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ORIGINAL ARTICLE

# Antibiotic resistance modulation by natural products obtained from *Nasutitermes corniger* (Motschulsky, 1855) and its nest



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**Abstract** Insects and their products are included in the traditional pharmacopoeia of various ethnic groups worldwide. In the Brazilian semiarid region can be highlighted the use of the termite *Nasutitermes corniger* for the treatment of various diseases. This study evaluated the ethanol extract of *N. corniger* and its nest as an antimicrobial agent and as a modulator of bacterial resistance against multidrug strains. The Minimum Inhibitory Concentration (MIC) of the extract on *Staphylococcus aureus* and *Escherichia coli* by microdilution was determined, as well as MIC of antibiotics in the presence and absence of extract. Despite having no significant antimicrobial activity (MIC  $\geq 1000 \mu\text{g mL}^{-1}$ ), the extract showed additive activity to the antibiotic efficacy, significantly

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reducing its MIC. These results suggest that *N. corniger* and its nest are promising natural products for use in antimicrobial therapy.

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## 1. Introduction

*Escherichia coli* is the most prevalent Gram-negative rod-shaped bacterium in human fecal flora optional, usually inhabiting the colon as a harmless commensal (Johnson, 1991); however, pathogenic variables have the ability to cause diarrhea and extra intestinal diseases. *Staphylococcus aureus* is an important pathogen that is part of the normal flora of the human skin and mucous membranes. Infection with this microorganism often occurs through a lesion on the skin which can lead to the appearance of wounds, but also *S. aureus* can infect any tissue of the body, causing potentially fatal diseases such as osteomyelitis, endocarditis, pneumonia and septicemia (O'Riordan and Lee, 2004; Fournier and Philpott, 2005).

The prevalence of resistant bacterial strains has increased in several countries, where it was found that a range of antibiotics including penicillins, cephalosporins and aminoglycosides no longer had efficacy on certain pathogens (Subramanian et al., 2009; Croxen et al., 2013). Such resistance increases the bacterial pathogenic potential and presents a serious challenge to human health, increasing morbidity and mortality, as well as the costs for the treatment of infections (Andersson and Hughes, 2010).

The use of natural products derived from animals, plants and microorganisms has been seen as an alternative to antibiotics. Apart from substances with direct antimicrobial activity, researchers have sought substances that can act as adjuvants by modifying the effectiveness of antimicrobial agents (Coutinho et al., 2010a; Veras et al., 2012).

The insect class is the largest and most widely distributed taxonomic group. It has about one million described species, comprising approximately three-quarters of organisms on Earth, which represents a colossal biomass in nature (Gullan and Cranston 2005; Chakravorty et al., 2011). These characteristics have made insects an important resource for many civilizations over the centuries, which have been using these animals for several purposes, including the use in healing rituals (Morris, 2004).

The use of insects and their products in traditional medicine has been documented by several authors in different parts of the world (Pemberton, 1999; Costa-Neto, 2002; Lehman et al., 2007). Extracts of insects have been considered important and studied by modern science, revealing important therapeutic functions, which consider these animals as an important tool for the discovery of new drugs (Feng et al., 2009).

In Brazil, among the species most commonly used for therapeutic purposes are the termites (Isoptera) (Alves and Rosa 2006, 2007), including *Nasutitermes corniger* (Motschulsky, 1855), whose natural products are listed in the traditional pharmacopoeia of the semiarid region for the treatment of asthma, cough, flu and sore throat (Ferreira et al., 2012).

This study aimed at evaluating, through experimental models *in vitro*, the extract of *N. corniger* and its nest as an antibacterial agent and as a modifier of resistance against pathogenic microorganisms.

## 2. Materials and methods

### 2.1. Zoological material

The collection of *N. corniger* along with the nest was performed on the farm Farinha, municipality of Pocinhos, semiarid region of the Paraíba state, (7°07'S, 36°07'W). The species was identified by Prof. Alexandre Vasconcellos from the Department of Systematics and Ecology at the Universidade Federal da Paraíba (UFPB). A sample was deposited to the Isoptera collection at the Center of Exact and Natural Sciences UFPB under the number CICB 69.

### 2.2. Extract preparation

Twenty grams of termites with nest were subjected to extraction by maceration with 100 mL of absolute ethanol for 5 days at room temperature ( $25 \pm 3^\circ\text{C}$ ) with occasional stirring. After filtration, the extracts were concentrated on a rotary evaporator at  $40^\circ\text{C}$ .

### 2.3. Determination of total polyphenols

The total polyphenol content of plant extracts was measured using spectrophotometry in the visible region by the method of Folin–Ciocalteu described by Chandra and Mejia (2004) with minor modifications. The extracts (25 mg) were dissolved in distilled water to obtain a final concentration of  $200 \mu\text{g mL}^{-1}$ . From each solution, a 1 mL aliquot was added to 1 mL of 1 mol/L Folin–Ciocalteu reagent. This mixture remained undisturbed for 2 min before the addition of 2 mL of 20% (w/v)  $\text{Na}_2\text{CO}_3$  solution and left undisturbed for 10 min. Thereafter the reading was performed using Spectrophotometer Shimadzu, model UV-mini 1240, at 757 nm. The calibration curve was obtained with a stock solution of gallic acid ( $1000 \mu\text{g mL}^{-1}$ ), from which dilutions were made at concentrations between 1 and  $40 \mu\text{g mL}^{-1}$ .

### 2.4. Determination of total flavonoids

The total flavonoids were determined by the method described by Meda et al. (2005). The extracts were diluted with methanol at  $1000 \mu\text{g mL}^{-1}$ . To 5 mL of each test solution was added the same volume of 2% (w/v)  $\text{AlCl}_3$  solution in methanol. This mixture remained undisturbed for 10 min before the UV spectrophotometric reading at 415 nm wavelength. The total flavonoids were determined by the calibration curve using quercetin (Sigma–Aldrich) as standard at concentrations between 2 and  $30 \mu\text{g mL}^{-1}$ .

### 2.5. Determination of condensed tannins

The content of condensed tannins was verified through the method of vanillin–HCl described by Makkar and Becker

(1993), where 0.25 mL of the sample was added to 1.5 mL of vanillin solution in methanol (4% w/v) and subsequently, 0.75 mL of concentrated HCl (37%). After HCl addition, the tube content was shaken in a water bath at 30 °C for 3–4 s before reading on a spectrophotometer at 500 nm wavelength. Catechin was used as a standard at concentrations between 10 and 100  $\mu\text{g mL}^{-1}$ .

### 2.6. Determination of saponins

The quantification of total saponins followed the method described by Makkar et al. (2007). 250  $\mu\text{L}$  of 8% vanillin solution in ethanol was added to 250  $\mu\text{L}$  extract solution in 80% methanol; then 2.5 mL of 72% sulfuric acid was added. The tubes were incubated at 60 °C in a water bath for 10 min and transferred to an ice bath, staying for 4 min. The absorbance reading at 544 nm was performed against a blank consisting of the vanillin solution, 80% methanol and sulfuric acid. The calibration curve was obtained from a diosgenin solution at concentrations between 100 and 500  $\mu\text{g mL}^{-1}$ .

### 2.7. Microbial strains

We used the clinical isolates of *Staphylococcus aureus* resistant to ampicillin, ciprofloxacin, cephalixin, erythromycin, penicillin and amoxicillin and *Escherichia coli* resistant to amoxicillin, cephalothin, levofloxacin, chloramphenicol, tetracycline and gentamicin. The tested bacterial strains were incubated at 37 °C for 24 h in Mueller–Hinton agar and maintained on agar slants in tubes on the same medium.

### 2.8. Drugs

All the drugs tested were obtained from Sigma Chemical Corp., St. Louis, MO, USA, and dissolved in sterile water before use.

### 2.9. Minimal Inhibitory Concentration (MIC) Determination and Modulation activity

The minimum inhibitory concentration (MIC) was determined by the microdilution method in 96-well plates (CLSI, 2012) using Mueller–Hinton broth. Colonies of microorganisms were suspended in 0.9% saline solution and the suspension was adjusted by the spectrophotometric method at 625 nm to a final concentration of  $5 \times 10^6$  CFU  $\text{mL}^{-1}$ . We performed serial dilutions of the extract solubilized in 10% DMSO in the range of 1000–3.9  $\mu\text{g mL}^{-1}$  and antibiotics in the range of 2500–2.4  $\mu\text{g mL}^{-1}$ . DMSO 10% was included as negative control. The plates were incubated at  $37 \pm 1$  °C for 24 h. Bacterial growth was indicated by the addition of 20  $\mu\text{L}$  of 0.01% resazurin aqueous (Sigma–Aldrich) with incubation at  $37 \pm 1$  °C for 2 h, and MIC values were identified as the lowest concentration in which no bacterial growth is visible. Evaluation of extracts as modulators of antibiotic resistance was performed according to Coutinho et al. (2010a). The MIC of the antibiotic was determined in the presence and absence of sub-inhibitory concentrations (125  $\mu\text{g mL}^{-1}$ ). Plates were incubated as described above and each assay was performed in triplicate.

### 2.10. Statistical analysis of microbiological tests

The test results were expressed as geometric means. The two-way analysis of variance followed by Bonferroni post-test was applied using the GraphPad Prism 5.0 software (Matias et al., 2013).

## 3. Results and discussion

Results of chemical tests are described in Table 1. The termite *N. corniger* and its nest have plant secondary metabolites, probably from their host plant. This is because this species of termite is xylophage and compounds present in the plant consumed may still be in the digestive system of the animals or the excrement used for making the nest (Noirot and Darlington, 2000; Thorne and Haverty, 2000).

With respect to antimicrobial activity, the extract showed no clinically significant activity against resistant strains of *S. aureus* and *E. coli*, with the Minimum Inhibitory Concentration (MIC) > 1000  $\mu\text{g mL}^{-1}$ . Ríos and Recio (2005) suggest that in the study on medicinal plants, the extract should be considered as possessing antimicrobial activity if the MIC is < 1000  $\mu\text{g mL}^{-1}$ . Despite the extracts tested in this study not being obtained from plant material, we take this value as a reference.

Moreover, by adding the extract of *N. corniger* and its nest in sub-inhibitory concentration (125  $\mu\text{g mL}^{-1}$ ) to the culture medium, there was a significant reduction ( $P < 0.001$ ) in the MIC of all antibiotics tested, except for cephalothin on *E. coli* (Figs. 1 and 2). For *S. aureus*, the best modulation effects were observed in association with erythromycin extract, which provided a more marked reduction of MIC, 2500–625  $\mu\text{g mL}^{-1}$ , while for *E. coli* there was a MIC decrease from 156.25 to 39.06  $\mu\text{g mL}^{-1}$  when combining the extract and gentamicin.

Similar results were obtained by Coutinho et al. (2010a,b) who evaluated the synergism between decoctions of *N. corniger* and aminoglycosides on resistant strains of *E. coli*. Although decoctions have not shown significant antimicrobial activity (MIC > 1024  $\mu\text{g mL}^{-1}$ ) when combined with antibiotics, they promoted significantly reduced MIC.

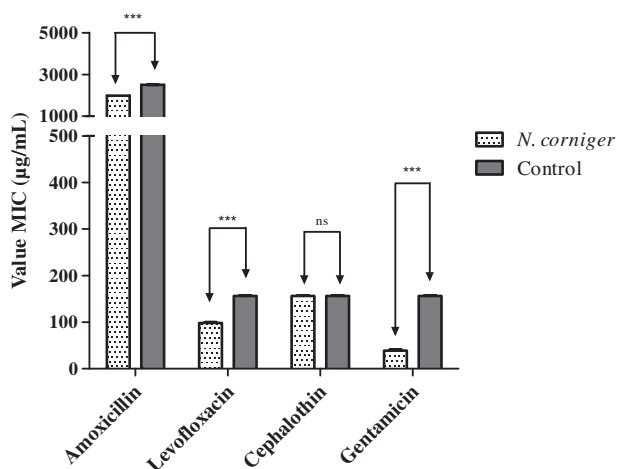
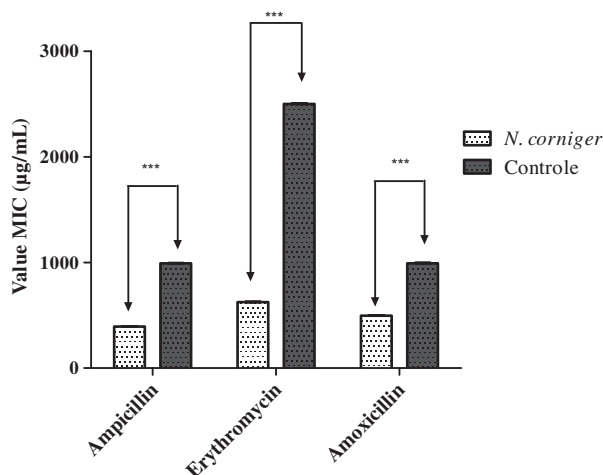
The biological activity exhibited both in this work as in those above should probably be related to the presence of the host plant secondary metabolites, peptides produced by the animals as a defense mechanism, or even substances produced by mound's microorganisms.

Regarding the substances of plant origin, tannins can act on the metabolism in microorganisms through inhibition of enzymes, oxidative phosphorylation and the electron transport system, inactivation of microbial adhesins and proteins of the cell envelope (Scalbert 1991; Cowan, 1999). Flavonoids can disrupt lipid bilayers by directly penetrating and disrupting the barrier function, but may also cause membrane fusion, resulting in leakage of intramembranous materials (Ikigai et al., 1993; Cushnie and Lamb, 2005). Saponins cause disturbance in the lipid component forming pores in membranes (Francis et al., 2002; Sung and Lee, 2008).

Termites have developed the ability to deal with a rich microbial community and are often infectious inhabiting their nests and feeding sites, developing, among other defense mechanisms, the synthesis of antimicrobial peptides (Traniello et al., 2002; Schmid-Hempel, 2005). Two peptides with antimicrobial

**Table 1** Concentration of secondary metabolites ( $\text{mg g}^{-1}$ ) determined for the extract of *N. corniger*.

Metabolites	Total polyphenols	Flavonoids	Condensed tannins	Total saponins
Concentration	$17.56 \pm 0.23$	$6.75 \pm 0.05$	$10.5 \pm 0.09$	$38.22 \pm 13.21$

**Figure 1** Graph demonstrating the modulatory activity of the ethanol extract of *N. corniger* on resistance of *E. coli* to antibiotics. \*\*\* – statistically significant with  $P$  value  $< 0.001$ ; ns – not statistically significant value with  $P > 0.05$ .**Figure 2** Graph demonstrating the modulatory activity of the ethanol extract of *N. corniger* on resistance of *S. aureus* to antibiotics. \*\*\* – statistically significant with  $P$  value  $< 0.001$ .

properties have been isolated from *Pseudacanthotermes spiniger* by Lambert et al. (2001), “termicin”, which showed essentially antifungal properties and “spinigerin”, which showed antifungal and antibacterial activities. Cysteine-rich peptides and  $\alpha$ -helical peptides, such as termicin and spinigerin respectively, have the ability to permeabilize microbial cytoplasmic membranes (Dimarcq et al., 1998), which was confirmed by Lee et al. (2003) when studying the antimicrobial properties of Spinigerin. Bulmer and Crozier (2004, 2006) in studies on the molecular biology of *Nasutitermes* termites identified genes responsible for the synthesis of antimicrobial peptides.

The nests of social insects, for its characteristics of temperature and humidity, have a rich associated microbiota, where some species live in symbiosis with insects, protecting them from infections by bacteria and fungi. Madden et al. (2013) reported the antimicrobial activity of actinobacteria isolated from the nest of *Polistes dominulus* (Christ). Visser et al. (2012) isolated actinobacteria from nests of the termites *Macrotermes natalensis*, *Microtermes* sp. and *Odontotermes* spp. and also confirmed their antimicrobial activity. Bonfim (2010) confirmed the antimicrobial activity of actinobacteria and *Bacillus* isolated from nests of *Nasutitermes* mainly on Gram-positive bacteria. Extracts, such as the one used in the present study, represent a complex mixture of substances, affecting and interfering in several mechanisms related to synergistic or additive effects when combined with antibiotics or other drugs (Imming et al., 2006; Schmidt et al., 2008; Wagner and Ulrich-Merzenich, 2009).

#### 4. Conclusion

The results of this study revealed *N. corniger* and its nest as having modulating activity of multidrug bacterial resistance to commercial antibiotics and can be used as adjuvants in antimicrobial therapy.

#### Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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