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Transference of bioactive compounds from support plants to the termites *Constrictotermes cyphergaster* (Isoptera)

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HIGHLIGHTS

- The use of animals for the treatment of different human diseases is common in traditional medicine.
- Termites are among the species most commonly used in folk medicine.
- Potential microbiological activities of termites may be associated with their relationships with plants.
- The antimicrobial potential of ethanol extracts of the bark of supporting plants is higher than the antimicrobial potential of the termite *C. cyphergaster* extracts;
- The combination of the extracts of *C. cyphergaster* and its nests with antibiotics produces a strong synergistic activity.

GRAPHICAL ABSTRACT

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ABSTRACT

This study aims to investigate the microbiological potential of the termite species *Constrictotermes cyphergaster* (Silvestri, 1901) and its support plants. We collected five *C. cyphergaster* nests from three different support plant species. Microbiological assays were performed on these extracts using the serial microdilution method in triplicate to measure the minimum inhibitory concentration (MIC) of each microorganism for the analysed extract. The ethanol extracts of the termite C. *cyphergaster* showed no significant activity against strains of *Staphylococcus aureus* and *Escherichia coli*, with an MIC >1000 μg mL-1. Only the extracts of the nests and termites with the nest had the same MICs. These results were in contrast to the extracts of *Spondias tuberosa* (Umbuzeiro), *Poincianella pyramidalis* (Catingueira), and *Amburana cearensis* (Cumaru), which demonstrated significant activity against *S. aureus* with MICs <1000 μg mL-1. The modulating activity of the extracts tested in the present study demonstrated potentiation of most antibiotics across the bacterial strains tested when combined with the extracts for both *S. aureus* and *E. coli*. These results indicate that the extracts tested in the present study may be composed of animal and vegetable origins with the potential to modify the activity of antibiotics and thus may aid in antimicrobial therapy.

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1. Introduction

Biodiversity is an invaluable source of information and bioactive chemicals that support human health (Chivian, 2002). Plants and animals have been documented in several geographical regions (WHO, 2002; Ferreira et al., 2012; Alves and Rosa, 2013; Van Vliet et al., 2017; Hajdari et al., 2018) as sources of remedies in traditional medicine worldwide. Although plants and their derivatives constitute most of the products used in traditional medicine, whole animals or their parts and animal sub-products are also important constituents of materia medica in different human societies (Marques, 1995; Alves and Rosa, 2013).

Among the invertebrates, insects have played an important role as sources of therapeutic products in traditional medicine (Costa Neto, 2005; Costa Neto et al., 2006; Dossey, 2010). Insects and the products derived from them have been used by human cultures for medicinal purposes in different geographical regions (Figueirêdo et al., 2015; Kritsky, 1987; Morris, 2004; Costa Neto, 2005; Dossey, 2010). For an array of reasons, insects and their biological defence systems offer a important source of chemicals with great potential for use as novel medicinal compounds (Dossey, 2010; Dettner, 2011; Alves and Albuquerque, 2013).

Termites (Isoptera) are an insect group that is commonly used in traditional folk medicine (Wilsanand, 2005; Coutinho et al., 2009; Figueirêdo et al., 2015). In Brazil, several termite species are commonly used for the treatment of human diseases, including bronchitis, influenza, whooping cough, asthma, sinusitis, hoarseness and tonsillitis (Alves et al., 2013). Studies with animal extracts have demonstrated the efficiency of some species against bacterial strains (Coutinho et al., 2009, 2010; Chaves et al., 2014). Termites feed on living and dead plant material. Some termite species use trees as support for nest construction, and some of these support trees are used in folk medicine (Wilsanand, 2005; Albuquerque et al., 2007). This scenario provides an good research opportunity in the field of bioprospection because the potential microbiological activity of animals may be associated with their relationships with plants. Possible correlations between medicinal flora and fauna need to be evaluated in pharmacological studies (World Resources Institute, 2000), and the use of animals and plants in folk medicine may help identify and further characterize useful species. Therefore, termites provide an excellent opportunity to evaluate the medicinal properties of biological resources and the importance of the interactions between the animals and plants used in traditional medicine. Additionally, the possible pharmacological activity of termites will contribute to a greater appreciation for these animals, which are usually known for their negative aspects associated with economic losses.

Bacterial infections are currently the focus of public health, mainly due to the significant growth of bacterial resistance. Infections caused by *Staphylococcus aureus* are the most common, showing a greater difficulty in treatment due to its resistance to various antibiotics (Tortora et al., 2008). The species *Pseudomonas aeruginosa* is the leading cause of nosocomial infections, attacking the skin, urinary tract, ear, and eye (Murray et al., 2004). *Escherichia coli* are the most common species of the genus *Escherichia*, associated with severe urinary tract infections, meningitis and gastroenteritis (Murray et al., 2004; Tortora et al., 2008).

In the present study, we investigated *Constrictotermes cyphergaster*, which is one of the most important termite species that build conspicuous nests (with visible structures) in ecosystems with sparse vegetation in South America (Melo and Bandeira, 2004). This species is considered dominant among other termite species in the caatinga (Neotropical dry forest). Additionally, *C. cyphergaster* actively participates in the decomposition of plant organic matter and nutrient cycling (Vasconcellos et al., 2007). Bezerra-Gusmão et al. (2013) observed that the supporting plants used by this termite species were shrubs that occurred at a high density, including *Poincianella pyramidalis* (Tul). LP Queiroz (Catingueira), which affected the distribution of termite mounds in arid environments. We investigated the plant species *P. pyramidalis* (Catingueira) of the Fabaceae family, *Spondias tuberosa* Arruda (Umbuzeiro) of the Anacardiaceae family, and *Amburana cearensis* (Allemo) AC Smith (Cumaru) of the Fabaceae family, which are used by *C. cyphergaster* as support. These plants are widely used in folk medicine for treating diseases (Silveira and Pessoa, 2005; Lins-neto et al., 2010; Almeida et al., 2010; Medeiros et al., 2012). Therefore, this study aimed to assess whether (i) the antimicrobial potential of termites depended on the supporting plants, (ii) the supporting plants had higher or lower antimicrobial potential than the termites and (iii) variation was present in the antimicrobial potential of the extracts of the supporting plants, nests and termites. In this context, this study investigated the microbiological potential of the termite species *Constrictotermes cyphergaster* and its supporting plants.

2. Materials and methods

2.1. Study site and sampling

The termite material was collected in the Private Reserve of Natural Heritage (Reserva Particular do Patrimônio Natural - RPPN) Fazenda Almas (7°28′S and 36°53′W), in São José dos Cordeiros, state of Paraíba, Brazil (Barbosa et al., 2007) (Fig. 1). During the study period, 15 samples were collected from nests that used *Poincianella pyramidalis* (Tul). LP Queiroz (Catingueira) (5 samples) and *Amburana cearensis* (Allemao) AC Smith (Cumaru) (5 samples), both of the Fabaceae family, and *Spondias tuberosa* Arruda (Umbuzeiro) (5 samples) of the Anacardiaceae family as supporting plants. The samples were collected from different specimens of each plant species. The specimens were randomly selected in the collection area, and the collected specimens were distanced at least 50 m from one another. We collected termites, the inner wall of the nest, and the stem bark from these specimens. The samples were collected using a hatchet. The nests and termites were transferred to sterilized glass containers, and samples of the stem bark of the supporting plants were stored in Kraft paper bags. The termite species was identified by Prof. Alexandre Vasconcellos from the Systematics and Ecology Department (Federal University of Paraíba - UFPB). Two samples were deposited in the Isoptera Collection of the Exact Sciences of Nature Center UFPB under the numbers 2047 and 2048. The botanical identification was carried out in the "Prof. Lauro Pires Xavier Herbarium" (JPB), Systematics and Ecology Department, Federal University of Paraíba, where vouchers specimens were deposited with following reference numbers: JPB 30.589 for *Amburana cearensis*, JPB 34.322 for *Spondias tuberosa* and JPB 41.167 for *Poincianella pyramidalis*.

2.2. Preparation of extracts

The collected animals were manually separated from the nest and divided into three samples as follows: i) termites only, ii) nests only, and iii) termites and nests at a 1:1 ratio. The collected stem bark was broken down into small fragments, dried in a forced air oven at 40 °C until the complete stabilization of moisture, ground in a knife mill, and sieved through a 10-mesh sieve to obtain bark powder, which was used to prepare the extracts.

Twenty grams was extracted from each sample by cold soaking using ethanol as the solvent for 5 days at room temperature. Subsequently, after filtration, the extracts were concentrated on a rotary evaporator at 40 °C until complete evaporation of the solvent was achieved. The 60 obtained samples included 15 samples of termites alone, 15 samples of nests alone, 15 samples of termites and nests, and 15 samples of stem bark, being isolated from each support plant in which they were collected.