INVESTIGATION OF ANTIOXIDANT CAPACITY OF EXTRACTS FROM MARINE SNAILS AND BIVALVES IN THE OFFSHORE ISLANDS IN SOUTH VIET NAM

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Abstract. The DPPH free radical scavenging activities of methanol and ethyl acetate extracts and fractions of these extracts from mollusk collected in the offshore islands in South Viet Nam were investigated. The extracts and theirs fractions were then characterized using TLC, FTIR and SDS-PAGE. The results showed that the methanol extracts from 3 snail species *Chicoreus torrefactus, Lambis lambis* and *Turbo bruneus* and 3 bivalve species *Ostrea* sp., *Pinctada margaritifera* and *Tridacna crocea* exhibited high antioxidant capacity. The ethyl acetate extracts from the snails *Angaria delphinus*, *C. torrefactus* and from the bivalves *Ostrea* sp., *Spondylus* sp., and *T. squamosa* also demonstrated this capacity. Several fractions from the active samples exhibited antioxidant activity. The highest antioxidant values were observed in the fraction F2 from *C. torrefactus,* F3 from *L. lambis* and *T. bruneus*, F1 from *Ostrea* sp., *P. margaritifera* and *T. crocea* of the methanol extracts. In case of the ethyl acetate extracts, the fraction F3 of *A. delphinus*, F2 of *C. torrefactus, Ostrea* Sp. and F1 of *Spondylus* sp. and *T. squamosa* samples showed the highest antioxidant values. The TLC and FTIR results of antioxidant samples revealed the presence of several groups of substances, in which the methanol extracts contained mainly proteins and polar compounds and the ethyl acetate extracts possesed mainly non- and less polar compounds. The molecular weights of proteins in the fractions of methanol extracts with high antioxidant capacity ranged from 19 kDa to 70 kDa.

Keywords: Mollusk, DPPH activity, TLC, FTIR, SDS-PAGE.

1. Introduction

Marine organisms are considered as a potential source for naturally bioactive compounds. In which, mollusk- the largest group possesses antioxidant, anti-inflammatory, cytotoxic, antibacterial, antifungal, and anticancer properties. Mediterranean mussel *Mytilus gallofwozvincialis,* European flat oyster *Ostrea edulk* and Pacific oyster *Crassostrea gigas* possessed several substances exhibiting activity against bacteria (Hubert et al., 1996). Indeed, myticin and aplysia compounds isolated from *M. galloprovincialis* (Mitta et al., 1999), substances from the snails *Thais tissoti, Babylonia spirata* (Kumaran et al., 2011), giant tun *Tonna galea* (Santhi et al., 2011), and *B. zeylanica* (Santhi et al., 2013) were effective against some pathogenic bacteria. Antimicrobial peptides have also been isolated from the bivalves *M. galloprovincialis, M. edulis, M. trosolus, C. virginica, Ruditapes philippinarum* and *Biomphalaria glabrata,* and abalones *Haliotis discus hannai, H. discus discus, H. laevigata* (Li et al., 2011). Black clam *Villorita cyprinoides,* backwater clam

Meretrix casta, and green mussel *Perna viridis* contained potent anti-HIV-1 compounds. *P. viridis* extracts also expressed the ability to prevent the replication of viruses causing influenza, skin and mucosal, and respiratory diseases (Chatterji et al., 2002), pathogenic bacteria and fungi such as strong suppression of bacteria *Staphylococcus aureus* and fungus *Aspergillus flavus*, weak suppression of bacteria *Salmonella paratyphi* and fungus *Mucor sp.* (Chandran et al., 2009), very strong suppression of bacteria *Pseudomonas aeruginosa* and low suppression of *Escherichia coli* (Kiran et al., 2014). Indian volute *Melo melo* contained compounds that were strong against bacteria *Klebsiella pneumoniae* and fungus *Trichophyton mentagarophytes*, and weak against *S. typhi* and *A. flavus* (Kanagasabapathy et al., 2011). Two clam species, *M. meretrix* and *M. casta* possessed also antibacterial and antifungal compounds (Sugesh and Mayavu, 2013). Most of gastropod species including abalones (*H. laevigata, H. rubra*, and *H. rufescens*), snails (*Littorina littorea, Buccinulum corneum, Tegula gallina, Rapana venosa* and *Buccinum undatum*) and bivalve species including clams (*Mercenaria mercenaria, Mya arenaria, R. philippinarum*), oysters (*Cerastoderma edule, C. virginica, C. gigas* and *O. edulis*), mussels (*M. galloprovincialis* and *Crenomytilus grayanus*), contained compounds showing activity against microbial and virus pathogens (Dang et al., 2015). Extracts from the snail tissue *Hemifusus pugilinus* had anti-inflammatory and antibacterial activities (Arumugasamy and Cyril, 2017).

Mollusk was also a source of anticancer and antioxidant compounds. Dolastatin 10 and Dolastatin 15 from the sea hare *Dollabella auricularia* were reported to have anti-tumor activity against breast and liver cancer cells (Luesch et al., 2002). Kahalalide F from the sacoglossan mollusk *Elysia rufescens* has been evaluated for its potential against colon, breast and lung cancers and been developed into a therapeutic drug by Pharmamar (Becerro et al., 2001). Keenamide A from the sea slug *Pleurobranchus forskalii* showed the ability to inactivate cancer cell lines P-388, A-549, MEL-20 and HT-29 (Wesson and Hamann, 1996). In recent years, some substances resistant to tested bacterial strains and a compound having inhibition on both MCF7 and HepG2 cell lines have been determined (Phan and Chau, 2016) and a number of new compounds have been discovered in marine mollusks. Two new substances, Monodontins A, B and the 12 known compounds were isolated from the sea snail *Monodonta labio*, some of them possessed the potential anti-toxicity against hepatocellular and human cervical carcinoma (Phan et al., 2017). Pham et al. (2018) isolated the compound Dendrodoristerol, a C20 steroid from the sea slug *Dendrodoris fumata*, which demonstrated the anti-toxicity against 6 human cancer cell lines. Also in 2018, Nguyen et al. discovered a new substance [7.7] paracyclophane from the sea snail *Planaxis sulcatus* exhibiting anti-toxicity on some human cancer cell lines. Recent results showed that the methanol and chloroform extracts from some snails and bivalves in Viet Nam were resistant to some bacterial strains (Pham et al., 2019). In addition, Pachaiyappan et al (2014) determined the methanol extracts from bivalves

exhibiting stronger antioxidant activity than those from gastropod and echinoderm. Other studies reported that oysters *C. virginica* (Roesijadi et al., 1989), *P. viridis* (Madhu et al., 2014), clam *Atactodea striata* (Hasan et al., 2015), snail *L. littorea*, clam *Galatea paradoxa* (Borquaye et al., 2015), and snail *Hemifusus pugilinus* (Arumugasamy and Cyril, 2017) contained effective antioxidant properties. In cephalopod, polysaccharides from cuttlebone of cuttlefish *Sepia aculeata* (Subhapradha et al., 2014), the methanol extracts from the tissues of *S. pharaonis*, *S. intermis* and *Octopus vulgaris* (Ponnusamy et al., 2016), the extracts & Naqash, 2013), the methanol extracts from *S. esculenta* and *Uroteuthis chinensis* (Nguyen et al., 2020) exhibited strong antioxidant activity. The ink of cuttlefish *S. officinalis* exhibited antioxidant, anti-inflammatory and cytotoxic activities (Fahmy & Soliman, 2012). Melanin in the squid *L. formosana* also had antioxidant activity through DPPH free radical scavenging ability (Vate and Benjakul, 2013). Sudhakar and Nazeer (2015) found that the squid peptides could be used as natural antioxidants in preventing oxidative reactions in food processing. Aubourg et al. (2016) suggested the ethanol extracts from the skin of the jumbo squid *Dosidicus gigas* as a promising source of antioxidants to slow down the lipid oxidation in fish.

Viet Nam has a long coastline and many islands with high biodiversity of mollusks. In this study, we investigated the antioxidant capacity of crude extracts from snails and bivalves collected in the offshore islands in South Vietnam to screen the species containing bioactive compounds.

2. Materials and methods

2.1. Sampling

Marine snails and bivalves were collected in the offshore islands (Phu Qui, Con Dao, Phu Quoc) in South Viet Nam. Animals were sampled by SCUBA divers during the joint investigation between Viet Nam and Rusia using the Akademik Oparin vessel in May-June 2018. After collecting, specimens were washed outside with marine and fresh water, and some were photographed for identification. Other specimens were deshelled to collect soft tissues, stored in nitrogen liquid, and then brought to laboratory at the Institute of Oceanography, Viet Nam for further analysis.

2.2. Extraction

Extraction was performed according to routine method for natural products. About 50-100g of homogenized soft tissue was extracted with methanol or ethyl acetate in a ratio of 1: 4 (g: ml), stored at 4 ^{o}C for 24 hours. The extract was then filtered through filter paper (Whatman No1) and the tissue was re-extracted for a second time with the same solvent at the above ratio. The combined solvents from filtrates were completely evaporated by vacuum rotary evaporator (Robota, Germany). The dried crude extracts were weighed and used for determination of antioxidant activity as well as physicochemical characterization.

2.3. Radical scavenging activity

Free radical diphenyl-picrylhydrazine (DPPH) for estimating the antioxidant activity of the extract was performed according to Nazeer and Naqash (2013). The extract was dissolved in methanol or ethyl acetate at a concentration of 12.5-100 µg/mL. Then 4 ml of DPPH 0.004% in methanol or ethyl acetate was added to 1ml of the above- sample solution. The mixture was shaken by hand for 10 seconds and incubated in the dark at room temperature for 30 min. The blank sample was methanol or ethyl acetate without extract. The absorbance of DPPH was measured using a spectrophotometer at 517 nm. The experiment for each sample was repeated 3 times. The scavenging effect was calculated according to the following formula:

% Inhibition = {(Blank absorbance – Sample absorbance)/Blank absorbance} x 100

Some extracts exhibiting high antioxidant activity were characterized using thin-layer chromatography (TLC), Fourier infrared spectroscopy (FTIR) and SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

2.4. Characterization of active extracts

2.4.1. TLC

Components of the extracts were separated on TLC silica gel 60 F254. The n-butanol: acetic acid: water system (5:1:4)(Merck) was used for isolation of several compounds in the methanol extracts. In case of the ethyl acetate extracts, the hexane: ethyl acetate (5:1) system was used (Merck).

The TLC sheets were then coloured with saturated iodine or 70 % H_2SO_4 or 1.5 % ninhydrin in ethanol (Merck), and allowed to air dry, and then dried at 45 ^oC for 5-10 minutes. Retardation factor of the compound (R_f) was equal to A/B, where A is the migration distance of the compound, B is the migration distance of the solvent.

The separated fractions were recovered based on the position on the chromatographic sheet through R_f and also examined under a UV lamp. The silica gel layer containing fractions were eluted with methanol/ethyl acetate solvent. The resulting solution was filtered through filter paper (Whatman No1) and evaporated under a vacuum to collect groups of substances that were used to evaluate antioxidant activity and determine some physicochemical characteristics.

2.4.2. FTIR

The functional group component of some extracts with antioxidant capacity was analyzed using an infrared spectrometer TENSOR 27- BRUKER Equinoxx 55- Germany with a resolution of 16cm^{-1} in 32 scans using the potassium bromide (KBr) substrate. 10 µg of the sample was mixed with 100 µg of dry KBr and compressed in a salt dish (10 mm diameter) for spectroscopy.

2.4.3. SDS-PAGE

Protein component of some fractions of methanol extracts were analysed by SDS-PAGE according to Laemmli (1970) and described by Pham et al (2019).

3. Results

3.1. Contents of crude extracts

The content of methanol extracts ranged from 2.22 to 5.37 % in snails and 1.57 to 6.18 % in bivalves, and that of ethyl acetate extracts were from 0.29 - 0.78% in snails and 0.19 - 4.16 % in bivalves (wet weight) (Data not shown).

3.2. Antioxidant activity of crude extracts

The antioxidant activity of methanol and ethyl acetate extracts from snails and bivalves is presented in Tables 1 and 2, respectively.

Table 1. DPPH radical scavenging activity of methanol extracts from snails and bivalves

Mollusk species	**DPPH (%)**			
	12.5µg/ml	25µg/ml	50µg/ml	100µg/ml
Ascorbic acid	77.07±3.2	82.36±4.1	84.89±3.8	88.70±5.3
Marine snail				
Angaria delphinus	8.58±0.9	9.05±0.8	12.83±1.1	19.26±1.5
Astralium rhodostoma	9.56±0.9	9.24±0.9	15.55±1.2	17.92±1.6
Chicoreus torrefactus	14.54±1.3	14.58±1.2	27.96±2.1	30.75±2.2
Lambis lambis	9.86±0.7	20.65±1.4	30.13±2.4	33.01±2.0
Mancinella siro	7.77±0.5	8.52±0.7	12.10±0.9	15.86±1.1
Mauritia arabica	6.28±0.4	7.96±0.5	8.30±0.7	11.87±0.9
Tectus pyramis	12.13±0.9	11.15±0.8	14.45±1.2	14.81±1.3
Turbo bruneus	19.02±1.6	26.94±2.2	27.54±2.5	37.44±2.6
Trochus histrio	9.26±0.9	8.93±0.7	10.68±1.1	11.06±1.2
Bivalve				
Atrina vexillum	7.48±0.4	8.24±0.8	8.04±0.7	15.68±1.3
Barbatia foliata	9.76±0.7	9.38±0.9	9.60±0.8	9.86±0.7
Beguina semiorbiculata	11.14±0.9	10.61±0.8	11.22±1.2	9.60±0.8
Chama sp.	8.53±0.7	9.89±0.8	12.05±1.3	12.43±1.1
Isognomon isognomum	7.52±0.5	8.29±0.5	8.49±0.4	8.07±0.6
Malleus malleus	7.74±0.4	8.37±0.5	8.52±0.4	6.74±0.3
Ostrea sp.	29.02±1.2	31.61±1.3	33.44	39.45±3.1
Pinctada margaritifera	19.29±1.2	21.27±1.9	28.47±2.2	38.16±3.3
Pinna bicolor	2.08±0.2	2.13±0.2	3.19±0.3	3.97±0.5
Pteria penguin	9.34±0.8	8.68±0.5	9.28±0.9	6.74±0.4

Spondylus sp.	12.16±1.2	12.64±1.3	12.76±1.1	12.18±1.2
Tridacna crocea	26.16±2.2	25.86±2.1	26.73±2.5	35.69±3.1
Tridacna squamosa	8.35±0.4	9.14±0.6	6.99±0.4	7.06±0.5
Mytilus spp.	9.65±0.6	10.37±0.9	8.80±0.7	9.01±0.5

The methanol extracts from 3 snail species *C. torrefactus*, *L. lambis* and *T. bruneus* exhibited antioxidant activities of 30.75, 33.01 and 37.44 (%), respectively, at the concentration of 100 µg/ml. In addition, those from 3 bivalve species *Ostrea* sp., *P. margaritifera, T. crocea* also showed high antioxidant capacity (39.45, 38.16 and 35.69 %, respectively at the concentration of 100 µg/ml).

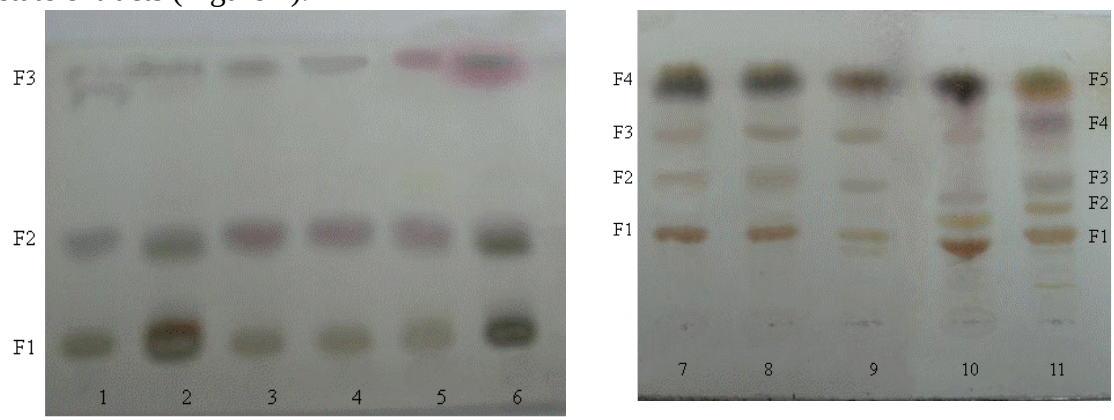
Table 2. DPPH radical scavenging activity of ethyl acetate extracts from snails and bivalves

Species	**DPPH (%)**			
	12.5µg/ml	25µg/ml	50µg/ml	100µg/ml
Marine snail				
Angaria delphinus	18.14±1.2	21.11±1.3	22.27±1.2	35.35±2.1
Astralium rhodostoma	10.74±0.9	5.99±0.3	16.13±1.1	31.80±2.0
Chicoreus torrefactus	19.79±1.1	22.79±1.6	24.90±1.8	30.73±2.3
Lambis lambis	10.04±0.9	11.80±0.9	22.88±1.4	12.23±0.8
Mancinella siro	10.47±0.6	10.92±0.7	22.12±1.8	18.92±1.6
Mauritia arabica	10.41±0.9	9.22±0.7	31.07±2.2	14.84±1.0
Tectus pyramis	6.70±0.4	8.02±0.6	19.31±1.4	21.80±1.8
Turbo bruneus	13.39±1.2	12.45±1.1	20.78±1.9	16.44±1.3
Trochus histrio	8.54±0.7	18.98±1.5	19.04±1.6	12.14±0.9
Bivalve				
Atrina vexillum	8.35±0.7	9.91±0.6	21.83±1.8	19.38±1.5
Barbatia foliata	6.69±0.4	17.61±1.2	17.50±1.3	20.00±1.4
Beguina semiorbiculata	10.04±0.9	12.52±0.9	17.45±1.4	17.65±1.2
Chama sp.	18.25±1.1	15.83±1.0	16.78±1.1	17.73±1.2
Isognomon isognomum	19.47±1.4	20.47±1.6	15.40±1.2	15.07±1.2
Malleus malleus	7.03±0.5	13.75±0.7	20.66±1.6	25.38±1.9
Ostrea sp.	17.98±1.2	18.41±1.1	19.89±1.4	23.77±1.7
Pinctada margaritifera	7.58±0.8	8.85±0.7	14.69±1.1	14.04±0.9
Pinna bicolor	12.38±0.9	11.47±1.2	16.38±1.2	12.90±0.9
Pteria penguin	12.69±1.1	10.36±0.9	16.49±1.1	17.75±1.4
Spondylus sp.	9.16±0.8	19.14±1.3	26.33±2.2	31.13±2.5
Tridacna crocea	10.75±0.8	14.11±0.9	18.53±1.4	15.24±1.2
Tridacna squamosa	12.20±1.0	11.85±0.8	23.97±1.7	32.90±3.1
Mytilus spp	11.70±0.9	10.29±1.1	18.12±1.8	12.83±1.2

The antioxidant activity of ethyl acetate extracts from two snail species *A. delphinus, C. torrefactus* was 35.35 and 30.73 %, respectively and from 3 bivalve species *Ostrea* sp., *Spondulus* sp., and *T. squamosa* were 23.77, 31.13 and 32.90 (%), respectively at the concentration of 100 µg/ml.

3.3. Component of extract by TLC

The TLC results showed that 3 fractions (F1-F3) with Rf 0.2-0.87 were separated from the methanol extracts and 4-5 fractions (F1-F5), Rf 0.42-0.89 were separated from the ethyl acetate extracts (Figure 1).



1. Ostrea sp., 2. Pinctada margaritifera, 3. Tridacna croce, 4. Chicoreus torrefactus, 5. Lambis lambis, 6. Turbo bruneus (A)

7. Ostrea sp., 8. Spondylus sp., 9. Tridacna squamosa, 10. Angaria delphinus, 11.Chicoreus torrefactus (B)

Figure 1. Component of methanol (A) and ethyl acetate (B) extracts of some snail and bivalve species by TLC

3.4. Antioxidant activity of the fractions

The antioxidant activities of the fractions separated from methanol and ethyl acetate extracts of some snail and bivalve samples are presented in Figures 2, 3.

For methanol extracts from snail, the F2 fraction from *C. torrefactus* and F3 fraction from *L. lambis* and *T. bruneus* displaced high antioxidant values of 34.52 %, 34.54 % and 40.16 % respectively. For bivalve, the F1 fraction of methanol extracts from *O.* sp., *P. margaritifera* and *T. crocea* exhibited relatively high DPPH scavenging activity at the values of 44.41, 43.10 and 38.87 %, respectively.

Among the fractions from ethyl acetate extracts, the highest antioxidant values were observed in the F3 of *A. delphinus* (36.26 %), F2 of *C. torrefactus* (31.91 %), and *O.* sp. (25.98 %) and F1 of *S.* sp. (37.29 %) and *T. squamosa* (32.99 %).

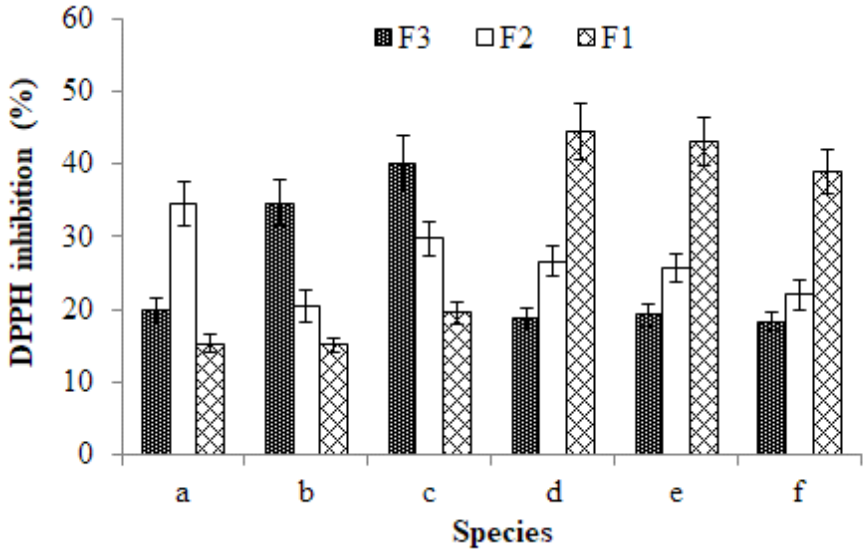


Figure 2. DPPH antioxidant activity of fractions (F1-F3) from the methanol extracts of some snails and bivalves at the concentration of 50 µg/ml. a: Murex snail *C. torrefactus, b:* Spider conch *L. lambis, c:* Dwarf turban *T. bruneus, d:* Oyster *Ostrea* sp., *e:* Pearl oysters *P. margaritifera, f:* Clam *T. crocea*

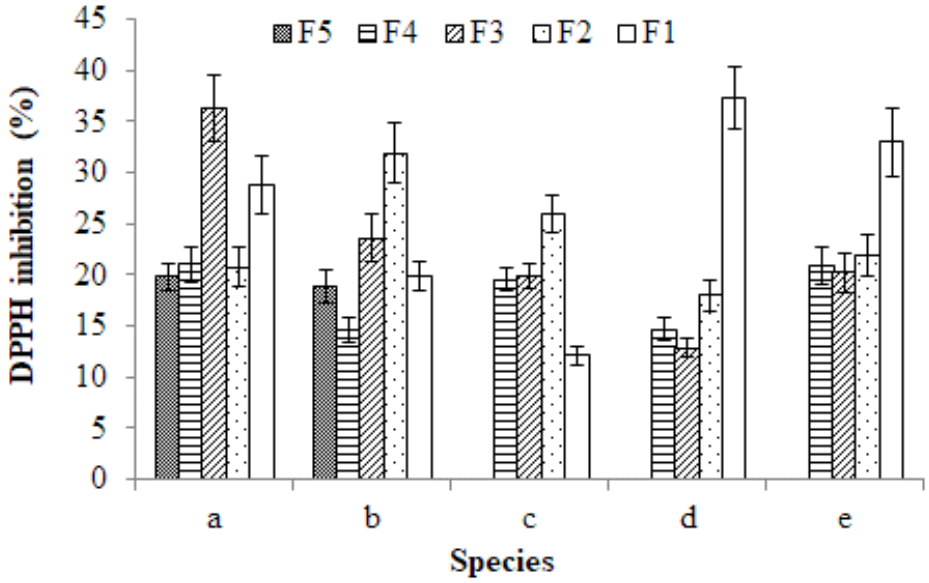


Figure 3. DPPH antioxidant activity of the fractions (F1-F5) from ethyl acetate extracts of some snail and bivalve species at the concentration of 50 µg/ml. a: Snail *A. delphinus, b:* Murex snail *C. torrefactus, c:* Oyster *Ostrea* sp., *d:* Spiny oyster *Spondylus* sp., *e:* Fluted giant clam *T. squamosa*

3.5. FTIR

The IR spectra of the representative samples displaced several substance groups, including proteins in the methanol extracts and nonpolar and less polar compounds in the ethyl acetate extracts (Figures 4 and 5).

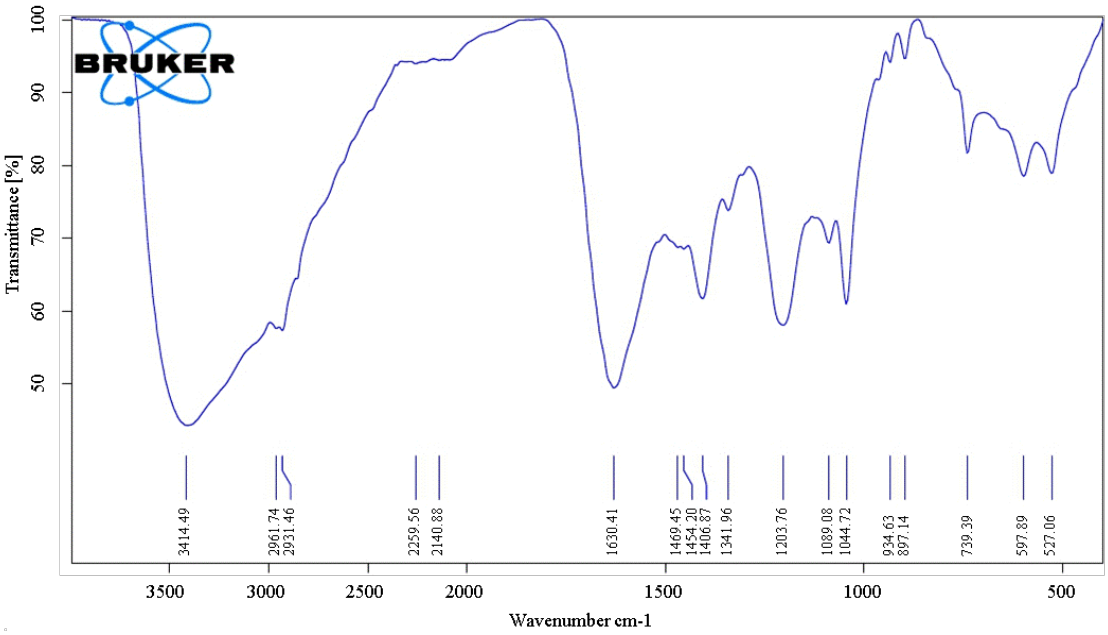


Figure 4. FTIR spectra of the methanol extract from the snail *Chicoreus torrefactus*

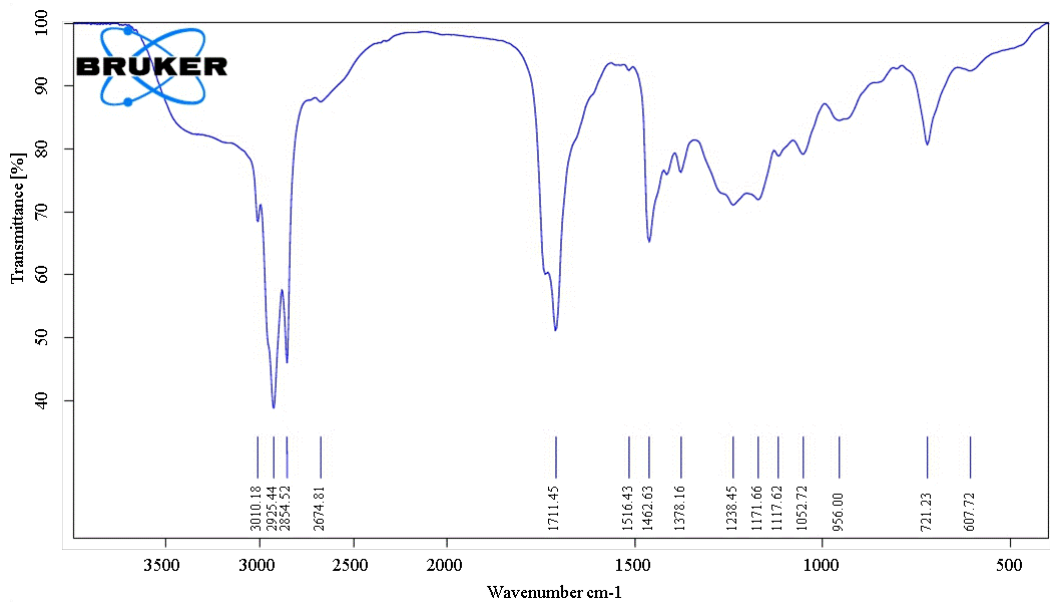


Figure 5. FTIR spectra of the ethyl acetate extract from the snail *Chicoreus torrefactus*

The IR spectra of the fractions from methanol extracts revealed the presence of proteins via the C-H functional group through stretching vibration at 2800-2900 cm^{-1}. For the amide I, the stretching vibration of N-H appeared at about 3440 cm^{-1} and C=O at 1630-1645 cm^{-1}. The C-N vibration of the amide II absorbed at 1406-1412 cm^{-1}. Meanwhile, for the amide III, the N-H bending vibrations were observed at 1203-1215 cm^{-1} and the C-N stretching vibrations at 1336-1341 cm^{-1}. Moreover, the spectra also showed the out-of-plane bending vibrations of N-H at 524-602cm^{-1} and 739-739.95 cm^{-1}, and the C-O stretching vibrations at 1045 and 1085-1115cm^{-1}. The peak at 900 cm^{-1} represented an aromatic or double bonds presented in substance.

3.6. Protein pattern

The fractions from methanol extracts exhibiting high antioxidant activity showed the presence of proteins with molecular weights ranging from about 19 to 70 kDa (Figure 6).

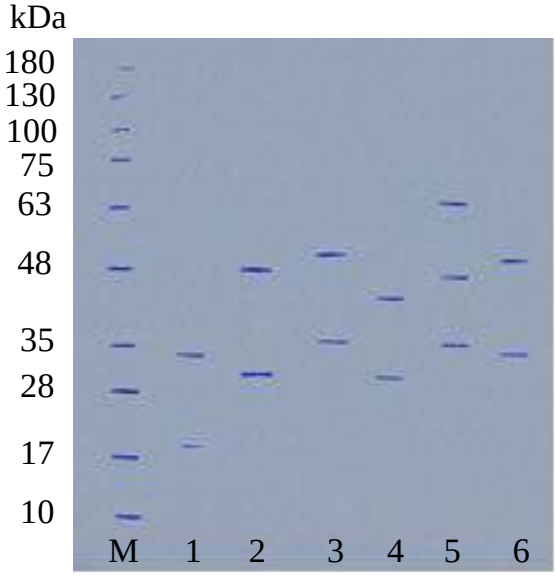


Figure 6. Protein pattern of the fractions from methanol extracts. M: protein maker, (1) F2 fraction from *C. torrefactus*, (2) F3 fraction from *L. lambis*, (3) F3 fraction from *T. bruneus*, (4) F1 fraction *Ostrea* sp., (5) F1 fraction from *P. margaritifera*, (6) F1 fraction from *T. crocea.*

4. Discussion

In this study, the antioxidant activity of extracts from some snails and bivalves was evaluated. The antioxidant capacity seemed to be different between solvent extracts, in which the antioxidant activity of methanol extracts tended to be stronger than that of ethyl acetate extracts. This result is similar to some previous studies, where the stronger antioxidant and antibacterial activity of methanol extracts from mollusks compared with

other solvent extracts was noted. For example, the methanol extract was more efficient in DPPH radical scavenging than the ethyl acetate extract from the clam *G. paradoxa* and snail *L. littorea*, with the IC50 of the methanol extract from *L. littorea* of 0.37 mg/mL (Borquaye et al., 2016). The highest DPPH radical scavenging of the methanol extract from the *P. viridis* was 76.9 % at the concentration of 100 µg/ml (Madhu et al., 2014). The methanol extract from the freshwater snail *Pila virens* had DPPH free radical scavenging activity ranging from 20.06 % to 74.83 % at concentrations of 0.1 - 2.0 µg/ml (Gayathri et al., 2017). This extract from bivalves exhibited stronger antioxidant activity than that from gastropods and starfish (Pachaiyappan et al., 2014). The other bivalve species such as the eastern oyster *C. virginica* (Roesijadi et al., 1989), clam *A. striata* (Hasan et al., 2015), clam *G. paradoxa* contained potent antioxidants whereas extracts from the snail *L. littorea* (Borquaye et al., 2015) and *H. pugilinus* tissue (Arumugasamy and Cyril, 2017) in addition to antioxidant capacity also showed some other biological activities. In addition, the methanol extracts from the cuttlefish *S. pharaonis* tissue, *S. intermis*, *O. vulgaris* and *D. gigas* skin exhibited strong antioxidant activity (Ponnusamy et al., 2016; Aubourg et al., 2016). In *P. viridis*, the methanol extract had strong antioxidant and antibacterial activities at low concentrations, indicating that the compound involved in the effect of these activities is probably a highly polar compound (Kiran et al., 2014). The methanol extracts from the *O. dolfusii* (Ramasamy et al., 2011) and from the sea slug *Armina babai* very well inhibited some pathogenic bacteria strains (Ramya et al., 2014). In general, several extracts with solvents improved activity and increased antibacterial or antioxidant capacity. Together with other results, this study suggests that the methanol is probably one of the suitable solvents for extraction of biologically active substances from mollusks.

In the present study, the results of TLC and electrophoresis revealed the presence of low molecular weight proteins in methanol active extracts from snail and bivalve tissues. In addition, the FTIR spectra displacing the appearance of some peaks of amides and proteins in methanol extracts were similar to the IR spectra of methanol extract from *P. viridis* with 14 peaks of polyamide and protein groups (Kiran et al., 2014). A result of Rayma et al (2014) also showed that the methanol extract from *A. babai* exhibiting antibacterial activity contained proteins with molecular weight from 13-72 kDa. Periyasamy et al (2012) determined the molecular weight of proteins in the extract exhibiting activity against bacteria from the snail *B. spirata* from 2 to 110 kDa, and the antibacterial protein extracted from the gills of *P. viridis* had the molecular weight of 9.7kDa (Chandran et al., 2009) and 29-63 kDa from the tissues of this species (Madhu et al., 2014). Whereas the molecular weight of proteins in the clam *M. meretrix* and *M. casta* ranged between 45 and 261 kDa and the active peptides 14-29 kDa were purified (Sumita et al., 2009). Sudhakar and Nazeer (2015) reported that the peptides extracted from the snail *Conus betulinus* and the cuttlefish *S. brevimana* exhibited strong antioxidant activity. In addition, among the

four isolated protein fractions extracted from the clam *A. striata*, one fraction had the strongest DPPH antioxidant activity at IC_{50} 183.75 µg/mL (Hasan et al., 2015). The crude peptides extracted from the clam *G. paradoxa* and rustic limpet *Patella rustica* showed the DPPH radical scavenging activity of 56.77% and 79.77% at 0.39 mg/mL, respectively (Borquaye et al., 2015). Proteins could be the main group of substances involved in biological activities, including antioxidant capacity in these animals. In which, proteins with small size (<10 kDa) have been proved to be substances with strong antioxidant and antibacterial activities, and are valuable medicinal sources that can be exploited from marine snails and bivalves instead of terrestrial plants. Normally, sea snails and bivalves contained proteins such as aglutinin and glycoproteins with potent biological activity. The results of this study indicate that marine snails and bivalves in Vietnam are potential sources of antioxidant compounds. Although the crude extracts and some fractions showed antioxidant activity, they have not been isolated and therefore need to be purified to elucidate their chemical structure in further studies.

Natural bioactive compounds from marine organism in general and mollusks in particular are increasingly attracting the attention of the scientific community because of their bioactive values. Some studies have shown that mollusk is considered as a potential drug cabinet because they possessed anti-inflammatory, antimicrobial, antioxidant and cytotoxic properties. For traditional medicine in some countries, some parts of gastropods are used to treat a number of diseases. The results obtained from our survey show the potential of a rich resource of natural medicine from sea snails and bivalves in Vietnam beyond nutritional value. The discovery of antioxidant along with the previous finding in antibacterial ability of substances from these animals (Pham et al., 2019) is to promote the search for an alternative natural source for a new generation of antibacterial and antioxidant agents.

Conclusion

The present results indicate that some marine snail and bivalve species possesses antioxidant properties. The study also suggests that these animals are a potential source for bioactive substances in Viet Nam.

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KHẢO SÁT HOẠT TÍNH KHÁNG OXI HÓA CỦA CHẤT CHIẾT TỪ ỐC BIỂN VÀ HAI MẢNH VỎ VÙNG BIỂN NAM VIỆT NAM

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Tóm tắt. Hoạt tính ức chế gốc tự do DPPH của các chất chiết methanol và ethyl acetate và các phân đoạn của một số chất chiết này từ động vật thân mềm vùng đảo xa bờ miền Nam Việt Nam đã được khảo sát. Tính chất của các chất chiết và phân đoạn có hoạt tính được xác định bằng TLC, FTIR và SDS-PAGE. Kết quả cho thấy chất chiết methanol từ 3 loài ốc, *Chicoreus torrefactus, Lambis lambis* và *Turbo bruneus* và 3 loài hai mảnh vỏ *Ostrea* sp., *Pinctada margaritifera* và *Tridacna crocea* có khả năng kháng oxy hóa cao. Các chất chiết ethyl acetate từ ốc *Angaria delphinus, C. torrefactus* và hai mảnh vỏ *Ostrea.* sp., *Spondylus* sp., và *T. squamosa* cũng có hoạt tính này. Một số phân đoạn của chất chiết từ mẫu có hoạt tính kháng oxy hóa. Giá trị kháng oxy hóa cao nhất ở phân đoạn F2

từ chất chiết methanol của loài *C. torrefactus*, F3 từ *L. lambis* và *T. bruneus*, F1 từ *Ostrea.* sp., *P. margaritifera* và *T. Crocea*. Đối với chất chiết ethyl acetate, phân đoạn F3 của chất chiết từ loài *A. delphinus*, F2 của *C. torrefactus, Ostrea* sp. và F1 của *Spondylus* sp. và *T. squamosa* có giá trị kháng oxy hóa cao nhất. Kết quả phân tích bằng TLC và FTIR các mẫu có khả năng kháng oxy hóa xác nhận sự hiện diện của một số nhóm chất, trong đó mẫu sử dụng chất chiết methanol chủ yếu chứa protein và các hợp chất phân cực, còn mẫu sử dụng chất chiết ethyl acetate chứa chủ yếu các hợp chất không và ít phân cực. Trọng lượng phân tử của protein ở các phân đoạn của chất chiết methanol có khả năng kháng oxy hóa cao dao động từ 19 - 70 kDa.

Từ khóa: Động vật thân mềm, hoạt tính DPPH, TLC, FTIR, SDS-PAGE.