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# SUSCEPTIBILITY OF SOME PATHOGENIC MICROBES TO SOFT TISSUE EXTRACT OF THE MUD CLAM, *Polymesoda expansa* (BIVALVIA: CORBICULIDAE)

## ABSTRACT

Antibiotic resistance of microbes has led to the search for potential sources of bioactive compounds with antimicrobial properties. The innate defense mechanisms of aquatic invertebrates against pathogenic organisms have made them prime candidates for extraction of microbicidal compounds. The mud clam (*Polymesoda expansa*) successfully thrives in an environment full of pathogenic microorganisms. An experiment on the antimicrobial activity of the clam was conducted to determine its potential as a source of antimicrobial compounds. Antimicrobial activity of various concentrations of the ethanolic crude extract (ECE) of *P. expansa* was tested against the bacteria *Escherichia coli* (gram-negative), *Staphylococcus aureus* (gram-positive), and the fungus *Candida albicans* using standard discs diffusion technique. The ECE showed 24-h activity on *E. coli* with inhibition zone (IZ) range of 27.65 to 32.57 mm. Similar IZ range (28.73–32.08 mm) was observed in *S. aureus* test cultures, however the efficacy time of the ECE was only 7 h. *C. albicans* test cultures showed low activity of ECE with IZ range of 0.00 to 12.75 mm and efficacy time of 8 h. The study showed that *P. expansa* is a potential source of antimicrobial compounds. Identification, extraction, and purification of such compounds are recommended for future studies.

**Keywords:** *Polymesoda expansa*, pathogens, antimicrobial activity, Philippines

## INTRODUCTION

In recent years, various reports have shown the alarming rise in antibiotic resistance of different types of microbes[1,2,3]. This has led to the search of more antimicrobial substances from other natural sources including the aquatic environment[4]. Invertebrates, including bivalves, are known to survive in their hostile aquatic habitat surrounded by pathogenic organisms due to their well-adapted immune system[5]. They have antibody-like materials that serve as defense from disease-causing organisms[6]. Hence, they are considered potential sources of bioactive compounds against harmful microbes.

There are about 7500 species of known bivalves in the aquatic environment[7]. These species are exposed to various types of pathogenic microbes, which also thrive in the same habitat[8,9]. Bivalves rely on their innate immune system to protect their body from such pathogens[10]. Therefore, bivalves are prime candidates for identification of novel metabolites with antimicrobial properties.

Mud clams such as *Polymesoda expansa* are commonly found in the Southeast Asian mangroves, including the Philippines[11,12]. Mangrove ecosystem is a well-known habitat that harbors various pathogenic organisms[13,14]. Thus, *P. expansa* is present in an environment containing a rich source of microbes, and some may be pathogenic to certain animals. However, *P. expansa* successfully thrives in such condition, which suggests that this clam might have natural immunity to harmful microorganisms. This study investigates the antimicrobial activity of *P. expansa* to assess its potential as a source of bioactive compounds.

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## MATERIALS AND METHODS

### Preparation of ethanolic crude extract (ECE)

Soft tissue samples were collected from the mangrove *Nypa* zone of Loay-Loboc River, Bohol, Philippines (9.60853°N, 124.01265°E). These tissue samples were brought to the Marine Biology Section laboratory of the University of San Carlos for the preparation of ECE.

ECE was prepared by using 300 g of soft tissue samples soaked and macerated in 300 mL of laboratory grade ethanol. The mixture of macerated soft tissue and ethanol was stored at room temperature (30°C) for 24 h and was centrifuged at 15000 rpm for 10 min and the supernatant was filtered through Whatman® grade GF/C glass microfiber filter. Finally, the solvent was concentrated to a gummy dark-brown residue in a rotary evaporator. The ECE was stored at 4°C until usage for antimicrobial activity.

### Test microorganisms

The pathogenic bacteria used for antibacterial assays were *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative). The yeast, *Candida albicans*, was used for antifungal assay.Bacterial and fungal strains were cultivated and maintained (37°C) in Nutrient Agar slants. After 24 h, the cultures were used for study.

### Antimicrobial activity

The antimicrobial activity of the ECE was determined using the standard disc diffusion method[15]. Microbial inoculums were prepared by picking 3–5 distinct colonies from the bacterial and fungal cultures, which were then homogenized in 5-mL sterile saline solution (0.85%). The turbidity of the bacteria and fungus saline solution was visually adjusted to that produced by a 0.5 McFarland standard. Test plates were prepared using 20 mL of Mueller-Hinton Agar medium for bacteria and Potato Dextrose Agar medium for fungus. Inoculations of pathogenic microbial strains were carried out by using sterile cotton swabs dipped in the prepared bacteria and fungus saline solution and swabbed several times on the surfaces of petri plates. Different concentrations of the ECE were applied (30 µL) to 6-mm sterile discs, allowed to dry at room temperature and placed on the top of each test plates seeded with pathogenic microbial strains. A range-finding test was conducted prior to the actual experiment to establish the concentrations of the crude extract (per 1 mL ethanol) used in the study. Three concentrations (500, 1000, 1500 $mg.mL^{-1}$) together with negative (ethanol) and positive (50 $mg.mL^{-1}$ ethanol of Tetracycline® for bacteria and Ketoconazole® for fungus) controls were used in the definitive test. Antimicrobial activity of the ECE of *P. expansa* was assessed by determining the susceptibility of the pathogenic microorganisms. Diameters of the inhibition zones (IZ) were measured in millimeters. Efficacy time was determined by observing the test plates on an hourly basis for 24 h. The antimicrobial activity assays were triplicated.

### Statistical treatment

A model I one-way analysis of variance with replications was used to determine the effects of various ECE concentrations on the susceptibility of the pathogenic microorganisms. Tukey's honestly significant difference test was used as post hoc test. The significance level was set at 95% ($p = 0.05$).

## RESULTS AND DISCUSSION

The antibacterial activity of *P. expansa*'s ECE on *E. coli* test cultures ranges from 27.65 to 32.57 mm mean IZ diameter (Fig. 1). No activity was observed on the pure ethanol discs (negative control). Tetracycline® discs (positive control) showed 30.92 mm mean IZ diameter. The mean IZ diameters of 1000 and 1500 $mg{\cdot}mL^{-1}$

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concentrations and Tetracycline® discs were statistically similar ($p > 0.05$), but showed significantly higher activity ($p < 0.05$) among all other treatments. No proliferation on the IZ of the *E. coli* test cultures was observed after 24 h of incubation.

*E. coli* is an indicator organism for water quality[16]. Studies have reported the proliferation and survival of *E. coli* in estuarine waters[17,18,19], suggesting that populations of these pathogenic bacteria are naturally occurring in such environment. Exposure to such condition may be fatal for other organisms but bivalves appeared to naturally coexist with such microbes[8]. This implies that bivalves have developed immunity against *E. coli*. This study confirmed such immunity by showing optimum antimicrobial activity of *P. expansa* extract against the tested pathogen. Similar results were reported in other bivalves such as *Lamellidens marginalis*, *Meretrix casta*, *M. meretrix*, and *Tridacna maxima*[20,21,22].

The mean IZ (Fig. 2) revealed that increasing concentrations of ECE on *S. aureus* strain ranged from 28.73–32.08 mm diameter. The pure ethanol discs did not show any activity while Tetracycline® discs recorded a 27.70 mm mean IZ diameter. Significantly high ($p < 0.05$) activity was observed with 1500 $mg.mL^{-1}$ concentration. It was noticed that *S. aureus* started to proliferate on the IZ after seven hours of observation. After 24 h, the IZs were still distinct but were already masked with *S. aureus*.

Moderate antimicrobial activity of *P. expansa* extract was observed against *S. aureus*. Although IZ was similar between *E. coli* and *S. aureus* test cultures, the efficacy time of the ECE against the latter was shortened. The bacterium *S. aureus* is a pathogen commonly occurring in the nasal tract of humans and other animals[23]. Yoshpe-Purer and Golderman[24] reported low occurrence (4.3%–8.1% of water samples) of *S. aureus* in coastal and estuarine waters and believed that it was just introduced through anthropogenic activities. In addition, Fujioka and Unutoa[25] observed that *S. aureus* will not proliferate in estuarine and seawater under normal environmental conditions. It appeared that *S. aureus* is not a common bacterium found in estuarine environment. Bivalves in this ecosystem may not have fully developed immunity for such bacterium. Similar to *P. expansa*, extracts from other estuarine bivalves such as *Villorita cyprinoides*, *Perna viridis*, and *Crassostrea madrasensis* showed low to moderate antibacterial activity against *S. aureus*[26,27].

The antifungal activity of the ECE of *P. expansa* against *C. albicans* test cultures recorded mean IZ ranges of 0.00 to 12.75 mm diameter (Fig. 3). Similar with bacteria, no activity was observed with the ethanol discs. However, highest mean IZ (23.45 mm) was observed in the Ketoconazole® discs. The mean IZ of the Ketoconazole® discs was significantly higher among all treatments. It's noteworthy to mention that among the ECE concentrations, the 1500 $mg.L^{-1}$ concentration recorded significantly high mean IZ. The efficacy of the ECE against the yeast lasted for eight hours beyond which proliferation of *C. albicans* was observed in the IZ.

The ECE of *P. expansa* showed low antimicrobial activity against the yeast, *C. albicans*. This yeast was reported to survive in tropical marine and fresh waters[28]. However, this dimorphic fungus exists as a commensal of warm-blooded animals including humans[29]. Bivalves are cold-blooded aquatic invertebrates. The host specificity of *C. albicans* may explain the low activity of the ECE of *P. expansa* against this microbe. Similar IZ results for *C. albicans* were reported in bivalve extracts of *P. viridis*, *M. casta* and *M. meretrix*[22,30].

Like other estuarine bivalves, *P. expansa* is exposed in an environment in which high concentrations of various pathogenic microorganisms exist. For this reason, the immune system of this filter-feeding mud clam should be based on nonspecific, rapid cellular and humoral responses. Various reports have demonstrated the important roles of defensins and cysteine-rich peptides in the immune defense system of different bivalves, including members of genus *Polymesoda*[26,31]. These peptides were reported to have microbicidal activity against bacteria and fungi[32].

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## CONCLUSION

The use of commercial antibiotics is effective in eliminating pathogenic microbes involved in several infections. However, the growing resistance to antibiotics by microbes poses a threat to humanity. The ECE of *P. expansa* showed antimicrobial activity, hence, a potential source of antimicrobial products. It is worth mentioning that products obtained from natural sources are good for the health and devoid of side effects. However, the use of the ECE of *P. expansa* as a drug source still needs further investigation. Other pathogenic microorganisms should be used for further analysis of its antimicrobial properties. Future researches should also focus on the extraction, identification and purification of the bioactive compounds on the ECE of *P. expansa*.

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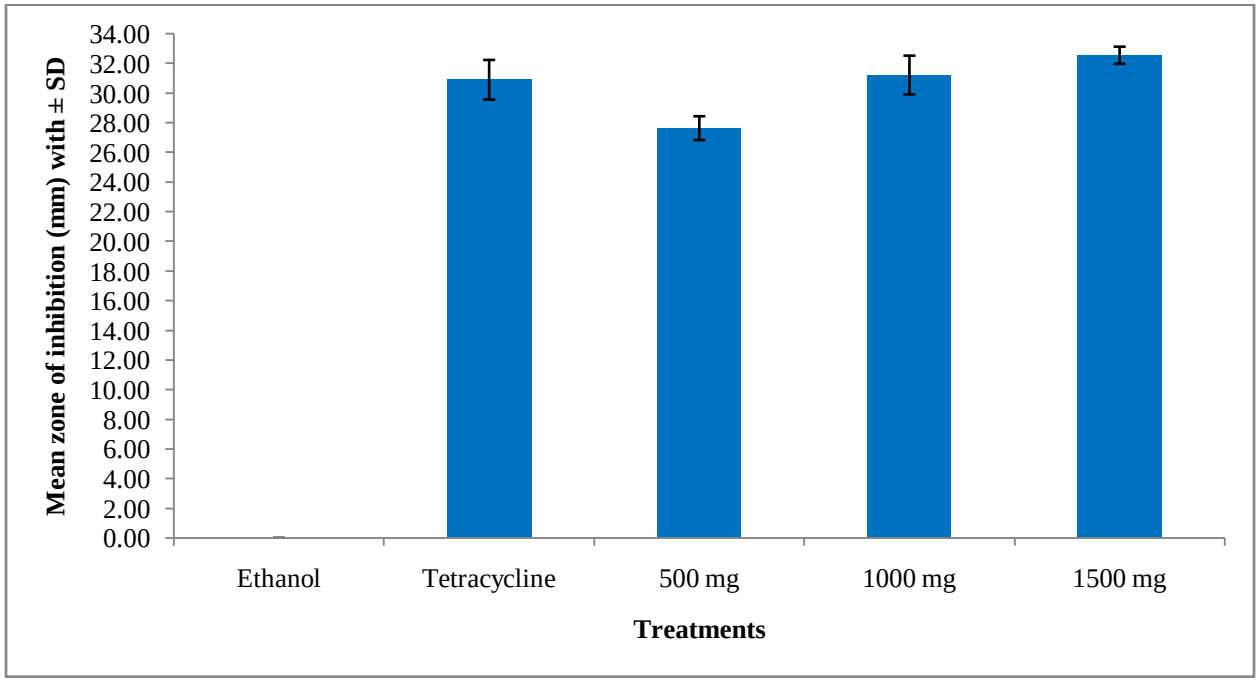


**Figure 1. Antibacterial activity of the ECE of *P. expansa* on *E. coli* test cultures.**

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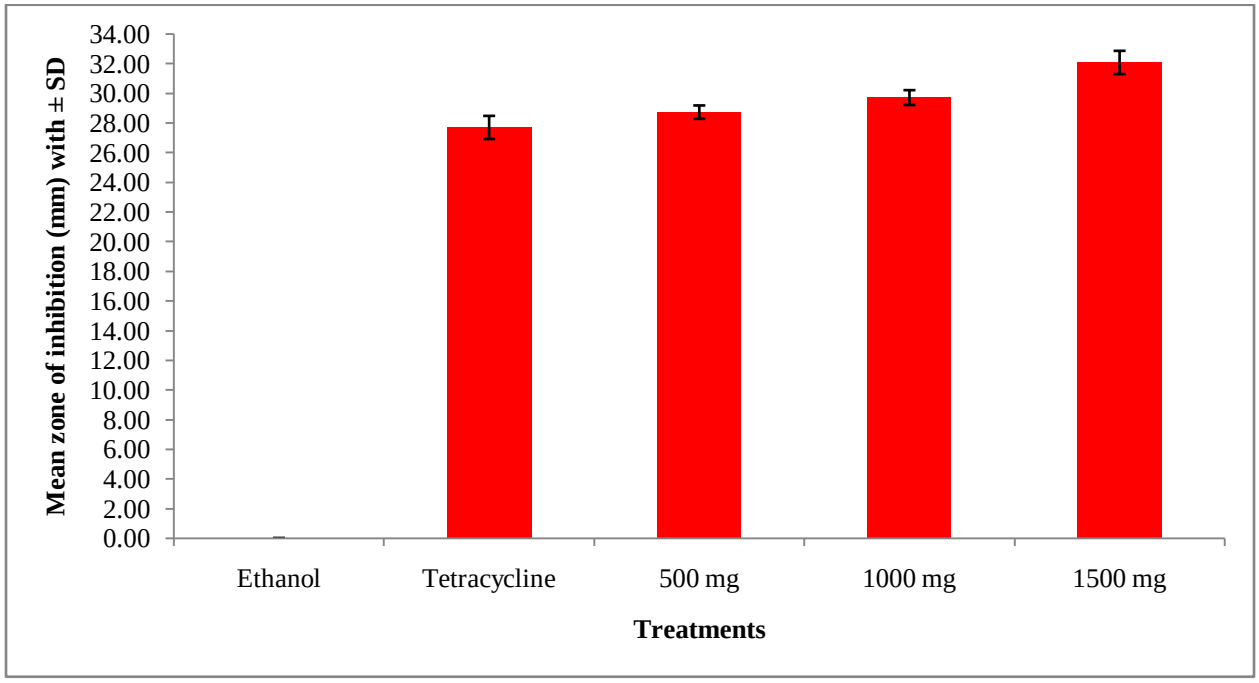


**Figure 2. Antibacterial activity of the ECE of *P. expansa* on *S. aureus* test cultures.**

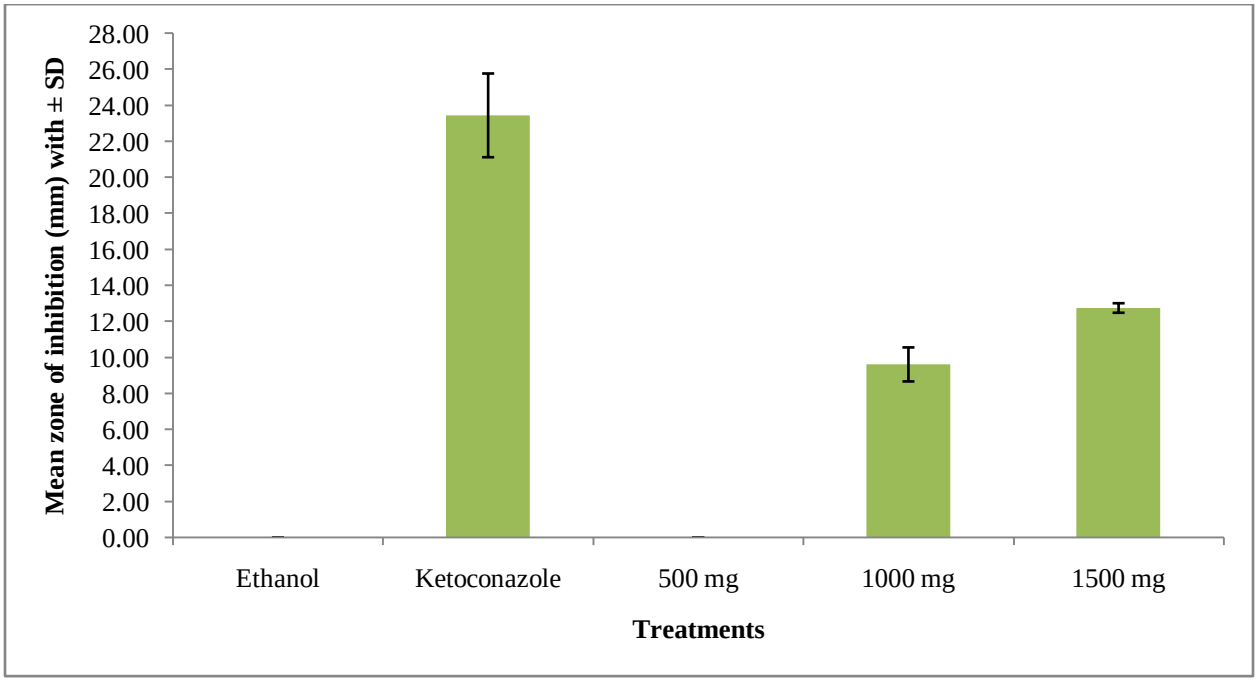


**Figure 3. Antifungal activity of the ECE of *P. expansa* on *C. albicans* test cultures.**

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