar and Henrique D.M. Coutinho

ABSTRACT

Several studies have shown that plants of plant origin are more relevant, where the antibacterial and modulating action of antibiotic resistance is highlighted. *Turnera subulata* is popularly known as “chanana”. This plant species is commonly used in Brazilian popular medicine to treat infections and In the search for new alternatives to treat infections caused by resistant microorganisms, many studies. The present study aimed to evaluate the antibacterial and modulating activity of the methyl extract obtained from *Turnera subulata* alone or in combination with aminoglycoside antibiotics, using the microdilution method. The methyl extract of *Turnera subulata* was used alone in the antibacterial test, and in combination with antibiotics in the modulation assay. All tests were performed in triplicate. The methyl extract of *Turnera subulata* presented both antibacterial and antibiotic-modulating effects *in vitro*, alone or in association with aminoglycosides. The activity of the extract depends on the bacterial strain and may be associated with the presence of tannins and flavonols. However, further studies are required to characterize the potential of *Turnera subulata* in the development of new drugs against multiresistant bacteria.

Keywords: Aminoglycosides; *Turnera subulata*; antibacterial; bacterial resistance

1. EXPERIMENTAL

1.1 BACTERIAL STRAINS

The microorganisms used in this research were provided by the Regional University of Cariri. The following standard strains were used: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. Multi-resistant strains of *Escherichia coli* 27, *Staphylococcus aureus* 35, *Pseudomonas aeruginosa* 31. The resistance profiles of these microorganisms are described in table S1. Before the assay, the cells were cultured for 24 h at 37 ° C in Brain Heart Infusion (BHI), Difco Laboratories LTDA.

1.2 PLANT MATERIAL

The leaves of *Turnera subulata* were collected, processed in exsiccates and deposited in the “Dárdano de Andrade Lima” herbarium at the Regional University of Cariri - URCA under number 12.787.

1.3 PREPARATION OF THE METHYL EXTRACT AND TEST SOLUTIONS

After collection, 422 g of the leaves of *Turnera subulata* were weighted, crushed and placed in a container with 1 liter of methyl alcohol (solvent) in which it was kept submerged for 72 h. After this period, the mixture was filtered and placed in a water bath until total evaporation of the solvent, yielding 5g of methyl extract, which corresponds to 1.3% of the total weight of the leaves used. The extract was prepared in a concentration of 10 mg / mL, dissolved in DMSO (dimethyl sulfoxide) and diluted with distilled water to a concentration of 1024µg / mL (Coutinho et al, 2008).

1.4 PHYTOCHEMICAL PROSPECTION

The phytochemical prospection aimed to identify the presence of secondary metabolites in the methyl extract of *Turnera subulata*. To this end, we used the methodology proposed by Matos (1997) which detects the presence of steroids, quinones, organic acids, triterpenes, coumarins, and alkaloids. The test is based on visual colorimetric observation or formation of precipitate after addition of specific reagents and induction of variation of pH and temperature.

1.5 EVALUATION OF ANTIBACTERIAL ACTIVITY AND DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The minimum inhibitory concentration (MIC) of the methyl extract of *Turnera subulata* was determined using the broth microdilution method (Ushimaru et al, 2007). Briefly, for each strain, 100 µL of the inoculum was prepared in BHI broth at a concentration of 10⁵ CFU / mL. This solution was transferred to 96-well microtiter

plates and serially diluted. In each well, 100µL of the sample solutions were added to obtain concentrations ranging from 512 - 8 µg / mL. The plates were incubated at 35 °C for 24 h and after this period, the readings were performed, using resazurin as indicator (Salvat; Antonnacci & Fortunato, 2001). The MIC was defined as the lowest concentration at which no growth was observed.

1.6 DETERMINATION OF THE MODULATING ACTIVITY OF THE METS IN ASSOCIATION WITH AMINOGLYCOSIDES

The antibiotic-modulating activity of the METS was analyzed according to the methodology proposed by Coutinho et al. (2008). In this test, extract was tested at sub-inhibitory concentrations (MIC / 8). The plates were filled with 100µL of a solution containing 10% BHI in each well. Then, 100µL of the drug were added to the first well and serially diluted, in a ratio of 1: 1 to the penultimate cavity. The last well was used as control. The concentrations of aminoglycosides varied gradually from 5000 to 2.44 µg / mL (Sato et al., 2004). Afterwards, the plates were incubated at 35 °C for 24 h and then, the readings were performed as described above (Javadpour et al., 1996). All antibiotics tested were obtained from Sigma.

1.7 STATISTICAL ANALYSIS

All tests were performed in triplicate and results were expressed as geometric mean. Data were analyzed by ANOVA followed by Bonferroni's test using GraphPad Prism software (GraphPad, San Diego, CA). Differences with $p < 0.05$ were considered significant.

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Table S1: Resistance Profile

Bacterium	N°	Collection site	Resistance Profile
<i>Staphylococcus aureus</i>	SA 358	Surgical wound	Oxa, Gen, Tob, Ami, Can, Neo, Para, But, Sis, Net
<i>Pseudomonas aeruginosa</i>	P-31	Nosal	Pol, Cpm, Ctz, Ptz, Ami Imi, Cip, Lev, Mer,
<i>Escherichia coli</i>	EC27	Surgical wound	Ast, Ax, Amp, Ami, Amox, Ca, Cfc, Cf, Caz, Cip, Clo, Imi, Can, Szt, Tet, Tob

Resistance Profile: Ami = amikacin; Sam = ampicillin-sulbactam; Cip = ciprofloxacin; Lev = levofloxacin; Cpm = cefepime; Ctz = ceftazidime; Pol = polymyxin b; Imi = imipenem; Mer = meropenem; Ptz = piperacillin; Tig = tigecycline. Ast = aztreonam; Ax = Amoxicillin; Amp = ampicillin; Amox = amoxilin, Ca = cefadroxil; Cfc = cefaclor; Cf = cephalothin; Caz = ceftazidime; Clo = chloramphenicol; Can = kanamycin; Szt = sulfametriz, Tet = tetracycline; Tob = tobramycin; Oxa = oxacillin; Gen = gentamicin; Neo = neomycin; Para = paramomycin; But = butyrosine; Sis = sisomycin; Net = netilmicin.

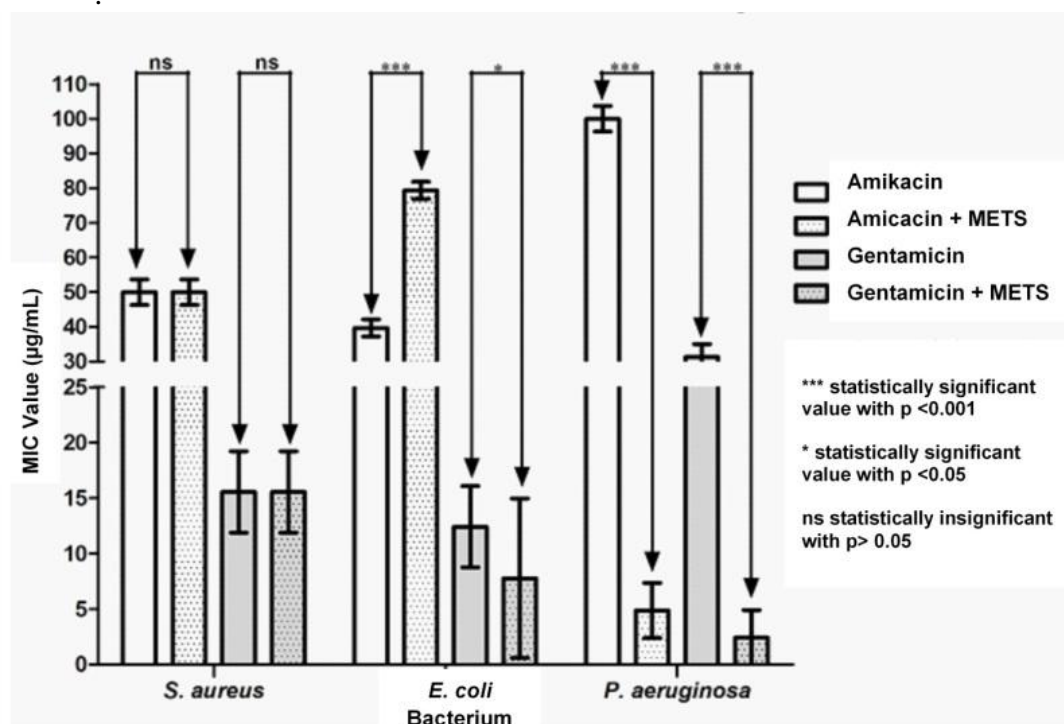
Table S2: Phytochemical Prospection of *Turnera subulata* Methyl Extract

Metabolite	1	2	3	4	5	6	7	8	9	10	11	12	13
Presence (+)	-	+	-	-	+	+	+	-	-	-	-	-	-
Absence (-)	-	+	-	-	+	+	+	-	-	-	-	-	-

1-Phenols; 2-Pyrogallol Tannins; 3-Anthocyanins; 4-Anthocyanidins; 5-Flavones; 6-Flavonols; 7-Xanthones; 8-Chalcones; 9-Aurones; 10-leucoanthocyanidins; 11-Catechins; 12-Flavones; 13- Alkaloid.

Table S3: Minimum Inhibitory Concentration (MIC) of the METS against Bacterial Strains

BACTERIUM	MIC
<i>Escherichia coli</i> ATCC 25922	406 µg / mL
<i>Escherichia coli</i> 27	512 µg / mL
<i>Staphylococcus aureus</i> ATCC 25923	512 µg / mL
<i>Staphylococcus aureus</i> 35	512 µg / mL
<i>Pseudomonas aeruginosa</i> 31	512 µg / mL
<i>Pseudomonas aeruginosa</i> ATCC 27853	512 µg / mL

**Figure S1:** Modulating effect of the methyl extract of *Turnera subulata* in association with aminoglycosides