Research Article

Evaluation of the Antimicrobial Activity of the Decoction of Tropidurus hispidus (Spix, 1825) and Tropidurus semitaeniatus (Spix, 1825) Used by the Traditional Medicine

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Tropidurus hispidus and Tropidurus semitaeniatus are two lizard species utilized in traditional medicine in Northeast Brazil. Their medicinal use includes diseases related with bacterial infections such as tonsillitis and pharyngitis. They are used in the form of teas (decoctions) for the treatment of illnesses. In this work, we evaluated the antimicrobial activity of the decoctions of T. hispidus (DTH) and T. semitaeniatus (DTS) against bacterial strains, namely, standard and multiresistant Escherichia coli, Staphylococcus aureus, and Pseudomonas aureuginosa. Chemical prospecting tests revealed the presence of alkaloids in DTS. This is the first study evaluating the medicinal efficacy of T. hispidus and T. semitaeniatus and contributes to the list of new sources of medicines from natural products of animal origin.

1. Introduction

Natural substances from animals, plants, and minerals have provided a continuous source of medications [1]. In Brazil, as in other countries, animals and plants have been widely utilized since antiquity by the traditional medicine [2, 3], and according to Alves and Rosa [4], they have played a significant role in the healing arts up to nowadays.

Despite their prevalence in the practice of traditional medicine throughout the world, the medicinal use of animals have often been neglected in research, compared to medicinal plants [5]. According to Alves et al. [6], emphasis has been placed for the most part on plant-based medications more than on those from animal origin. Besides, plants are easier to collect, store, and sell. However, recent publications have demonstrated the importance of zootherapy in different sociocultural environments worldwide, and examples of the use of remedies derived from animals can currently be found in many urban and semiurban localities, particularly in developing countries [2, 3, 7, 8].

Reptiles are among the species most utilized in popular medicine, and their role in practices and beliefs related to the treatment and/or prevention of diseases has been reported by different traditional communities worldwide [4, 7–11].
Despite the extensive use of reptiles for medicinal purposes, there is a general lack of detailed information about the exploitation of these animals and their impact on the species involved [11].

Among the species utilized in traditional medicine in Brazil, we can cite *Tropidurus hispidus* and *T. semitaeniatus*. *Tropidurus semitaeniatus* (Spix, 1825) is endemic to the “Caatinga” biome. Popularly known as the “outcrop lizard,” it is a small lizard with a diurnal habit. It is found on broad rocky surfaces (outcrops), and with a dorsoventral flattened body, specialized at getting into small cracks in the rocks, where it is protected and probably remains during the warmest hours of the day [12]. *T. semitaeniatus* is indicated for the treatment of measles, asthma, alcoholism, dermatomycosis, and chickenpox [6].

The animals were collected in the municipality of Crato (7°14’03”S × 39°24’34”W), Ceará, Brazil in April 2010. They were caught manually and with air pistols by rummaging through habitats where these animals can be found (Permission for collection: 154/2007 no. 23544-1 process no. 17842812). Once the lizards were collected and sacrificed, their skins were removed and dried in a drying oven to prepare extracts. Control specimens were fixed in 70% alcohol and deposited in the zoology collection of the Universidade Regional do Cariri/LAZ-URCA (Table 1).

### 2. Material and Methods

#### 2.1. Zoological Material. The animals were collected in the municipality of Crato (7°14’03”S × 39°24’34”W), Ceará, Brazil in April 2010. They were caught manually and with air pistols by rummaging through habitats where these animals can be found (Permission for collection: 154/2007 no. 23544-1 process no. 17842812). Once the lizards were collected and sacrificed, their skins were removed and dried in a drying oven to prepare extracts. Control specimens were fixed in 70% alcohol and deposited in the zoology collection of the Universidade Regional do Cariri/LAZ-URCA (Table 1).

#### 2.2. Preparation of Decoctions of *T. hispidus* (DTH) and *T. semitaeniatus* (DTS). The decoctions of *T. hispidus* and *T. semitaeniatus* were prepared by submersing the whole lizards, already oven-dried, in boiling distilled water for 2 h. Afterward, the decoction was filtered, frozen, and later lyophilized. A concentrated form was used in the antimicrobial assays. The yields for the decoctions are shown in Table 2. The decoctions were then stored in a freezer for future analyses.

#### 2.3. Strains. The experiments were carried out using the following bacteria: clinical isolates of *Escherichia coli* (EC27), *Staphylococcus aureus* 358 (SA358), and *Pseudomonas aeruginosa* PA RB1 and the standard strains *E. coli* ATCC 10536, *S. aureus* ATCC 25923, and *P. aeruginosa* ATCC.

### Table 1: Species utilized in the antimicrobial analyses.

| Species               | University/Archive no. |
|-----------------------|------------------------|
| *Tropidurus hispidus* | Universidade Regional do Cariri-URCA LZ-847 |
| *Tropidurus semitaeniatus* | Universidade Regional do Cariri-URCA LZ-926 |

### Table 2: Fresh weight, dry weight, and yield of decoctions of *Tropidurus hispidus* and *Tropidurus semitaeniatus* (g).

| Species               | Fresh weight | Dry weight | Solvent         | Yield of crude extract |
|-----------------------|--------------|------------|-----------------|------------------------|
| *T. hispidus* Whole animal | 193.5        | 52.00      | Distilled water (DTH) | 11.0539 |
| *T. semitaeniatus* Whole animal | 94.68        | 27.7067   | Distilled water (DTS) | 4.4885 |

DTH: decoction of *Tropidurus hispidus*; DTS: decoction of *Tropidurus semitaeniatus*.
Table 3: Results of chemical prospecting of decoctions of Tropidurus hispidus and Tropidurus semitaeniatus.

| Classes of secondary metabolite | DTH | DTS |
|--------------------------------|-----|-----|
| Phenols                        |     |     |
| Pyrogallic tannins             |     |     |
| Flobatenic tannins             |     |     |
| Anthocyanins                   |     |     |
| Anthocyanidines                |     |     |
| Flavones                       |     |     |
| Flavonols                      |     |     |
| Xanthones                      |     |     |
| Chalcones                      |     |     |
| Aurones                        |     |     |
| Flavanonols                    |     |     |
| Leucoanthocyanidins            |     |     |
| Catechins                      |     |     |
| Flavanones                     |     |     |
| Terpenes                       |     |     |
| Alkaloids                      |     | +   |

DTH: decoction of Tropidurus hispidus; DTS: decoction of Tropidurus semitaeniatus. (+) presence and (−) absence.

15692 [25]. All the strains were maintained on heart infusion agar slants (HIA, Difco), and, before the assays, the cells were grown overnight at 37°C in brain heart infusion broth (BHI, Difco).

2.4. Drugs. The antibiotics utilized, gentamicin, kanamycin, amikacin, and neomycin, were obtained from Sigma Chemical Corp., St. Louis, Mo, USA. All the drugs were dissolved in sterile water before use.

2.5. Drug Susceptibility Tests. The test solution of the decoctions of the two species was prepared by dissolving 10 mg of the samples in 1 mL of dimethylsulfoxide (DMSO-Merck, Darmstadt, Germany), obtaining an initial concentration of 10 mg/mL. This solution was then diluted to 1024 µg/mL using sterile water. The minimal inhibitory concentrations (MIC) of the extracts were determined using microdilution assays in BHI broth with bacterial suspensions of 10⁶ CFU/mL and drug concentrations varying from 1024 to 1 µg/mL (in 2-fold serial dilutions) [26]. MIC was defined as the lowest concentration of drug at which no bacterial growth was observed. For the evaluation of extracts for antibiotic-modifying activity, MICs of the antibiotics were determined in the presence and absence of each decoction at subinhibitory concentrations (128 µg/mL), and the plates were incubated for 24 h at 37°C.

2.6. Chemical Prospecting. The chemical tests to determine the presence of heterosides, saponins, tannins, flavonoids, steroids, triterpenes, cumarins, quinones, organic acids, and alkaloids were performed according to the method described by Matos [27]. The tests are based on visual inspection for a color change or formation of a precipitate after the addition of specific reagents. The results obtained are presented in Table 3.

3. Results

The decoctions of the lizards T. hispidus and T. semitaeniatus did not show a clinically relevant antibacterial activity, presenting a MIC ≥ 1024 µg/mL against all bacterial strains tested, suggesting that these lizards are ineffective in traditional medicine against bacterial infections. DTH and DTS were tested for possible antibacterial activity when combined with commonly used antibiotics. No effect of any decoction was observed against the multiresistant strain of E. coli-EC27. Against the strain SA358, DTS combined with a kanamycin and amikacin significantly reduced the MIC of these antibiotics as observed in Table 4. DTH also enhanced the action of the kanamycin against the same bacterial strain. Against the multiresistant clinical isolate and Pseudomonas aeruginosa RB1, both DTH and DTS showed synergy with the aminoglycosides neomycin and gentamicin (Table 4). The chemical prospecting tests demonstrated the presence of alkaloids in the decoction of T. semitaeniatus, but not in the case of T. hispidus, as seen in Table 3.

4. Discussion

The present study demonstrated that decoctions of T. hispidus and T. semitaeniatus did not present clinically relevant antibacterial activity, with MIC of ≥ 1024 µg/mL against all the strains used. Similar results were obtained in a study by Ferreira et al. [28], demonstrating the lack of in vitro antimicrobial activity of body fat from Tupinambis merianae, which is used in popular medicine against bacterial infections caused by E. coli and S. aureus, besides, several proteins and peptides from animals present antibacterial activity [29].

On the other hand, a synergistic effect was observed between the extracts with aminoglycosides, reducing the MIC of the antibiotics was also observed in other studies with natural products isolated from animals and plants [30–32]. Therefore, there is a need to understand how these substances act in order to increase the activity of conventional antibiotics, since a substantial decrease in the concentration of aminoglycosides would be a promising improvement in the chemotherapy of infections. According to Matias et al. [33], several components of the extracts can act as cell permeabilizers, increasing the cellular uptake of antibiotics [34]. Interference with bacterial enzyme systems can also be a potential mechanism of action [35]. These mechanisms of action can be involved in the combination of an antibiotic with a natural product at a subinhibitory concentration [36, 37].

The presence of alkaloids in the decoction of T. semitaeniatus used in these antimicrobial assays can be a strong indication that these substances present the antibiotic-modifying activity, since these extracts potentiated the antibiotic action (Table 4). Studies have demonstrated different pharmacological activities of alkaloids [38, 39]. In the case of DTH, which does not contain alkaloids but still showed synergism
with particular aminoglycosides, other possible bioactive substances not detected may be responsible for its synergistic effect, necessitating further studies to identify these natural products.

It is important to note that the use of natural products combined with conventional drugs has been previously described. Calvet-Mir et al. [40] reported the use of traditional medicine products in combination with Western medicine for the treatment of diarrhea, vomiting, and stomachache. Vandeboek et al. [41] reported on the use of natural products and commercial medications together for the treatment of diseases of the respiratory and digestive tracts.

According to Ferreira et al. [42], studies of substances from reptiles must be stimulated to determine their pharmacological activities. Ciscotto et al. [43] described the antibacterial and antiparasitic activities of l-amino acid oxidase from the venom of this snake. Morais et al. [44] reported the anticoagulant activity of antithrombin factor from Bothrops jararaca (Wied, 1924) venom. Products from other species of reptiles were also studied in attempt to elucidate their pharmacological proprieties. Liu et al. [45] demonstrated the antitumor effect of extracts of the lizard Gecko japonicas (Schlegel, 1836), which is widely utilized in Chinese traditional medicine. The lysozymes of the turtles Trionyx sinensis (Wiegmann, 1835), Amyda cartilaginea (Boddart, 1770), and Chelonia mydas (Linnaeus, 1758) demonstrated a strong antibacterial activity [46].

5. Conclusion

The decoctions of T. hispidus and T. semitaeniatus, alone, did not show antimicrobial activity, suggesting the ineffectiveness of products derived from these animals for the treatment of bacterial infectious diseases in traditional medicine. However, the decoctions were found to be effective when combined with aminoglycoside, demonstrating a pharmacological potential to enhance the antibiotic activity. Further studies with natural products of animal origin are needed since this field still remains few explored compared to phytotherapeutic substances, and the medicinal potential of products derived from animals can lead to notable advances in conventional medicine, as well as to the development of management techniques in the conservation of species with potential medicinal use.

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