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Chemical characterization, antibacterial, antibiofilm, and antioxidant activities of the methanolic extract of *Paratapes undulatus* clams (Born, 1778)

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ABSTRACT

Mollusks represent the second-largest animal phylum in the marine realm. They have direct and indirect commercial and medical importance worldwide. *Paratapes undulatus* is a species of saltwater clam, a marine bivalve mollusk in the family Veneridae. The current study aimed to examine the chemical profile of the methanolic extract of *P. undulatus* soft parts using Gas chromatography mass spectroscopy (GC-MS) analysis and then assessing its antibacterial, antibiofilm, and antioxidant activities. GC-MS investigations led to the identification of major components including 7-hexadecenoic acid, methyl ester, (*Z*) (32.01%), hexadecanoic acid, methyl ester (14.03%), 9-octadecenoic acid, methyl ester (8.43%), palmitoleic acid (5.75%), 5,8,11,14,17-eicosapentaenoic acid, methyl ester (5.25%), tridecanoic acid, 4,8,12-trimethyl-, methyl ester (3.44%), tetradecanoic acid, methyl ester (3.34%), and *cis*-9-hexadecenoic acid (3.23%). Moreover, the results revealed that the extract showed remarkable antibacterial, antibiofilm, and antioxidant activities. In conclusion, the present results might be helpful for pharmaceutical companies to develop natural supplements from these clams.

INTRODUCTION

Mollusks represent the second-largest invertebrate animal phylum after Arthropoda (Baldwin, 2003; Rusmore-Villaume, 2008). They are also considered one of the most important elements in food chains as a balanced source of protein (Haszprunar and Wanninger, 2012) and represent the most prominent members of marine faunal ecosystems. Some species have direct and indirect commercial importance and even medical benefits to humans (Rusmore-Villaume, 2008) and are utilized as prospective resources of bioactive products in numerous cultures worldwide (Khan *et al*., 2009).

Recently, the molluscan natural products (high content of proteins, vitamins, and trace elements) have become very valuable foodstuffs, and their synthetically formulated structural analogs have been recognized in clinical trials as promising therapeutic agents for many diseases (Alves and Diederich, 2021; Tabakaeva *et al*., 2018). Marine bivalves are considered a rich source of antimicrobial peptides that possess various biological activities such as antibacterial, antioxidant, anticoagulant, anti-inflammatory, and anticarcinogenic (Galdiero *et al*., 2015). The main cause of their bioactivities is the presence of biologically active peptides (Alves and Diederich, 2021). Bivalves occurring in Egypt represent a neglected animal group, and little is known about them or their diversity and importance (Temraz, 2016). Therefore, more light should be shed on their chemical and biological aspects.

In particular, *Paratapes undulatus*, common name undulate Venus, is a species of saltwater clams. These clams are a popular food in Egypt, inhabiting the inshore shallow sandy seabed in Timsah Lake, located near Ismailia City at the midpoint of the Suez Canal and about 80 km south of Port Said,

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Egypt (Loftus *et al*., 2015). Lately, a broad spectrum of naturally occurring bioactive molecules separated from the mollusks was utilized in the manufacture of several medications (Alves and Diederich, 2021). The strong impact of antimicrobial resistance on healthcare and the economy has led to a crucial demand to secure unique molecules with novel modes of action to reduce the harmful effects of infectious diseases (Ghareeb *et al*., 2015). Several antimicrobial compounds have been isolated from peptides subunits of marine bivalves due to their thorough capability to resist infection with different types of viruses (Khan *et al*., 2009). They also exhibit a wide range of antibacterial activities against Gram-positive and Gram-negative bacteria, as well as yeast (Burge *et al*., 2014; Schmitt *et al*., 2012). The rapid and increasing development of antimicrobial resistance is one of the current global health challenges. Pathogenic microorganisms have the capability to hinder the effect of antimicrobial agents, which leads to raising the accompanying hazards of infections via pathogenic microorganisms like bacteria, fungi, viruses, and parasites. Furthermore, the appearance of novel pathogens like SARS, H1N1, and various types of influenza has become a vital communal health risk. To overcome this phenomenon, scientists have worked to discover novel antimicrobial and antiviral pharmaceuticals from natural resources like plants, marine organisms, mollusks, and fungal extracts (Abdel-Aziz *et al*., 2018, 2021; Elkhouly *et al*., 2021a; Ghareeb *et al*., 2014a, 2019; Hamed *et al*., 2020).

On the other hand, overproduction of reactive species led to the initiation of oxidative stress that in turn initiates several dangerous disorders such as "cancer, cardiovascular, and inflammation." Moreover, the accompanying destructive side effects can be reduced using naturally occurring antiradical ingredients that act as strong free radical scavengers (Ghareeb *et al*., 2018; Sobeh *et al*., 2018).

In the current study, we aimed to investigate for the first time the chemical and biological profiles of the soft parts of the marine clams *P. undulatus* and the scientific evidence regarding their chemical composition and associated antibacterial, antibiofilm, and antioxidant activities.

MATERIALS AND METHODS

Chemicals, reagents, and instruments

All solvents, standards, and reagents are of high analytical grade. Methanol and sulfuric acid were obtained from El-Nasr Pharmaceutical Chemicals Company (Cairo, Egypt). Nutrient agar and Nutrient Broth media were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, ascorbic acid, gallic acid, Folin–Ciocalteu's reagent, sodium carbonate, sodium phosphate, and ammonium molybdate were purchased from Sigma-Aldrich (Steinheim, Germany). For antioxidant assays, the absorbance was measured using a spectrophotometer (UV-VS spectrophotometer, Milton Roy 601, CO). The obtained extract was concentrated under vacuum using a rotary evaporator, Buchi R-300 (Flawil, Switzerland).

Collection of *P. undulatus*

Venus clams (*P. undulatus*) were collected from Timsah Lake which is located close to Ismailia City at the midpoint of the Suez Canal and about 80 km south of Port Said, Egypt (Fig. 1) (El-Serehy *et al*., 2018). The collected samples were cleaned with distilled water to eliminate any sands or contaminations and then kept in a refrigerator at −18°C till being used.

Preparation of the methanolic extract of *P. undulatus* clams (Born, 1778)

Paratapes undulatus clams are a popular food in Egypt, so after being washed with distilled water, their shells were opened with stainless steel knives and their soft bodies were separated from these shells by forceps. The collected soft bodies (250 g) were homogenized and then were extracted four times with methanol (2 l) at room temperature (25°C ± 2°C). The combined extracts were concentrated to afford 19.0 g methanol extract.

Antibacterial activity assay

The antibacterial activity of the tested extract was evaluated against some pathogenic microbial strains including four Gram-negative bacteria (*Escherichia coli* ATCC 25955, *Pseudomonas aeruginosa* ATCC 10145, *Proteus vulgaris*, and *Klebsiella pneumoniae*), one Gram-positive bacterium (*Staphylococcus aureus* NRRL B-767), and one yeast (*Candida albicans* ATCC 10231) according to the reported procedures (Elkhouly *et al*., 2021b). Ciprofloxacin and nystatin were utilized as a control.

Antibiofilm activity assay

The biofilm inhibitory potential was determined against four pathogenic microbial strains, including *S. aureus* and *Bacillus subtilis* as Gram-positive bacteria as well as *P. aeruginosa* and *E. coli* as Gram-negative bacteria, according to the reported procedures (El-Shazly *et al*., 2021; Hamed *et al*., 2020).

Total phenolic content (TPC) using Folin–Ciocalteu's assay

The TPC was evaluated using Folin–Ciocalteu's reagent according to the reported procedures (El-Neekety *et al*., 2016; Hathout *et al*., 2016). Briefly, 100 µl of the tested sample (500 µg/ml) was mixed with 500 µl of Folin–Ciocalteu's reagent and 1.5 ml of sodium carbonate (20%). The reaction mixture was shaken and completed to 10 ml using distilled water. The mixture was allowed to stand for 120 minutes. Afterward, the absorbance was recorded at 765 nm. All measurements were performed in triplicate. The TPC was presented as mg gallic acid equivalent (GAE) per g extract.

Antioxidant activity evaluation

Free radical scavenging activity using DPPH

Free radical scavenging activity was evaluated using the DPPH assay according to the reported procedures (Ghareeb *et al*., 2016; Shirwaikar *et al*., 2006). Briefly, various serial concentrations from the tested sample (1.5 ml) were added to a (1.5 ml) solution of 0.1 mmol/l DPPH. Equivalent volumes of methanol and DPPH acted as the control. After incubation for 20 minutes at 37°C in the absence of light, the absorbance was recorded at 517 nm. The test was carried out in triplicate. The free radical scavenging activity was expressed in IC_{50} value (concentration from tested sample required to scavenge 50% of the radical).

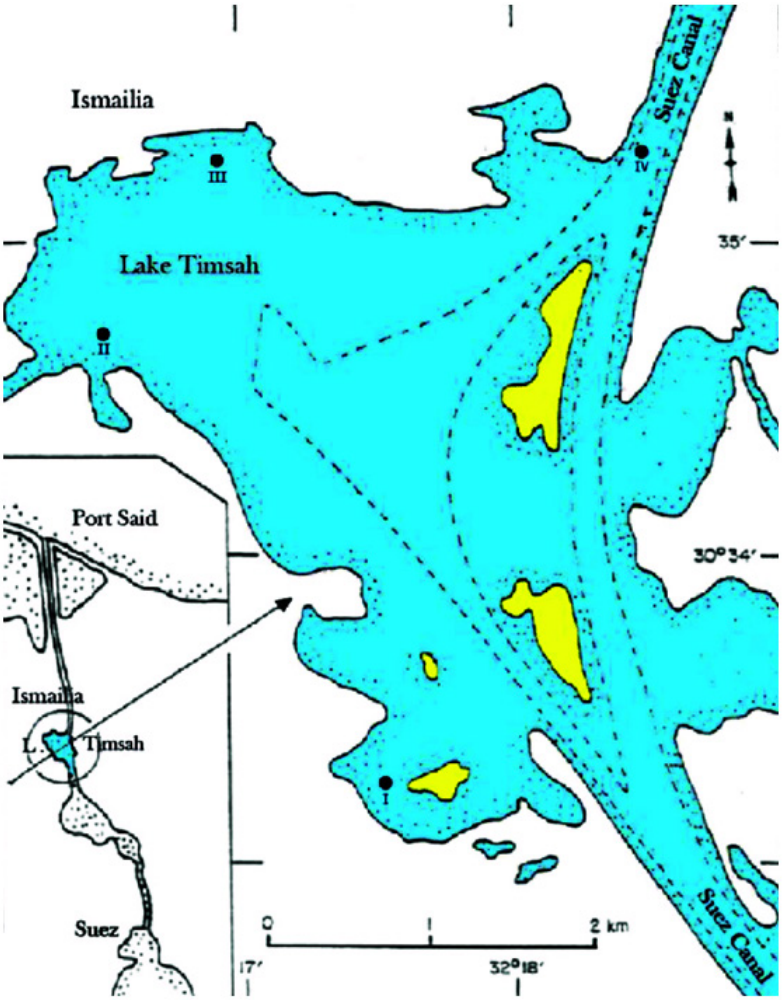


Figure 1. A map of Timsah Lake with the inset showing the position of the lake on the Suez Canal.

Total antioxidant capacity (TAC) using phosphomolybdenum assay

The TAC was evaluated using the phosphomolybdenum assay based on the reported procedures (Prieto *et al*., 1999). Basically, this assay is based on the reduction of molybdenum (VI) to molybdenum (V) by the tested sample and consequent development of a green color [phosphate = molybdenum (V)] complex at low pH with maximum absorption at 695 nm. Briefly, 0.5 ml of the tested sample (500 µg /ml) in methanol was mixed with 5 ml from the test reagent [0.6 M H_2SO_4, 28 mM NaH_2PO_4, and 4 mM $(NH_4)6Mo_7O_{24}$]. The tubes containing the tested samples and reagents were capped and incubated in a water bath at 95°C for 1.5 hours. After cooling, the absorbance was recorded at 695 nm against a blank. The blank comprised all reagents and solvents without the tested sample. All experiments were carried out in triplicate. The TAC was presented as the number of ascorbic acid equivalent (AAE) (Elkhouly *et al*., 2021; Prieto *et al*., 1999).

Chemical characterization using GC-MS analysis

GC-MS examination was conducted according to the reported procedures (Khalaf *et al*., 2020), using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 m, 0.251 mm, and 0.1 mm film thickness). An electron ionization system with ionization energy of 70 eV was utilized for GC/MS recognition. Helium gas was utilized as the carrier gas at a regular flow rate of 1 ml/minute. The injector and MS transfer line temperature was set at 280°C. The oven temperature was instructed to an initial temperature of 50°C (hold 2 minutes) to 150°C at an increasing rate of 7°C/minute, then to 270°C at an increasing rate of 5°C/minute (hold 2 minutes), and subsequently to 310°C as the definitive temperature at a growing level of 3.5°C/minute (hold 10 minutes). The quantitative determination of all the identified compounds was examined using a percent relative peak area. A tentative recognition of the components was accomplished based on the comparison of the irrelative retention time and mass spectra with those of the National Institute of Standards and Technology (NIST), Wiley library data of the GC-MS technique.

Statistical analysis

All investigations were carried out thrice, and the obtained results are recorded as the average and standard deviation

(SD). Significant variations were explored using the one-way analysis of variance. Variances at $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Marine bivalves represent a basic source of highly nutritional, low-calorie, and easily digestible food with high protein contents (Panayotova *et al*., 2020; Nair *et al*., 2015). Recently, their consumption has highly increased as they contain many bioactive molecules with biomedical importance (Eghianruwa *et al*., 2019). The extracts of the marine mollusks showed numerous biological activities like anti-infectious disease, antimicrobial, anticancer, antioxidant, and cardiovascular protection activities (Odeleye *et al*., 2019). Clams are edible bivalve mollusks that represent the most important seafood resources in the coastal regions. Previous reports revealed that the extract of these clams, either ethanolic or methanolic, showed anti-inflammatory, antioxidant, antihypertensive, and antidiabetic activities (Joy *et al*., 2016). Bivalves could be utilized as naturally occurring antioxidant supplements to relieve oxidative stress-induced diseases, as the clams' tissues are not damaged after exposure to high water salinity or any toxic chemical compounds (González *et al*., 2015).

GC-MS investigation of the methanolic extract of *P. undulatus*

GC-MS examination of the crude methanol extract of *P. undulatus* (*Born, 1778*) led to the identification of 38 compounds (Fig. 2). The recognized ingredients comprise 88.54% of the total extract composition; the characterized compounds are listed in Table 1. The major detected ingredients are 7-hexadecenoic acid, methyl ester, (*Z*) (32.01%), hexadecanoic acid, methyl ester (14.03%), 9-octadecenoic acid, methyl ester (8.43%), palmitoleic acid (5.75%), 5,8,11,14,17-eicosapentaenoic acid, methyl ester (5.25%), tridecanoic acid, 4,8,12-trimethyl-, methyl ester (3.44%), tetradecanoic acid, methyl ester (3.34%), and *cis*-9-hexadecenoic acid (3.23%) (Fig. 3). The present investigations highlight for the first time the chemical constituents of the marine clam *P. undulatus* and study the possible activities of its methanolic extract. The characterization was achieved using "computer search user-generated reference libraries" and integrating mass/mass spectra (Abdel-Wareth *et al*., 2019; Khalaf *et al*., 2020; Madkour *et al*., 2017; Shawky *et al*., 2019).

Concomitantly, Ibrahim *et al*. (2020) stated that GC-MS analysis of the methanol extract from *Holothuria atra* (sea cucumber) revealed the presence of 15 major bioactive constituents including methanesulfonylacetic acid (34.70%), 2,3-dihydro-6-hydroxy-3-oxo-2-(piperidinomethyl) pyridazine (39.23%), acetic acid butyl-methyl-phosphinoyl methyl ester (14.13%), S-methyl methanethiosulfonate (85.34%), 1,2,4-trithiolane (62.85%), 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1carboxaldehyde (5.83%), 4,25-secoobscurinervan-4-one, *O*-acetyl-22-ethyl-15,16-dimethoxy-,(22à) (29.54%), N,N'-bis(carbobenzyloxy)-lysine methyl(ester) (23.68%), 2-propen-1-ol, 2-methyl-3-(2,6,6-trimethyl-2-cyclohexen-1-yl)-,(E) (8.49%), 2-amino-3-(4-hydroxyphenyl)-propanoic acid (7.64%), cyclohexasiloxane, dodecamethyl (91.46%), Z,Z,Z-4,6,9-nonadecatriene (6.81%), cycloheptasiloxane, tetradecamethyl (72.91%), cyclooctasiloxane, hexadecamethyl (43.78%), and ergosta-5,22-dien-3-ol, acetate, (3á,22E) (6.81%). These compounds were categorized as organic and fatty acids as well as their derivatives, in addition to organic alcohols, steroids, terpenoids, amino acids, esters, and benzene derivatives. However, most of these constituents act as antibacterial and antifungal agents (Hussein *et al*., 2016; Ibrahim, 2012; Ibrahim *et al*., 2018).

Recently, Ibrahim *et al*. (2018) detected the principal components in the sponge *Ciocalypta penicillus* extracts by GC-MS analysis including fatty acids and their esters (hexadecanoic acid and octadecanoic acid), as well as steroids and terpenoids, which have antimicrobial effects. Fatty acids are able to act as anionic surfactants; they also possess antifungal and antibacterial characteristics at low pH (Donia and Hamann, 2003; Ibrahim and Abd-Elnaby, 2010). More specifically, diterpene isonitriles isolated from the tropical marine sponge *Cymbastela hooperi* and

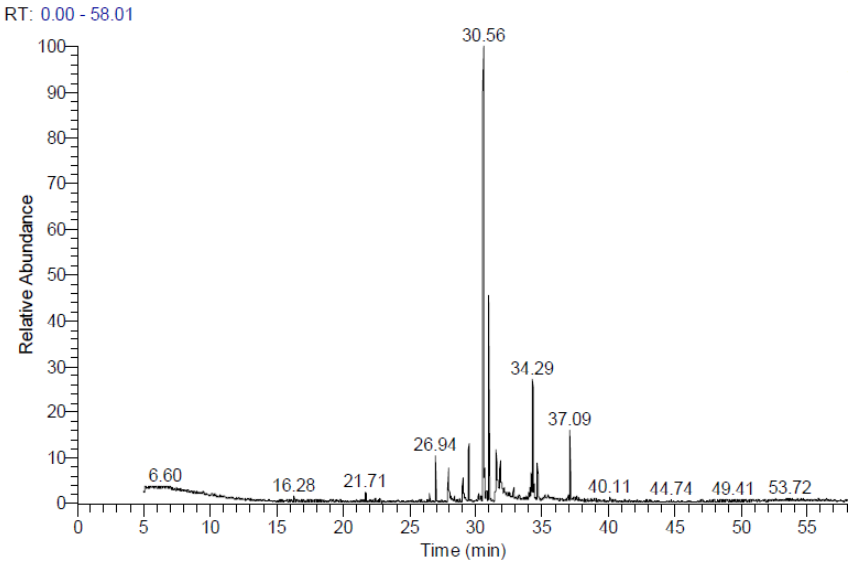


Figure 2. GC-MS chromatogram of the methanolic extract of P. undulatus clams.

Table 1. Chemical compositions of the methanolic extract of *P. undulatus* clams.

No.	R_t	Area%	MW	MF	Main fragments	Identified compounds	Class/category
1	5.22	0.45	248	$C_{16}H_{24}O_2$	84, 91, 121, 149	1,3-Dioxolane, 2-heptyl-4-phenyl	Benzene derivatives
2	10.09	0.20	242	$C_{16}H_{34}O$	57, 69, 71, 84	1-Hexadecanol	Fatty alcohol
3	16.28	0.43	296	$C_{21}H_{44}$	57, 71, 85, 99, 155	Heptadecane, 2,6,10,14-tetramethyl	Long-chain alkane
4	17.51	0.31	268	$C_{19}H_{40}$	43, 57, 71, 91, 119	Octadecane, 2-methyl	Branched alkanes
5	21.71	0.64	198	$C_{14}H_{30}$	41, 43, 57, 70, 85, 99, 113, 155	Decane, 2,3,5,8-tetramethyl	Alkane
6	22.78	0.30	310	$C_{22}H_{46}$	43, 57, 71, 85, 99, 310	Docosane	Straight-chain alkane
7	24.13	0.32	214	$C_{14}H_{30}O$	29, 41, 43, 55, 57, 69, 71, 111, 196	2-Hexyl-1-octanol	Fatty alcohol
8	26.52	0.56	324	$C_{23}H_{48}$	43, 55, 57, 69, 71, 85, 99, 127, 141	Tricosane	Straight-chain alkane
9	26.94	3.34	242	$C_{15}H_{30}O_2$	43, 55, 74, 87, 97, 143, 199, 242	Tetradecanoic acid, methyl ester	Fatty acid esters
10	27.43	0.24	366	$C_{26}H_{54}$	29, 41, 43, 55, 57, 71, 85, 95, 113	Hexacosane	Straight-chain alkane
11	27.96	3.44	270	$C_{17}H_{34}O_2$	57, 74, 87, 99, 111, 115, 143, 157, 213	Tridecanoic acid, 4,8,12-trimethyl-, methyl ester	Sesquiterpenoids
12	28.38	0.23	256	$C_{17}H_{36}O$	41, 43, 57, 69, 71, 83, 97, 111, 125, 238	1-Hexadecanol, 2-methyl	Long-chain fatty alcohol
13	29.03	2.47	282	$C_{18}H_{34}O_2$	43, 55, 57, 73, 83, 97, 113, 129, 198	9-Octadecenoic acid (Z)-	Unsaturated fatty acid
14	30.36	0.48	282	$C_{20}H_{42}$	43, 55, 57, 71, 85, 97, 134	Hexadecane, 2,6,10,14-tetramethyl	Diterpene
15	30.56	32.01	268	$C_{17}H_{32}O_2$	55, 69, 74, 83, 96,111, 152, 194, 237	7-Hexadecenoic acid, methyl ester, (Z)-	Fatty acid esters
16	30.82	0.82	338	$C_{24}H_{50}$	43, 55, 57, 69, 71, 85, 99, 113, 127, 155	Tetracosane	Straight-chain alkane
17	30.96	14.03	270	$C_{17}H_{34}O_2$	43, 55, 57, 74, 87, 97, 129, 143, 171, 227	Hexadecanoic acid, methyl ester	Fatty acid esters
18	31.53	5.75	254	$C_{16}H_{30}O_2$	55, 59, 83, 97, 111, 123, 152, 207, 236	Palmitoleic acid	Fatty acid
19	31.85	3.23	254	$C_{16}H_{30}O_2$	29, 41,55, 69, 83, 97, 111, 137, 236, 254	*cis*-9-Hexadecenoic acid	Fatty acid
20	32.16	0.46	296	$C_{19}H_{36}O_2$	41, 43, 55, 69, 71, 87, 97, 111, 143, 210	Cyclopropanecarboxylic acid, pentadecyl ester	Fatty acid esters
21	32.42	0.24	352	$C_{23}H_{44}O_2$	41, 55, 67, 74, 81, 95, 109, 123	13-Docosenoic acid, methyl ester, (Z)	Fatty acid methyl ester
22	32.55	0.24	338	$C_{22}H_{42}O_2$	29, 41, 43, 69, 71, 97, 115, 224	Cyclopentanecarboxylic acid, 4-hexadecyl ester	Fatty acid esters
23	32.85	0.71	326	$C_{21}H_{42}O_2$	41, 43, 55, 69, 74, 87, 97, 129, 143, 213	Eicosanoic acid, methyl ester	Fatty acid methyl ester
24	33.98	0.40	152	$C_{10}H_{16}O$	27, 39, 41, 55, 67, 81, 93, 108, 134, 152	4-Cyclohexylidenebutyraldehyde	Alpha-hydrogen aldehyde
25	34.10	0.52	294	$C_{19}H_{34}O_2$	41, 55, 67, 81, 95, 109, 123, 178, 294	9,12-Octadecadienoic acid (Z,Z), methyl ester	Fatty acid methyl ester
26	34.29	8.43	296	$C_{19}H_{36}O_2$	41, 55, 69, 74, 83, 97, 111, 123, 137, 264	9-Octadecenoic acid, methyl ester	Fatty acid esters
27	34.39	1.04	294	$C_{19}H_{34}O_2$	41, 55, 67, 81, 95, 109, 123, 149, 294	9,12-Octadecadienoic acid, methyl ester, (E,E)	Fatty acid esters
28	34.65	2.77	298	$C_{19}H_{38}O_2$	57, 74, 87, 129, 143, 199, 213, 255, 298	Octadecanoic acid, methyl ester	Fatty acid esters
29	35.18	0.23	330	$C_{19}H_{38}O_4$	55, 57, 73, 98, 129, 171, 213, 239, 256	Hexadecanoic acid, 2,3-dihydroxypropyl ester	1-Monoacylglycerols
30	35.24	0.19	268	$C_{17}H_{32}O_2$	43, 55, 69, 81, 95, 109, 123, 137, 193, 268	*E*-11-Methyl-12-tetradec en-1-ol acetate	Fatty alcohol esters
31	35.46	0.28	506	$C_{36}H_{74}$	43, 57, 71, 85, 99, 113, 141, 156, 239	Hexatriacontane	Straight-chain alkane

Continued

No.	R_t	Area%	MW	MF	Main fragments	Identified compounds	Class/category
32	36.97	0.29	180	$C_{12}H_{20}O$	39, 41, 55, 67, 79, 91, 105, 119, 133, 180	Z3,Z6,E8-dodecatrien-1-ol	Fatty alcohols
33	37.09	5.25	316	$C_{21}H_{32}O_2$	39, 41, 67, 79, 91, 105, 119, 147, 201. 316	5,8,11,14,17-Eicosapentaenoic acid, methyl ester	Fatty acid esters
34	37.58	0.37	170	$C_{10}H_{18}O_2$	41, 43, 56, 67, 71, 81, 95, 111, 121,	Pulegol	Monoterpenoids
35	37.74	0.26	212	$C_{12}H_{20}O_3$	41, 43, 55, 57, 70, 83, 98, 111, 152, 181	Methyl 11-oxo-9-undecenoate	Fatty acid esters
36	38.29	0.19	400	$C_{28}H_{48}O$	69, 81, 95, 105, 121, 175, 203, 315, 329	Cholestan-3-ol, 2-methylene-, (3á,5à)	Steroid
37	38.95	0.19	268	$C_{17}H_{32}O_2$	41, 43, 55, 69, 87, 113, 127, 182, 199	2-Cyclopropylcarbonyloxy tridecane	Alkane derivatives
38	40.10	0.33	262	$C_{18}H_{30}O$	41, 55, 67, 70, 95, 108, 121, 135, 206, 262	9,12,15-Octadecatrienal	Fatty aldehydes
		T% 88.54					

R_t: Retention time; MW: Molecular weight; MF: Molecular formula.

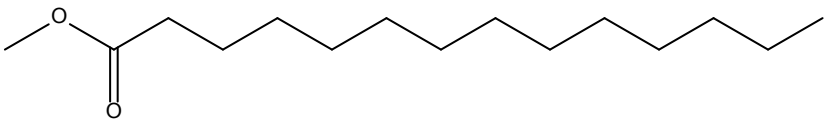
the sesquiterpene axisonitrile-3 isolated from the tropical marine sponge *Acanthella kletra* were evaluated as antifouling, antialgal, antiphotosynthetic, antibacterial, antifungal, and antitubercular agents (Wright *et al*., 2011).

Antibacterial and antibiofilm activities

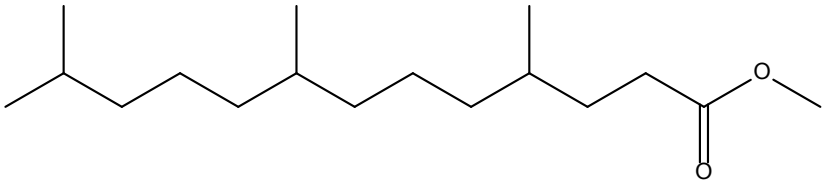
The *in vitro* antibacterial ability of the methanolic extract of *P. undulatus* clams was estimated against numerous pathogenic microbes including four Gram-negative and one Gram-positive bacteria and one yeast. Our findings revealed that the tested extract exhibited variable antibacterial activity against tested microbial strains in the (blank/tested extract) manner: (0.449/0.196), (0.503/0.319), (1.048/0.174), (1.077/0.221), (1.067/0.102), and (0.792/0.285), respectively, for *E. coli*, *P. aeruginosa*, *P. vulgaris*, *K. pneumoniae*, *S. aureus*, and *C. albicans* (Table 2, Fig. 4). On the other side, the antibiofilm activity was evaluated using the Media Transfer Protocol (MTP) assay at concentration (100 µg/ml). In this assay, the tested extract was evaluated for its biofilm inhibition activity versus some investigated pathogenic microbial strains as well as their malice factor from the direction of biofilm development. The obtained results revealed that the tested extract exhibited a potent inhibition percentage of 91.98% against *S. aureus*, followed by 53.01% against *B. subtilis*, 31.90% against *P. aeruginosa*, and 24.20% against *E. coli* (Table 3). Conclusively, the crude methanol extract of *P. undulatus* clams is likely to be a good candidate against biofilm colonization and cell adherence. It is worth mentioning that bacterial infection leads to a high risk in both human and animal populations (Kandhasamy and Arunachalam, 2008). Antibiotics usage has highly increased during the last decades, and this has led to the development of strong resistance from pathogenic bacteria against drugs (Lavanya and Veerappan, 2011). Due to the high cost of these drugs (Idsoe *et al*., 1968), there is an urgent need to discover alternative antimicrobials from natural sources (Abubakar *et al*., 2012; Smith *et al*., 1994). The marine environment is a huge source of bioactive substances that are widely used in biomedical applications (Omar, 2012). The marine mollusks collected from the Kanyakumari coast comprised numerous biologically active ingredients that could be used as promising antibacterial agents (Giftson and Patterson, 2016). Also, Ibrahim *et al*. (2020) stated that many of the marine mollusks create their antibacterial factors as a first line of defense versus pathogenic microbial infection. They concluded that the crude extracts of the marine sea cucumber and sponges caused inhibition of different human pathogens such as *S. aureus, Enterococcus faecalis*, *P. aeruginosa,* and *E. coli* and could be represented as a promising source of new bioactive substances that are applied in the clinical field. Additionally, Youssef *et al*. (2013) confirmed the presence of three types of alkaloids from the Red Sea sponges which have antioxidant, antimicrobial, and anticancer activities.

TPC and antioxidant activity

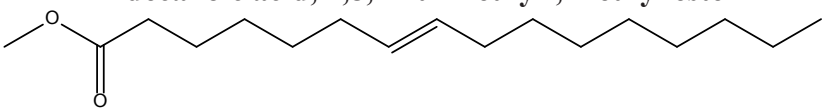
The methanolic extract was evaluated for its TPC, free radical scavenging activity (DPPH), and TAC. The extract exhibited a TPC value of 177.23 mg GAE/g dry extract. Also, it had the capability to scavenge DPPH free radical with an IC_{50} value of 14.59 µg/ml in comparison with ascorbic acid as a reference compound with an IC_{50} value of 7.52 µg/ml. Moreover, it showed a TAC value of 371.66 mg AAE/g dry extract, reflecting its reduction capability to convert molybdenum (VI) to molybdenum (V) (Table 4). Joy *et al*. (2016) confirmed the antioxidant, antidiabetic, anti-inflammatory, and antihypertensive potential of ethyl acetate-methanol extract of bivalve clams. They stated that the EtOAc-MeOH extract of *Paphia malabarica* showed total phenolics significantly greater (88.6 mg GAE/g) than those in *Villorita cyprinoides* (73.9 mg GAE/g). The polyphenolic ingredients exhibited free radical scavenging activities and were ensured to shield the delicate bodied mollusks from photooxidation (Karawita *et al*., 2005; Rockenbach *et al*., 2011). Polyphenolics showed high redox potential and act as strong electron donors due to their unique heavy hydroxylation pattern (Ghareeb *et al*., 2014b). The crude methanol and ethyl acetate extracts of *Perna viridis* exhibited notable free radical scavenging activity against DPPH free radical, which reinforces our current findings (Sreejamole and Radhakrishnan, 2013). Also, *P. undulatus* extracts have bioactive molecules called hydrolysates that have shown potential hepatoprotective roles through their antioxidant activities (Nair *et al*., 2015).



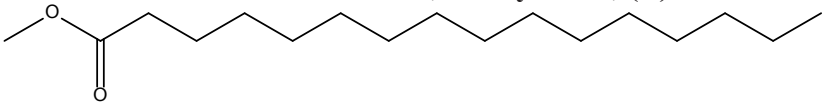
Tetradecanoic acid, methyl ester



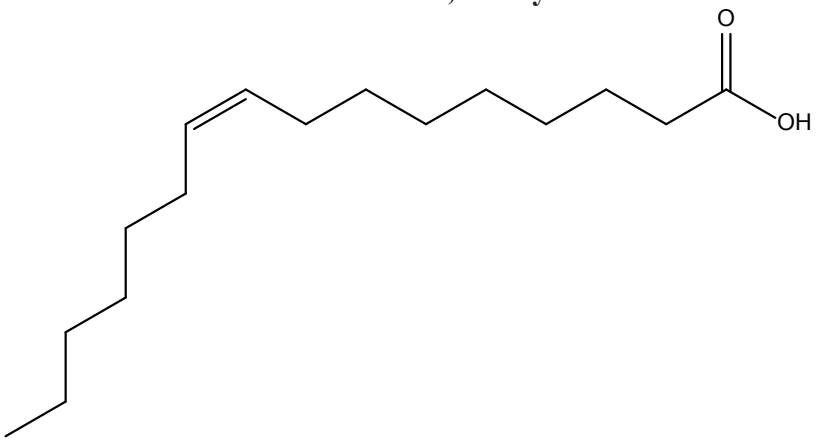
Tridecanoic acid, 4,8,12-trimethyl-, methyl ester



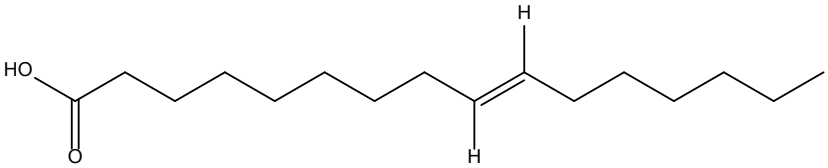
7-Hexadecenoic acid, methyl ester, (Z)



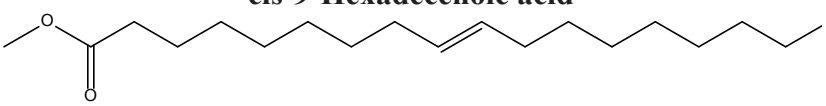
Hexadecanoic acid, methyl ester



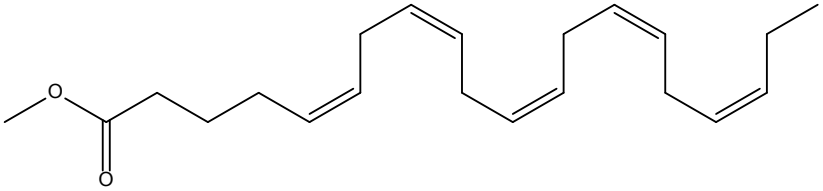
Palmitoleic acid



cis-9-Hexadecenoic acid



9-Octadecenoic acid, methyl ester



5,8,11,14,17-Eicosapentaenoic acid, methyl ester

Figure 3. Chemical structures of the major identified compounds.

Table 2. *In vitro* antibacterial activity of the methanolic extract of *P. undulatus* clams against some pathogenic microbial strains.

Tested extract/ blank	Pathogenic microbial strains					
	Escherichia coli	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Candida albicans*
Blank	0.449	0.503	1.048	1.077	1.067	0.792
Methanol extract	0.196	0.319	0.174	0.221	0.102	0.285

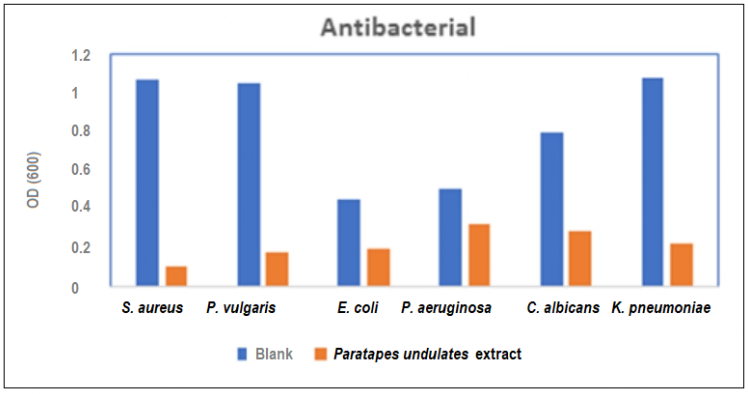


Figure 4. *In vitro* antibacterial activity of the methanolic extract of *P. undulatus* clams against some pathogenic microbial strains.

Table 3. Antibiofilm activity of the methanolic extract of *P. undulatus* clams against some pathogenic microbial strains.

Tested extract	Microbial strains/biofilm inhibitory (%)			
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
Methanol extract	91.98413	53.01587302	24.20635	31.90476

Table 4. TPC, free radical scavenging activity (DPPH), and TAC of the methanolic extract of *P. undulatus* clams.

Tested extract	TPC (mg GAE/g dry extract)[a,b]	TAC (mg AAE/g dry extract)[c]	DPPH IC_{50} (µg/ml)[5]
Methanol extract	177.23 ± 2.44	371.66 ± 2.08	14.59 ± 1.39
Ascorbic acid	-	-	7.52 ± 1.49

[a] Results are means ± SD (n = 3).
[b] GAE: gallic acid equivalent.
[c] AAE: ascorbic acid equivalent.
[d] IC_{50}: the amount of extract needed to scavenge 50% of DPPH radicals.

CONCLUSION

The obtained results disclosed that the methanolic extract of the marine clam *P. undulates* consists of many bioactive molecules like hexadecenoic acid, methyl ester, hexadecanoic acid, 9-octadecenoic acid, and palmitoleic acid. Additionally, the results showed that the extract has remarkable antibacterial, antibiofilm, and antioxidant activities. The present results might be helpful for pharmaceutical companies to develop natural supplements from these clams. Subjecting the extract to further chromatographic isolation and identification is recommended to identify its main ingredients using developed chromatographic and spectroscopic tools.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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This study does not involve experiments on animals or human subjects.

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DATA AVAILABILITY

All the data obtained during the study are presented in this manuscript. Any further inquiries for additional information are available upon request from the corresponding author.

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