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Optimization of Different Extraction Methods in Determining the Bioactive Potential of Crown-of-Thorn Starfish (COTS)

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Abstract. The COTS is a giant corallivorous predatory starfish that consumes large areas of coral, causing damage to the marine ecosystem, and has potential applications for medicinal and pharmaceutical purposes. This study sought to investigate the effect of different extraction methods in determining the bioactive potential of *A. planci*. Ethanol solvents were used to obtain the crude extracts of AD, FD, and GD samples of *A. planci*. The phytochemical screening revealed that the FD extract of *A. planci* had the presence of flavonoids, phenolics and tannins, glycosides, coumarin, terpenoids, reducing sugars, amino acids, and proteins. While AD and GD extracts were positive in the presence of flavonoids, phenolics and tannins, saponins, glycosides, coumarin, terpenoids, reducing sugars, amino acids, and proteins. All extracts showed no antibacterial potential against *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *S. aureus* ATCC 1582. But, zoochemical analysis shows a promising innovative source of unique bioactive compounds for developing marine-derived drugs and other purposes.

Keywords: air-dried; bioactive compounds; COTS; freeze-dried; gonads

1. Introduction

As an archipelagic nation with a vast coastline, the Philippines is in the heart of the Coral Triangle, a region recognized as the worldwide center of marine biodiversity (Carpenter & Springer, 2005). It is characterized by more than 470 species of reef-building corals and over 2,200 species of reef-associated fishes. This high level of coral reefs provides a habitat for approximately one-third of all marine fish species, contributing to coastal protection, carbon sequestration, and tourism (Hughes et al., 2017). Hence, conserving and preserving coral reefs are crucial to maintaining marine ecosystems' health and diversity (White et al.,2011).

Despite the effort to maintain a healthy marine ecosystem, overpopulated coral predators, namely the crown-of-thorn starfish (COTS), significantly

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increased the mortality of corals and fish populations (Kayal et al., 2012). The issue concerning COTS can consume large areas of coral, leading to massive declines in coral cover (De'ath et al., 2012) that are detrimental to marine ecosystems. Several studies demonstrated the pharmaceutical application of COTS to reduce its detrimental effect on the marine ecosystem and use it as a beneficial source of natural bioactive compounds. This includes the anti-inflammatory effects (Hafez et al., 2021), antioxidative activities (Lee et al., 2014), anti-enzymatic activity of crude venom extract against casein, gelatin, and starch (Siro, 2022), and a potential candidate for inducing apoptosis in human cervical cancer lines (Islamiah et al., 2021).

To date, there are limited or no known reports about the antibacterial potential and phytochemical constituents of COTS, and no method-optimized protocol for crude extraction. Thus, the present study aimed to investigate the effect of different extraction methods in determining the bioactive potential of the whole COTS and its gonads.

2. Methodology

2.1. Collection and Identification of Samples

Before collection, a letter of permission was secured from the LGU and MENRO of Dingalan, Aurora. The live specimen of *A. planci* was randomly collected from the intertidal zone of White Beach, located in Barangay Paltic, Dingalan, Aurora, Philippines. Each sample was carefully collected with the assistance of the local fishermen using the non-destructive method by utilizing modified extended tongs for carrying the *A. planci* underwater and rinsed with water to remove the sedimentary particles or organisms, then weighed individually to acquire the wet weight using a mechanical scale. Subsequently, each sample was stored individually in a clean microwavable container and placed in an icebox for immediate transportation to the laboratory. On arrival at the laboratory, the *A. planci* diameter was measured from arm tip to arm tip individually using a tape measure and immediately processed. Samples were identified with the aid of previous literature describing the morphology of *Acanthaster* spp. (Humphreys, 1981; Clark & Rowe, 1971).

2.2. Isolation and Crude Extraction of Gonad

The subsample of gonads from *A. planci* was isolated by following only the dissection procedure described by Mendoza-Porras et al. (2022) with some modifications, as the sample was observed to be devoid of signs of life. And the extraction of gonads was conducted following the method described by

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Abubakar et al. (2012) with some modifications. The subsamples of gonads were homogenized for 2 minutes using a commercial blender in 70% ethanol with a ratio of 1g:2.5mL at room temperature. Rotary evaporation was performed at the Philippine Rice Research Institute (PhilRice) in Science City of Muñoz, Nueva Ecija. The extract was placed in clean amber bottles with aluminium foil covers and refrigerated until further analysis.

2.3. Optimization of Sample for Crude Extraction

a) Air-dried (AD): Each sample of *A. planci* collected was dissected into smaller pieces using clean blades and sorted with a similar amount of different body parts without gonads. The fragments intended for air-drying were initially hung in shaded areas outdoors using mosquito nets for five days to remove the dripping water from the sample. The fragments were then transferred to a set-up of well-ventilated boxes covered with mosquito nets. The setup was kept in shaded areas outdoors with ambient temperature for five days. The dried sample was pulverized using a commercial blender until fine powders were obtained. The powdered sample weight was measured, stored in a sterile container, and refrigerated until extraction.

b) Freeze-dried (FD): Each sample collected was dissected into smaller pieces using sterile blades and sorted with a similar amount of different body parts without gonads. The sample was initially stored in a freezer at a temperature of -40°C overnight. The freeze-drying process was conducted at PhilRice in Muñoz, Nueva Ecija. *A. planci.* One temperature was applied in the chamber (-110°C); the process was carried out for 17 days until the moisture content of the COTS was removed. The dehydrated sample was pulverized using a commercial blender until fine powders were obtained and stored in a clean plastic container covered with aluminium foil and a Ziploc bag. The powdered sample was refrigerated until further analysis.

2.4. Ethanol Crude Extraction

The freeze-dried and air-dried samples were subjected to the maceration procedure with some modifications and placed in 95% ethanol following the ratio of (10mL:1g) (Andrés et al., 2020) for 72 hours. The extraction setting was a lighted room at room temperature, under stirring for 60 minutes. The filtrate was collected by passing through filter paper and concentrated under reduced pressure at 40°C using a rotary evaporator in PhilRice in Muñoz, Nueva Ecija. The concentrated ethanolic extract was placed in separate sterile amber bottles with aluminium covers and kept refrigerated until further analysis.

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2.5. Phytochemical Analysis of Ethanolic Crude Extracts

The ethanolic crude extracts of air-dried, freeze-dried, and gonad samples of COTS were screened for phytochemical tests using standard procedures of Agunos et al. (2020), Deshmukh & Theng (2018), Singh & Kumar (2017), Ajayi et al. (2011), and Firdouse & Alam (2011). Triplicates were followed to all qualitative tests for detecting the presence of flavonoids (Alkaline Reagent, Ammonia Solution, and Shinoda tests), tannins and phenolic compounds (Ferric chloride 5% and Dilute Iodine Solution tests), carotenoids, saponins (Frothing test), glycosides (Glycoside,10% NaOH, and Keller-Killani tests), coumarin, anthocyanin (Sulfuric Acid test), terpenoids (Salkowski test), reducing sugars (Fehling and Benedict tests), proteins and amino acids (Biuret and Ninhydrin tests).

2.6. Antibacterial Assay of A. planci Crude Extracts

The antibacterial analysis of *A. planci* ethanolic crude extracts against *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 6538 was conducted by the DOST- National Capital Region, Taguig, using the disc diffusion method as described by USP (2007). Ethanol (95%) served as a negative control, Amikacin (30 µg) for *P. aeruginosa,* and Oxacillin for *S. aureus* were used as positive controls.

In contrast, the antibacterial activity of ethanolic crude extracts of air-dried, freeze-dried, and gonad samples was reevaluated against *Escherichia coli* ATCC 25922 and *S. aureus* ATCC 1582 at the Biology Laboratory, College of Arts and Sciences, Nueva Ecija University of Science and Technology, following the protocol of Padilla (2022). Ethanol (95%) served as a negative control, and Chloramphenicol (30 µg) was used as a positive control. Three plate replicates were prepared for each ethanolic crude extract of COTS against the test pathogens. The plates were then incubated inverted overnight at 35°C. If there are no zones surrounding the paper disc, aseptically lift the paper disc and observe the area under the sample.

3. Results and Discussion

3.1. Collected Crown-of-Thorns Starfish

A total of 11 samples of *A. planci* were collected, and the average measurement for all samples was 545.45 g for weight26.27 cm for length, and 14.38 g for the total gonad weight. The low weight of isolated gonad samples may be due to the gender

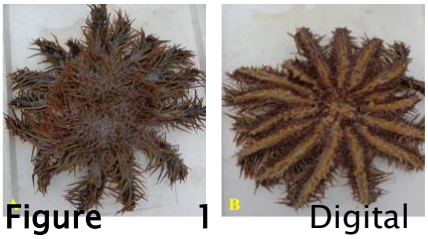

Figure 1 Digital photograph of *A. planci* showing the aboral (a) and oral (b) views

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of *A. planci* collected in this study and the size of individual starfish.

The *A. planci* identified belongs to subclass Acanthasteridae, due to its deep brown color above with red tips to spines and tube feet fawn; adults generally measure 25 cm in diameter (Humphreys, 1981), as shown in Figure 1. They typically have between 8 and 21 arms with long and conspicuous aboral spines that generally range from 15 – 30 mm in length (Clark & Rowe, 1971). The coloration varies with hues ranging from red and orange to purple, which is believed to be influenced by their diet (Ault et al., 2011). Several studies reported the presence of *A. planci* in different coastal areas in the Philippines, including Agusan del Norte (Walag et al., 2018), Davao del Norte (Fortaleza et al., 2021), Mindoro (Vine, 1973), Batangas (White et al., 2011), Pangasinan (Endean & Chesher, 1973), Leyte (Benliro et al., 1999), and Misamis Oriental (Llacuna et al., 2016).

3.2. Phytochemical Analysis of Acanthaster planci Crude Extracts

The phytochemical screening results were carried out on various ethanolic crude extracts of the *A. planci* sample. FD, AD, AND GD extract of *A. planci* was composed of flavonoids, phenolics and tannins, glycosides, coumarin, terpenoids, reducing sugars, amino acids, and proteins, except saponins were absent in FD. Flavonoid components in the extracts were consistent with the previous reports of Walag and Kharwar (2021), with a promising therapeutic potential as an anticancer agent against human malignant melanoma (Lee et al., 2014) and human cervical cancer (Islamiah et al., 2021). Phenolic compounds are known as natural sources of antioxidants that were found present in echinoderms (Hossain et al., 2022). In addition, Lee et al. (2014) reported that the ethanol fraction of *A. planci* contains better phenolics compared to the petroleum ether fraction, ethyl acetate fraction, and butanol fractions. The presence of saponin in AD and GD extracts was similar to a previous report by Walag et al. (2019), and *A. planci* consists of potent saponins Lee et al., (2024). It can be utilized as an environmentally friendly insecticide to control Kalotermitidae pest (Wijanarko et al., 2017).

Glycosides were found present in echinoderms, which contribute to their toxicity (Kim & Himaya, 2012). Also, specific types of glycosides were found isolated in different extracts of *A. planci* (Komori et al., 1983). The presence of coumarin in the extracts conforms to the results of Walag et al. (2019), wherein their results show that a modified oven-dried sample of *A. planci* has no presence of coumarins. Additionally, the result was consistent with the findings of Rahim

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(2012), who found that the ethyl acetate and n-hexane fractions of *Acanthaster sp*. contained terpenoids. A dried sample of *A. planci* from a modified oven drying method shows a moderate presence of terpenoids (Walag et al., 2019). The study detecting the presence of reducing sugars in *A. planci* using the Benedict Test was limited. Finally, studies show the presence of high essential amino acids in *A. planci* flour that can be utilized as fish feed ingredients (Safir et al., 2022). In addition, Luo (2011) described the amino acid composition of *A. planci* as similar to that of fish meal.

3.3. A. planci Crude Extracts against Antibiotic-Resistant Bacteria

The antibacterial activity of various ethanolic crude extracts of *A. planci* was presented in Figure 2. Compared to the positive control, the ethanolic crude extracts from *A. planci* have no antibacterial activity against all test pathogens. This indicates the possibility that the bioactive compounds extracted are low in concentration to exhibit antibacterial activity. According to Andriani et al. (2018), the methanolic crude extract of *A. planci* showed no antibacterial activity against both Gram-positive and Gram-negative bacterial strains, but inhibited the growth of *Micrococcus sp*. Several studies indicated the antibacterial potential of *Acanthaster sp.* by isolating specific compounds from the sample, including the steroid derivatives (Hafez et al., 2021), spine venom (Siro, 2022), and phospholipase A2 from the spines (Ibrahim et al., 2013).

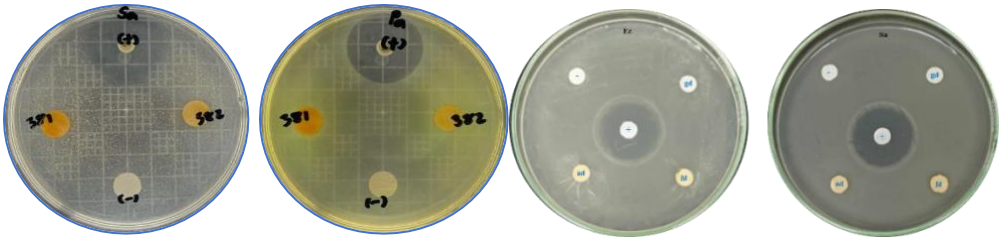

Figure 2 Antibacterial activity of freeze-dried (381), air-dried (382), and gonads ethanol crude extracts of *A. planci* against *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *S. aureus* ATCC 1582

4. Conclusions

The *A. planci* was collected using a non-destructive method. An air-dried, freeze-dried, and gonad crude extract of *A. planci* using an optimized procedure was able to extract bioactive compounds. Crude extracts were found to contain flavonoids, phenolics and tannins, glycosides, coumarin, terpenoids, reducing sugars, saponins, amino acids, and proteins. However, extracts showed no antibacterial activity against all pathogenic bacteria. Yet, zoochemical analysis

shows a promising innovative source of unique bioactive compounds for developing marine-derived drugs and other purposes.

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