Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(5): 625 – 650 (2022)
www.ejabf.journals.ekb.eg

GC-MS analysis of *Anadara uropigimelana* and screening of its *in vitro* antioxidant and antiproliferative effect against MCF-7 cells

Aalaa M. El-Sadat *, Mohamed K. Hassan, Samya H. Mohammad
Zoology Department, Faculty of Science, Port Said University, Egypt
***Corresponding Author:** alaaelsadat@sci.psu.edu.eg

ARTICLE INFO
Article History:
Received: July 27, 2022
Accepted: Aug. 9, 2022
Online: Oct. 3, 2022

Keywords:
Marine bivalves,
GC-MS,
DPPH,
MCF-7,
Antioxidant,
MTT assay,
Flavonoid

ABSTRACT

In recent years, many researchers have shifted their attention away from synthetic bioactive compounds to naturally existing ones for the purpose of novel therapeutic drugs. Such studies focused on the bioactive compounds from plants or animal sources including marine bivalves. *Anadara uropigimelana* is one of the most abundant marine bivalves along the coastal zones of the Mediterranean Sea, Port Said, Egypt. In the present study, live samples of *A. uropigimelana* (*Au*) were collected from the intertidal zones during winter to avoid elevated water temperature and gametogenesis during summer. GC-MS analysis was used to identify and characterize the bioactive compounds in *Au* extract. The protein content, flavonoid, and antioxidants of the *Au* extract were assessed. Its DPPH radical scavenging activity and its cytotoxicity (MTT assay) against the human breast cancer cells (MCF-7) were performed. Twenty-eight compounds were identified with a variety of referenced pharmacological activities. The MTT results showed that *Au* extract has a cytotoxic effect against MCF-7 (IC_{50} value was 0.579 mg/ml after 72 h of treatment). *Au* extract exhibited higher DPPH scavenging activity, with IC_{50} of 59.61 µg/ml, than that of the ascorbic acid. The present results indicate that *Au* extract showed rich content of flavonoids and proteins with appreciated antioxidant activity and cytotoxic effect against a representative cancer cell model. The present study spotlighted one of the ecologically neglected marine bivalves, that offers a valuable tissue extract, which could be beneficially used as a natural source of bioactive compounds with its considerable content of protein, flavonoids, and antioxidants.

INTRODUCTION

Many natural bioactive compounds from living organisms are more biologically friendly than synthesized ones **(Babar *et al.*, 2016)**. This makes them excellent choices for further discovery of therapeutic drugs **(Atanasov *et al.*, 2021)**. However, marine invertebrates are believed to have advanced biochemical and physiological mechanisms. Such mechanisms yield distinctive and unique biochemical compounds that differ from those found in the land animals **(Babar *et al.*, 2016)**. These unique marine bioactive compounds exhibit a broad variety of anti-inflammatory, antitumor, antiviral,

antimicrobial, and cytotoxic properties, serving as a basis for developing new therapies to treat many other serious human diseases **(Jha *et al*., 2004; Babar *et al*., 2016)**.

Marine mollusks are considered an excellent source of bioactive compounds among marine invertebrates **(Odeleye *et al*., 2019)**. Anticancer drugs derived from marine mollusks are characterized by their efficiency and specificity in the mechanisms of action with digestive behavior. In addition to these properties, their capability to target biological features of the cancer cells together with an effective performance to suppress the chemotherapy resistance **(Ciavatta *et al*. 2017)** are recognized.

Marine bivalves such as oysters, clams, and mussels are soft, tasty, and easily digested seafood. These qualities make them attractive to the consumers and highly dominates the farmed species and traded food items **(Wijsman *et al*., 2019)**. Marine bivalves represent the highest item in the overall edible Mollusca hierarchy. Some recent reports demonstrated that marine bivalves have bioactive compounds with high pharmaceutical and nutraceutical values for human health **(Chakraborty & Joy, 2020)**. Furthermore, they provide an inexpensive source of high protein content with significant nutritional values **(Venugopal & Gopakumar, 2017)**. Moreover, they can provide the human body with vitamin B12 **(Wright *et al*., 2018)**, flavonoid **(Krishnamoorthy *et al*., 2019; Martins *et al*., 2019)**, and a high omega-3/omega-6 ratio compared to that found in most marine fishes **(Tan *et al*., 2020)**. Furthermore, they are considered a promising source of potent anti-inflammatory, antioxidant, and antidiabetic agents **(Joy *et al*., 2016)**. In addition, their anti-cancer effect against human prostate, breast, cervical, lung, and colon cancer cells **(Cheong *et al*., 2013; Umayaparvathi *et al*., 2014; Wu *et al*., 2014; Liao *et al*., 2016)** is remarkable. It is noteworthy that, marine bivalves can produce a variety of unique antiviral compounds that provide them with an inborn defensive system against a wide range of viruses. Hence, prospect-specified target drug-like compounds can be engineered to fend off viral infections, especially current SARS-CoV-2 infections **(Yap, 2020)**.

Blood cockle "*Anadara uropigimelana* **(Bory de Saint-Vincent, 1827)**" is one of the most abundant species in Port said, Egypt. It inhabits the shallow intertidal mudflats to deep water along the coastal zones. It has neither commercial value nor great success in Egypt due to its bloody color **(EL-Agamy *et al*., 2005)**.

The aims of the present study were to: 1) identify and characterize the potential bioactive compounds in the tissue of *A. uropigimelana (Au)*, since no study has investigated the bioactive compounds in this species, 2) investigate vital constituents such as proteins, flavonoids, antioxidants. 3) evaluate its potential anticancer effect against the MCF-7 human breast cancer cell line.

MATERIALS AND METHODS

Sample collection

Live samples of *Au* were collected in 2017 from the intertidal mudflats along the coastal zones of Port Said governorate, Egypt. Samples were collected during winter to

avoid elevated water temperature, hypoxia, or gametogenesis during the summer season. They were immediately brought in containers with seawater inside to the invertebrate laboratory of the Zoology Department, Faculty of Science, Port Said, Egypt.

Preparation of the tissue extract

Preparation of the tissue extract was performed according to the method of García-Morales **(García-Morales *et al*., 2016)** with some modifications. Bivalve shells were removed and the whole soft tissues were dissected to remove viscera. They were then cleaned in 0.9% NaCl saline, cut into small pieces, and homogenized by a 1000-watt hand-blender with 10 mM phosphate buffer saline (PBS; 0.1 M NaCl, pH 7.2) in a ratio of 1:3 (w/v). The homogenate was centrifuged (3615 x g for 10min at 4°C). The supernatant was neglected, and the precipitate was re-homogenized and centrifuged (3615 x g for 10min at 4°C) as its tissues require an efficient homogenization to easily liberate its vital tissue contents. The supernatant was collected, filtered by a Millipore filter 0.22 μm, and lyophilized for efficient storage at -20° C to be readily used as *Au* extract.

Gas chromatography – Mass spectrum (GC-MS)

Sample preparation for GC-MS analysis was prepared according to the method of **Ramasamy and Balasubramanian (2012)**. Chemical composition analysis of the *Au* extract was carried out using a GC (Agilent Technologies 7890A, Santa Clara, CA, USA) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm I.D. and 0.25 μm film thickness). The carrier gas was helium with the linear velocity of 1.0 ml/min. The injector and detector temperatures were 200ºC and 250ºC, respectively. Volume injected was 1.0 μl of the sample. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250ºC, and acquisition mass range 50–800. Accordingly, retention time is a measure of the time taken for a solute to pass through a chromatography column. It is calculated as the time from injection to detection.

Total antioxidant capacity

It was measured by a commercial colorimetric assay kit obtained from Bio-diagnostic Co., Giza, Egypt. The method depends on the reaction of antioxidants in the sample, with a specified quantity of an exogenously provided hydrogen peroxide **(Koracevic *et al*., 2001)**. Briefly, the potential antioxidants in the sample remove a definite amount of H_2O_2, while the residual H_2O_2 is calorimetrically defined. Accordingly, the absorbance of the blank and sample was read using UV-visible spectrophotometer (ST-UV-D1901PC, USLAB, Colorado Springs, Co, USA) and recorded separately against distilled water at 510 nm. The total antioxidant content was measured according to the following equation:

Total antioxidant concentration (mM / L) = $(A_{\mathbf{B}} - A_{\mathbf{SA}}) \times 3.33$.

Where, A_B represents the absorbance of the blank content, and A_{SA} represents the absorbance of the sample (*Au* extract).

DPPH radical scavenging assay

The capacity of *Au* extract to directly react with and quench free radicals was evaluated by the conventional DPPH radical scavenging assay as described by **Hossain *et al*. (2014),** with minor modification. Briefly, a volume of 2ml of the DPPH methanolic solution (4 mg/100 ml) was mixed with 2ml of different concentrations (12.5, 25, 50, 100, and 200 µg/ml) of *Au* extract. Ascorbic acid was used as a reference standard. The reaction flasks, in triplicates, were vigorously shaken and then incubated in a dark place for 45min at 37ºC. A control sample was prepared, containing 2ml of DPPH solution with 2ml of absolute methanol, which was used as the blank. The absorbance of *Au* extract and ascorbic acid concentrations were measured at 517nm using a UV-visible spectrophotometer. The DPPH scavenging activity (%) was calculated using the following formula:

DPPH scavenging activity (%) = $[(A_C - A_S) / A_C] \times 100$.

Where, A_C represents the absorbance of the control sample, and A_S represents the absorbance in case of *Au* extract or standard.

IC_{50} values (the concentration of the sample that could scavenge 50% of DPPH free radical) for *Au* extract and ascorbic acid were obtained by linear regression analysis of dose-response curve plotted between inhibition (%) and concentrations.

MTT assay

Cell viability was determined using a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay **(Mosmann, 1983)**. Briefly, human breast cancer cells (MCF-7) were seeded into 96-well plates, with 5×10^3 cells per well and left to attach for 24 h at standard culture conditions, 37°C and 5% CO_2. Next day, cells were treated with *Au* extract at different concentrations (0, 0.16, 0.32, 0.64, 1.28, 2.56, and 5.12 mg/ml) for 72h. After that, culture media were removed and 20µl of MTT solution (5 mg/ml, Sigma-Aldrich, USA) was added to each well and incubated for 4h at 37°C. Then, 100µl of dimethyl sulfoxide (DMSO, Sigma-Aldrich) was added to each well to dissolve the resulting formazan crystals. The produced color was detected by determining the absorbance at 570nm, using a FLUOstar Omega-microplate reader (BMG Labtech, NC, USA). The percentage of cell viability was calculated as follows:

Cell viability (%) = (Mean $OD_{treatment}$ / Mean $OD_{control}$) ×100.

Where, the absorbance values are displayed as optical density (OD), which depends on the number of cells per well. Then, IC_{50} value (inhibitory concentration; a

concentration of a compound inhibiting 50% of the cell growth) was calculated and compared to that of the untreated control group.

Protein content

Au extract was weighed (10 g), homogenized in phosphate buffer saline (PBS; 0.1 M NaCl, pH 7.2) in a ratio of 1:3 (w/v), and centrifuged (3615 x g for 10 min at 4°C). The total protein concentration of *Au* extract was determined in 10 µl of the prepared stock using the method of **Bradford (1976)**. Its value was estimated by using the bovine serum albumin (BSA) standard curve and expressed as µg/µl. The absorbance was recorded at 595 nm using a UV-visible spectrophotometer and measured in triplicate.

SDS-PAGE analysis

Banding pattern of protein content in *Au* extract was monitored by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) **(Laemmli, 1970)**. Briefly, *Au* extract was boiled with sample buffer; 65.8 mM Tris-HCl, pH 6.8, 2.1% SDS, 26.3% (w/v) glycerol, and 0.01% bromophenol blue for five minutes at 95ºC, cooled on ice then loaded on 10% acrylamide gel. Electrophoresis was carried out at 200 V for 35 – 40min. The sample was loaded in parallel with protein marker (250 – 20 kDa). After fixation in 30% methanol, gels were stained with Coomassie Brilliant Blue (R-250; Bio-Rad, USA) to visualize the potential proteins bands, and images were captured by a documentation system (ChemiDoc, Bio-Rad, USA).

Total flavonoid content

Total flavonoid content in *Au* extract was determined as described by **Al-Saeedi *et al*. (2015)**, with slight modification. The absorbance of all working solutions was measured against a methanol blank at 510 nm using a UV-visible spectrophotometer. Flavonoids content in *Au* extract was estimated by using the quercetin standard calibration curve, and the result was expressed as a microgram of quercetin equivalent (Qu) per 1.0 g of dry extract. The measurements were recorded in triplicate.

Statistical analysis

Statistical analysis was performed by IBM® SPSS® Statistics 20.0. The data were expressed as mean value ± standard deviation (SD) and analyzed using one-way ANOVA and two-way ANOVA followed by Dunnett's test and Duncan's multiple rang test (DMRT), respectively. Differences between means were considered significant at $P < 0.05$. Graphics of data were done using Microsoft 365® Excel® App.

RESULTS

GC-MS analysis of *Au* extract

The present study revealed a detection of twenty-eight compounds in *Au* extract through GC-MS analysis. This was based on a comparison of their mass spectra and retention time with those of the authentic compounds. In addition, computer matching with NIST and WILEY library were used together with the comparison of the

fragmentation pattern of the mass spectral data with those reported in the literature. The identified compounds along with their molecular formula, molecular weights, retention time (RT), and peak area (%) are presented in Table (1), while their GC-MS chromatogram is shown in Fig. (1).

Table 1. GC-MS analysis of *Anadara uropigimelana* extract

No	Name	Molecular formula	Molecular weight	Retention time	Peak area (%)
1	Methyl 15-methoxyhexadecanoate	$C_{18}H_{36}O_3$	300.5	3.427	0.98
2	4-Nonenoic acid, methyl ester	$C_{10}H_{18}O_2$	170.25	4.754	0.54
3	6,4′-Dimethoxy-3-hydroxyflavone	$C_{17}H_{14}O_5$	298.29	8.594	2.01
4	19,20-DiHDPA	$C_{22}H_{34}O_4$	362.5	9.858	0.83
5	Astilbin (ATN)	$C_{21}H_{22}O_{11}$	450.4	10.226	0.8
6	4-Ethoxy-7-methylcoumarin	$C_{12}H_{12}O_3$	204.22	10.433	1.9
7	9-Octadecen-12-yonic acid, methyl ester	$C_{19}H_{32}O_2$	292.5	11.4	0.52
8	Octacosanoic acid, ethyl ester	$C_{37}H_{66}O_4$	574.9	12.573	0.51
9	Hexadecenoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5	13.171	0.5
	Table 1. ***(continued)***				
10	13(Z)-Docosenoic acid	$C_{22}H_{42}O_2$	338.6	13.491	1.37
11	10-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296.5	13.815	1.46
12	β-Sitosterol	$C_{29}H_{50}O$	414.7	14.413	0.63
13	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322.5	14.642	1.12
14	5,7,3′,4′,5′-Pentahydroxyflavone	$C_{15}H_{10}O_7$	302.23	15.402	8.38
15	Methyl copalate	$C_{21}H_{34}O_2$	318.5	15.951	6.87
16	Methyl eicosa-7,10,13-trienoate	$C_{21}H_{36}O_2$	320.5	17.538	2.1
17	Stearidonic acid ethyl ester	$C_{20}H_{32}O_2$	304.5	18.091	8.48

18	Arachidonic acid	$C_{20}H_{32}O_2$	304.5	18.163	5.74
19	6,7-Dimethoxy-4-ethylcoumarin	$C_{13}H_{14}O_4$	234.25	18.242	2.55
20	2α,3α-Epoxy-5α-Cholestane	$C_{27}H_{46}O$	386.7	18.336	20.17
21	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.4	19.517	0.93
22	Methyl 2-hydroxystearate	$C_{19}H_{38}O_3$	314.5	20.171	1.14
23	Quercetin 3,5,7,3′,4′, -Pentamethyl ether	$C_{20}H_{20}O_7$	372.4	20.57	0.5
24	8-Carboxy-3-methylflavone	$C_{17}H_{12}O_4$	280.27	21.428	1.02
25	Cis-4,7,10,13,16,19 Docosahexaenoic acid	$C_{22}H_{32}O_2$	328.5	21.853	1.65
26	(±)-δ-Cadinene	$C_{15}H_{24}$	204.35	22.293	15.19
27	Thunbergol	$C_{20}H_{34}O$	290.5	22.59	11.86
28	4′,5,6,7- Tetramethoxy flavanone	$C_{19}H_{18}O_6$	342.3	23.158	0.22

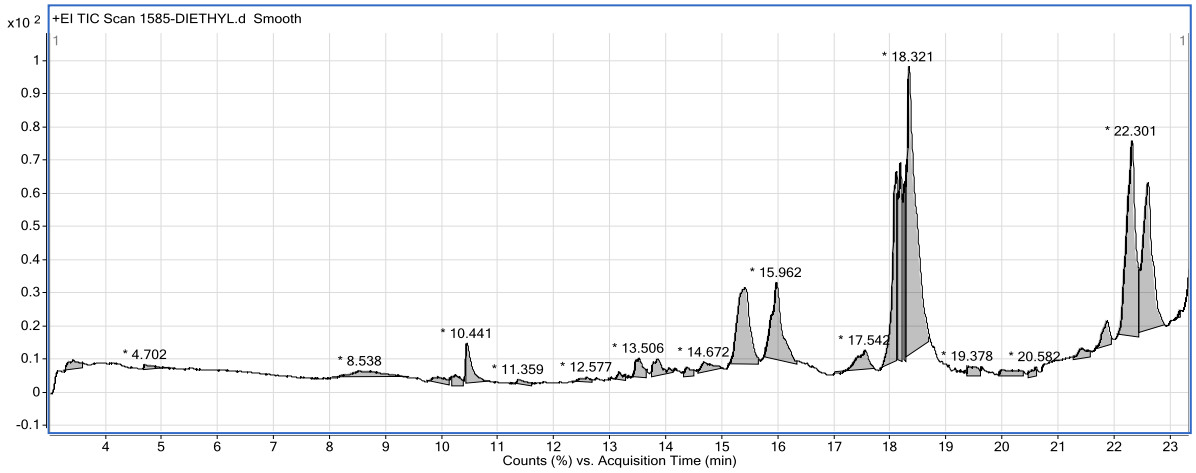

Fig. 1. GC-MS chromatogram of *A. uropigimelana* extract

Antioxidant capacity and scavenging activities of the *Au* extract

Total antioxidant capacity of *Au* extract was measured (2.57 ± 0.10 mM/L). DPPH scavenging activity of *Au* extract was then assessed in parallel with that of the reference scavenging agent, ascorbic acid, as shown in Fig. (2). Interestingly, *Au* extract showed appreciated concentration-dependent scavenging activity (R^2 = 0.9795). *Au* extract showed maximum free radical scavenging activity (96.86% ± 0.06) at 200 µg/ml and minimum activity (28.39% ± 0.62) at 12.5 µg/ml. This value exceeded that of the ascorbic acid (85.34 % ± 0.03 and 25.86% ± 0.43) at the same concentration. The estimated IC_{50} value of *Au* extract was 59.61 µg/ml, while that of ascorbic acid was 78.05 µg/ml, with a highly significant difference (P = 0.000).

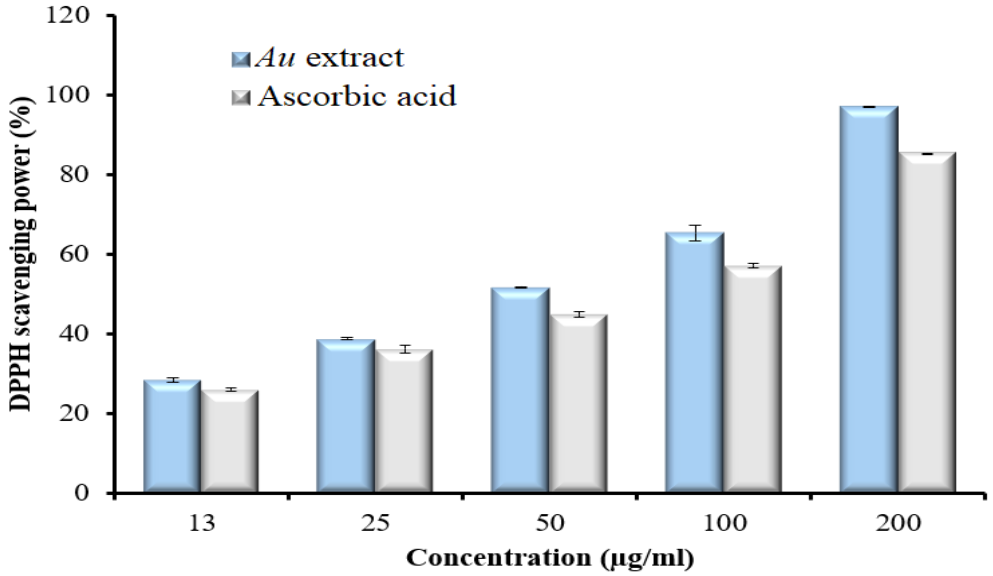

Fig. 2. DPPH scavenging activity of *A. uropigimelana* extract and ascorbic acid

Cytotoxic effect of *Au* extract against MCF-7 cells

To evaluate the anticancer activity of the *Au* extract on the viability of a representative human cancer cells, breast cancer cells (MCF-7) were used, and the viability was tested using MTT assay within 72h. It was found that, *Au* extract significantly inhibited the growth of MCF-7 cells in a concentration-dependent manner (Fig. 3). The estimated IC_{50} value was 0.579 mg/ml. It was noticed that, a concentration of 0.08mg didn't show an antiproliferative effect against MCF-7 cells, while the concentrations of 0.16 mg/ml and higher ones inhibited significantly ($P \leq 0.001$) the cell viability compared to the control. Furthermore, concentrations of 0.64, 1.28, and 2.56 mg/ml displayed the same antiproliferative effect against MCF-7 cells, while the concentration of 5.12 mg/ml showed the highest inhibitory effect with cell viability of 20.42 %.

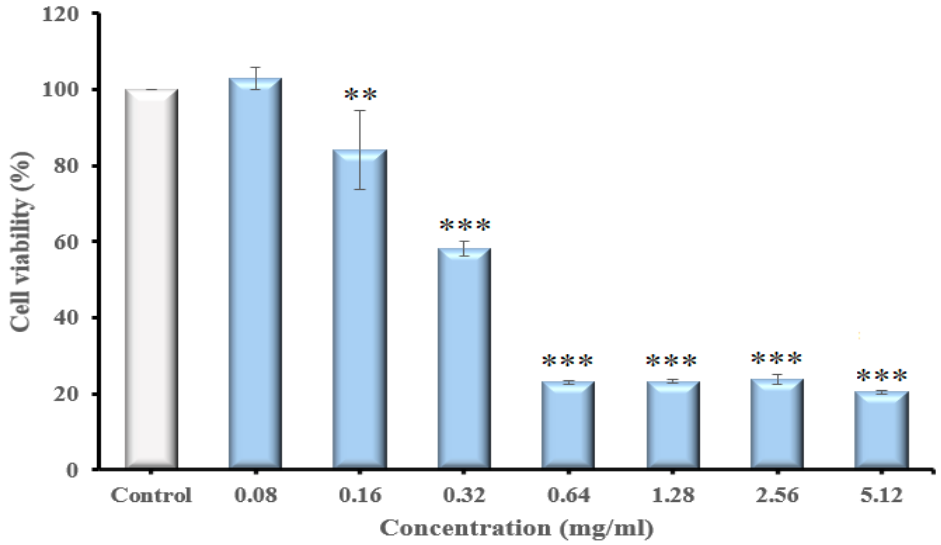

Fig. 3. Cell viability of MCF-7 breast cancer cell line after treatment for 72h at multiplier concentrations of *A. uropigimelana* extract.

Each value is represented as Mean ± SD, n=3. The columns marked with (**) and (***) are significantly different referred to the control at $P < 0.01$ and $P < 0.001$ using one- way ANOVA test followed by Dunnett's multiple comparison test.

Protein content of *Au* extract

The present results revealed that the total protein concentration in *Au* extract was 63.85 ± 1.17 µg/µl. Then SDS–PAGE was used to study the variability of the protein bands/molecular size in the extract. The abundance of different protein bands in *Au* extract is shown in Fig. (4). Condensed protein bands reflected high concentrations of these proteins in the high molecular weight bands approximately at 130, 190, and 250 kDa; while in the low molecular weight bands, they were observed at approximately 37, 25, and 18 kDa. It was also noticed that, about 5 bands with less condensed protein had molecular masses between 150 - 49 kDa.

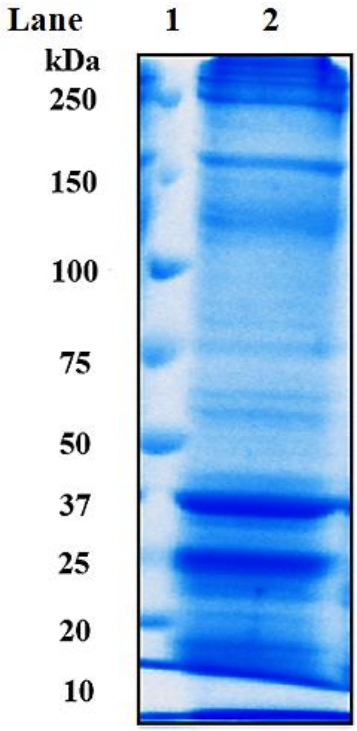

Fig. 4. SDS-PAGE gel electrophoresis of the protein profile in *A. uropigimelana* extract, stained with Coomassie brilliant blue (R-250; Bio-Rad, USA).

Lane 1: marker, and Lane 2: *Au* extract.

Flavonoid content of *Au* extract

The present result exhibited a considerable amount of flavonoid with 361.59 ± 3.82 µg QU / g dry extract.

DISCUSSION

The present study is the first one, to the best of our knowledge, that identifies the chemical compositions of *Au*. Earlier research has shown that marine bivalves harbor a variety of bioactive compounds that are characterized by valuable bioactivities, including anti-microbial, anti-inflammatory, antioxidant and anticancer **(Chakraborty & Joy, 2020)**. Notably, all the identified compounds, 28 compounds, possess promising biological activities, except the methyl eicosa-7,10,13-trienoate, (Table 2). Most (> 75%) of the detected compounds have fatty acids nature. Meanwhile, a considered ratio (21%) has pure and mixed flavonoids such as 6,4′-dimethoxy-3-hydroxyflavone, astilbin (ATN), 5,7,3′,4′,5′-pentahydroxyflavone, quercetin 3,5,7,3′,4′, -pentamethyl ether, 8-carboxy-3-methylflavone, and 4′,5,6,7-tetramethoxy flavanone. The lowest ratio (7.1%) was recorded for the compounds with coumarin nature such as 4-ethoxy-7-methylcoumarin and 6,7-dimethoxy-4-ethylcoumarin. However, comparing the present result with that of

Anadara granosa, it was found that *Au* extract contains bioactive compounds that exceeded those detected in *A. granosa* by twofolds **(Ramasamy & Balasubramanian, 2012)**.

Table 2. Nature and referenced bioactivity of the identified compounds in the *Anadara uropigimelana* extract through GC-MS analysis

No	Name	**Compound nature	Bioactivity
1	Methyl 15-methoxyhexadecanoate	Fatty acid methyl ester	Antimicrobial **(Shady *et al*., 2019)**.
2	4-Nonenoic acid, methyl ester	Fatty acid methyl ester	Antimicrobial **(Matejić *et al*., 2018)**.
3	6,4′-Dimethoxy-3-hydroxyflavone	Flavonoid	Antioxidant, antihepatotoxic, and nephroprotective **(Gutierrez, 2013)**. Antitumor **(Jasril *et al*., 2003)**.
4	19,20-DiHDPA	Oxylipin	Ventricular arrhythmias marker and anti- high Ca_2^+ sensitivity in human lung **(Zhang *et al*., 2016)**.
5	Astilbin (ATN)	Flavonoid glycoside	Anti-inflammatory and anti-arthritic **(Cai *et al*., 2003)**. Antioxidant **(Petacci *et al*., 2010)**. Antibacterial **(Moulari *et al*., 2006)**. Anti-hepatic injury **(Wang *et al*., 2004)**. Anti-renal injury **(Chen *et al*., 2011)**. Antidiabetic **(Li *et al*., 2009)**. Neuroprotective **(Wang *et al*., 2017)**.

Table 2. ***(continued)***

6	4-Ethoxy-7-methylcoumarin	Coumarin (polyphenolic)	Anti-human lung cancer cells (A549) **(Musa *et al*., 2012)**. Anti-chronic myelogenous leukemia, anti-colon cancer cells (LS180), and anti-breast cancer (MCF-7) **(Miri *et al*., 2016)**. Antioxidant **(Sancheti *et al*., 2013).**
7	9-Octadecen-12-yonic acid, methyl ester	Fatty acid methyl ester	Antioxidant **(Yadav *et al*., 2018)**.
8	Octacosanoic acid, ethyl ester	Fatty acid ethyl ester	Antioxidant **(Shi *et al*., 2009)**. Anti-leukemic (HL60) **(Rashid *et al*., 2018)**.
9	Hexadecenoic acid, ethyl ester	Palmitic acid ethyl ester	Antioxidant, and hypocholesterolemic agent **(Balamurugan, 2017)**. Anti-inflammatory **(Joshua *et al*., 2020)**.
10	13(Z)-Docosenoic acid	Monounsaturated omega-9 fatty acid	Inflammatory modulator **(Johnson *et al*., 2014)**.
11	10-Octadecenoic acid, methyl ester	Fatty acid methyl ester	Antibacterial, antifungal, antioxidant and, hypocholesterolemic agent **(Belakhdar *et al*., 2015)**.
12	β-Sitosterol	Phytosterol	Anti-diabetic **(Zeb *et al*., 2017)**. Hypocholesterolemic, and antibacterial **(Yinusa *et al*., 2015)**. Anti-inflammatory **(Phatangare *et al*., 2017)**. Antioxidant **(Gupta *et al*., 2011)**. Hepatoprotective **(Balamurugan *et al*., 2017)**. Chemo preventive against colon, prostate, cervical, and breast cancers **(Cheng *et al*., 2015)**.

Table 2. ***(continued)***

13	11,14-Eicosadienoic acid, methyl ester	Polyunsaturated omega-6 fatty acid	Antimicrobial **(Suresh *et al*., 2014)**. Anti-inflammatory **(Pereira *et al*., 2014)**. Antioxidant, antiarthritic, and anti- coronary heart disease **(Gomathi *et al*., 2015)**.
14	5,7,3′,4′,5′-Pentahydroxyflavone	Flavonoid lipid molecule	Anti-breast cancer (MCF-7) **(Hsu *et al*., 2009)**. Antioxidant **(Singh *et al*., 2017)**.
15	Methyl copalate	Diterpenoid	Antileishmanial **(Arruda *et al*., 2019)**.
16	Methyl eicosa-7,10,13-trienoate	Fatty acid methyl ester	No activity reported **(Diab *et al*., 2021)**.
17	Stearidonic acid ethyl ester	Rare polyunsaturated omega-3 fatty acid	Anti-inflammatory and antioxidant **(Sung *et al*., 2017)**. Antithrombotic and anticancer **(Guil- Guerrero, 2007)**. Cardioprotective **(Jump *et al*., 2012)**. Anti-breast cancer (MDA-MB-231) **(Horia *et al*., 2005)**. Synergetic effect with doxorubicin in prostatic cancer **(Mansour *et al*., 2018)**.
18	Arachidonic acid	Polyunsaturated omega-6 fatty acid	Anti-Pneumococcal **(Das, 2018)**. Anti-colon cancer **(Zhang *et al*., 2015)**.
19	6,7-Dimethoxy-4-ethylcoumarin	Coumarin derivative	Antimicrobial and antioxidant **(Tataringa and Zbancioc, 2020)**.
20	2α,3α-Epoxy-5α-Cholestane	Epoxy steroids	Antibacterial **(Knölker *et al*.,**

			2008).

Table 2. ***(continued)***

21	9,12-Octadecadienoic acid (Z, Z)-	Polyunsaturated omega-6 fatty acid (Linoleic acid)	Anti-inflammatory and anti-cancer **(Krishnamoorthy and Subramaniam, 2014)**. Antioxidant **(Kim *et al*., 2020)**.
22	Methyl 2-hydroxystearate	Stearic acid	Delivery of anti-HIV agents **(Gangadhara *et al*., 2014)**.
23	Quercetin 3,5,7,3′,4′, Pentamethyl ether	Polyphenol flavonoid	Antioxidant and anti-inflammatory **(Batiha *et al*., 2020)**.
24	8-Carboxy-3-methylflavone	Flavone	Anti-ulcerogenic, and hepatoprotective **(Atta *et al*., 2018)**.
25	Cis-4,7,10,13,16,19 Docosahexaenoic acid	Polyunsaturated omega-3 fatty acid	Neuroprotective **(Lukiw *et al*., 2005)**. Cardioprotective **(Diab *et al*., 2021)**. Anti-breast cancer (MCF-7 MDA-MB-231, and 4T1) **(Xue *et al*., 2014; Pizato *et al*., 2018)**. Breast cancer chemotherapy enhancer **(Newell *et al*., 2017)**.
26	(±)-δ-Cadinene	Unsaturated hydrocarbon	Anti-ovary cancer (OVACR-3) **(Hui, 2015)**. Antimicrobial **(González *et al*., 2012)**.
27	Thunbergol	Monocyclic diterpene alcohol	Antibacterial **(Mitić *et al*., 2019)**.
28	4′,5,6,7- Tetramethoxy flavanone	Flavonoid	Anti-inflammatory **(Pandith *et al*., 2013)**. Antibacterial **(Maia *et al*., 2011)**.

******Source: PubChem, SpectraBase™ and NIST Spectrometry Data Cent.

GC-MS data also elucidated that the *Au* extract contains omega 3,6,9 fatty acids as monounsaturated omega-9 fatty acid (13 (Z)-docosenoic acid), polyunsaturated omega-6

fatty acids (11,14-eicosadienoic acid methyl ester, arachidonic acid, and 9,12-octadecadienoic acid (Z, Z)-), and omega-3 fatty acids (stearidonic acid ethyl ester and Cis-4,7,10,13,16,19- docosahexaenoic acid).

Generally, it was noticed that, 17 compounds out of the detected ones have more than one bioactivity. Consequently, there were 15 compounds with antimicrobial activity, 14 compounds with antioxidant activity, and 13 compounds with anticancer activity. Additionally, other bioactivities such as anti- inflammatory, cardioprotective, chemopreventive, neuroprotective, hepatoprotective, antidiabetic, nephroprotective, and compounds with a synergistic effect with different chemotherapy drugs were related to the remaining compounds. This result may encourage further investigations such as testing its anti-proliferative / cytotoxic effect in a cellular model. In addition, it may be benefit in the comparison with other organisms.

It has been reported that, any sort of oxidative stress plays a major role in the pathophysiology of numerous diseases, such as heart disease, cancer, diabetes, and Alzheimer's disease **(Hossain *et al.*, 2019)**. In this study, the data revealed that antioxidants of *Au* extract were able to react with the DPPH solution and change its purple color, indicating the radical scavenging in a concentration-dependent pattern. These results agree with those which demonstrated the active free radical scavenger response by the whole tissue of marine bivalves **(Krishnamoorthy *et al.*, 2019; Yang *et al.*, 2019; Karaulova *et al.*, 2021)**. This effect might be attributed to the detected USFA (omega-9) and PUSFA (omega-3 and omega-6) in *Au* extract as other marine bivalves that represent an important source of omega 3,6,9 fatty acids **(Tan *et al.*, 2020; Moniruzzaman *et al.*, 2021)**. Such fatty acids offer a healthful diet and protective effect against oxidative stress and other bioactivities.

The abundance of many natural bioactive compounds in *Au* extract encouraged the study of its protein content. Bradford assay revealed that *Au* extract had high protein content. This was in consistent with that **Venugopal and Gopakumar (2017)**, who stated that shellfish such as mussels, oysters, clams, scallops, and others have high protein content. Moreover, SDS-PAGE analysis did not only confirm the high protein contents but also showed the wide range of molecular size of the proteins in *Au* extract.

Marine bivalves are a good source of natural flavonoids **(Minsas *et al.*, 2020)**. The natural flavonoid is considered by versatile bioactive compounds that act as anticancer, antioxidant, anti-inflammatory, cardioprotective, hepatoprotective, nephroprotective, neuroprotective **(Clark *et al.*, 2015; Xu *et al.*, 2018)**. Importantly, the total flavonoid content in *Au* extract in the present study was 361.59 µg Qu/g. This was in contrast with the finding of **Krishnamoorthy *et al.* (2019)** who reported the absence of flavonoids in extracts of the marine bivalve *Perna viridis*. Furthermore, it was in

consistent with the finding of **Minsas *et al*. (2020)**, who confirmed the presence of flavonoids in the methanolic extract of Meretrix *meretrix*. This flavonoid content might be the reason behind the antioxidant effect observed here. This is supported by the fact that flavonoid compounds have an excellent antioxidant activity **(Xu *et al*., 2018; Clark *et al*., 2015)**. In addition, **Minsas *et al*. (2020)** demonstrated the active free radical scavenger response of the whole tissue of marine bivalves.

Marine bivalves exhibit promising anticancer effects against several types of cancer, especially breast cancer **(Cheong *et al*., 2013; liao *et al*., 2016)**. **Wu *et al*. (2017)** and **Chi *et al*. (2015)** reported the anticancer effect of some extracts obtained from marine bivalves such as *Meretrix Meretrix* and *Tegillarca granosa*. **Lee *et al*. (2017)** also assured the anticancer effect of anticancer *Patinopecten yessoensis* extract on MCF-7 human breast cancer cells. These studies were in line with the current study. As the extract of *Au* proved cytotoxic effects on the representative cancer cell line, MCF-7. The possible cytotoxic effect of *Au* extract against MCF-7 cell line, in the present study, could be explained by several reasons. First, about ten out of the detected compounds have several referenced anticancer activities, especially against breast cancer cells. Second, the presence of high content of flavonoids, which have an anticancer effect as reported by **Martins *et al*. (2019)**. Finally, its protein content may enhance, as that of other bivalves, the abnormal cell toxicity against several types of cancer include breast cancer **(Wu *et al*., 2017)**. Based on the above-mentioned results and according to **Carlisi *et al*. (2016)** who stated that the anti-cancer drugs trigger reactive oxygen species generation, it may be proposed that *Au* extract may be used as a chemopreventive agent, as well as a cancer cytotoxic one. So, this article highlights the potential pharmacological benefits of *Au* extract and opens the gate for further investigation with each category of compounds from *Au*.

REFERENCES

Al-Saeedi, A. H. and Hossain, M. A. (2015). Total phenols, total flavonoids contents and free radical scavenging activity of seeds crude extracts of *pigeon pea* traditionally used in Oman for the treatment of several chronic diseases. Asian Pacific Journal of Tropical Disease., 5: 316-321. https://doi.org/10.1016/S2222-1808(14)60790-8

Arruda, C.; Mejía, J. A. A.; Ribeiro, V. P.; Borges, C. H. G.; Martins, C. H. G.; Veneziani, R. C. S. and Bastos, J. K. (2019). Occurrence, chemical composition, biological activities, and analytical methods on *Copaifera* genus—A review. Biomedicine and Pharmacotherapy., 109: 1-20. https://doi.org/10.1016/j.biopha.2018.10.030

Atanasov, A. G.; Zotchev, S. B.; Dirsch, V. M. and Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. Nature Reviews Drug Discovery., 20: 200-216. https://www.nature.com/articles/s41573-020-00114-z

Atta, A. H.; Nasr, S. M.; Mouneir, S. M.; Ibrahim, S. I. and Atta, S. A. (2018). *Alhagi Maurorum* Methanol Extract ameliorates gastric ulceration and liver injury in a rat model. Wulfenia Journal., 25: 20-40.

Babar, A. G.; Pande, A. and Kulkarni, B. G. (2016). Anti-fungal activity and investigation of bioactive compounds of marine intertidal bivalve *Gafrarium divaricatum* from West coast of India. Int J Pure App Bio., 4: 211-217. http://dx.doi.org/10.18782/2320-7051.2273

Batiha, G. E. S.; Beshbishy, A. M.; Ikram, M.; Mulla, Z. S.; El-Hack, M. E. A.; Taha, A. E. and Elewa, Y. H. A. (2020). The pharmacological activity, biochemical properties, and pharmacokinetics of the major natural polyphenolic flavonoid: quercetin. Foods., 9: 374. https://doi.org/10.3390/foods9030374

Balamurugan, A.; Evanjaline, M.; Parthipan, B. and Mohan, V. (2017). GC-MS analysis of bioactive compounds from the ethanol extract of leaves of *Neibuhriaapetala Dunn*. International Research Journal of Pharmacy., 8: 72-78. DOI: 10.1016/0003-2697(76)90527-3

Belakhdar, G.; Benjouad, A. and Abdennebi, E. H. (2015). Determination of some bioactive chemical constituents from *Thesium humile Vahl*. J Mater Environ Sci., 6: 2778-2783.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry., 72: 248-254. DOI:10.1006/abio.1976.9999

Cai, Y.; Chen, T. and Xu, Q. (2003). Astilbin suppresses collagen-induced arthritis via the dysfunction of lymphocytes. Inflammation Research., 52: 334-340. https://doi.org/10.1007/s00011-003-1179-3

Carlisi, D.; Buttitta, G.; Di Fiore, R.; Scerri, C.; Drago-Ferrante, R.; Vento, R. and Tesoriere, G. (2016). Parthenolide and DMAPT exert cytotoxic effects on breast cancer stem-like cells by inducing oxidative stress, mitochondrial dysfunction, and necrosis. Cell Death and Disease., 7: e2194-e2194. DOI:10.1038/cddis.2016.94

Chakraborty, K. and Joy, M. (2020). High-value compounds from the molluscs of marine and estuarine ecosystems as prospective functional food ingredients: An overview. Food Research International., 137: 109637. https://doi.org/10.1016/j.foodres.2020.109637

Chen, L.; Lan, Z.; Zhou, Y.; Li, F.; Zhang, X.; Zhang, C. and Li, P. (2011). Astilbin attenuates hyperuricemia and ameliorates nephropathy in fructose-induced hyperuricemic rats. Planta Medica., 77: 1769-1773. DOI: 10.1055/s-0030-1271135

Cheong, S. H.; Kim, E. K.; Hwang, J. W.; Kim, Y. S.; Lee, J. S.; Moon, S. H. and Park, P. J. (2013). Purification of a novel peptide derived from a shellfish, *Crassostrea gigas*, and evaluation of its anticancer property. Journal of Agricultural and Food Chemistry., 61: 11442-11446. https://doi.org/10.1021/jf4032553

Cheng, D.; Guo, Z. and Zhang, S. (2015). Effect of β-sitosterol on the expression of HPV E6 and p53 in cervical carcinoma cells. Contemporary Oncology., 19: 36. https://doi.org/10.5114/wo.2015.50011

Chi, C. F.; Hu, F. Y.; Wang, B.; Li, T. and Ding, G. F. (2015). Antioxidant and anticancer peptides from the protein hydrolysate of blood clam (*Tegillarca granosa*) muscle. Journal of Functional Foods., 15: 301-313. https://doi.org/10.1016/j.jff.2015.03.045

Ciavatta, M. L.; Lefranc, F.; Carbone, M.; Mollo, E.; Gavagnin, M.; Betancourt, T. and Kiss, R. (2017). Marine Mollusk- Derived Agents with Antiproliferative Activity as Promising Anticancer Agents to Overcome Chemotherapy Resistance. Medicinal Research Reviews., 37: 702-801. https://doi.org/10.1002/med.21423

Clark, J. L.; Zahradka, P. and Taylor, C. G. (2015). Efficacy of flavonoids in the management of high blood pressure. Nutrition Reviews., 73: 799-822. https://doi.org/10.1093/nutrit/nuv048

Das, U. N. (2018). Arachidonic acid and other unsaturated fatty acids and some of their metabolite's function as endogenous antimicrobial molecules: A review. Journal of Advanced Research., 11: 57-66. https://doi.org/10.1016/j.jare.2018.01.001

Diab, T. A.; Donia, T. and Saad-Allah, K. M. (2021). Characterization, antioxidant, and cytotoxic effects of some Egyptian wild plant extracts. Beni-Suef University Journal of Basic and Applied Sciences., 10: 1-13. https://doi.org/10.1186/s43088-021-00111-0

El-Agamy, A. E.; Mohammed, S. Z.; El-Shahawi, A. M. and Kamel, Z., Z. (2005). Reproductive biology of the cockle *Anadara uropigimelana* from the Port-Said beaches. Zag. Vet. J., 33: 232- 247.

Gangadhara, K. L.; Lescrinier, E.; Pannecouque, C. and Herdewijn, P. (2014). Hydroxy fatty acids for the delivery of dideoxynucleosides as anti-HIV agents. Bioorganic and Medicinal Chemistry Letters., 24: 817-820. https://doi.org/10.1016/j.bmcl.2013.12.093

García-Morales, H.; Gutiérrez-Millán, L. E.; Valdez, M. A.; Burgos-Hernández, A.; Gollas-Galván, T. and Burboa, M. G. (2016). Antiproliferative activity of protein extracts from the black clam (*Chione fluctifraga*) on human cervical and breast cancer cell lines. African Journal of Biotechnology., 15: 214-220. DOI: 10.5897/AJB2014.13783

Gomathi Rajashyamala, L. and Elango, V. (2015). Identification of bioactive components and its biological activities of *Evolvulus alsinoides linn*. A GC–MS study. International Journal of Chemical Studies., 3: 41-44.

González, A. M.; Tracanna, M. I.; Amani, S. M.; Schuff, C.; Poch, M. J.; Bach, H. and Catalán, C. A. (2012). Chemical composition, antimicrobial and antioxidant properties of the volatile oil and methanol extract of *Xenophyllum poposum*. Natural Product Communications., 7: 1934578X1200701230. https://doi.org/10.1177/1934578X1200701230

Guil- Guerrero, J. L. (2007). Stearidonic acid (18: 4n- 3): Metabolism, nutritional importance, medical uses, and natural sources. European Journal of Lipid Science and Technology., 109: 1226-1236. https://doi.org/10.1002/ejlt.200700207

Gupta, R.; Sharma, A. K.; Dobhal, M. P.; Sharma, M. C. and Gupta, R. S. (2011). Antidiabetic and antioxidant potential of β- sitosterol in streptozotocin- induced experimental hyperglycemia. Journal of Diabetes., 3: 29-37. https://doi.org/10.1111/j.1753-0407.2010.00107.x

Gutierrez, R. M. P. (2013). Antihepatotoxic, nephroprotective, and antioxidant activities of phenolic compounds from *Satureja macrostema* leaves against carbon tetrachloride-induced hepatic damage in mice. Medicinal Chemistry Research., 22: 1846-1855. https://doi.org/10.1007/s00044-012-0176-x

Horia, E. and Watkins, B. A. (2005). Comparison of stearidonic acid and α-linolenic acid on PGE2 production and COX-2 protein levels in MDA-MB-231 breast cancer cell cultures. The Journal of Nutritional Biochemistry., 16: 184-192. https://doi.org/10.1016/j.jnutbio.2004.11.001

Hossain, M. A.; Al Kalbani, M. S. A.; Al Farsi, S. A. J.; Weli, A. M. and Al-Riyami, Q. (2014). Comparative study of total phenolics, flavonoids contents and evaluation of antioxidant and antimicrobial activities of different polarities fruits crude extracts of *Datura metel L*. Asian Pacific Journal of Tropical Disease., 4: 378-383. https://doi.org/10.1016/S2222-1808(14)60591-0

Hossain, M. A.; Weli, A. M. and Ahmed, S. H. I. (2019). Comparison of total phenols, flavonoids, and antioxidant activity of various crude extracts of *Hyoscyamus gallagheri* traditionally used for the treatment of epilepsy. Clinical Phytoscience., 5: 1-8. https://doi.org/10.1186/s40816-019-0114-2

Hsu, Y. L.; Uen, Y. H.; Chen, Y.; Liang, H. L. and Kuo, P. L. (2009). Tricetin, a dietary flavonoid, inhibits proliferation of human breast adenocarcinoma mcf-7 cells by blocking

cell cycle progression and inducing apoptosis. Journal of Agricultural and Food Chemistry., 57: 8688-8695. https://doi.org/10.1021/jf901053x

Hui, L. M.; Zhao, G. D. and Zhao, J. J. (2015). δ-Cadinene inhibits the growth of ovarian cancer cells via caspase-dependent apoptosis and cell cycle arrest. International Journal of Clinical and Experimental Pathology., 8: 6046.

Jasril, J.; Mooi, L. Y.; Ali, A. M.; Sukari, M. A.; Rahman, A. A.; Othman, A. G. and Nakatani, N. (2003). Antioxidant and antitumor promoting activities of the flavonoids from *Hedychium thyrsiforme*. Pharmaceutical Biology., 41: 506-511. https://doi.org/10.1080/13880200308951344

Jha, R. K. and Zi-Rong, X. (2004). Biomedical compounds from marine organisms. Marine Drugs., 2: 123-146. https://doi.org/10.3390/md203123

Johnson, M. A. C. B. and Bradford, C. (2014). Omega-3, omega-6 and omega-9 fatty acids: implications for cardiovascular and other diseases. J Glycomics Lipidomics., 4: 2153-0637. DOI: 0.4172/2153-0637.1000123

Joshua, P. E.; Anosike, C. J.; Asomadu, R. O.; Ekpo, D. E.; Uhuo, E. N. and Nwodo, O. F. C. (2020). Bioassay-guided fractionation, phospholipase A2-inhibitory activity and structure elucidation of compounds from leaves of *Schumanniophyton magnificum*. Pharmaceutical Biology., 58: 1078-1085. https://doi.org/10.1080/13880209.2020.1839510

Joy, M.; Chakraborty, K. and Pananghat, V. (2016). Comparative bioactive properties of bivalve clams against different disease molecular targets. Journal of Food Biochemistry., 40: 593-602. https://doi.org/10.1111/jfbc.12256

Jump, D. B.; Depner, C. M. and Tripathy, S. (2012). Omega-3 fatty acid supplementation and cardiovascular disease: thematic review series: new lipid and lipoprotein targets for the treatment of cardiometabolic diseases. Journal of Lipid Research., 53: 2525-2545. https://doi.org/10.1194/jlr.R027904

Karaulova, E. P.; Yakush, E. V.; Slutskaya, T. N. and Shulgina, L. V. (2021). Proximate Composition, Antioxidant Properties, and Hepatoprotective Activity of Three Species of Shellfish of the Pacific Coast of Russia. Molecules., 26: 3397. https://doi.org/10.3390/molecules26113397

Kim, B. R.; Kim, H. M.; Jin, C. H.; Kang, S. Y.; Kim, J. B.; Jeon, Y. G. and Han, A. R. (2020). Composition and antioxidant activities of volatile organic compounds in radiation-bred Coreopsis cultivars. Plants., 9: 717. https://doi.org/10.3390/plants9060717

Koracevic, D.; Koracevic, G.; Djordjevic, V.; Andrejevic, S. and Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. Journal of Clinical Pathology., 54: 356-361. http://dx.doi.org/10.1136/jcp.54.5.356

Knolker, H. J.; Agarwal, S.; Schlechtingen, G.; Brammeier, T. and Schroeder, C. (2008). CHOLESTERYLAMINES FOR THE TREATMENT AND PREVENTION OF INFECTIOUS DISEASES (WO/2008/068037). WIPO. https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2008068037

Krishnamoorthy, K. and Subramaniam, P. (2014). Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC-MS. International Scholarly Research Notices., 2014: 1-13. http://doi.org/10.1155/2014/567409

Krishnamoorthy, V.; Chuen, L. Y.; Sivayogi, V.; Kathiresan, S.; Bahari, M. B.; Raju, G. and Parasuraman, S. (2019). Exploration of antioxidant capacity of extracts of *Perna viridis*, a marine bivalve. Pharmacognosy Magazine., 15: 402. DOI: 10.4103/pm.pm_301_19

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature., 227: 680-685. https://doi.org/10.1038/227680a0

Lee, C.; Chun, W.; Zhao, R.; Kim, Y. D.; Nam, M. M.; Jung, D. H. and An, W. G. (2017). Anticancer effects of an extract from the scallop *Patinopecten yessoensis* on MCF 7 human breast carcinoma cells. Oncology Letters., 14: 2207-2217. https://doi.org/10.3892/ol.2017.6424

Li, G. S.; Jiang, W. L.; Yue, X. D.; Qu, G. W.; Tian, J. W.; Wu, J. and Fu, F. H. (2009). Effect of astilbin on experimental diabetic nephropathy in vivo and in vitro. Planta Medica., 75: 1470-1475. DOI: 10.1055/s-0029-1185802

Liao, J. H.; Chien, C. T. H.; Wu, H. Y.; Huang, K. F.; Wang, I.; Ho, M. R. and Wu, S. H. (2016). A multivalent marine lectin from *Crenomytilus grayanus* possesses anti-cancer activity through recognizing globotriose Gb3. Journal of the American Chemical Society., 138: 4787-4795. https://doi.org/10.1021/jacs.6b00111

Lukiw, W. J.; Cui, J. G.; Marcheselli, V. L.; Bodker, M.; Botkjaer, A.; Gotlinger, K. and Bazan, N. G. (2005). A role for docosahexaenoic acid–derived neuroprotectin D1 in neural cell survival and Alzheimer disease. The Journal of Clinical Investigation., 115: 2774-2783. https://doi.org/10.1172/JCI25420

Maia, G. L. D. A.; Falcão-Silva, V. D. S.; Aquino, P. G. V.; Araújo-Júnior, J. X. D.; Tavares, J. F.; Silva, M. S. D. and Barbosa-Filho, J. M. (2011). Flavonoids from

Praxelis clematidea RM King and Robinson modulate bacterial drug resistance. Molecules., 16: 4828-4835. https://doi.org/10.3390/molecules16064828

Mansour, M.; Van Ginkel, S.; Dennis, J. C.; Mason, B.; Elhussin, I.; Abbott, K. and Morrison, E. (2018). The combination of omega-3 stearidonic acid and docetaxel enhances cell death over docetaxel alone in human prostate cancer cells. Journal of Cancer., 9: 4536. DOI: 10.7150/jca.26681

Martins, B. T.; Correia da Silva, M.; Pinto, M.; Cidade, H. and Kijjoa, A. (2019). Marine natural flavonoids: chemistry and biological activities. Natural Product Research., 33: 3260-3272. https://doi.org/10.1080/14786419.2018.1470514

Matejić, J. S.; Stojanović-Radić, Z. Z.; Ristić, M. S.; Veselinović, J. B.; Zlatković, B. K.; Marin, P. D. and Džamić, A. M. (2018). Chemical characterization, in vitro biological activity of essential oils and extracts of three *Eryngium L*. species and molecular docking of selected major compounds. Journal of Food Science and Technology., 55: 2910-2925. https://doi.org/10.1007/s13197-018-3209-8

Minsas, S.; Nurdiansyah, S. I.; Prayitno, D. I.; Sofiana, M. S. J.; Kalija, T. A. and Fadly, D. (2020). Screening of Bioactive Compounds and Antioxidant Activity of Ale-ale Shellfish (*Meretrix meretrix*) Crude Extracts from West Kalimantan, Indonesia. Systematic Reviews in Pharmacy., 11: 222-227.

Miri, R.; Nejati, M.; Saso, L.; Khakdan, F.; Parshad, B.; Mathur, D. and Firuzi, O. (2016). Structure–activity relationship studies of 4-methylcoumarin derivatives as anticancer agents. Pharmaceutical Biology., 54: 105-110. https://doi.org/10.3109/13880209.2015.1016183

Mitić, Z. S.; Jovanović, B.; Jovanović, S. Č.; Stojanović-Radić, Z. Z.; Mihajilov-Krstev, T.; Jovanović, N. M. and Stojanović, G. S. (2019). Essential oils *of Pinus halepensis* and *P. heldreichii*: Chemical composition, antimicrobial, and insect larvicidal activity. Industrial Crops and Products., 140: 111702. https://doi.org/10.1016/j.indcrop.2019.111702

Moniruzzaman, M.; Sku, S.; Chowdhury, P.; Tanu, M. B.; Yeasmine, S.; Hossen, M. N. and Mahmud, Y. (2021). Nutritional evaluation of some economically important marine and freshwater mollusc species of Bangladesh. Heliyon., 7: e07088. https://doi.org/10.1016/j.heliyon.2021.e07088

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods., 65: 55-63. https://doi.org/10.1016/0022-1759(83)90303-4

Moulari, B.; Pellequer, Y.; Lboutounne, H.; Girard, C.; Chaumont, J. P.; Millet, J. and Muyard, F. (2006). Isolation and in vitro antibacterial activity of astilbin, the bioactive

flavanone from the leaves of *Harungana madagascariensis Lam*. ex Poir. (*Hypericaceae*). Journal of Ethnopharmacology., 106: 272-278. https://doi.org/10.1016/j.jep.2006.01.008

Musa, M. A.; Badisa, V. L.; Latinwo, L. M.; Patterson, T. A. and Owens, M. A. (2012). Coumarin-based benzopyranone derivatives induced apoptosis in human lung (A549) cancer cells. Anticancer Research., 32: 4271-4276.

Newell, M.; Baker, K.; Postovit, L. M. and Field, C. J. (2017). A critical review on the effect of docosahexaenoic acid (DHA) on cancer cell cycle progression. International Journal of Molecular Sciences., 18: 1784. https://doi.org/10.3390/ijms18081784

Odeleye, T.; White, W. L. and Lu, J. (2019). Extraction techniques and potential health benefits of bioactive compounds from marine molluscs: a review. Food and Function., 10: 2278-2289. DOI: 10.1039/c9fo00172g

Pandith, H.; Zhang, X.; Thongpraditchote, S.; Wongkrajang, Y.; Gritsanapan, W. and Baek, S. J. (2013). Effect of Siam weed extract and its bioactive component scutellarein tetramethyl ether on anti-inflammatory activity through NF-κB pathway. Journal of Ethnopharmacology., 147: 434-441. https://doi.org/10.1016/j.jep.2013.03.033

Pereira, D. M.; Correia-da-Silva, G.; Valentão, P.; Teixeira, N. and Andrade, P. B. (2014). Anti-inflammatory effect of unsaturated fatty acids and ergosta-7, 22-dien-3-ol from *Marthasterias glacialis*: Prevention of CHOP-mediated ER-stress and NF-κB activation. PLoS One., 9: e88341. https://doi.org/10.1371/journal.pone.0088341

Petacci, F.; Freitas, S. S.; Brunetti, I. L. and Khalil, N. M. (2010). Inhibition of peroxidase activity and scavenging of reactive oxygen species by astilbin isolated from *Dimorphandra mollis* (*Fabaceae, Caesalpinioideae*). Biological Research., 43: 63-74. http://dx.doi.org/10.4067/S0716-97602010000100008

Phatangare, N. D.; Deshmukh, K. K.; Murade, V. D.; Naikwadi, P. H.; Hase, D. P.; Chavhan, M. J. and Velis, H. E. (2017). Isolation and characterization of β-sitosterol from *Justicia gendarussa burm*. F.-An anti-inflammatory compound. International Journal of Pharmacognosy and Phytochemical Research., 9: 1280-1287. DOI: 10.25258/phyto.v9i09.10317

Pizato, N.; Luzete, B. C.; Kiffer, L. F. M. V.; Corrêa, L. H.; de Oliveira Santos, I.; Assumpção, J. A. F. and Magalhães, K. G. (2018). Omega-3 docosahexaenoic acid induces pyroptosis cell death in triple-negative breast cancer cells. Scientific Reports., 8: 1-12. https://doi.org/10.1038/s41598-018-20422-0

Ramasamy, M. and Balasubramanian, U. (2012). Identification of bioactive compounds and antimicrobial activity of marine clam *Anadara granosa* (Linn.). Int. J. Sci. Nat., 3: 263-266.

Rashid, Z. M.; Ali, A. M.; Douzenel, P.; Bourgougnon, N.; Shaari, K.; Andriani, Y. and Mohamad, H. (2018). Phenolics, fatty acids composition and biological activities of various extracts and fractions of Malaysian *Aaptos aaptos*. Asian Pacific Journal of Tropical Biomedicine, 8: 554. DOI: 10.4103/2221-1691.245971

Sancheti, S.; Sancheti, S. and Seo, S. Y. (2013). Ameliorative effects of 7-methylcoumarin and 7-methoxycoumarin against CCl4-induced hepatotoxicity in rats. Drug and Chemical Toxicology., 36: 42-47. https://doi.org/10.3109/01480545.2011.648329

Shady, N. H.; Fouad, M. A.; Salah Kamel, M.; Schirmeister, T. and Abdelmohsen, U. R. (2019). Natural product repertoire of the genus *Amphimedon.* Marine Drugs., 17: 19. https://doi.org/10.3390/md17010019

Shi, H.; Wang, H.; Wang, M. and Li, X. (2009). Antioxidant activity and chemical composition of *Torreya grandis* cv. Merrillii seed. Natural Product Communications., 4: 1934578X0900401122. https://doi.org/10.1177/1934578X0900401122

Singh, B.; Sahu, P. M. and Sharma, R. A. (2017). Flavonoids from *Heliotropium subulatum* exudate and their evaluation for antioxidant, antineoplastic and cytotoxic activities II. Cytotechnology., 69: 103-115. https://doi.org/10.1007/s10616-016-0041-8

Sung, J.; Jeon, H.; Kim, I. H.; Jeong, H. S. and Lee, J. (2017). Anti- Inflammatory Effects of Stearidonic Acid Mediated by Suppression of NF- κB and MAP- Kinase Pathways in Macrophages. Lipids., 52: 781-787. https://doi.org/10.1007/s11745-017-4278-6

Suresh, A.; Praveenkumar, R.; Thangaraj, R.; Oscar, F. L.; Baldev, E.; Dhanasekaran, D. and Thajuddin, N. (2014). Microalgal fatty acid methyl ester a new source of bioactive compounds with antimicrobial activity. Asian Pacific Journal of Tropical Disease., 4: S979-S984. https://doi.org/10.1016/S2222-1808(14)60769-6

Tan, K.; Ma, H.; Li, S. and Zheng, H. (2020). Bivalves as future source of sustainable natural omega-3 polyunsaturated fatty acids. Food Chemistry., 311: 125907. https://doi.org/10.1016/j.foodchem.2019.125907

Tataringa, G. and Zbancioc, A. M. (2020). Coumarin Derivatives with Antimicrobial and Antioxidant Activities. In: "Phytochemicals in Human Health." Rao, V., Mans, D. and Rao, L. (Eds.). IntechOpen., London, United Kingdom, pp. 1-19.

Umayaparvathi, S.; Meenakshi, S.; Vimalraj, V.; Arumugam, M.; Sivagami, G. and Balasubramanian, T. (2014). Antioxidant activity and anticancer effect of bioactive

peptide from enzymatic hydrolysate of oyster (*Saccostrea cucullata*). Biomedicine and Preventive Nutrition., 4: 343-353. https://doi.org/10.1016/j.bionut.2014.04.006

Venugopal, V. and Gopakumar, K. (2017). Shellfish: nutritive value, health benefits, and consumer safety. Comprehensive Reviews in Food Science and Food Safety., 16: 1219-1242. https://doi.org/10.1111/1541-4337.12312

Wang, J.; Zhao, Y. and Xu, Q. (2004). Astilbin prevents concanavalin A-induced liver injury by reducing TNF-α production and T lymphocyte adhesion. Journal of Pharmacy and Pharmacology., 56: 495-502. https://doi.org/10.1211/0022357023033

Wang, D.; Li, S.; Chen, J.; Liu, L. and Zhu, X. (2017). The effects of astilbin on cognitive impairments in a transgenic mouse model of Alzheimer's disease. Cellular and Molecular Neurobiology., 37: 695-706. https://doi.org/10.1007/s10571-016-0405-9

Wijsman, J. W. M.; Troost, K.; Fang, J. and Roncarati, A. (2019). Global production of marine bivalves. Trends and challenges. In: "Goods and services of marine bivalves." Smaal, A. C., Ferreira, J. G., Grant, J., Petersen, J. K. and Strand, O. (Eds). Springer Open., New York, United states, pp. 7-26.

Wright, A. C.; Fan, Y. and Baker, G. L. (2018). Nutritional value and food safety of bivalve molluscan shellfish. Journal of Shellfish Research., 37:695-708. https://doi.org/10.2983/035.037.0403

Wu, Y.; Hu, X.; Song, L.; Zhu, J. and Yu, R. (2014). The inhibitory effect of a novel polypeptide fraction from *Arca subcrenata* on cancer-related inflammation in human cervical cancer HeLa cells. The Scientific World Journal., 2014: 1-8. https://doi.org/10.1155/2014/768938

Wu, J.; Wang, P. and Zhou, M. (2017). Study on the anti-tumor mechanism of Meretrix meretrix glycopeptide. In AIP Conference Proceedings. AIP Publishing LLC., 1820: 030014. https://doi.org/10.1063/1.4977271

Xu, H.; Luo, J.; Huang, J. and Wen, Q. (2018). Flavonoid's intake and risk of type 2 diabetes mellitus: A meta-analysis of prospective cohort studies. Medicine., 97: e0686. DOI: 10.1097/MD.0000000000010686

Xue, M.; Wang, Q.; Zhao, J.; Dong, L.; Ge, Y.; Hou, L. and Zheng, Z. (2014). Docosahexaenoic acid inhibited the Wnt/β-catenin pathway and suppressed breast cancer cells in vitro and in vivo. The Journal of Nutritional Biochemistry., 25: 104-110. https://doi.org/10.1016/j.jnutbio.2013.09.008

Yadav, A.; Yadav, M.; Kumar, S.; Sharma, D. and Yadav, J. P. (2018). In vitro antioxidant activities and GC-MS analysis of different solvent extracts of *Acacia nilotica* leaves.

Indian Journal of Pharmaceutical Sciences., 80: 892-902. DOI: 10.4172/pharmaceutical-sciences.1000436

Yang, X. R.; Qiu, Y. T.; Zhao, Y. Q.; Chi, C. F. and Wang, B. (2019). Purification and characterization of antioxidant peptides derived from protein hydrolysate of the marine bivalve mollusk *Tergillarca granosa*. Marine Drugs., 17: 251. https://doi.org/ 10.3390/ md17050251

Yap, C. K. (2020). Antiviral compounds from marine bivalves for evaluation against SARS-CoV-2. J. PeerSci., 2: e1000015.

Yinusa, I.; George, N. I.; Ayo, R. G. and Rufai, Y. (2015). Evaluation of the pharmacological activities of beta-sitosterol isolated from the bark of *sarcocephalus latifolius* (smith bruce). Global Journal of Pure and Applied Chemistry Research., 3: 7-14.

Zeb, M.; Khan, S.; Rahman, T.; Sajid, M. and Seloni, S. (2017). Isolation and Biological Activity of β-Sitosterol and Stigmasterol from the Roots of *Indigofera heterantha.* Pharmacy and Pharmacology International Journal., 5: 139. DOI: 10.15406/ppij.2017.05.00139

Zhang, C.; Yu, H., Shen, Y.; Ni, X.; Shen, S. and Das, U. N. (2015). Polyunsaturated fatty acids trigger apoptosis of colon cancer cells through a mitochondrial pathway. Archives of medical science: AMS., 11: 1081. DOI: 10.5114/aoms.2015.54865

Zhang, Y.; Guallar, E.; Blasco-Colmenares, E.; Harms, A. C.; Vreeken, R. J.; Hankemeier, T. and Cheng, A. (2016). Serum-based oxylipins are associated with outcomes in primary prevention implantable cardioverter defibrillator patients. PloS one., 11: e0157035.