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

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**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\textsubscript{50} value was 0.579 mg/ml after 72 h of treatment). Au extract exhibited higher DPPH scavenging activity, with IC\textsubscript{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 10 min at 4°C). The supernatant was neglected, and the precipitate was re-homogenized and centrifuged (3615 x g for 10 min 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 200°C and 250°C, respectively. Volume injected was 1.0 μl of the sample. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250°C, 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 H₂O₂, while the residual H₂O₂ 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:

\[
\text{Total antioxidant concentration (mM / L)} = (A_B - A_{SA}) \times 3.33.
\]
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 45 min 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 517 nm using a UV-visible spectrophotometer. The DPPH scavenging activity (%) was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = \left( \frac{(A_C - A_S)}{A_C} \right) \times 100.$$  

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 $5 \times 10^3$ cells per well and left to attach for 24 h at standard culture conditions, 37ºC 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 37ºC. 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:

$$\text{Cell viability (\%)} = \left( \frac{\text{Mean OD}_{\text{treatment}}}{\text{Mean OD}_{\text{control}}} \right) \times 100.$$  

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**

Bandung 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 95°C, cooled on ice then loaded on 10% acrylamide gel. Electrophoresis was carried out at 200 V for 35 – 40 min. 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

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Substance</th>
<th>Molecular Formula</th>
<th>Mass</th>
<th>Retention Index</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Arachidonic acid</td>
<td>C_{20}H_{32}O_{2}</td>
<td>304.5</td>
<td>18.163</td>
<td>5.74</td>
</tr>
<tr>
<td>19</td>
<td>6,7-Dimethoxy-4-ethylcoumarin</td>
<td>C_{13}H_{14}O_{4}</td>
<td>234.25</td>
<td>18.242</td>
<td>2.55</td>
</tr>
<tr>
<td>20</td>
<td>2α,3α-Epoxy-5α-Cholestanate</td>
<td>C_{27}H_{46}O</td>
<td>386.7</td>
<td>18.336</td>
<td>20.17</td>
</tr>
<tr>
<td>21</td>
<td>9,12-Octadecadienoic acid (Z, Z)-</td>
<td>C_{18}H_{32}O_{2}</td>
<td>280.4</td>
<td>19.517</td>
<td>0.93</td>
</tr>
<tr>
<td>22</td>
<td>Methyl 2-hydroxystearate</td>
<td>C_{19}H_{38}O_{3}</td>
<td>314.5</td>
<td>20.171</td>
<td>1.14</td>
</tr>
<tr>
<td>23</td>
<td>Quercetin 3,5,7,3′,4′-Pentamethyl ether</td>
<td>C_{20}H_{20}O_{7}</td>
<td>372.4</td>
<td>20.57</td>
<td>0.5</td>
</tr>
<tr>
<td>24</td>
<td>8-Carboxy-3-methylflavone</td>
<td>C_{17}H_{12}O_{4}</td>
<td>280.27</td>
<td>21.428</td>
<td>1.02</td>
</tr>
<tr>
<td>25</td>
<td>Cis-4,7,10,13,16,19 Docosahexaenoic acid</td>
<td>C_{22}H_{32}O_{2}</td>
<td>328.5</td>
<td>21.853</td>
<td>1.65</td>
</tr>
<tr>
<td>26</td>
<td>(±)-δ-Cadinene</td>
<td>C_{15}H_{24}</td>
<td>204.35</td>
<td>22.293</td>
<td>15.19</td>
</tr>
<tr>
<td>27</td>
<td>Thunbergol</td>
<td>C_{20}H_{34}O</td>
<td>290.5</td>
<td>22.59</td>
<td>11.86</td>
</tr>
<tr>
<td>28</td>
<td>4′,5,6,7- Tetramethoxy flavanone</td>
<td>C_{19}H_{18}O_{6}</td>
<td>342.3</td>
<td>23.158</td>
<td>0.22</td>
</tr>
</tbody>
</table>

**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 (R² = 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 IC₅₀ 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).

![Graph showing DPPH scavenging activity of Au extract and ascorbic acid](image)

**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 IC₅₀ 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 ≤ 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 $63.85 \pm 1.17 \mu g/\mu l$. 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.
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

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th><strong>Compound nature</strong></th>
<th>Bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methyl 15-methoxyhexadecanoate</td>
<td>Fatty acid methyl ester</td>
<td>Antimicrobial (Shady et al., 2019).</td>
</tr>
<tr>
<td>2</td>
<td>4-Nonenoic acid, methyl ester</td>
<td>Fatty acid methyl ester</td>
<td>Antimicrobial (Matejić et al., 2018).</td>
</tr>
<tr>
<td>3</td>
<td>6,4′-Dimethoxy-3-hydroxyflavone</td>
<td>Flavonoid</td>
<td>Antioxidant, antihepatotoxic, and nephroprotective (Gutierrez, 2013). Antitumor (Jasril et al., 2003).</td>
</tr>
<tr>
<td>4</td>
<td>19,20-DiHDPA</td>
<td>Oxylipin</td>
<td>Ventricular arrhythmias marker and anti- high Ca(_2^+) sensitivity in human lung (Zhang et al., 2016).</td>
</tr>
<tr>
<td></td>
<td>Chemical Name</td>
<td>Type and Characteristics</td>
<td>Biological Activity</td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------------</td>
<td>---------------------------</td>
<td>---------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>6</td>
<td>4-Ethoxy-7-methylcoumarin Coumarin</td>
<td>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).</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9-Octadecen-12-yonic acid, methyl ester</td>
<td>Fatty acid methyl ester</td>
<td>Antioxidant (Yadav et al., 2018).</td>
</tr>
<tr>
<td>8</td>
<td>Octacosanoic acid, ethyl ester</td>
<td>Fatty acid ethyl ester</td>
<td>Antioxidant (Shi et al., 2009). Anti-leukemic (HL60) (Rashid et al., 2018).</td>
</tr>
<tr>
<td>9</td>
<td>Hexadecenoic acid, ethyl ester</td>
<td>Palmitic acid ethyl ester</td>
<td>Antioxidant, and hypocholesterolemic agent (Balamurugan, 2017). Anti-inflammatory (Joshua et al., 2020).</td>
</tr>
<tr>
<td>10</td>
<td>13(Z)-Docosenoic acid</td>
<td>Monounsaturated omega-9 fatty acid</td>
<td>Inflammatory modulator (Johnson et al., 2014).</td>
</tr>
<tr>
<td>11</td>
<td>10-Octadecenoic acid, methyl ester</td>
<td>Fatty acid methyl ester</td>
<td>Antibacterial, antifungal, antioxidant and, hypocholesterolemic agent (Belakhdar et al., 2015).</td>
</tr>
</tbody>
</table>
Table 2. (continued)

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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Anti-inflammatory and anti-cancer (Krishnamoorthy and Subramaniam, 2014). Antioxidant (Kim et al., 2020).</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>9,12-Octadecadienoic acid (Z, Z)-Polyunsaturated omega-6 fatty acid (Linoleic acid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Methyl 2-hydroxystearate</td>
<td>Stearic acid</td>
<td>Delivery of anti-HIV agents (Gangadhara et al., 2014).</td>
</tr>
<tr>
<td>23</td>
<td>Quercetin 3,5,7,3′,4′,Pentamethyl ether Polyphenol flavonoid</td>
<td></td>
<td>Antioxidant and anti-inflammatory (Batiha et al., 2020).</td>
</tr>
<tr>
<td>24</td>
<td>8-Carboxy-3-methylflavone</td>
<td>Flavone</td>
<td>Anti-ulcerogenic, and hepatoprotective (Atta et al., 2018).</td>
</tr>
<tr>
<td>26</td>
<td>(+)-δ-Cadinene</td>
<td>Unsaturated hydrocarbon</td>
<td>Anti-ovary cancer (OVACR-3) (Hui, 2015). Antimicrobial (González et al., 2012).</td>
</tr>
<tr>
<td>27</td>
<td>Thunbergol</td>
<td>Monocyclic diterpene alcohol</td>
<td>Antibacterial (Mitić et al., 2019).</td>
</tr>
<tr>
<td>28</td>
<td>4′,5,6,7- Tetramethoxyflavanone Flavonoid</td>
<td></td>
<td>Anti-inflammatory (Pandith et al., 2013). Antibacterial (Maia et al., 2011).</td>
</tr>
</tbody>
</table>

**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


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


GC-MS analysis of *Anadara uropigimelana* and its effect against MCF-7 cells


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]


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


