Rendiconti Lincei. Scienze Fisiche e Naturali (2021) 32:149–161
https://doi.org/10.1007/s12210-020-00964-0

RESEARCH PAPER

Antimicrobial activity, cytotoxic effect and characterization of marine bivalve extracts *Cerastoderma glaucum*

Mohamed H. Mona[1] · Gihan M. El-Khodary[2] · Nahla E. Omran[1] · Karolin K. Abd El-Aziz[2] · Salwa A. El-Saidy[2]

Received: 2 June 2020 / Accepted: 17 October 2020 / Published online: 31 October 2020

Abstract
Cerastoderma glaucum, a marine bivalve inhabiting Lake Timsah, is surrounded by different pathogenic organisms. The present study evaluates the antimicrobial activities, cytotoxicity and characterization of *C. glaucum* extracts. Chloroform, methanol and acidic tissue extracts were prepared from *C. glaucum* collected during winter and summer seasons. Winter acidic extract exhibits potent antimicrobial activities against 21 bacterial, 2 yeast and 2 viral strains. The inhibition zone of this extract ranges from 10 mm against *Klebsiella oxytoca, Pseudomonas stutzeri, Globicatella sulfidifaciens* and *Bacillus* (*B. badius*, *B. amyloliquefaciens* and *B. pumilus*) to 24 mm against *Shigella flexneri*. Also, the inhibition viral activities of this extract at a concentration of 62.5 µg ml^{-1} against *Hepatitis A* virus and *Herpes simplex* virus type 1 (HSV-1) are 62.383% and 57.035%, respectively, with low cytotoxicity of 24.030%. Furthermore, winter acidic extract of *C. glaucum* has the highest total protein contents (9.8 mg ml^{-1}) compared with the other extracts. Moreover, the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) indicates the presence of four clear low molecular weight peptides bands; 8.588, 7.237, 4.423 and 2.692 kDa. Fourier transform-infrared (FT-IR) analysis indicates the presence of 12 functional groups of proteins in winter acidic extract of *C. glaucum* at appropriate wavelengths.

Keywords Bivalves · Antimicrobial · Antiviral · Gel electrophoresis · Fourier transform infrared · Bioactive compounds

1 Introduction

Marine bivalves live under the highest microbial concentrations in seawater, approximately 10^6 bacteria/ml and 10^9 virus/ml of seawater (Diaz 2010). So, they need to have vigorous immune system strategies. The antimicrobial peptides (AMPs) constitute the first line of these defense strategies against invading microorganisms to survive in this marine environment (Falanga et al. 2016; Zannella et al. 2017). Marine bivalves are considered one such rich source of these AMPs that possess various biological activities, such as antibacterial, antioxidant, anticoagulant, etc. (Galdiero et al. 2015). The AMPs show antimicrobial properties, and provide a rapid and immediate response against the invading microbes (Boman 1995; Bartlett et al. 2002). They are small peptides, less than 60 amino acids and less than 10 kDa in mass, and are triggered immediately after microbial infection. Their value in innate immunity lies on their ability to function without either high specificity or memory. Also, they can be synthesized without dedicated cells or tissues, and they rapidly diffuse to the point of infection (Relf et al. 1999; Chandran et al. 2009). AMPs are effective against dormant bacteria that can survive in high concentrations of antibiotics and need an extensive treatment (Lai and Gallo 2009; Hurdle et al. 2011). Also, AMPs have remarkable specificity for prokaryotes, such as bacterial pathogens without cytotoxicity to eukaryotic host cells (Matsuzaki 2009).

These AMPs are characterized by their high cysteine content, and they have been organized into four groups according to their primary structure, especially cysteine array; defensins, mytilins, myticins and mytimycin (Parisi et al. 2009). Despite variations in structure and size of AMPs, most of them have cationic and amphiphilic characters, and possess affinity towards both hydrophilic and hydrophobic surfaces. These peptides generally act by forming pores in microbial membranes disrupting membrane integrity (Tam

✉ Salwa A. El-Saidy
salwa_elseedy@yahoo.com

[1] Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt

[2] Department of Zoology, Faculty of Science, Damanhour University, Damanhour, Egypt

et al. 2000). Microbes can not develope resistance against this action mode (Falanga et al. 2016; Zannella et al. 2017).

Moreover, some AMPs can be effective in inhibiting viral infections by many mechanisms, including prevention of viral attachment or penetration into the host cells by interaction with the specific cellular receptor, blocking early steps of viral entry by surface carbohydrate interaction, inactivation of viral envelope glycoproteins, inhibition of viral DNA and/or RNA synthesis, blocking intracellular expression of viral genes and/or production of viral proteins and modulation of antiviral responses of host cell (Dang et al. 2015; Zannella et al. 2017).

Marine bivalves *Cerastoderma glaucum* (Bruguière 1789) survive in a hostile environment in Lake Timsah, Egypt, where they are surrounded by different pathogenic organisms, including human pathogens. So, the present study was conducted to evaluate and compare the efficacy of whole-body soft tissue extracts of the marine bivalve *C. glaucum* using different types of polar and nonpolar solvents as an antimicrobial. Further study is extended to detect the antimicrobial substances of *C. glaucum* using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Fourier transform-infrared (FT-IR).

2 Materials and methods

2.1 Study site and sample collection

Specimens of *Cerastoderma glaucum* (the common name is cockle) were collected during winter and summer, 2018 from Lake Timsah, Ismailia, Egypt. This lake is small and shallow, situated between 30° 33′3″ and 30° 35′ 31″ North latitude and 32° 16′ 30″ and 32° 18′ 50″ East longitude (Ibrahim and El-Regal 2014; Kandeel 2018) (Fig. 1). The whole-body soft tissues of cockle samples (31.58 ± 0.485 mm long, 18.67 ± 0.334 mm width and 25.02 ± 0.278 mm height) were collected in polyethylene bags and freeze-dried as a whole in isothermal boxes.

2.2 Preparation of the tissue extracts of *Cerastoderma glaucum*

Three different solvents, chloroform, methanol and hydrochloric acid, were used. The chloroform and methanol extracts were prepared by keeping 150 g of the homogenated tissues in 150 ml of these solvents overnight at 4 °C and then centrifugation at 10,733 × *g* for 20 min. The supernatant was concentrated using a vacuum rotary evaporator (35–55 °C) to give predominantly an aqueous suspension which was lyophilized to give yellow gummy mass (Abirami et al. 2014; Madhu et al. 2014).

Acidic extraction was performed by incubating 150 g of wet weight of soft tissue in one liter of 1 N HCl for 10 min at 100 °C (Zatylny et al. 2000). After homogenization, they were centrifuged at 20,000 × g for 30 min at 4 °C. The supernatant was lyophilized to give the acidic extract (Defer et al. 2009; Abirami et al. 2014). All three crude extracts were stored at − 20 °C until antimicrobial assays were performed. In each test, all extracts were used in the same weight volume ratio.

2.3 In vitro antibacterial and antifungal assay

The antibacterial and antifungal activities of these three crude extracts of *C. glaucum* were determined by means

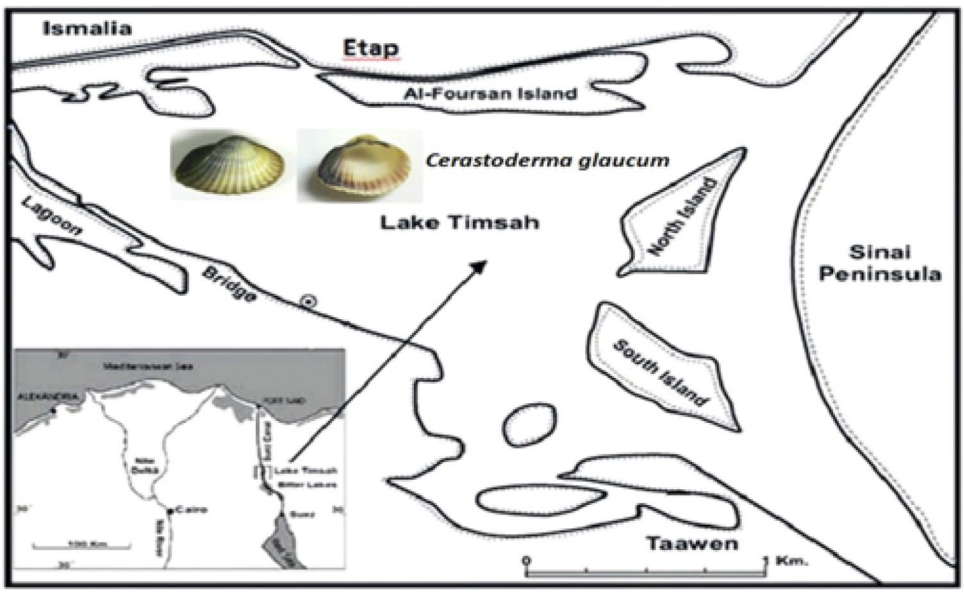


Fig. 1 Map of Lake Timsah, Ismailia, Egypt (Kandeel 2018)

of the standard agar disc diffusion method (NCCLS 1993; Bizuye et al. 2013; CLSI 2017). The extracts were tested against 16 g-negative bacteria, such as *Acinetobacter* (*A. haemolyticus* and *A. lwoffii*), *Brevundimonas diminuta, Escherichia coli*, *Klebsiella* (*K. oxytoca* and *K. pneumoniae*), *Lelliottia amnigena, Ochrobactrum anthropi, Pseudomonas* (*P. alcaligenes, P. aeruginosa* and *P. stutzeri*), *Salmonella typhimurium*, *Shewanella algae*, *Shigella flexneri*, *Stenotrophomonas maltophilia* and *Vibrio alginolyticus,* 7 g-positive bacterial strains, such as *Bacillus* (*B. amyloliquefaciens*, *B. badius*, *B. pumilus* and *B. subtilis*), *Globicatella sulfidifaciens, Staphylococcus aureus* and *Streptococcus pyogenes*, two yeast strains, such as (*Candida albicans* and *Trichosporon asahii*) and two fungal strains belong to *Aspergillus* (*A. braselienses* and *A. fumigates*).

One-hundred µl of each dissolved extract in dimethyl sulfoxide (DMSO) (100 µg ml^{-1}) was loaded separately into each disc (6 mm diameter) for antimicrobial and antifungal assay, and left until dry. The loaded discs were added to the surface of cultured Petri dishes, and kept for 2 h at 4 °C to allow the diffusion of dissolved compounds into the agar. Then, the plates were incubated for 24 h at 37 °C for bacteria and 48–72 h at 28 °C for fungi. The present study used DMSO as a negative control. After incubation, the inhibition zones diameters were measured in mm using a Vernier caliper scale.

2.4 Measurement of the cytotoxicity of winter acidic crude tissue extract of *Cerastoderma glaucum* using 3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

This assay was performed according to Bahuguna et al. (2017). VERO (Verda reno) cells from passage number 76 were used in this assay, and grown as a monolayer in Eagle's Minimum Essential Media (EMEM) with hanks balanced salt solution (HBSS) supplemented with 10% fetal bovine serum and 1% antibiotic solution mixture of penicillin G-sodium salt and streptomycin sulfate was added to avoid bacterial contamination (Olicard et al. 2005a, b). Eight different concentrations of the acidic extract (1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8 µg ml^{-1}) were applied in four replicates. Fifty µl of MTT reagent were added to wells of blank, negative cell control and different concentrations of the acidic extract. After that, the plates were incubasted in a CO_2 incubator at 37 °C for 4 h for the reduction of MTT into formazan by the mitochondrial dehydrogenase activity of viable cells. One-hundred µl of dimethyl sulfoxide (DMSO) was added to each well and incubated in a CO_2 incubator at 37 °C for 30 min to solubilize the purple needle crystals of formazan. Finally, the absorbance at 570 nm was measured with an enzyme-linked immunosorbent assay (ELISA) microplate reader to determine the cellular viability. The percentage of cell survival rate was calculated by the following equation, and the maximum non-toxic concentration (MNTC) was determined.

$$\text{Survival rate } \% = \frac{A_{\text{Ex}} - A_b}{A_c - A_b} \times 100$$

where, A_{Ex}: Absorbance of different concentrations of the acidic extract. A_b: Absorbance of the blank. A_c: Absorbance of the negative control.

$$\text{Cytotoxicity } \% = 100 - \text{Survival rate } \%$$

2.5 In vitro antiviral activity of winter acidic crude tissue extract of *Cerastoderma glaucum* using MTT assay

The antiviral activity of winter acidic extract was evaluated in vitro against two viral strains; *Hepatitis A* virus H-10 strain and *Herpes simplex* virus type 1 (HSV-1, sensitive to Acyclovir that was used as a positive control of HSV-1 infection and as a reference for HSV-1 inhibition) infecting VERO cells. The determination of antiviral activity was based on the cytopathic effect inhibition assay (Langois et al. 1986). This assay was performed in eight replicates. The percentage of inhibition viral activity of winter acidic extract was calculated using the following equation:

$$\frac{A_{\text{virus - extract}} - A_{\text{control virus}}}{A_{\text{cellular control}} - A_{\text{control virus}}} \times 100$$

where $A_{\text{virus-extract}}$: absorbance of infected treated (virus-extract) well. $A_{\text{control virus}}$: absorbance of the control virus. $A_{\text{cellular control}}$: absorbance of the cellular control.

2.6 Characterization of the different crude soft tissue extracts of *Cerastoderma glaucum*

2.6.1 Estimation of total protein concentration

One gram of each extract was added to water-soluble extraction buffer (a ratio of 1:2 *w:v*) for protein purification from the different crude tissue extracts and estimation of total protein content. The total protein concentration in all different crude soft tissue extracts of *C. glaucum* was determined according to Bradford (1976) method. This assay was performed in three replicates.

2.6.2 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE was used to distinguish the total soluble protein fragments and their molecular weights distribution

according to the method of Laemmli (1970). Slab gel (15%) and stacking gel (4%) were prepared. After gel polymerization, 30 µg proteins of each sample were loaded, and unstained broad range protein ladder (250–5 kDa) (Thermo Fisher, 26,630) was applied as a molecular marker. Electrophoresis was performed at 75 V through the stacking gel followed by 125 V for approximately 2 h. The gel was stained 0.1% coomassie brilliant blue R-250 for 2 h, then destained with a solution of glacial acetic acid, methanol and water (1:3:6, respectively). Gel documentation system (Gel doc-it, UVP, England) was applied for data analysis using Totallab analysis software, www.totallab.com, (Version 1.0.1). The samples were compared with the standard protein molecular weight marker (250–5 kDa).

2.6.3 Fourier transform-infrared (FT-IR) spectral analysis

One part of the lyophilized sample was mixed with 80 parts of dried potassium bromide (KBr), and compressed to prepare as a salt disc with 10 mm diameter. Then, the absorption of this disc was read using the FT-IR spectrophotometer (Germany Bruker tensor 27) between 400 and 4000 cm^{-1}. The frequencies of different components in each sample were analyzed (Periyasamy et al. 2012; Gayathri et al. 2017).

2.7 Statistical analysis of data

One way analysis of variance (ANOVA) and post-hoc multiple-comparison (Tukey) tests were used to compare the total protein concentrations among the different crude soft tissue extracts of *C. glaucum*. The results were presented as means ± standard deviations (SD). Statistical analysis of data was performed using the statistical package for the social sciences IBM-SPSS software (Version 20.0). The statistical significance was set at level $P \leq 0.05$.

3 Results

3.1 Antimicrobial activities of the different crude soft tissue extracts of *Cerastoderma glaucum*

Results shown in Table 1 indicate that winter acidic tissue extract of *Cerastoderma glaucum* demonstrated the highest antimicrobial activities compared to winter chloroform and methanol extracts. The maximum zone of inhibition of winter acidic tissue extract was recorded against *Shigella flexneri* (24 mm), and the minimum zone of inhibition was observed against *Klebsiella oxytoca, Pseudomonas stutzeri, Globicatella sulfidifaciens* and *Bacillus* (*B. amyloliquefaciens*, *B. badius* and *B. pumilus*) (10 mm). On the other hand, winter chloroform and methanolic extracts of *C. glaucum* showed activities against 4 bacterial strains with inhibition zones ranging from 12 to 13 mm and 9 to 13 mm, respectively. Furthermore, winter acidic extract of *C. glaucum* has antifungal activity against 2 yeast strains *Candida albicans* (11 mm) and *Trichosporon asahii* (22 mm), with no recorded activity against *Aspergillus* (*A. fumigatus* and *A. braselienses*).

Also, the very low antimicrobial activity of different tissue extracts of *C. glaucum* during summer season when compared with winter extracts. During summer season, antibacterial activities of the acidic extract (10 mm) were observed against 3 bacterial strains; *Klebsiella pneumoniae*, *P. stutzeri* and *G. sulfidifaciens*, while the activities of a chloroform extract (10 mm) appeared only against *P. stutzeri* and *G. sulfidifaciens*. But, no detectable antimicrobial activity of methanol extract was observed against all tested bacterial and fungal strains during summer season.

3.2 Cytotoxicity and antiviral activity of winter acidic extract of *Cerastoderma glaucum*

The winter acidic tissue extract (62.5 µg ml^{-1}) of *C. glaucum* presented cytotoxicity about 24.030%. This concentration (62.5 µg ml^{-1}) was considered to be the MNTC used in the antiviral assay of winter acidic extract of *C. glaucum* (Table 2). At the antiviral level, inhibition viral activities of this extract against *Hepatitis A* virus H-10 strain and HSV-1 virus are 62.383% and 57.035%, respectively. Also, 1.25 µg ml^{-1} of acyclovir which is the positive control for HSV-1 showed a low percentage of cell destruction (5.147%) (Table 3).

3.3 Characterization of the different crude soft tissue extracts of *Cerastoderma glaucum*

3.3.1 Quantification of protein

The present results in Fig. 2 indicate that all winter extracts of *C. glaucum* exhibited high significance in the total protein contents compared with summer extracts ($P = 0.000$). Moreover, the total protein content increased significantly in winter acidic extract of *C. glaucum* when compared with the other extracts ($P \leq 0.01$). Also, the acidic extract of *C. glaucum* exhibited the highest total protein contents during winter and summer seasons (9.8 and 6.3 mg ml^{-1}, respectively) compare with chloroform and methanolic extracts.

3.3.2 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis

The electrophoretic profile of SDS-PAGE of different crude tissue extracts of *C. glaucum* showed active fractions of low molecular weights of peptides less than 10 kDa (Fig. 3). Four clear bands were detected in the gel of winter acidic

Table 1 Inhibition zones (mm) of the three different crude soft tissue extracts of *Cerastoderma glaucum* collected from Lake Timsah during winter season

Microorganisms strains	Collection number	Inhibition Zones (mm) of different tissue extracts		
		Chloroform	Methanol	Acidic
Bacterial strains				
Gram-negative bacteria				
Acinetobacter haemolyticus		13	13	12
Acinetobacter lwoffii		–	–	11
Brevundimonas diminuta		12	12	13
Escherichia coli	ATCC: 25922	–	–	13
Klebsiella oxytoca		–	–	10
Klebsiella pneumoniae	ATCC: 10031	–	–	15
Lelliottia amnigena		-	–	18
Ochrobactrum anthropi		12	12	–
Pseudomonas alcaligenes		–	–	13
Pseudomonas aeruginosa	ATCC: 9027	–	–	15
Pseudomonas stutzeri		–	–	10
Salmonella typhimurium	ATCC: 19430	-	–	16
Shewanella algae		–	–	14
Shigella flexneri	ATCC: 12022	–	-	24
Stenotrophomonas maltophilia		–	9	–
Vibrio alginolyticus		–	–	11
Gram–positive bacteria				
Bacillus amyloliquefaciens		-	–	10
Bacillus badius		12	–	10
Bacillus pumilus		–	–	10
Bacillus subtilis	ATCC: 19659	–	–	14
Globicatella sulfidifaciens		-	–	10
Staphylococcus aureus	ATCC: 29213	–	–	13
Streptococcus pyogenes	ATCC: 19615	–	–	16
Yeast strains				
Candida albicans	ATCC: 10231	–	–	11
Trichosporon asahii		14	14	22

Table 2 Cytotoxicity of winter acidic extract of *Cerastoderma glaucum* at different concentrations and determination of the maximum non-toxic concentration (MNTC)

Concentration of winter acidic extract (μg ml^{-1})	1000	500	250	125	62.5	31.25	15.625	7.8
Cytotoxicity %	96.013	94.265	51.993	34.844	24.030	18.350	14.855	0.873

Table 3 Antiviral activities and cytotoxicity at the maximum non-toxic concentration (MNTC) of winter acidic extract of *Cerastoderma glaucum* and acyclovir against viral strains

	Viral strains	Inhibition viral activity %	Cytotoxicity %	MNTC μg ml^{-1}
Winter acidic extract of *Cerastoderma glaucum*	*Hepatitis A* virus H-10 strain	62.383	24.030	62.5
	Herpes simplex virus type 1	57.035		
Acyclovir	*Herpes simplex* virus type 1	83.726	5.147	1.25

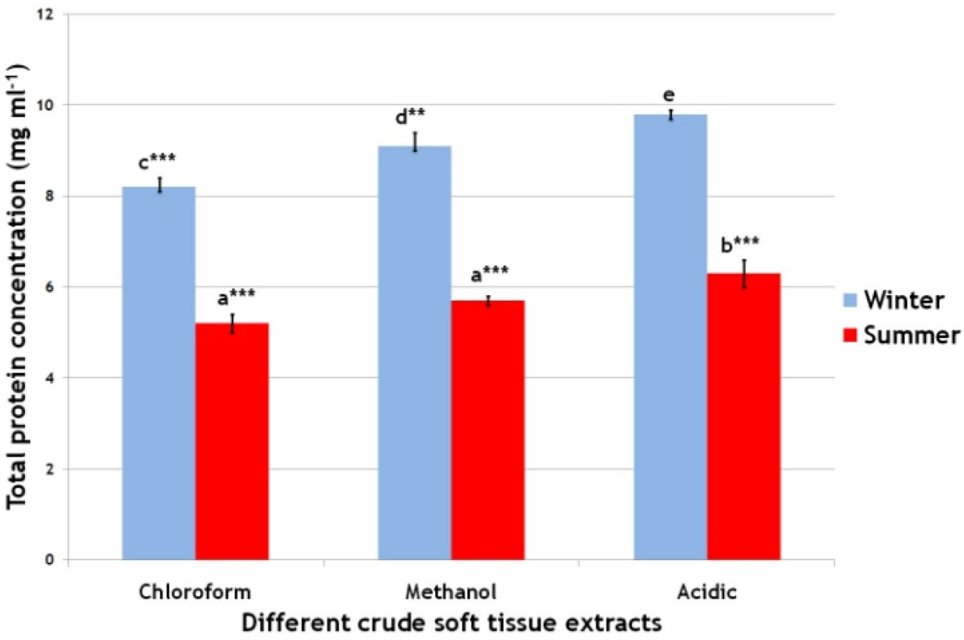


Fig. 2 Total protein concentrations (mg ml^{-1}) of different crude soft tissue extracts of *Cerastoderma glaucum* collected during winter and summer seasons. Values are means ± standard deviation (SD). Different letters (a, b, c, d and e) are significant, *Significant at P value ≤ 0.05, **Significant at P value ≤ 0.01 and ***Significant at P value ≤ 0.001 (one-way ANOVA)

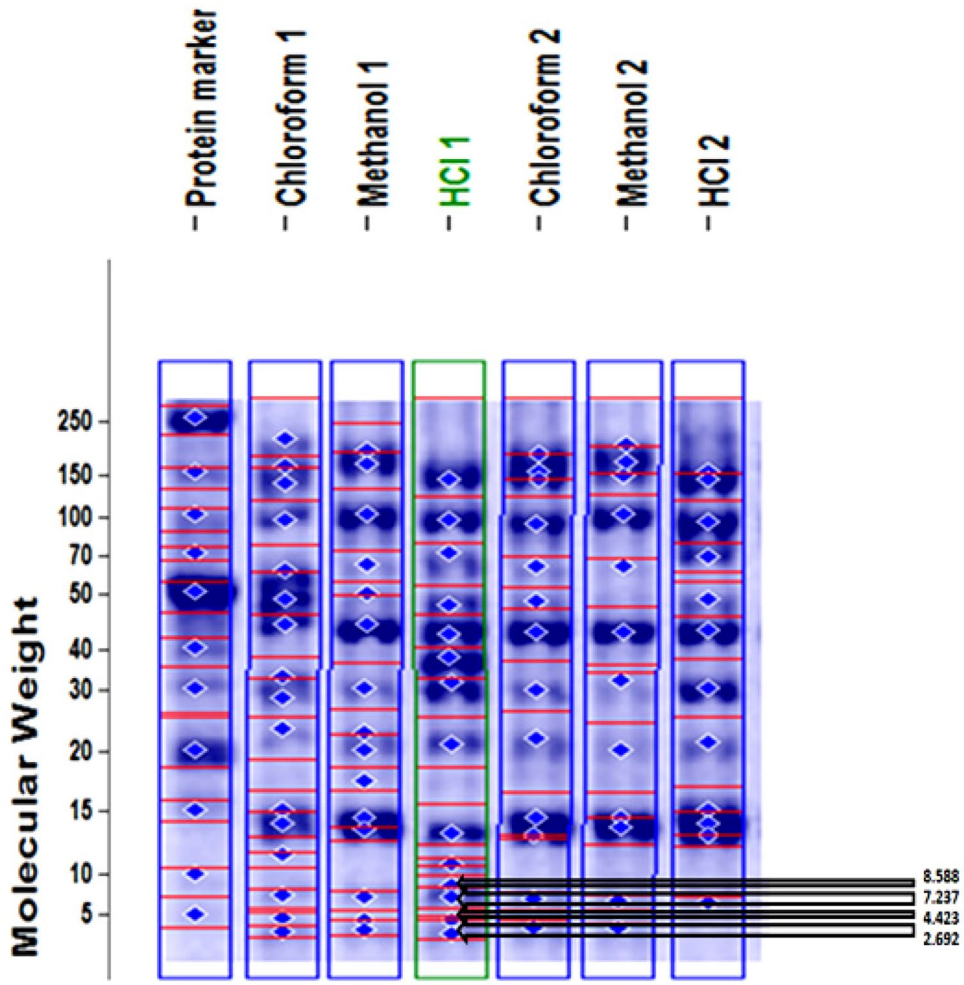


Fig. 3 An electrophoretic profile of 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) showing the computerized detection of protein patterns in the different crude soft tissue extracts of *Cerastoderma glaucum* collected during winter and summer seasons. The gel was stained with coomassie brilliant blue stain. Where, Lane 1: corresponds to the position of marker molecular masses ranging from 250 to 5 kDa; Lanes 2, 3 and 4: are different winter extracts; Lanes 5, 6 and 7: are different summer extracts

tissue extract which represented molecular weights of proteins of 8.588, 7.237, 4.423 and 2.692 kDa. While, winter chloroform and methanol soft tissue extracts showed 3 bands with molecular weights less than 10 kDa (7.237, 4.615 and 3.654 kDa) and (7.237, 4.423 and 3.654 kDa), respectively. Also, there were 3 active peptides with molecular masses of 8.401, 6.231 and 3.654 kDa in summer chloroform extract, while only two clear bands were observed along the gel in summer methanol and acidic tissue extracts at (6.231 and 3.654 kDa) and (8.401 and 6.231 kDa), respectively.

Also, the results indicated that the protein contents of the bands with molecular masses; 7.237 and 4.423 kDa were high in winter acidic extract when compared with winter chloroform and methanol extracts. Moreover, there were two bands of molecular weights; 8.588 and 2.692 kDa in winter acidic extract, not found in the other extracts, containing protein content 1.69% and 1.56%, respectively.

3.3.3 Fourier transform-infrared (FT-IR) spectral analysis

FT-IR characterization of all crude tissue extracts of *C. glaucum* showed the presence of functional groups of protein like HN–C=O, ketone C=O (Amide V) and C–O stretching vibration in carboxylic group (COOH), C–N stretching vibration in primary and secondary amines, C-S linkage and C–H stretching vibration in the methylene group at appropriate wavelengths (Fig. 4). Winter acidic extract of *C. glaucum* had a large number of functional groups (12 groups) compare with the other extracts. All extracts, except winter and summer chloroform extracts, had peaks at 1486–1546 cm^{-1} which are attributed to N–H bending vibration coupled with the C–N stretching vibration (Amide II) group. Also, the S–S linkage stretching vibrations were recorded in all extracts, except summer methanol extract, at wavenumbers ranging from 529 to 598 cm^{-1}. Furthermore, the additional bands were observed in winter acidic extract in 1651–1737 cm^{-1}, 3385 cm^{-1} and 1193–1205 cm^{-1} peaks indicating the presence of C=O stretching vibration in COOH (Amide I), N–H stretching vibration in aliphatic primary amine and C–N stretching vibration in aliphatic tertiary amine, respectively (Table 4).

4 Discussion

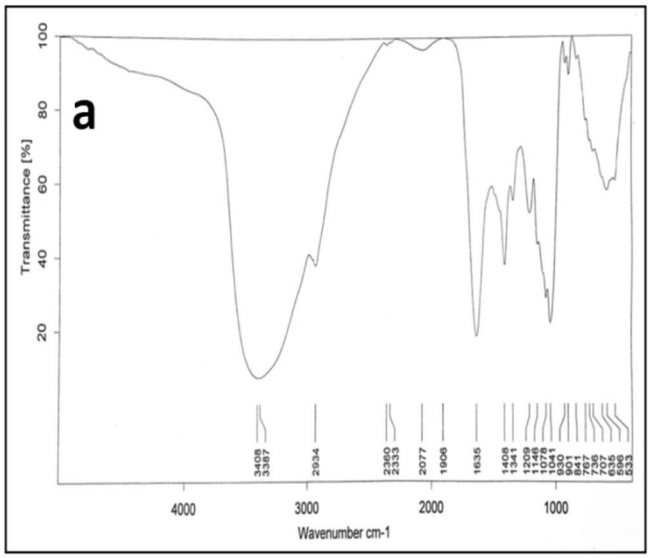
The marine bivalves *Cerastoderma glaucum* are greatly dispersed in Lake Timsah, Ismailia, Egypt. The purpose of using different solvents in preparing a tissue extract is to provide inclusive information on the properties of the bioactive substances and their activity (Fernández et al. 1996).

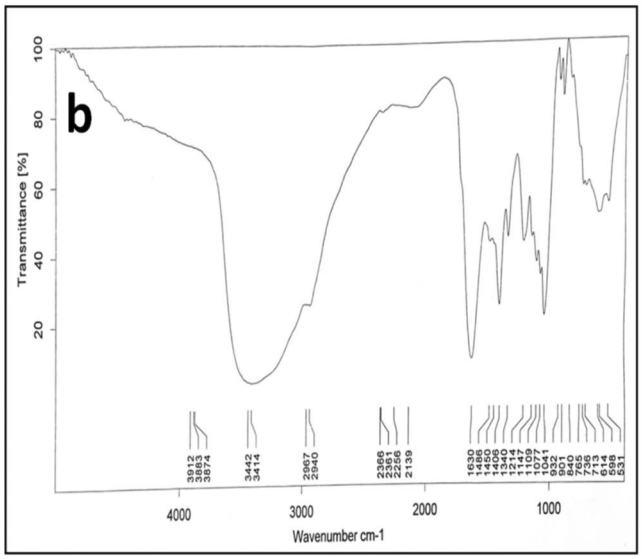
The present study shows high antimicrobial activity of different winter extracts compared with summer extracts. This may be due to high significance in total protein content in all winter crude extracts compare with summer crude extracts Gayathri et al. (2017). indicated that bivalve extracts are usually complex mixtures of bioactive molecules, mainly proteins and peptides which are responsible for antimicrobial and antiviral activities. Also, Sharma et al. (2009), Estari et al. (2011) and Dhanalakshmi and Sanjeevi (2016) returned the low antimicrobial activity during summer to illness stress, hypoxia, increasing water temperature and salinity and gametogenesis process during summer season. Moreover, free ions produced by the high salt concentrations in the surrounding medium, typical of some diseases, could effectively decrease the electrostatic interactions of AMPs with the negatively-charged surface of bacterial membrane and thus antimicrobial activity (Jenssen et al. 2006; Zannella et al. 2017).

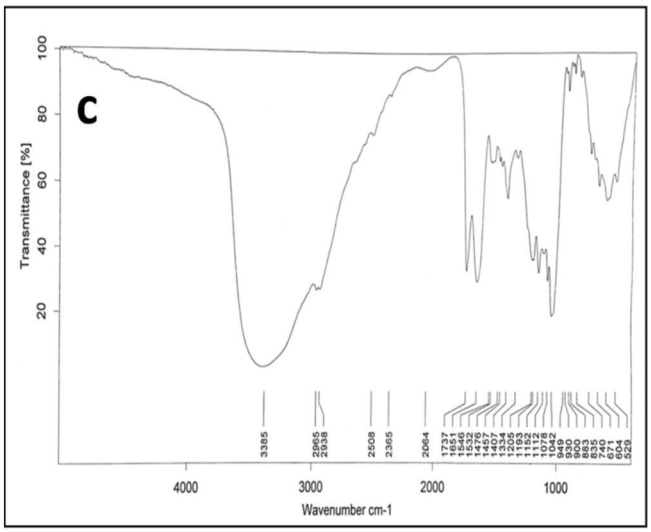
The acidic extract of *C. glaucum* demonstrated the highest antimicrobial activities. These results are confirmed by Abirami et al. (2014) who found that the acidic extract of *Meretrix casta* exhibited prominent antibacterial activity. The differences in the antimicrobial activity of bivalve extracts may depend on the extraction method, extracting capacity of solvents, compound extracted and bacteria and bivalve species (Dhanalakshmi and Sanjeevi 2016; Ghorbanalizadeh et al. 2018).

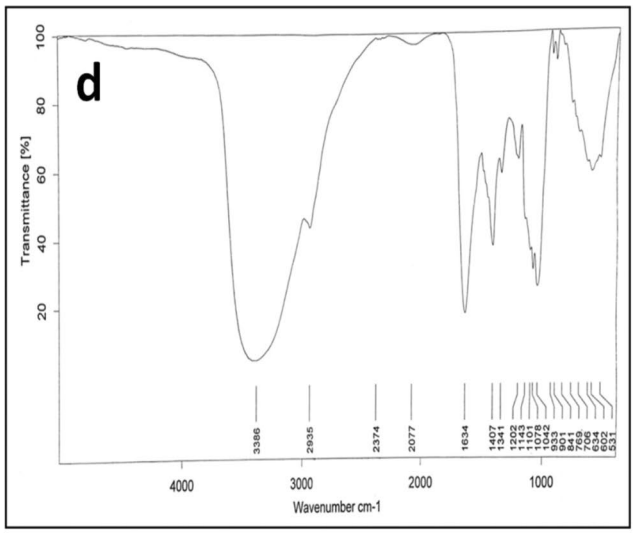
The antimicrobial activity of winter acidic extract of *C. glaucum* ranged from 10 to 24 mm. This extract had inhibition zones against *Escherichia coli, Salmonella typhimurium*, *Shigella flexneri* and *Streptococcus pyogenes*. These observations agree with Arputha et al. (2013), Sugesh and Mayavu (2013) and Bibiana et al. (2014). Arputha et al. (2013) recorded the inhibitory effect of 9–12 mm, 8–13 mm, 8–16 mm, 10–23 mm and 16–26 mm with 5% acetic acid crude extracts of *Donax cuneatus* phenotypes (P1, P2, P3, P4 and P5, respectively). Also, the maximum inhibition zones of the acetic acid extract of *D. cuneatus* phenotype (P5) were observed against *Streptococcus* (18 mm), *Salmonella* (17 mm) and *Shigella* (17 mm) Sugesh and Mayavu (2013). recorded that the acetic acid extract of *M. casta* showed a 13 mm inhibition zone against *E. coli*. Bibiana et al. (2014) reported that the maximum inhibitory effects against *S. flexneri* and *S. typhimurium* were 25 and 19 mm, respectively in case of 5% acetic acid crude extract of *D. cuneatus,* and 21 and 17 mm, respectively in case of *Pitar erycina* extract.

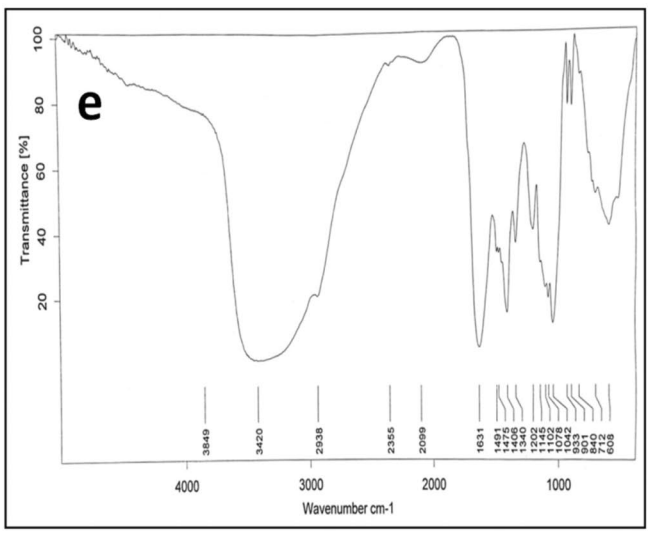
Furthermore, the present study indicated that winter acidic extract of *C. glaucum* had antifungal activity against 2 yeast strains; *Candida albicans* (11 mm) and *Trichosporon asahii* (22 mm), with no recorded activity against *Aspergillus* (*A. fumigatus* and *A. braselienses*). Similar results were reported against *C. albicans* in the previous studies, such as Sharma et al. (2009) and Sugesh and Mayavu (2013) supporting the present antifungal results of winter acidic extract of *C. glaucum*. Sharma et al. (2009) found that the

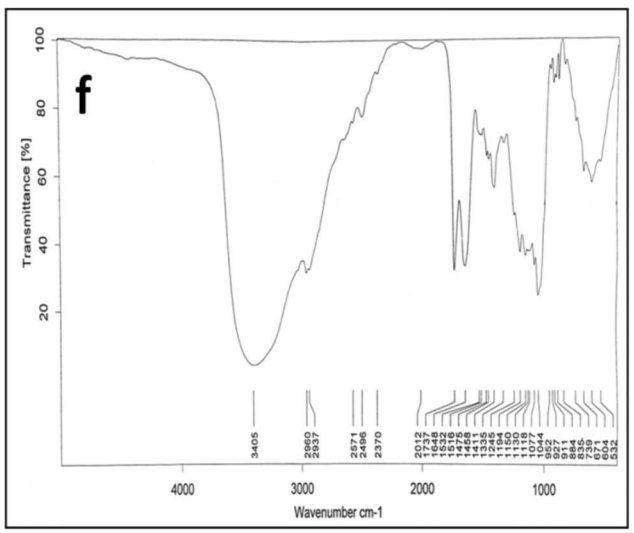

a
Transmittance [%]
Wavenumber cm-1


b
Transmittance [%]
Wavenumber cm-1


c
Transmittance [%]
Wavenumber cm-1


d
Transmittance [%]
Wavenumber cm-1


e
Transmittance [%]
Wavenumber cm-1


f
Transmittance [%]
Wavenumber cm-1

◂**Fig. 4** Fourier transform-infrared (FT-IR) spectral analysis of the different crude tissue extracts of *Cerastoderma glaucum* collected during winter and summer seasons: **a** Winter chloroform extract; **b** Winter methanol extract; **c** Winter acidic extract; **d** Summer chloroform extract; **e** Summer methanol extract; **f** Summer acidic extract

acid-enzyme hydrolysis aqueous (AEH-Aq) extract of *Villorita cyprinoides* had antifungal activity against *C. albicans* (13 mm) Sugesh and Mayavu (2013). reported that the methanolic, ethanolic and acetic acid extracts of *M. meretrix* had inhibition zones with 5, 7 and 9 mm, respectively against *C. albicans*.

On studying the effect of winter acidic extract against viruses, the obtained results show that 62.5 µg ml^{-1} concentration caused inhibition of viral activities about 62.383% and 57.035% against *Hepatitis A* virus and HSV-1 virus, respectively with low cytotoxicity (24.030%). These results are in accordance with Defer et al. (2009) who found that the antiviral activities of 80% solid-phase extraction (SPE)-fractions of the acidic extract from *Cerastoderma edule* (85 µg ml^{-1}), *Ruditapes philippinarum* (125 µg ml^{-1}), *Ostrea edulis* digestive gland (100 µg ml^{-1}) and *O. edulis* (gills + mantle) (1280 µg ml^{-1}) were 59.3% (24.3% of cytotoxicity), 48.7% (22.8% of cytotoxicity), 40% (34% of cytotoxicity) and 28% (20% of cytotoxicity), respectively against HSV-1.

The current study showed that winter extracts of *C. glaucum* exhibited high significance in the total protein contents, compared with summer crude tissue extracts. Moreover, the total protein content increased significantly in winter acidic extract of *C. glaucum* when compared with the other extracts. These results are confirmed with Abirami et al. (2014) who reported that methanol and acidic extracts of *M. casta* exhibited high amounts of protein; 786 and 745 µg mg^{-1}, respectively, whereas the hexane and chloroform extracts exhibited low protein content of 304 and 147 µg mg^{-1}, respectively.

From the present SDS gel electrophoresis results, all crude tissue extracts showed active fractions of low molecular weights of peptides, less than 10 kDa, representing AMPs (Hancock and Scott 2000; Mercado et al. 2005). Winter acidic extract had a higher number of low molecular weight bands compare with the other extracts. It presented four clear bands of proteins exhibited molecular weights of 8.588, 7.237, 4.423 and 2.692 kDa. Also, the results indicated that the protein contents of the bands with molecular masses; 7.237 and 4.423 kDa were high in winter acidic extract when compared with winter chloroform and methanol extracts. Moreover, there were two bands of molecular weights; 8.588 and 2.692 kDa in winter acidic extract, not found in the other extracts, containing protein contents; 1.69% and 1.56%, respectively. Chandran et al. (2009) observed only one clear band of molecular weight 9.7 kDa in the gill extraction of *P. viridis* using SDS gel electrophoresis, which represented a low molecular weight peptide. Also, Arputha et al. (2013) observed the crude extract of 5% acetic acid of *D. cuneatus* phenotype P5 to SDS-PAGE analysis, and showed bands at molecular weights of 3 and 6 kDa.

The low molecular weight peptides that were observed in *C. glaucum* tissue extracts, such as 6.231, 4.423, 4.615 and 3.654 kDa are very close to the molecular weights of AMPs, such as mytimycin (6.2335 kDa), myticin-A (4.438 kDa) or defensin-B (4.3924 kDa) or MGD-1 (4.418 kDa), myticin-B (4.562 kDa) and mytilin-A (3.7737 kDa), respectively. All of these AMPs are cysteine-rich polypeptides with intramolecular disulfide bonds isolated from the plasma and haemocyte granules of mussels *Mytilus* (*M. galloprovincialis* and *M. edulis*) (Charlet et al. 1996; Hubert et al. 1996; Mitta et al. 1999a, b, 2000a). Also, defensins have been described in oysters, such as *Crassostrea gigas* and *C. virginica* (Seo et al. 2005; Gueguen et al. 2006; González et al. 2007) and abalone *Haliotis discusdiscus* (De Zoysa et al. 2010). While, mytilins have been described in clam *Ruditapes decussatus* (Gestal et al. 2007). These AMPs display antibacterial activity against Gram-negative bacteria and Gram-positive bacteria. Mytimycin is active against *Micrococcus luteus* (Epand and Vogel 1999). Also, myticins A and B exhibit activity against *Micrococcus* (*M. lysodeikticus*, *M. luteus*), *Bacillus megaterium* and *Enterococcus viridans*, whereas only myticin-B is active against the Gram-negative bacteria, such as *E. coli* D31, *P. aeruginosa* and *S. typhimurium* (Mitta et al. 1999a, b, 2000b; Tincu and Taylor 2004). Moreover, Charlet et al. (1996), Romestand et al. (2003) and Gueguen et al. (2006) showed that defensins A and B had high inhibitory growth activity against the Gram-positive strains *M. lysodeikticus* and *M. luteus* than the Gram-negative strain *E. coli*. Furthermore, native MGD-1 has biological activity against both Gram-positive bacteria, such as *B. Megaterium, M. lysodeikticus* and *Staphylococcus* (*S. aureus* and *S. epidermidis*) and Gram-negative bacteria, such as *E. coli* 363, *Vibrio P1*, *Vibrio* (*V. alginolyticus*, *V. splendidus, V. Metschnikowii*) and *Salmonella newport* (Hubert et al. 1996; Yang et al. 2000; Romestand et al. 2003; Li et al. 2011). Mytilin-A shows considerable activity against both Gram-positive bacteria (*Aerococcus viridans*, *Enterococcus faecalis, M. lysodeikticus, M. luteus*, *S. aureus* and *B. megaterium*) and Gram-negative bacteria (*E. coli*, *V. splendidus*, *V. anguillarum* and *S. typhimurium*) (Charlet et al. 1996). Mytimycin was reported as antifungal, inhibiting the growth of *Fusarium culmorum* and *Neurospora crassa* (Charlet et al. 1996). Myticin-B and native MGD-1 are active against the fungus *Fusarium oxysporum* (Mitta et al. 1999a; Romestand et al. 2003). Mytilin-A showed antiviral activity on the model HSV-1 virus/VERO cells, suggesting a direct interaction of the peptide with the virus membrane (Carriel-Gomes et al. 2007).

Table 4 Functional groups of the different crude tissue extracts of *Cerastoderma glaucum* collected during winter and summer seasons

Characteristic functional groups	Frequency cm^{-1}					
	Winter extracts			Summer extracts		
	Chloroform	Methanol	Acidic	Chloroform	Methanol	Acidic
HN–C=O	1635	1630	1532 1546 1651	1634	1631	1532 1648
C=O stretching vibration in COOH (Amide I)	–	–	1651 1737	–	–	1737
Ketone C=O (Amide V)	707 736 767	713 736 765	671 740	706 769	712	671 739
C–O stretching vibration in COOH	1341	1214 1340	1334	1341	1340	1245 1335
N–H stretching vibration (aliphatic primary amine)	3387	–	3385	3386	–	–
C–N stretching vibration (primary amine)	1041 1078	1041 1077	1042 1078	1042 1078	1042 1078	1044 1077
C–N stretching vibration (secondary amine)	1146	1147	1152	1143	1145	1130 1150
C–N stretching vibration (aliphatic tertiary amine)	1209	–	1193 1205	1202	1202	1194
Amine (NH) bending vibration coupled with cyanide (C–N) stretching vibration (Amide II)	–	1486	1532 1546	–	1491	1516 1532
Disulfide S–S linkage stretching vibrations	533 596	531 598	529	531	–	532
C–S linkage	635	614	604 671	602 634	608	604 671
C–H stretching vibration in the methylene group	2934	2940 2967	2938 2965	2935	2938	2937 2960

FT-IR spectral analysis was used to detect and identify the function groups of compounds (El-Sheekh and El Kassas 2014; Abd El-Hamid et al. 2018). The present FT-IR spectral analysis revealed the occurrence of antimicrobial compound signals at different wavelengths in the crude extracts of *C. glaucum*. FT-IR characterization of all crude extracts of *C. glaucum* showed the presence of functional groups of protein like HN–C=O (Stuart et al. 1996), ketone C=O (Amide V) (Sjahfirdi and Nasikin 2012) and C-O stretching vibration (Al-Sheibany et al. 2005) in the carboxylic group (COOH), C–N stretching vibration in primary and secondary amines (Coates 2006), C–S linkage (El-Hag and Dahab 2016) and C–H stretching vibration in the methylene group (Sharma 1981; Sharma et al. 2018) at appropriate wavelengths. Winter acidic extract of *C. glaucum* had a large number of functional groups (12 groups) compare with the other extracts. As, there were some additional functional groups found in this extract, such as C=O stretching vibration in COOH (Amide I) (Sjahfirdi and Nasikin 2012), N–H stretching vibration in aliphatic primary amine, C–N stretching vibration in aliphatic tertiary amine (Coates 2006), N–H bending vibration coupled with C–N stretching vibration (Amide II) (Sjahfirdi and Nasikin 2012) and S–S linkage stretching vibration (Biswas et al. 2007; El-Hag and Dahab 2016) at different wavelengths.

Moreover, the present FT-IR spectral results obtained from *C. glaucum* tissue extracts are very close to FT-IR spectra recorded by Arputha et al. (2013) and Madhu et al. (2014). Arputha et al. (2013) noticed aldehyde C=O, amino (NH) and amine at 1707, 3396 and 1560 cm^{-1}, respectively in the acetic acid crude extract of *D. cuneatus*. Also Madhu et al. (2014), observed peaks at 1654 and 1500 cm^{-1}in the methanol tissue extract of *P. viridis*, which indicate the presence of the characteristic amide groups, such as C=O stretching vibration in COOH (Amide I) and N–H bending vibration coupled with C–N stretching vibration (Amide II), respectively. The bands in the range of 1520 to 1700 cm^{-1} are associated with HN–C=O which is the peptide group of proteins, and provides information about protein structures (Pauling and Corey 1951; Stuart et al. 1996). The bands at 1647 and 1654 cm^{-1}, that occur at similar wavelengths in polyamides and proteins, are commonly assigned to stretching of C=O group bonded to N–H of the neighboring infra sheet chain (Focher et al. 1992).

The presence of S–S linkage and C-S linkage stretching vibrations in the different *C. glaucum* extracts at bands

529–598 cm^{-1} and 602–671 cm^{-1}, respectively indicate the presence of cystine peptide. Baranska (2013) found that cystine peptide and cysteine amino acid containing sulfur showed an intensive band area of 500 cm^{-1} (S–S stretching band) as well as in the range between 2550–2580 cm^{-1} (SH stretching vibration band), respectively. Ameri et al. (2017) revealed that the antibacterial activity of *Thais savignyi* gastropod extract may be due to the presence of a disulfide (S–S) functional group (534 cm^{-1}). Therefore, the present study suggests that the absence of antimicrobial activity of summer methanol extract is due to the absence of the S–S functional group in this extract.

5 Conclusion

In conclusion, winter acidic extract of *Cerastoderma glaucum* collected from Lake Timsah, Ismailia, Egypt may be a source of a large group of low molecular weight peptides, and has antimicrobial activities against both Gram-negative and Gram-positive bacteria, yeast and virus strains due to these peptides. Consequently, it could be effectively used as an alternative source of antimicrobial and antiviral drugs with subsequent health benefits. The purification and identification of these AMPs from *C. glaucum* will help researchers in the development of novel antibiotics.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abd El-Hamid MS, Anis A, Elbawab RH, Mohammed AA, Orabi SH, Fathalla SI (2018) Distinctive antagonistic role of new *Enterococcus faecium* ER-3M strain and its bacteriocin effect against *Staphylococcus aureus* pneumonia. Rend Fis Acc Lincei 29(3):675–690

Abirami P, Giji S, Mohan K, Arumugam M (2014) Antibacterial activity of different solvent extracts of marine bivalve *Meretrix casta*. Curr Bio 8(3):270–277

Al-Sheibany IS, Kadhim KH, Abdullah AS (2005) Qualitative and quantitative evaluation of some organic compounds in Iraqi Thyme. Iraqi Nat J Chem 19:366–379

Ameri A, Shushizadeh MR, Nabavi SMB, Espere F, Ahmady AZ (2017) Antibacterial evaluation and biochemical characterization of *Thais savignyi* gastropod extracts from the Persian Gulf. Jundishapur J Nat Pharm Prod 12(2)

Arputha BM, Gayathri K, Selvamani P, Latha S (2013) Appraisal of five different phenotypic edible bivalves crude protein extract for microbicidal potency. IJPRBS 2(6):20–30

Bahuguna A, Khan I, Bajpai VK, Kang SC (2017) MTT assay to evaluate the cytotoxic potential of a drug. Bangladesh J Pharmacol 12(2):115–118

Baranska M (2013) Optical spectroscopy and computational methods in biology and medicine. Springer Sci Bus Med 14

Bartlett TC, Cuthbertson BJ, Shepard EF, Chapman RW, Gross PS, Warr GW (2002) Crustins, homologues of an 11.5-kDa antibacterial peptide, from two species of penaeid shrimp, *Litopenaeus vannamei* and *Litopenaeus setiferus*. Mar Biotechnol 4(3):278–293

Bibiana MA, Selvamani P, Latha S (2014) Identification and appraisal of crude protein extracts from south Indian marine edible bivalves for their potential bactericidal property. Asian J Pharm Clin Res 7(1):233–236

Biswas N, Waring AJ, Walther FJ, Dluhy RA (2007) Structure and conformation of the disulfide bond in dimeric lung surfactant peptides SP-B1–25 and SP-B8–25. Biochim Biophys Acta 1768(5):1070–1082

Bizuye A, Moges F, Andualem B (2013) Isolation and screening of antibiotic-producing actinomycetes from soils in Gondar town, North West Ethiopia. Asian Pacific J Trop Dis 3(5):375–381

Boman HG (1995) Peptide antibiotics and their role in innate immunity. Annu Rev Immunol 13(1):61–92

Bradford MM (1976) A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1–2):248–254

Bruguière JG (1789) Encyclopédie méthodique ou par ordre de matières; par une société de gens de lettres, de savans et d'artistes… Histoire naturelle des vers. 1. Tome sixieme: Paris, chez Panckoucke, libraire, Liege, chez Plomteux, Imprimeur des Etats 1:1–344

Carriel-Gomes MC, Kratz JM, Barracco MA, Bachère E, Monte Barardi CR, Oliveira Simões CM (2007) In vitro antiviral activity of antimicrobial peptides against Herpes simplex virus 1, adenovirus, and rotavirus. Mem Inst Oswaldo Cruz 102:469–472

Chandran B, Rameshkumar G, Ravichandran S (2009) Antimicrobial activity from the gill extraction of *Perna viridis* (Linnaeus, 1758). Glob J Biotech Biochem 4(2):88–92

Charlet M, Chernysh S, Philippe H, Hetru C, Hoffmann JA, Bulet P (1996) Innate Immunity. Isolation of several cysteine-rich antimicrobial peptides from the blood of a mollusc, *Mytilus edulis*. J Biol Chem 271(36):21808–21813

CLSI (2017) Clinical and laboratory standards institute: performance standards for antimicrobial susceptibility testing. M100, 27th Ed. Replaces M100-S26

Coates J (2006) Interpretation of infrared spectra, a practical approach. Encyclopedia of analytical chemistry. Appl Theory Instrum. https://doi.org/10.1002/9780470027318.a5606

Dang VT, Benkendorff K, Green T, Speck P (2015) Marine snails and slugs: a great place to look for antiviral drugs. J Virol 89(16):8114–8118

De Zoysa M, Whang I, Lee Y, Lee S, Lee JS, Lee J (2010) Defensin from disk abalone *Haliotis discusdiscus*: molecular cloning, sequence characterization and immune response against bacterial infection. Fish Shellfish Immunol 28(2):261–266

Defer D, Bourgougnon N, Fleury Y (2009) Screening for antibacterial and antiviral activities in three bivalve and two gastropod marine molluscs. Aquacult 293(1–2):1–7

Dhanalakshmi M, Sanjeevi SB (2016) Antibacterial activity of freshwater bivalve *Lamellidens marginalis* (Lamarck, 1819) from lower anaicut reservoir. India Int J Pure App Biosci 4(1):287–290

Diaz GA (2010) Defensins and cystein rich peptides: two types of antimicrobial peptides in marine molluscs. Invert Surviv J 7(2):157–164

El-Hag DA, Dahab AA (2016) Identification and characterisation of disulphide bonds in therapeutic proteins by using Raman Spectroscopy. Adv J Pharm Life sci Res 4(3):50–59

El-Sheekh MM, El Kassas HY (2014) Biosynthesis, characterization and synergistic effect of phytogenic gold nanoparticles by marine picoeukaryote *Picochlorum sp.* in combination with antimicrobials. Rend Fis Acc Lincei 25(4):513–521

Epand RM, Vogel HJ (1999) Diversity of antimicrobial peptides and their mechanisms of action. Biochim Biophys Acta 1462(1–2):11–28

Estari M, Satyanarayana J, Kumar BS, Bikshapathi T, Reddy AS, Venkanna L (2011) *In vitro* study of antimicrobial activity in freshwater mussel (*Lamellidens marginalis*) extract. Biol Med 3(2):191–195

Falanga A, Lombardi L, Franci G, Vitiello M, Iovene M, Morelli G, Galdiero S (2016) Marine antimicrobial peptides: Nature provides templates for the design of novel compounds against pathogenic bacteria. Inter J Mol Sci 17(5):785

Fernández MJR, Labarta U, Babarro JMF (1996) Comparative allometries in growth and chemical composition of mussel (*Mytilus galloprovincialis* Lmk) cultured in two zones in the Ria sada (Galicia, NW Spain). J Shellfish Res 15:349–353

Focher B, Naggi A, Torri G, Cosani A, Terbojevich M (1992) Structural differences between chitin polymorphs and their precipitates from solutions evidence from CP-MAS 13C-NMR. FT-IR and FT-Raman Spectrosc Carbohyd Polym 17(2):97–102

Galdiero S, Falanga A, Berisio R, Grieco P, Morelli G, Galdiero M (2015) Antimicrobial peptides as an opportunity against bacterial diseases. Curr Med Chem 22(14):1665–1677

Gayathri M, Ramasamy M, Santhiya N (2017) Extraction, identification of bioactive compounds and *in vitro* antioxidant activity potential in freshwater ampullariidae snail *Pila virens*. Extraction 2(2)

Gestal C, Costa M, Figueras A, Novoa B (2007) Analysis of differentially expressed genes in response to bacterial stimulation in hemocytes of the carpet shell clam *Ruditapes decussatus*: identification of new antimicrobial peptides. Gene 406(1–2):134–143

Ghorbanalizadeh A, Moshfegh A, Setorki M (2018) Evaluation of antimicrobial activity of peptides isolated from *Cerastoderma* and *Didacta* bivalves habitat in the southern shores of the Caspian Sea. Iranian J Aquat Anim Health 4(1):1–12

González M, Gueguen Y, Desserre G, De Lorgeril J, Romestand B, Bachère E (2007) Molecular characterization of two isoforms of defensin from hemocytes of the oyster *Crassostrea gigas*. Dev Comp Immunol 31(4):332–339

Gueguen Y, Herpin A, Aumelas A, Garnier J, Fievet J, Escoubas JM, Bulet P, Gonzalez M, Lelong C, Favrel P, Bachere E (2006) Characterization of a defensin from the oyster *Crassostrea gigas*: Recombinant production, folding, solution structure, antimicrobial activities, and gene expression. J Biol Chem 281(1):313–323

Hancock RE, Scott MG (2000) The role of antimicrobial peptides in animal defenses. P Natl Acad Sci USA 97(16):8856–8861

Hubert F, Noël T, Roch P (1996) A member of the arthropod defensin family from edible Mediterranean mussels (*Mytilus galloprovincialis*). Eur J Biochem 240(1):302–306

Hurdle JG, O'Neill AJ, Chopra I, Lee RE (2011) Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections. Nat Rev Microbiol 9(1):62–75

Ibrahim NK, El-Regal MAA (2014) Heavy metals accumulation in marine edible molluscs, Timsah Lake, Suez Canal. Egypt ARPN J Sci Tech Inter 4(4):282–288

Jenssen H, Hamill P, Hancock RE (2006) Peptide antimicrobial agents. Clin Microbiol Rev 19(3):491–511

Kandeel KES (2018) Population dynamics of *Venerupis aurea* (Bivalvia: Veneridae) in two different clam's beds in Lake Timsah, Suez Canal. Egypt Thalassia Salentina 40:67–94

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259):680–685

Lai Y, Gallo RL (2009) Amped up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol 30(3):131–141

Langois M, Allard JP, Nugier F, Aymard M (1986) A rapid and automated colorimetric assay for evaluating the sensitivity of *Herpes simplex* strains to antiviral drugs. J Biol Stand 14(3):201–211

Li H, Parisi M, Parrinello N, Cammarata M, Roch P (2011) Molluscan antimicrobial peptides, a review from activity-based evidences to computer-assisted sequences. ISJ 8

Madhu V, Sivaperumal P, Kamala K, Ambekar AA, Kulkarni BG (2014) Antibacterial and antioxidant activities of the tissue extract of *Perna viridis* Linnaeus, 1758 (Mollusca: Bivalvia) from Versova coast, Mumbai. Int J Pharm Pharmaceutical Sci 6:704–707

Matsuzaki K (2009) Control of cell selectivity of antimicrobial peptides. Biochim Biophys Acta 1788(8):1687–1692

Mercado L, Schmitt P, Marshall S, Arenas G (2005) Gill tissues of the mussel *Mytilus edulis chilensis*: a new source for antimicrobial peptides. Electron J Biotechn 8(3):284–290

Mitta G, Hubert F, Noel T, Roch P (1999a) Myticin, a novel cysteine-rich antimicrobial peptide isolated from haemocytes and plasma of the mussel *Mytilus galloprovincialis*. Eur J Biochem 265(1):71–78

Mitta G, Vandenbulcke F, Hubert F, Roch P (1999b) Mussel defensins are synthesized and processed in granulocytes then released into the plasma after bacterial challenge. J Cell Sci 112(23):4233–4242

Mitta G, Hubert F, Dyrynda EA, Boudry P, Roch P (2000a) Mytilin B and MGD2, two antimicrobial peptides of marine mussels: Gene structure and expression analysis. Dev Comp Immunol 24(4):381–393

Mitta G, Vandenbulcke F, Roch P (2000b) Original involvement of antimicrobial peptides in mussel innate immunity. FEBS Lett 486(3):185–190

NCCLS (1993) National committee for clinical laboratory standards: Methods for determining bactericidal activity of antimicrobial agents. Tentative guidelines. Villanova PA M26-T 12(19)

Olicard C, Didier Y, Marty C, Bourgougnon N, Renault T (2005a) *In vitro* research of anti-HSV-1 activity in different extracts from Pacific oysters *Crassostrea gigas*. Dis Aquat Org 67(1–2):141–147

Olicard C, Renault T, Torhy C, Benmansour A, Bourgougnon N (2005b) Putative antiviral activity in haemolymph from adult Pacific oysters *Crassostrea gigas*. Antiviral Res 66(2–3):147–152

Parisi MG, Li H, Toubiana M, Parrinello N, Cammarata M, Roch P (2009) Polymorphism of mytilin B mRNA is not translated into mature peptide. Mol Immunol 46(3):384–392

Pauling L, Corey RB (1951) Configurations of polypeptide c Chains with favored orientations around single bonds: two new pleated sheets. Proc Natl Acad Sci USA 37(11):729–740

Periyasamy N, Srinivasan M, Balakrishnan S (2012) Antimicrobial activities of the tissue extracts of *Babylonia spirata* Linnaeus, 1758 (Mollusca: Gastropoda) from Thazhanguda, southeast coast of India. Asian Pacific J Trop Biomed 2(1):36–40

Relf JM, Chisholm JR, Kemp GD, Smith VJ (1999) Purification and characterization of a cysteine-rich 11.5-kDa antibacterial protein from the granular haemocytes of the shore crab, *Carcinus maenas*. Eur J Biochem 264(2):350–357

Romestand B, Molina F, Richard V, Roch P, Granier C (2003) Key role of the loop connecting the two beta-strands of mussel defensin in its antimicrobial activity. Eur J Biochem 270(13):2805–2813

Seo JK, Crawford JM, Stone KL, Noga EJ (2005) Purification of a novel arthropod defensin from the American oyster *Crassostrea virginica*. Biochem Biophys Res Commun 338(4):1998–2004

Sharma BK (1981) Spectroscopy. Krishna Prakashan Media, pp 240

Sharma S, Chatterji A, Das P (2009) Effect of different extraction procedures on antimicrobial activity of marine bivalves: a comparison. Pertanika J Trop Agric Sci 32(1):77–83

Sharma D, Parveen K, Oza A, Ledwani L (2018) Synthesis of anthraquinone-capped TiO2 nanoparticles using *R. emodi* roots:

preparation, characterization and cytotoxic potential. Rend Fis Acc Lincei 29(3): 649–658

Sjahfirdi L, Nasikin M (2012) Protein identification using Fourier transform-infrared (FT-IR). IJRRAS 10(3):3–12

Stuart B, Ando DJ, George WO, McIntyre PS (1996) Modern infrared spectroscopy. ACOL. University of Greenwich by John Wiley and Sons, Chichester, pp 1–18

Sugesh S, Mayavu P (2013) Antimicrobial activities of two edible bivalves *M. meretrix* and *M. casta*. Pakistan J Biol Sci 16(1):38–43

Tam JP, Lu YA, Yang JL (2000) Marked increase in membranolytic selectivity of novel cyclic tachyplesins constrained with an antiparallel two-β strand cystine knot framework. Biochem Biophys Res Commun 267(3):783–790

Tincu AJ, Taylor SW (2004) Antimicrobial peptides from marine invertebrates. Antimicrob Agents Chemother 48(10):3645–3654 www.totallab.com

Yang YS, Mitta G, Chavanieu A, Calas B, Sanchez JF, Roch P, Aumelas A (2000) Solution structure and activity of the synthetic four-disulfide bond Mediterranean mussel defensin (MGD-1). Biochem 39(47):14436–14447

Zannella C, Mosca F, Mariani F, Franci G, Folliero V, Galdiero M, Galdiero M (2017) Microbial diseases of bivalve mollusks: infections, immunology and antimicrobial defense. Mar Drugs 15(6):182

Zatylny C, Gagnon J, Boucaud-Camou E, Henry J (2000) The sepovotropin: a new ovarian peptide regulating oocyte transport in *Sepia officinalis*. Biochem Biophys Res Commun 276(3):1013–1018