

Absence of antimicrobial activity of *Euphorbia milii* molluscicidal latex

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Abstract

Introduction: *Euphorbia milii* latex has been used as herbal medicine and proved to be a potent plant molluscicide. **Materials and Methods:** The susceptibility of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* to *E. milii* molluscicidal latex was evaluated by assays in the agar well diffusion method and tubes dilution, to determine the minimal inhibitory concentration and minimal bactericidal concentration. **Results:** Latex *in natura* (fresh) and lyophilized in a maximum viable concentration of 100 mg/L did not show any inhibitory effect on the growth of the bacterial strains tested. **Conclusion:** Although it has been effective as a molluscicide, this latex presented a total absence of antimicrobial activity in the two forms and concentrations tested.

Key words: Euphorbiaceae, euphorbia latex, non-target species, plant molluscicides

INTRODUCTION

The latex of the ‘Crown-of-Thorns’ (*Euphorbia milii*) has been reported as a wart remover^[1] and an anti-inflammatory,^[2] and in China, parts of this plant, including the latex, have been used as herbal remedies for hepatitis and abdominal edema.^[3] In Brazil, the latex of *E. milii* was discovered to be a potent plant molluscicide and an interesting alternative to synthetic chemical products. Several studies have demonstrated its molluscicidal potency in the laboratory^[4,5] and in the field.^[6,7] The selectivity toward target aquatic snails seems to be one of the main advantages of this natural product.^[5] Studies on the chemical composition of *E. milii* have demonstrated the presence of triterpenes and flavonoids,^[8] lasiodiplodin,^[3] milliamines A-G,^[9] milliamines H and I,^[10] milliamine L,^[11] and euphorbin.^[12]

Data on the bacteriological effects of latex are scarce, and so the present study was performed to investigate its biological activity on pathogenic microorganisms, evaluating its potential antimicrobial properties.

MATERIALS AND METHODS

Euphorbia milii Desmoul. ex Boiss (syn *E. splendens*, Euphorbiaceae) used in the present study was identified by Dr. Ivete Maria da Silva and a voucher specimen (reference number R202859) was deposited in the herbarium of the National Museum of the Federal University of Rio de Janeiro (UFRJ). Latex was obtained from plants grown in the experimental gardens located within the FIOCRUZ campus, Rio de Janeiro, Brazil. After collection, the material was transported to laboratory and separated, in part to be tested *in natura* and in part to undergo a lyophilization process. Latex forms were diluted in distilled water at a maximum viable aqueous concentration of 100 mg/L. Simultaneously with the microbial tests the latex forms were tested against *Biomphalaria glabrata* snails to confirm their molluscicidal activity. The antimicrobial activity was evaluated by the well-diffusion agar method and the tube dilution broth test, for investigation of the minimal inhibitory concentration (MIC) and minimal

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bactericidal concentration (MBC). The two assays were carried out according to the method of Bauer *et al.*^[13] under strict adherence to CLSI, formerly known as NCCLS, criteria.^[14] In a well-diffusion agar method, wells were made in Muller-Hinton agar medium and received latex in the two different forms tested (fresh and lyophilized — 100 mg/L in distilled water). Positive control was evaluated in another plate with paper disks (5 mm in diameter) containing the antibiotic gentamicin (10 µg). Both plates were inoculated with 100 µL of a suspension containing 10⁸ CFU/mL of each bacteria and incubated overnight at 37°C. The tests were performed in triplicate. After 16 hours the inhibition zones of bacterial growth around the disks and the wells were measured and recorded. In the tube dilution assay, 100 µL of a suspension containing 10⁸ CFU/mL of each bacterial strain were inoculated in tubes with 10 mL of 100 mg/L lyophilized and fresh latex diluted in nutrient broth. The latex solutions were sterilized by filtration in 0.5 µm Millipore membrane. The tubes were incubated at 37°C for 24 hours and visually evaluated for signs of growth. The microorganisms used were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25902, and *Pseudomonas aeruginosa* ATCC 27853, and to confirm the molluscicidal activity of the latex, a 24-hour test was performed with *Biomphalaria glabrata* in dechlorinated water at pH 7.0 – 7.4.

To calculate the 24 hour-LC_{50s} for molluscicidal activity the Trimmed Spearman Karber method was used.^[15]

RESULTS AND DISCUSSION

The results showed that the positive control tubes and plate disks (gentamicin) had a clear inhibitory effect on the growth of the three bacterial strains tested. On the other hand, the tubes and plate wells with 100 mg/L of fresh and lyophilized *E. milii* latex did not show any inhibitory effect, disabling the calculations of MIC and MBC. In comparison with the previous data of latex, the molluscicidal effect was sustained with lethal concentrations to 50% of the snails in 24 hours (24 hours-LC₅₀ and 95% confidence intervals) of 0.37 (0.24 – 0.49) mg/L and 0.29 (0.19 – 0.45) for fresh and lyophilized latex, respectively.

The absence of microbial inhibitory effects of Euphorbiaceae was observed in another study with *E. coli*.^[16] In a genotoxicity study, the latex of *E. splendens* did not present a mutagenic effect in the Ames test with *Salmonella typhimurium* or cytotoxicity in the Chinese hamster ovary cell assay.^[17] In an ecotoxicity evaluation of lyophilized latex by different toxicological endpoints, adverse effects were not observed on the respiratory rate of *Pseudomonas putida* or on the

bioluminescence of the *Vibrio fischeri* - Microtox Test System.^[5] Although Hood *et al.*^[18] affirmed that the well diffusion and agar dilution methods were capable of producing inconsistent results with essential oils, in the present study, a negative result was obtained in the tube dilution method too, validating the negative data that occurred in the first one method.

Recent studies have been conducted to find microorganisms with a potential to control snails^[19], but currently plant molluscicides are still the most studied.

In conclusion, data from the present study indicate that the aqueous solution of fresh and lyophilized *E. milii* latex (100 mg/L) has no antimicrobial effect in the methods tested, showing that although latex is a potent molluscicide, it is not toxic to microorganisms. Anyway from the medical point of view, new experiments could be carried out to look for other pharmacological endpoints, as per Jyothi *et al.*^[20] who investigated effects on the liver and antioxidant activities of *Euphorbia antiquorum*.

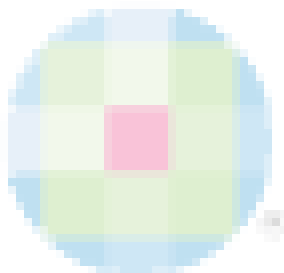
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