Research Article

Modulation of the Antibiotic Activity by Extracts from *Amburana cearensis* A. C. Smith and *Anadenanthera macrocarpa* (Benth.) Brenan

Fernando G. Figueredo,¹ Emerson O. Ferreira,¹ Bruno F. F. Lucena,¹ Cícero M. G. Torres,¹ Daniel L. Lucetti,¹,² Elaine C. P. Lucetti,¹,² João Marcos F. L. Silva,¹,² Francisco A. V. Santos,¹,² Cássio R. Medeiros,¹ Gardênia M. M. Oliveira,¹ Aracélio V. Colares,¹,³ José G. M. Costa,¹,³ Henrique D. M. Coutinho,⁴,⁵ Irwin R. A. Menezes,⁶ Júlio C. F. Silva,¹,² Marta R. Kerntopf,⁶ Patricia R. L. Figueiredo,⁶ and Edinardo F. F. Matias¹,⁴

¹Faculdade Leão Sampaio, 63100-000, Juazeiro do Norte, CE, Brazil
²Faculdade de Medicina Estácio de Juazeiro do Norte, 63100-000, Juazeiro do Norte, CE, Brazil
³Laboratório de Pesquisa em Produtos Naturais, Universidade Regional do Cariri, 63105-000, Crato, CE, Brazil
⁴Departamento de Química Biológica, Universidade Regional do Cariri, 63105-000, Crato, CE, Brazil
⁵Departamento de Medicina, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil
⁶Laboratório de Farmacologia e Química Molecular, Universidade Regional do Cariri, 63105-000, Crato, CE, Brazil

Correspondence should be addressed to
Henrique D. M. Coutinho; hdmcoutinho@gmail.com

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The aim of this study was to verify the possible interactions between ethanol extracts of *Amburana cearensis* A. C. Smith and *Anadenanthera macrocarpa* (Benth.) Brenan, combined with six antimicrobial drugs against multiresistant strains of *Staphylococcus aureus* and *Escherichia coli* isolated from humans. The antibacterial activity of the extracts was determined using the minimum inhibitory concentration (MIC). The microdilution assay was performed to verify the interactions between the natural products and the antibiotics using a subinhibitory concentration. The activity of amikacin associated with the extract of *Anadenanthera macrocarpa* against EC27 was enhanced, demonstrating an MIC reduction from 128 to 4 μg/mL. Among the β-lactams, no potentiation on its activity was observed, with exception to the antagonism of the natural products with ampicillin against *S. aureus* 358.

1. Introduction

The research for new antibacterial substances becomes necessary due to increase of the antibiotic resistance of clinically important pathogens [1]. Due to this fact, substances derived from plants could be attractive alternatives [2, 3]. Natural products from plant may change or modulate the action of the antibiotic, enhancing or reducing the activity of this drug [4]. In recent years, many plants have been evaluated not only for the direct antibacterial action, but also as a modulator of the antibiotic activity [5, 6].

The use of natural products, mainly the chemical components of plants with antimicrobial properties have contributed to significant results in therapeutic treatments [7–9].

The *Amburana cearensis* (German) A. C. Smith, Fabaceae, is a tree which reaches 10–12 m of height [10]. Also known as “Cumaru”, it has been explored for use in fine furniture making, sculpture and carpentry, being listed as a threatened species [11]. Moreover, due to their medicinal properties, the bark and seeds are used to produce popular drugs for the treatment of cough, asthma, bronchitis, and pertussis. The species is still used in the perfume
industry [12]. Medical trials have demonstrated preclinical anti-inflammatory, bronchodilator and analgesic activity for the hydroalcoholic extract, being possible to associate these effects of coumarin and flavonoidic fraction [13, 14].

The *Anadenanthera macrocarpa* is a species belonging to Mimosoideae [15]. This is a species of ‘angico’ with larger geographic areas, occurring from southern Bolivia to northern Argentina, in Brazil, and is not only found in southern region [16]. Popular medicine has been used against several diseases through the preparation of syrups andlickers, it is used for the treatment of coughs, bronchitis, fads, external wounds and inflammation [17].

The objective of this study was to realize the phytochemical prospecting and assay of the *in vitro* ethanolic extracts of leaves of *A. Cearensis* and *A. macrocarpa* to determine the antibacterial activity and the modifying antibiotic activity of aminoglycosides and beta-lactams against the *Escherichia coli* and *Staphylococcus aureus*.

## 2. Material and Methods

### 2.1. Bacterial Material

The bacterial strains used were *E. coli* (EC-ATCC10536 and EC27) and *S. aureus* (SA-ATCC25923 and SA358) with a resistance profile identified in Table 1. All strains were maintained on heart infusion agar (HIA, Difco Laboratories Ltd.). Before the tests, the strains were grown for 18 h at 37°C in broth brain heart infusion (BHI, Difco Laboratories Ltd.).

### 2.2. Plant Material

Leaves of *Amburana cearensis* and *Anadenanthera macrocarpa* were collected at Penaforte, Ceara, Brazil. The plant material was identified and a voucher specimen was placed in the respective herbal collections (Table 2).

### 2.3. Preparation of Ethanol Extracts of Amburana Cearensis and Anadenanthera Macrocarpa

For the preparation of extracts, leaves were collected and weighed (Table 3). The material was powdered and wrapped in a container with an amount of solvent to submerge the plant material by 72 hours. After this time, the eluent was filtered and concentrated in a rotary vacuum condenser (model Q-344B-Quimis, Brazil) and in an ultrathermal bath (model Q-214M2-Quimis, Brazil) [18]. For the tests, the solutions used were prepared from extracts in a concentration of 10 mg/mL dissolved in DMSO (dimethyl-sulfoxide), then diluted with distilled water to a concentration of 1024 μg/mL.

### 2.4. Phytochemical Prospecting

The phytochemicals tests to detect the presence of heterosides, tannins, flavonoids, steroids, triterpenes, coumarins, quinones, organic acids, and alkaloids were performed according to the method described by Matos [19]. The tests were based on visual observation of the change in color or formation of precipitate after the addition of specific reagents.

### 2.5. Antibacterial Activity Test

The MIC (minimal inhibitory concentration) was determined in a microdilution assay utilizing an inoculum of 100 μL of each strain, suspended in brain heart infusion (BHI) broth up to a final concentration of 10⁵ CFU/mL in 96-well microtiter plates, using twofold serial dilutions. Each well received 100 μL of each extract solution. The final concentrations of the extracts varied from 512 to 8 μg/mL. MICs were recorded as the lowest concentrations required to inhibit growth. The minimal inhibitory concentration for the antibiotics was determined in BHI by the microdilution assay utilizing suspensions of 10⁵ CFU/mL and a drug concentration range from 2.5 to 0.0012 mg/mL (twofold serial dilutions). MIC was defined as the lowest concentration at which no growth was observed. For the evaluation of the extracts as modulators of the resistance to the antibiotics, MIC of the antibiotics was determined in the presence or absence of EEAC and EEAM at subinhibitory concentrations (8 μg/mL) and the plates were incubated for 24 h at 37°C. Each antibacterial assay for MIC determination was carried out in triplicate.

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### Table 1: Origin of the bacterial strains and profile of resistance to antibiotics.

| Bacteria                | Origin         | Profile of resistance |
|-------------------------|----------------|-----------------------|
| *Escherichia coli* 27   | Surgical wound| Ast, Ax, Amp, Ami, Amox, Ca, Cfc, Cf, Caz, Cip, Chlo, Im, Kan, Szt, Tet, Tob |
| *Escherichia coli* ATCC10536 | ATCC           | —                     |
| *Staphylococcus aureus* 358 | Surgical wound | Oxa, Gen, Tob, Ami, Kan, Neo, Para, But, Sis, Net |
| *Staphylococcus aureus* ATCC25923 | ATCC       | —                     |

*Ast*: Aztreonam; *Ax*: Amoxicillin; *Amp*: Ampicillin; *Ami*: Amikacin; *Amox*: Amoxicillin; *Ca*: Cefadroxil; *Cfc*: Cefaclor; *Cf*: Cefalotin; *Caz*: Ceftazidime; *Cip*: Ciprofloxacin; *Chlo*: Chloramphenicol; *Im*: Imipenem; *Kan*: Kanamycin; *Szt*: Sulfametim; *Tet*: Tetracycline; *Tob*: Tobramycin; *Oxa*: Oxacillin; *Gen*: Gentamicin; *Neo*: Neomycin; *Para*: Paramomycin; *But*: Butirosin; *Sis*: Sisomicin; *Net*: Netilmicin; (—): sensitivity. ATCC: american type culture collection.

### Table 2: Botanical families, species, and number of the title of the plants used in this study.

| Family       | Species                  | Number HCDAL | Herbarium                      |
|--------------|--------------------------|---------------|-------------------------------|
| Leguminosae  | *Amburana cearensis*     | 5545          | Vale do São Francisco-UNIVASF |
| Fabaceae     | *Anadenanthera macrocarpa* | 6490          | Dárduo Andrade Lima-URCA      |
2.6. Evaluation of the Modulation of Extracts on the Resistance to Aminoglycosides and β-Lactams Antibiotics. To evaluate the extracts as modulators of antibiotic action, the MICs of antibiotics of the class aminoglycoside and beta–lactams, were evaluated in the presence and absence of the extracts in sterile microplates. The antibiotics were evaluated at concentrations ranging from 512 to 0.5 μg/mL. All antibiotics tested were obtained from Sigma. The extracts were mixed in BHI broth at 10% sub—inhibitory concentrations obtained and determined after the test evaluation of MIC, and for the modulation test concentration was used a concentration of extract referring the MIC diluted 8 times (MIC/8). The preparation of the antibiotic solutions was performed by adding sterile distilled water in a double concentration (1024 μg/mL) in relation to the initial concentration set volume of 100 μL and serially diluted 1 : 1 in 10% BHI broth. In each well with 100 μL of culture medium containing the bacterial suspension diluted (1 : 10). The same controls used in the evaluation of MIC for the extracts were used for the modulation [4]. The plates were filled and incubated at 35°C for 24 hours, and after that the reading was evidenced by the use of resazurin as previously mentioned in the test of determination of the MIC.

Table 4: Phytomedicine of the extracts of Anadenanthera macrocarpa and Amburana cearensis.

| Extracts | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|----------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|
| EEAC     | − | + | − | + | + | + | + | + | + | +  | +  | −  | +  | +  | −  |
| EEAM     | − | + | − | + | + | + | + | + | + | +  | +  | +  | +  | +  | −  |

1: phenols; 2: tannin pyrogallates; 3: tannin phlobaphenes; 4: anthocyanins; 5: anthocyanidins; 6: flavones; 7: flavonols; 8: xanthones; 9: chalcones; 10: aurones; 11: flavonoids; 12: leucoanthocyanidins; 13: catechins; 14: flavonones; 15: alkaloids. (+): presence; (−): absence. EEAC: ethanolic extract of Amburana cearensis; EEAM: Ethanol Extract of Anadenanthera macrocarpa.

Table 5: Minimal inhibitory concentration (MIC) of the ethanolic extracts of Anadenanthera macrocarpa and of Amburana cearensis (μg/mL).

| Extracts and antimicrobials | EC 27 | EC-ATCC 10536 | AS 358 | SA-ATCC 25923 |
|----------------------------|-------|---------------|--------|---------------|
| EEAM                       | ≥1024 | ≥1024         | ≥1024  | ≥1024         |
| EEAC                       | ≥1024 | ≥1024         | ≥1024  | 512           |

EEAM: ethanolic extract of Anadenanthera macrocarpa; EEAC: ethanolic extract of Amburana cearensis. EC: Escherichia coli; SA: Staphylococcus aureus.

3. Results and Discussion

The extracts evaluated in this work showed the yields demonstrated in Table 3, where we observed a higher yield of extract A. macrocarpa compared to A. cearensis. To perform the microdilution assay, the extracts were diluted in DMSO obtaining a solution of concentration of 10 mg/mL. A pilot study was conducted using only DMSO, but no antibacterial or modulatory activity was observed, indicating nontoxic effect.

Table 4 shows the presence of various potentially bioactive compounds in the extracts evaluated, like phenols, tannin pyrogallatos, tannin phlobaphenes, anthocyanins, anthocyanidins, flavones, flavonols, xanthones, chalcones, aurones, flavononols, leucoanthocyanidins, catechins, flavonones, and alkaloids. Through phytomedicine of prospecting of extracts, it was possible to identify the presence of several classes of secondary metabolites that exhibit a wide variety of biological activities such as antimicrobial [20–22], antioxidant [23], antitumor and antiophidian [24].

Table 5 shows the determination of minimum inhibitory concentration (MIC) of ethanol extracts tested against E. coli and S. aureus of reference and multiresistant. Comparatively, the extracts EEAM and EEAC showed the same MIC with the exception of EEAC against SA-ATCC 25923 which showed a better MIC of 512 μg/mL.

Several medicinal plants were used as a source of many antimicrobial drugs used in the treatment of infectious diseases, including against bacteria multiresistant to antibiotics [25]. It is known that the synergistic action of the natural products with antimicrobial agents is commonly used in the therapeutic treatment [26, 27].

Table 6 shows the interference of the extracts on the activity of aminoglycosides, demonstrating a modulation in the activity of antibiotics, reducing the MICs. The more representative effect was observed with the association of EEAM and amikacin, an increase being observed in the antibiotic activity against EC27, reducing the MIC of the antibiotic from 128 to 4 μg/mL.

Due to absorption into the intracellular space, the cell toxicity is common to all aminoglycosides (except to streptomycin). Nephrotoxicity, ototoxicity, and neuromuscular blockade are the most important toxic effects of aminoglycosides [7, 28]. The reported frequency of these side effects
is highly variable due to different criteria used for diagnosis [29]. The combination of the aminoglycosides with natural products can be an alternative to minimize the side effects of this class of antibiotics, since the association leads to a synergistic effect significantly reducing the MIC of these drugs, decreasing the dose needed for therapeutic usage.

Many β-lactam antibiotics can penetrate Gram-negative bacteria via protein channels present in the outer membrane. Through these channels, the drug can reach its receptor on the cell wall and exert its bactericidal action [30]. Although extracts have in its constitution secondary metabolites such as tannins and flavonoids, which are synthesized by plants, the presence of several antibacterial, which can be responsible for the observed modulatory effects, indicating the possibility of using natural products combined with aminoglycosides to increase the antimicrobial potential of these drugs against multiresistant microorganisms.

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