Supplementary material

Additive effect of *Lygodium venustum* SW. in association with gentamicin

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Running title: *Lygodium venustum* and gentamicin

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Abstract

The aim of this work was evaluate the interactions between gentamicin and the ethanol extract of the fern *Lygodium venustum* SW (EELV). The ethanol extract of *Lygodium venustum* was obtained, the phytochemicals were identified and the EELV was assayed by the checkerboard method with gentamicin against two bacterial strains multiresistant to antibiotics. The antibiotic activity of gentamicin, when associated with the extract, was enhanced in a additive manner against both strains. The results indicated that *L. venustum* can be a source of secondary metabolites to be used in association with antibiotics as aminoglycosides in the antibiotic chemotherapy against resistant bacteria.

Keywords: *Lygodium venustum*, ethanol extract, checkerboard method, gentamicin.

1. Experimental

1.1. Plant Material

Leaves of *L. venustum* were collected in the Crato county, Ceará, Brazil. The plant material was identified by Dr. Álamo Feitosa Saraiva and a voucher specimen was deposited with the number #5569 HCDAL in the “Herbário Caririense Dárdano de Andrade-Lima”, at URCA.

1.2. Obtention of the Ethanol extract of *Lygodium venustum* (EELV)

211.18 g of powdered leaves were immersed in 2 L of 92% ethanol at room temperature for 72 h. The extract was filtered and concentrated under vacuum in a rotary evaporator at 60 ° C and 760 mm / Hg, yielding 12.4 g (Brasileiro et al. 2006). The extract was first diluted in 1 mL of DMSO and then in sterile distilled water to obtain the desired concentration for testing (pH 6,8).

1.3. Phytochemical prospection

The phytochemical assays were used for the qualitative analysis of the presence of secondary metabolites. The detection tests to evaluate the presence of heterosides, saponines, tannins, flavonoids, steroids, triterpens, cumarins, quinones, organics acids and alkaloids were performed allowing the method described for Matos (1997). The tests are based in the visual observation of color modifications and formation of precipitate after the addition of specific reagents (Table S1).

1.4. Bacterial strains

The strains used in this work were the multiresistant clinical isolates *Staphylococcus aureus* 358 and *Escherichia coli* 27 (Table S2). The strains were obtained from the

Clinical Mycology Laboratory, UFPB, Brazil. All strains were maintained in Heart Infusion Agar slants (HIA; Difco), and prior to the assays, the cells were grown for 24 h at 37°C in Brain Heart Infusion (BHI, Difco).

1.5. Antibiotic

Gentamicin was obtained from SIGMA and dissolved in sterile water.

1.6. Checkerboard Method

The strains and the interactions between EELV and antibiotics were tested by the microdilution checkerboard technique (Eliopoulos and Moellering, 1991). Suspensions of 10^5 CFU/mL (0.5 McFarland) of bacterial culture were prepared and distributed with the medium into microtiter trays containing varying concentrations of the different drugs. The inoculated trays were incubated at 37°C for a period of 24 h, and then evaluated for bacterial growth. In order to evaluate the activity of combinations of drugs, fractional inhibitory concentration (FIC) indices were calculated as $FIC^A + FIC^B$, where FIC^A and FIC^B represent the minimum concentrations that inhibited the bacterial growth for drugs A and B, respectively: $FIC^A = MIC^A \text{ combination} / MIC^A \text{ alone}$ and $FIC^B = MIC^B \text{ combination} / MIC^B \text{ alone}$. A mean FIC index was calculated based on the following equation: $FIC^A + FIC^B$, and the interpretation made as follows: synergistic (< 0.5), additive (0.5-1.), indifferent (> 1), or antagonistic (> 4).

Tabela 1. Phytochemical prospection of the ethanol extract from *Lygodium venustum* SW.

METABOLLITES														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
-	-	+	-	-	+	+	+	-	-	+	-	-	+	+

1)phenols; 2) tannin pyrogallates; 3) tannin phlobaphenes; 4) anthocyanins; 5) anthocyanidins; 6) flavones; 7) flavonols; 8) xanthones; 9) chalcones; 10) aurones; 11) flavononols; 12) leucoanthocyanidins; 13) catechins; 14) flavonones; 15) alkaloids.

Table 2. Origin of bacterial strains and resistance to antibiotics.

Bacteria	Origin	Resistance
<i>Escherichia coli</i> 27	Surgical wound	Ast, Ax, Amp, Ami, Amox, Ca, Cfc, Cf, Caz, Cip, Clo, Im, Can, Szt, Tet, Tob
<i>Staphylococcus aureus</i> 358	Surgical wound	Oxa, Gen, Tob, Ami, Can, Neo, Para, But, Sis, Net

Ast - Aztreonan; Ax - amoxicillin, Amp - ampicillin; Ami - Amikacin; Amox - Amoxicillin, Ca - Cefadroxil; Cfc - Cefaclor; Cf - Cephalothin; Caz - Ceftazidime, CIP - Ciprofloxacin; Chl - Chloramphenicol; Im - Imipenem; Can - Kanamycin; SZT - sulphametrim, Tet - tetracycline; Tob - tobramycin; Oxa - Oxacillin; Gen - gentamicin; Neo - neomycin; To - Paramomicina; But - Butirosin; Sis - sisomicin; Net - Netilmicin;

Table 3. Minimal inhibitory concentration (MIC) of gentamicin and the combinatory effect of ethanol extract of *Lygodium venustum* (EELV) against bacterial isolates multiresistant to antibiotics.

Natural Product + Antibiotics	<i>Escherichia coli</i> 27		<i>Staphylococcus aureus</i> 358	
	MIC (□g/mL)	FIC index	MIC (□g/mL)	FIC index
EELV	≥ 1024	-	≥ 1024	-
Gentamicin	39.06	-	39.06	-
EELV + Gentamicin	4.88/19.53	0.5 (add)	4.88/19.53	0.5 (add)

FIC – Fractional Inhibitory Concentration; Syn – Synergism; Add – Additivity.

References

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