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Investigation of antioxidant activity of *Zoanthus vietnamensis*'s crude extract and dichloromethane extract

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ABSTRACT

Zoanthid is a potential natural resource with many biological activities, such as antioxidant, antiviral, anti-inflammatory, and cytotoxic activity. Therefore, this study aimed to evaluate the antioxidant activity of 70% ethanol crude extract and dichloromethane fraction from Zoanthus vietnamensis. The total polyphenol content of ethanol and dichloromethane extracts were 19.36 ± 1.37 and 24.95 ± 0.63 mg GAE/g extract, respectively. The DPPH free radical scavenging capacity increased slightly from low to high concentration. The IC50 values reached 9.974 µg/mL and 6.424 µg/mL for ethanol crude and dichloromethane extract. The potassium ferricyanide-reducing power assay result was meager, and the optical density value ranges from 0.015 to 0.105.

Keywords: Antioxidant activity, ethanol crude extract, dichloromethane extract, *Zoanthus vietnamensis*.

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INTRODUCTION

Marine resources in coral reefs are abundant, especially in the phylum Cnidaria. This group has more than 10,000 species, such as Anthozoa (sea anemones, corals, and zoanthids), Scyphozoa (jellyfishes), Cubozoa (box jellyfishes), Hydrozoa and Staurozoa [1]. They are considered potential raw materials in medicine, chemistry, and cosmetics.

The biological activities of this group have been studied, for example, anti-inflammatory, anti-microbial, antioxidant, cytotoxic, and anti-cancer. According to Roy et al., (2012), the secondary metabolites from an Okinawan soft coral *Cespitularia* sp. were identified as five novel diterpenoids and one alcyonolide. The results showed that the alcyonolide was cytotoxic to HCT 116 cell line (IC_{50} value was about 5.85 µM), and diterpenoids were less active than the previous compound (IC_{50} value ranged from 28.2 to 91.4 µM) [2]. In addition, almost all active compounds from Vietnamese sponges, for example, *Aaptos suberitoides*, *Petrosia nigricans*, *Dysidea cinerea*, *Dysidea fragilis*, *Smenospongia cerebriformis*, and *Haliclona oculate* were alkaloids, terpenoids, and steroids with biological activities, such as anti-inflammatory, antibacterial, antitumor, and cytotoxic activity [3–5].

Besides, the antioxidant activity from marine organisms was also mentioned. The natural antioxidants were divided into five types: organosulfur, flavonoids, phenolics, carotenoids, and vitamins. They were low molecular weight compounds that prevent the generation of ROS (reactive oxygen species) or damage oxidation of biomolecules such as DNA, RNA, and proteins, leading to the promotion of aging, as well as dangerous diseases, for example, atherosclerosis, cancer, diabetes, cardiovascular and neurological diseases [6, 7]. For instance, MAAs (mycosporine-like amino acids) have been recognized as new antioxidant compounds. These compounds could be soluble in water and absorb UV-A and UV-B rays in the wavelength range from 268 to 362 nm. They are usually isolated from highly photosynthetic organisms, such as cyanobacteria, microalgae, fungi, seaweeds (green and red algae), corals, and lichens. The chemical structure of MAAs has changed the position of amino acids to reach some MAAs with different physical and chemical properties leading to adapting to harsh environmental conditions and becoming new antioxidant compounds [8]. According to Dunlap and Yamamoto (1995), the antioxidant activity of aqueous extracts from four marine species, including grouper *Plectropomus leopardus*, red seaweed *Porphyra tenera*, squid *Lissoclinum patella*, and coral *Palythoa tuberculosa*, was determined by inhibition of phosphatidylcholine peroxidation. The results showed that the most potent antioxidant activity was the extract from *Palythoa tuberculosa* that the mycosporine-glycine was in their structure [9]. Moreover, Xuan Cuong et al. evaluated antioxidant activity on some fractions, such as n-butanol, ethyl acetate, n-hexane, ethanol, and chloroform from *Aaptos suberitoides*. The chloroform fractions had the strongest antioxidant activity, three times higher than the crude ethanol extract (75.36%) [5].

The zoanthid was a typical group in coral reefs, in which the dominant genus like as, Zoanthus, Protopalythoa, and Palythoa because of the strong competitiveness, high adaptability, combination of asexual and sexual reproduction, and rapid growth. This group was evaluated as a rich source with new secondary metabolites and potential biological activities. Most of these compounds were fatty acids, ceramides, steroids, prostaglandins, and glycerol derivatives [10, 11]. According to the research by Almeida et al., the sulfonylated-ceramide compound isolated from *Palythoa caribaeorum* and *Protopalythoa variabilis* that possessed anti-proliferative activity against the colon adenocarcinoma cell line HCT-116 [12]. In addition, the ecdysones, isolated from *Palythoa mutuki*, inhibited dengue virus production [13]. The alkaloid compounds extracted from *Zoanthus kuroshio* inhibited superoxide anion generation and elastase release [14]. The tuberazines C isolated from the ethanol extract of *Palythoa tuberculosa* had antitumor and anti-lympholytic activity by inhibition of LEC production (IC_{50} value was about 33 ± 1 µg/mL) [15]. In addition, the polyhydroxylated steroid isolated from

Palythoa tuberculosa was a selective inhibitor of MCF-7 human breast cancer cells [16]. This compound was also a potent inhibitor of cell replication, so it took a role in regulating cell proliferation or developing a new anti-cancer drug. Moreover, Alencar et al. (2015) reported the antioxidant activity of *Palythoa caribaeorum*'s extracts, in which the main group causing antioxidant activity was polyphenols. The total phenolic contents of the crude extract of 70% ethanol and their fractions with dichloromethane, ethyl acetate, and distilled water got 12.33, 18.17, 10.53 and 3.18 mg GAE/gram, respectively. Their IC_{50} of DPPH free radical scavenging activity were also recorded as 11.13, 11.25, 11.74, and 11.28 µg/mL, respectively. The antibacterial activity was not found in these extracts. For cytotoxic activity, the LC_{50} values of the dichloromethane, crude extract, ethyl acetate and water were 52.10, 83.06, 86.34, and 117.45 µg/mL, respectively [7]. Therefore, the above results showed that the antioxidant activity of the crude extracts and dichloromethane fractions was higher than the other fractions. According to the latest publications of Chen et al., the biological activities of compounds from *Zoanthus vietnamensis* in Taiwan, for instance, the anti-lymphangiogenic activity in 2019 [17], the antimetastatic activity in 2020 [18] and the anti-angiogenic activity in 2021 [19] have been investigated.

In addition, the zoanthid possessed toxic nematodes called palytoxin. This toxin was first isolated from *Palythoa toxica* and studied by Moore and Scheuer [13] or *Palythoa caribaeorum* by Ramos and Vasconcelos [20]. According to Domínguez-Pérez et al., palytoxin was isolated from *Zoanthus sociatus* and was lethal to rats at 792 µg/kg [21].

Conversely, there was limited studies on Vietnamese zoanthids, and they focused only on their taxonomy, biodiversity, and distribution. Specifically, a taxa list of this group was described clearly by Pax and Muller, Reimer et al. in the Southeast Asian region [22, 23]. Some species have been recorded in Vietnam. For example, *Palythoa tuberculosa*, *P*. cf. *toxica*, *Zoanthus sansibaricus* and *Z. Robustus* in Hon Lon, Nam Du, Kien Giang; *Z. sansibaricus* in Tri Nguyen, Nha Trang; *P. tuberculosa* and *Isaurus tuberculatus* in Hon Tre, Nha Trang; *P. tuberculosa*, *Z. sansibaricus* and *Z. vietnamensis* in Nha Trang Bay. However, studies on bioactive compounds of the zoanthids in the country have yet to be published.

This study tested the antioxidant activity of the crude ethanol and dichloromethane fraction extract from *Zoanthus vietnamensis* (Pax & Müller, 1957).

MATERIALS AND METHODS

Materials

Zoanthids were collected in Nha Trang Bay, Khanh Hoa Province, by scuba diving. They were stored in cool and dark conditions using black plastic bags with seawater at room temperature (about 26^{o}C).

Samples were identified by Mr. Thai Minh Quang (the staff of the Institute of Oceanography). Through the previous description [22, 23], *Zoanthus vietnamensis* had grey-green tentacles and a white aperture rim. The coenenchyma forms a thick cushion layer, about 3 mm. The distance of polyps was small, about 0–2 mm. The proximal sphincter with 40 mesh muscles was more than twice as long as the distal sphincter. The tentacle ectoderm had few spirocysts, about 13–14 µm long.

Preparation of materials: The polyps will be separated from the attachment, washed, and dried to remove epiphytes. Samples were lyophilized with a Christ Beta 1-8 LSC basic machine. Next, they were ground and stored at -30^{o}C.

Extraction of materials: Samples were extracted with ethanol (70%) and dichloromethane: The powder mixture (about 20 g) was soaked in 70% ethanol at 1:20 (w/v) and gently shaken overnight. Then, a volume of the extract (200 mL) was filtered and evaporated with a Buchi Rotavopor R-300 to get the ethanol crude extract. After that, the remaining volume (200 mL) was fractionated with dichloromethane at 1:2 (v/v). This fraction was then evaporated to get the dichloromethane fraction.

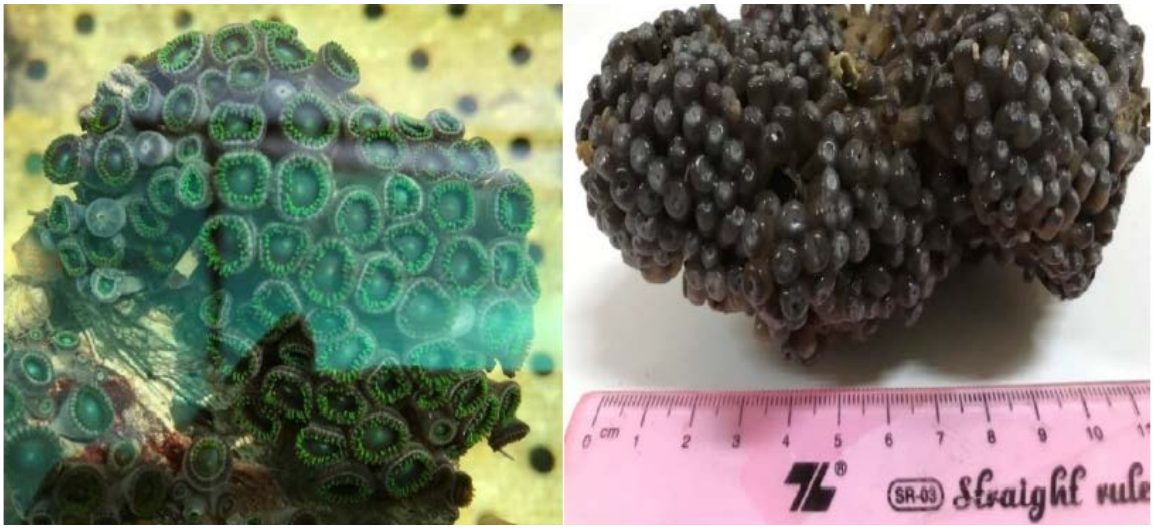

Figure 1. The morphology of *Zoanthus vietnamensis* (Pax and Müller (1957))
[*Source:* Phan Bao Vy et al., (2021)]

Determination of the antioxidant activity

DPPH free radical scavenging assay

The experiment was performed according to Duan et al. [24] with modifications:

1 mL of DPPH (0.16 mM) in methanol was mixed with 1 mL of extract. The mixture was incubated for 30 min in the dark, then measured at 517 nm using a Hitachi U-2900 spectrophotometer. The optical density value reflects the oxidation resistance of the sample. The blank did not contain crude extract, and the positive control was ascorbic acid. The percentage of DPPH free radical scavenging was determined according to the formula (1):

$$Scavenging\ effect\,(\%) = \left[1 - \frac{\left(Abs_{sample} - Abs_{black}\right)}{Abs_{black}}\right] \times 100\%$$

According to the graph of the percentage of DPPH radical scavenged and the equation of the percentage of free radical scavenging, the scavenging effect at 50% (IC_{50} value) was calculated.

Potassium ferricyanide reducing power assay

The experiment was performed according to Ganesan et al. and Alencar et al., [7, 25] with modifications:

The solution composed of 2.5 mL of 0.2 M phosphate buffer (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide solution was added to 1 mL of the crude extract. This mixture was incubated at 50^{o}C for 20 minutes. After cooling, a volume of 2.5 mL of 10% trichloroacetic acid was added. Next, this volume was mixed well and centrifuged at 3000 rpm for 10 minutes. Then, 2.5 mL of supernatant was added to a mixture of 2.5 mL of double distilled water and 0.5 mL of 0.1% ferric chloride solution. As to be continued, the final mixture was incubated for 10 min at room temperature and measured the absorbance at 700 nm using a Hitachi U-2900 spectrophotometer. Ascorbic acid was used as a positive control. The antioxidant effect of the extract was compared with that of the standard by optical densitometry at the same concentration value.

Total polyphenol of the dichloromethane fraction

Experiments were carried out according to Kumar et al., [26] with modifications:

The 100 µL of extract was added to a mixture of 900 µL of distilled water, 1 mL of 10% Folin-Ciocalteu reagent, and 2.5 mL of 20% sodium carbonate. The mixture was shaken well and incubated for 30 minutes in the dark at room temperature. Then, the optical density at 760 nm was measured using a Hitachi U-2900 spectrophotometer. The results are expressed in the extract's milligrams of gallic acid equivalent (mg GAE/g).

Statistical analyses

The experiments were repeated 3 times. The results were calculated on Microsoft Excel 2010 software and expressed as mean value ±*SD*. The mean values were compared using one-way ANOVA followed by Turkey's HSD at $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidant activity by DPPH

The DPPH free radical scavenging results are shown in Figure 2, showing that the free radical scavenging activity of the crude ethanol extract (EtOH) and the dichloromethane fraction (DCM) reach the same effect. The free radical scavenging activity increases slightly following both extracts' 12.5 µg/mL to 150 µg/mL concentration. At a small concentration of 12.5 µg/mL, the percentage of free radical scavenging of DCM and EtOH extracts was 54.37% and 53.96%, respectively. At the 150 µg/mL concentration, this value was 71.5% and 70.79%. Meanwhile, the DPPH free radical scavenging activity of the standard acid ascorbic, at 5 µg/mL, reached the highest value, 97.05%.

Similarly, in another study on sponge *Aaptos suberitoides*, the percentage of free radical scavenging DPPH for ethanol was about 62.13%. Meanwhile, for other solvents, such as chloroform, *n*-hexane, ethyl acetate, and *n*-butanol, this percentage was between 61% and 72% [5]. For the group of phenolic compounds of seaweed, the results showed that *Sargassum* sp.'s free radical scavenging activity was about 85.82 ± 1.89% for the aqueous extract at 50 mg/mL [27]. Besides, other results on the activity of *Padina* sp. of the aqueous extract got much lower, about 95.90 ± 1.88% at 50 mg/mL [28].

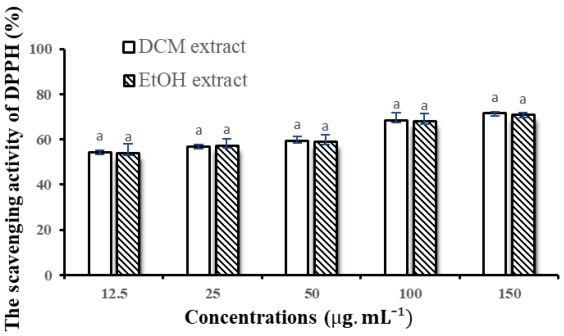

Figure 2. Scavenging activity (%) of DPPH of the EtOH crude extract and DMC fractional extract at different concentrations from the zoanthid Zoanthus vietnamensis. Same lowercase letters- no statistically significant difference ($p > 0.05$)

According to the graph of the percentage of DPPH radical scavenged and the equation of the percentage of free radical scavenging (Figures 3a, 3b, 4), the scavenging effect at 50% (IC_{50} value) of ascorbic acid, DCM fraction, and crude extract was calculated.

The IC_{50} values of ascorbic acid, DCM fraction, and crude extract were 0.096, 6.424, and 9.974 µg/mL, respectively. Therefore, the extract's antioxidant capacity of the EtOH was lower than that of the DCM fraction.

Another study showed that the IC_{50} values of the 70% EtOH crude extract and the DCM fraction of *Palythoa caribaeorum* were 11.13 and 11.25 µg/mL, respectively [7]. In addition, the IC_{50} values of n-butanol and *n*-hexane extracts of coral *Labophytum* sp. were 150 µg/mL and 70 µg/mL, respectively. These extracts showed no antioxidant activity for the other solvents, such as ethyl acetate or water [29].

Thus, the antioxidant activity of our study's extracts isolated from *Zoanthus vietnamensis* was high.

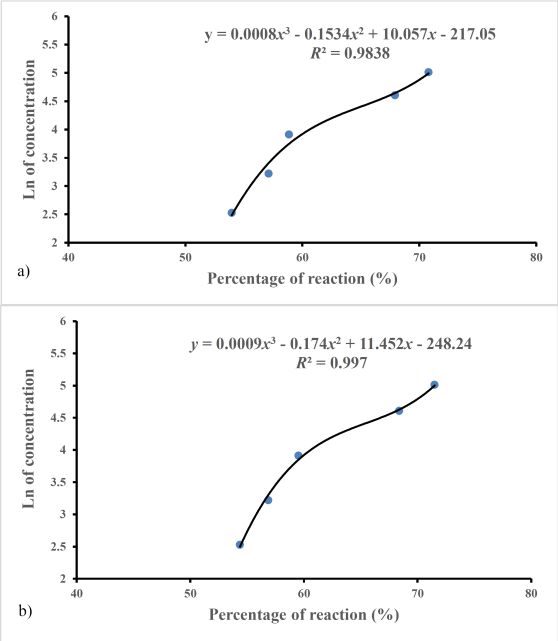

Figure 3. Percentage of reaction at different concentrations of DCM fractional and EtOH crude extracts

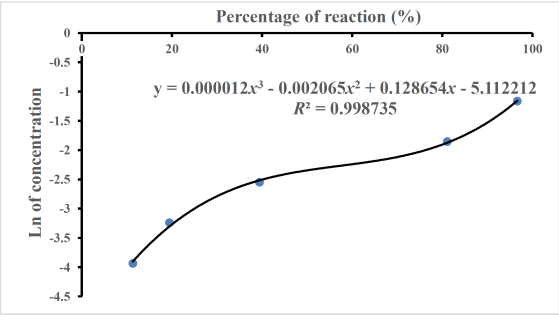

Figure 4. Percentage of reaction at different concentrations of the ascorbic acid

Antioxidant activity by potassium ferricyanide reducing power assay

The results show that the iron ion reduction capacity of the extracts is low (Figure 5). For the crude extract of ethanol, the optical density value ranged from 0.023 ± 0.008 at 12.5 µg/mL to 0.093 ± 0.001 at 150 µg/mL. Similarly, the optical density values at the lowest to the highest concentrations of the dichloromethane fraction ranged from 0.015 ± 0.002 to 0.105 ± 0.008.

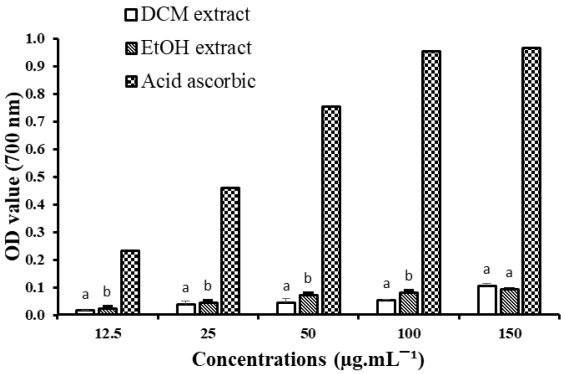

Figure 5. Reducing power of the 70% EtOH crude extract and DCM fractional extract at different concentrations of the zoanthid Zoanthus vietnamensis. In which, the same lowercase letters- no statistically significant difference ($p > 0.05$) or the different lowercase letters- statistically significant difference ($p < 0.05$)

Similarly, the study of Alencar et al., (2015) also showed that the ability to reduce iron ions of extracts isolated from *Palythoa caribaeorum* was very low [7]. The optical density value at 700 nm was only in the range of 0.051 to 0.061 at different concentrations from 12.5 µg/mL to 100 µg/mL. Moreover, the correlation coefficient between iron ion reduction capacity and total polyphenol content was investigated, and these results showed a low r value ($r = 0.454$, $p < 0.05$). According to Xuan Cuong et al., (2019), the antioxidant activity of different extracts, such as n-hexane, ethyl acetate, ethanol, and n-butanol, was evaluated by the iron reduction method with $FeSO_4$ as the positive control. The results showed that the iron deionization activity of the extracts isolated from *Aaptos suberitoides* ranged from 1.12 to 3.41 mg $FeSO_4$ equivalent/mL extract [5]. Therefore, the antioxidant activity of the crude ethanol extract and the dichloromethane fraction determined by the iron reduction was very low.

Total polyphenol content

The total polyphenol was calculated by equation (2): $y = 0.0515x - 0.0111$ ($R^2 = 0.9965$) at 1 mg/mL of the extract and a

standard concentration between 0.01 and 0.05 mg/mL. The results (Table 1) showed that the total polyphenol of the dichloromethane fraction was higher than the crude extract.

Table 1. Total phenolic content of the 70% EtOH crude extract and DCM fraction at 1 mg.mL^{-1} of the zoanthid *Zoanthus vietnamensis*

Extracts	TPC (mg GAE.g^{-1} extract)
Ethanol 70%	19.36 ± 1,37
Dichloromethane	24.95 ± 0,63

Another result on *Palythoa caribaeorum* showed that the total phenol of EtOH 70% extract and DCM fraction were 12.33 ± 0.53 and 18.17 ± 0.62 mg GAE/g extract, respectively [7]. Therefore, the total polyphenol of the extracts isolated from *Zoanthus vietnamensis* was higher than that of *Palythoa caribaeorum*. This value explains that the antioxidant activity of the extract from *Zoanthus vietnamensis* in our study was also higher than that of *Palythoa caribaeorum*. For *Aaptos suberitoides*, the phenol content of the ethanol extract was about 5 mg GAE/mL extract. The highest value was chloroform fraction, about 122.68 mg GAE/mL extract [5].

Therefore, the extract from *Zoanthus vietnamensis* has relatively high antioxidant activity, and this species can be considered a potential source of raw materials in medical and pharmaceutical research.

CONCLUSION

The crude extract of 70% ethanol and the dichloromethane fraction reach high antioxidant activity with IC_{50} values of 9.974 µg/mL and 6.424 µg/mL, respectively. The total polyphenol of the crude extract of 70% ethanol and the dichloromethane fraction got 19.36 ± 1.37 and 24.95 ± 0.63 mg GAE/g of the extract, respectively.

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