Chemical constituents of ostracod *Heterocypris salina* extract, anticancer and antimicrobial activity: *in silico* supported *in vitro* study

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Background
The majority of bioactive compounds derived from the sea have been extracted from aquatic invertebrates. While Ostracods are a diverse class of crustaceans that inhabit marine, brackish, and freshwaters, there are no available studies testing Ostracoda in the medical field.
Objective
The aim of this work was to produce H. salina on a large scale to evaluate its crude extract for anticancer and antimicrobial activities.
Materials and methods
Ostracod, *Heterocypris salina* was collected from its habitat in the eastern side of Lake Manzalla, Egypt, and the species was cultured in Soaba System of the National Institute of Oceanography and Fisheries (NIOF), Al-Khairia City, Egypt). Crude extract of Heterocypris salina was prepared and the extracr was tested as an anticancer against different human cancer cell lines. Also, the extract was investigated as antimicrobial against different Gram-positive and Gram-negative bacteria. In addition, chemical composition of the extract by GC-MS analysis and molecular docking were also studies.
Results and conclusion
Ostracod *Heterocypris salina* was cultured, at day 50 of the culturing, about 500 L of the culturing pond was harvested using plankton to collect the biomass of *H. salina*. A methanol extract of *H. salina* was prepared and showed a moderate to good anticancer effect against liver (HepG2), lung (A549), colon (HCT), and breast (MCF7) cancer cell lines with IC_{50} values in the range of 12.8–23.2 μg/ml), being safe for the healthy cells of the same organs. Also, the *H. salina* extract showed activity against three tested Gram-negative (*Escherichia coli, Klebsiella pneumonia*, and *Salmonella typhi*) and two Gram-positive (*Sarcina lutea* and *Bacillus cereus*) bacteria. GC-MS analysis of *H. salina* extract revealed the presence of 11 compounds including ethyl iso-allocholate (23.1%), 9(Z)-octadecenamide, (Z)-(20.6%), oleic acid (12.3%), palmitic acid (11.65%), and α-amyrin (10.73%). Moreover, a molecular docking study was performed in order to determine the possible binding interactions of the test compounds with the essential amino acids in the binding site of thymidylate kinase (TMK).

Keywords:
anticancer, antimicrobial, bioactive compounds, docking, Heterocypris salina, ostracoda, thymidylate kinase

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List of abbreviations: A549, Human lung cancer cell line; **ANOVA**, Analysis of variance; **ATCC**, American type culture collection; **ATP**, Adenosine triphosphate; **DMSO**, Dimethyl sulfoxide; **DNA**, Deoxyribonucleic acid; **dTDP**, Deoxythymidinediphosphate; **dTMP**, Deoxythymidine monophosphate; **dTTP**, Deoxythymidine triphosphate; **FHC**, Non-cancerous colon epithelial cells; **GC/MSD**, Gas chromatograph/mass selective detector; **GC-MS**, Gas chromatography-mass spectrometry; **HCT**, Human colon cancer cell line; **HepG2**, Human liver cancer cell line; **HPLC**, High performance liquid chromatography; **IC_{50}**, The half-maximal inhibitory concentration; **M.F.**, Molecular formula; **M.wt.**, Molecular weight; **MCF10a**, Non-cancerous breast epithelial cells; **MCF7**, Human breast cancer cell line; **MOE**, Molecular operating environment; **MTT**, 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide; **N/A**, Not active; **NIOF**, Institute of Oceanography and Fisheries; **NIST**, National institute of standards and technology; ***p***, Probability.**RMSD**, Root-mean-

square deviation; **Rt**, Retention time; **RTL**, Retention time locked; **SD**, Standard deviation; **SI**, Selectivity index; **THLE2**, Non-cancerous liver epithelial cells; **TMK**, Thymidylate kinase; ***Vs***, Versus; **WI38**, Non-cancerous lung fibroblast cell

Introduction

Natural products have been the source of many medicinal drugs throughout human history, and about 40% of all marketed drugs come from natural compounds [1,2]. The main sources of the natural compounds are microbes and plants from terrestrial and marine environments, where plants have been a prime source of highly effective conventional drugs for the treatment of many types of cancer [3]. Marine natural products have been particularly highlighted due to their extraordinary bioactivities developed through the adaptation of marine organisms to sometimes extremely harsh conditions [4]. The resultant secondary metabolites find applications as sources of drug discovery and development against many diseases [5,6]. The growing interest in discovering bioactive compounds from marine organisms led to the introduction of several important drugs into the clinic, further spurring the exponential growth of marine-based drug discovery [7].

Large no. of bioactive compounds have been extracted from aquatic invertebrates [8,9]. The chemical defense of secondary metabolites of invertebrates and their ability to adapt to difficult conditions are the main catalysts for the production of unique compounds that could be of interest for medical applications. Among aquatic invertebrates, marine (but not freshwater) invertebrates, particularly Ascidians and sponges, have been of most interest in the medical field [10]. In contrast, studies on small invertebrates and zooplankton as potential sources in medical applications are very limited; most of applied studies on these organisms have so far addressed them as food for fish cultivation due to their high nutritional value. However, promising opportunities exist to isolate compounds of medical interest from these organisms [11,12].

Ostracods are small crustaceans, usually about 1 mm in size. Their body consists of two parts, the hard outer part (the carapace) and the soft inner part (the whole body). Ostracods are a diverse class that inhabits marine, brackish, and freshwaters. Of the 70 000 identified species of Ostracods, there are 2000 identified species of freshwater habitats [13]. *Heterocypris salina* is an ostracod species inhabiting slightly salty and freshwaters. It can tolerate harsh environmental conditions and feed on a variety of food sources. This species is easy to culture; although investigations on the culturing conditions of *H. salina* are scarce [14]. Little studies have so far been performed to test ostracod extracts or natural products thereof in the medical field.

The expansion of antibiotic resistance has made bacterial infections a constant and growing concern to human health [15]. Antibiotic resistance is considered one of the biggest problems facing the infectious disease community today, as it has made many prevalent bacterial infections less susceptible to the effects of marketed antibiotics. The lack of new classes of antibiotics is only making the issue of widespread and rising antibiotic resistance worse, leaving a critical medical need in the treatment of severe bacterial infections [16,17]. Novel antibacterial agents targeting specifically the bacterial thymidylate kinase (TMK) have been developed through structure-based drug design. This enzyme is a nucleotide kinase essential in the DNA synthesis pathway; it transfers phosphate from ATP to thymidine monophosphate (dTMP) to form thymidine diphosphate (dTDP) and is an essential component of the thymidine triphosphate (dTTP) biosynthetic pathway. Disruption of TMK function is lethal to the cell because DNA replication is blocked. Because TMK sits at the juncture of the de novo and salvage metabolic pathways leading to dTMP synthesis, its inhibition negatively affects dTTPsynthesis.This means that, TMK is a necessary enzyme and a very desirable target for therapeutic strategy [18,19]. Therefore, the current study aims to produce *H. salina* on a large scale to evaluate its crude extract for potential anticancer and antimicrobial activities and to characterize the major compounds of this small crustacean. Also, a docking study was achieved with the intention of looking into potential antibacterial mechanism of compounds under investigation based on the aforementioned, also by using the structure of the bacterial target enzyme, it is possible to forecast and grade the states of protein-ligand interaction.

Materials and methods

Mass-production of *H. salina*

H. salina was collected from the eastern side of Lake Manzalla, Egypt, at a salinity of less than 1%0. The species was identified based on keys described by Ali *et al.* (2018) [20]. The species was cultured in a large ceramic pond of Soaba System (about 6000 L of

groundwater in the National Institute of Oceanography and Fisheries (NIOF), Al-Khairia city, Egypt) at 25±2°C, with daily photoperiod of 12 h. *H. salina* was fed daily on live *Chlorella vulgaris* (50×10^3 live cells/individual) as described by Hegab *et al*., (2019) [21]. *C. vulgaris* was cultivated at the Freshwater Hydrobiology Laboratory, NIOF using the BG-11 medium. The culture was started with a fixed number (1 individual/L) of *H. salina* (gravid female). *H. salina* was counted every 10 days to add the respective quantities of *C. vulgaris* for feeding the cultured species. For counting, three samples of 5 L were filtered through a plankton net, mesh size 55 µm. The collected samples were immediately fixed with 4% formaldehyde; 5 ml of each sample were examined under a binocular microscope. The number of *H. salina* individuals was expressed as the mean of the three samples (Fig. 1). After 50 day, about 500 L of the culture were filtered through a plankton net of 400 microns to collect the bio-mass of *H. salina*. The harvested biomass (Fig. 2) was concentrated as much as possible and washed with tab water many times through the plankton net (mesh size 500 microns) to remove any matters from the collective biomass.

Preparation of crude extract

The wet mass of the cultured species was dried in oven at 50°C for 24 h. Approximately 500 mg of the dried mass was exhaustively extracted by 10 ml of absolute methanol (HPLC grade, Merck, catalogue No. 107018) for 3 day in Checker (ThermoFisher Scientific, catalog No. 88881110) at room temperature. Extraction was repeated three times until no color was obtained to ensure the complete extraction. The combined extracts were filtered and concentrated through Whatman no.1 filter paper and evaporated in vacuum at 40°C by using a rotary evaporator (IKA Rotary Evaporator, IKA rv10) and stored at −20°C until the use for GC-MS analysis, antimicrobial, and cytotoxicity tests [22].

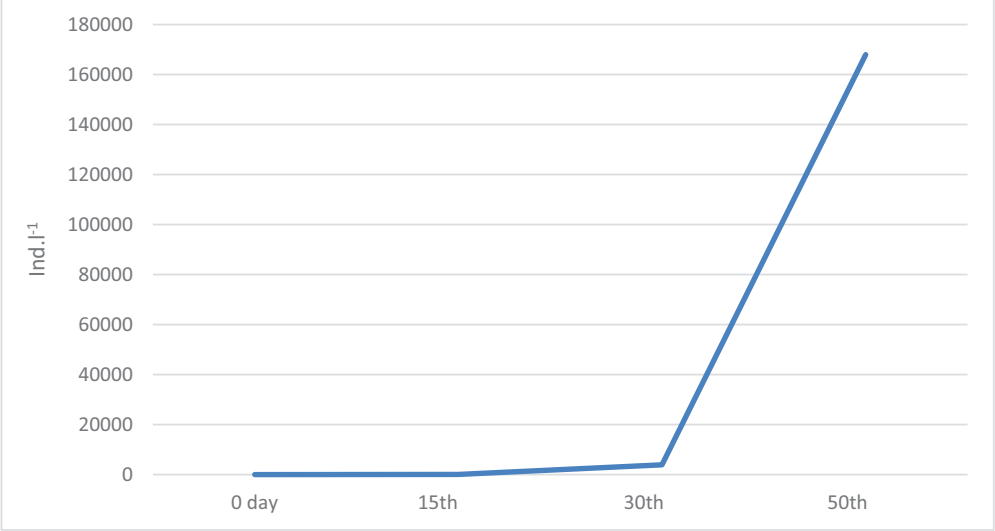
Anti-proliferation assay

The 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) protocol was performed for the *in vitro* determination of the anti-proliferative activity of the *H. salina* extract against the following human cancer cell lines; HepG2 (liver), A549 (lung), HCT (colon), and MCF7 (breast), in addition to THLE2 (non-cancerous liver epithelial cells), WI38 (non-cancerous lung fibroblast cell), FHC (non-cancerous colon epithelial cells), and MCF10a (non-cancerous breast epithelial cells) [23].

Antimicrobial activity

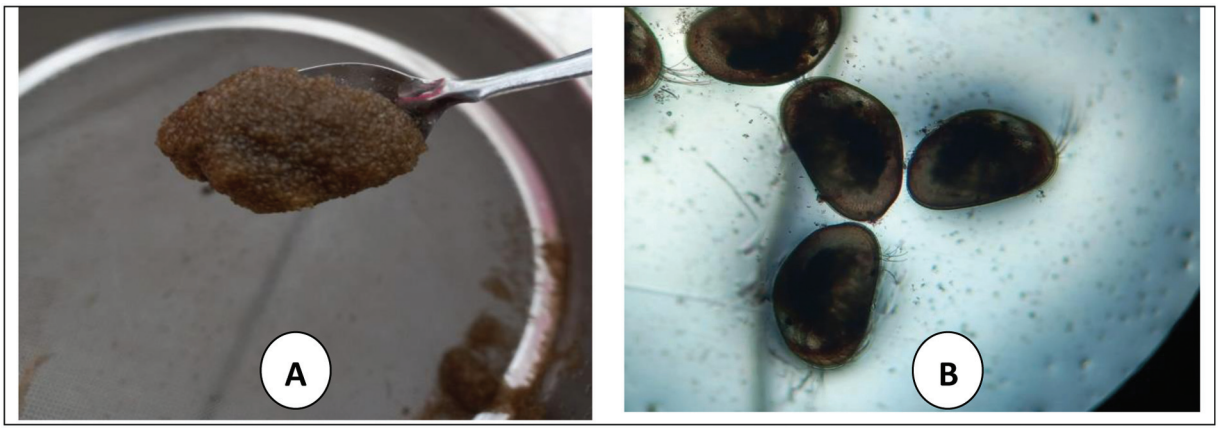
Assessment of the antimicrobial activity of the *H. salina* extract against Gram-negative bacteria (*Escherichia coli* (*ATCC 25922*), *Klebsiella pneumonia* (*ATCC 10031*), and *Salmonella typhi* (*ATCC 14028*)) and Gram-positive bacteria (*Sarcina lutea*, *Staphylococcus aureus (ATCC 29213)*, *Bacillus cereus*, *Bacillus subtilis (ATCC 6633)*, and *Enterococcus fecalis (ATCC 29212)*) was performed using the well diffusion agar method [24]. The antimicrobial activity of *H. salina* extract was tested at a concentration of 15 mg/ml. Commercial antibiotics (Amoxicillin, Amikacin, and Ciprofloxacin) were applied as positive controls, while 5% DMSO was used as a negative control.

Figure 1



The population growth rate curve of the *H. salina*.

Figure 2



The harvesting of *H. salina* from the culturing pond (A: The biomass of *H. salina* individuals, and B: *H. salina* individuals under binuclear microscopy).

Chemical composition of the extract (GC-MS analysis)

Chemical composition of the *H. salina* extract was analyzed using the Agilent 8860 Gas Chromatograph system and the Agilent 5977B GC/MSD system at the National Research Center, Cairo, Egypt. Bioactive compounds in the extract were identified as the closest (highest probability) matches with compounds recorded in the NIST MS Spectrum Library and Agilent's Retention Time Locked (RTL) database (ag-ilent.com/en/product/gas-chromatography-mass-spectrometry-gc-ms/gc-ms-application-solutions/gc-ms-libraries).

Molecular docking study

The chemical structures of identified compounds were built in their 2D and 3D conformations via the builder tool in MOE 2014.0901. At that time, energy minimization was performed by MMF94FX force field. A molecular docking of tested compounds in the active binding site of the enzyme was then performed. The water molecules were deleted during docking, and the absent hydrogen atoms were reserved to correct the ionization states to be assigned to the protein structure. The 'Docking' module in MOE program was run. The 'Ligand Interactions' MOE tool for analysis of docking results was employed by visualization of the protein-ligand interactions in the binding site.

Data analysis

A one-way statistical ANOVA test was applied to compare the variance of the cytotoxic crude extract activity on tumor cells and non-cancerous cells of the same organ by using XLSTAT 2016. Also, ANOVA test was applied to compare the activity of *H. salina* extract and the commercial antimicrobial against the investigated bacterial strains.

Results and discussions

Mass-production of *H. salina*

The mass culture of *H. salina* was started by inoculating the ceramic pond with 1 individual/L, and the density of the cultured Ostracods increased over time. After 50 days, the density of *H. salina* attained ca. 170 000 individuals/L, following the population growth rate (Fig. 1). At day 50, about 500 L of the culturing pond was harvested using plankton to collect the biomass of *H. salina*, following by washing and wringing out (Fig. 2). From the results of culturing, we conclude that growth during 50 days is optimal to achieve high production of the *H. salina* biomass. Known for its high egg productivity, *H. salina* is able to grow in a variety of environmental conditions [25], reproduces by parthenogenesis and reaches the adult stage in about 15 days [26]. *C. vulgaris* is a suitable food for promoting the growth of *H. salina* [14]. A methanol extract was prepared from the *H. salina* biomass as detailed in Methods/Experimental.

Antitumor activities of the *H. salina* extract

The cytotoxic/anti-proliferative activity of the *H. salina* crude extract against different human cancer cell lines originating from different organs-liver (HepG2), lung (A549), colon (HCT), and breast (MCF7)-was assessed as described in Materials and Methods. As shown in Table 1, the *H. salina* extract showed moderate to good growth inhibition against the cancer cell lines (IC_{50}=23.2, 17.6, 13.8, and 12.8 μg/ml, respectively). Staurosporine, a natural product with potent anticancer effects due to inhibition of multiple protein kinases [27] was used as a positive control and produced comparable growth inhibition (Table 1). We further tested for the growth inhibition effects against

Table 1 IC_{50} values of the growth-inhibition effect of *H. salina* and Staurosporine on cancer and non-cancerous cells, *P* values, and the Selectivity Index (SI) values. Data are presented as mean±SD, *n*=3

Organ	Liver		Lung		Colon		Breast	
Cell line	HepG2	THLE2	A549	WI38	HCT	FHC	MCF7	MCF10a
IC_{50} (μg/ml), *H. salina* extract	23.25±1.03	105.65±5.15	17.65±0.78	39.25±1.91	13.76±0.61	62.35±3.04	12.81±0.57	47.04±2.29
IC_{50} (μg/ml), Staurosporine (control)	7.79±0.35	31.50±1.53	6.25±0.28	27.14±1.32	8.82±0.39	24.86±1.21	6.30±0.28	21.71±1.06
SI	4.5		2.2		4.5		3.9	
P-value (cancer cells vs. normal cell)	<0.0001		<0.0001		<0.0001		<0.0001	
Significance	Yes		Yes		Yes		Yes	

healthy, non-cancerous cells from the same organs: THLE2 (liver epithelial cells), WI38 (lung fibroblast cells), FHC (colon epithelial cells), and MCF10a (breast epithelial cells), revealing a significantly lower sensitivity of the non-cancerous cells to the *H. salina* extract (Table 1). The cytotoxicity selectivity index (SI) calculated by dividing the IC_{50} of a non-cancerous cell line by IC_{50} of a cancer cell line of the same organ is used to find the degree of selectivity of a tested agent towards cancer cells [28]. SI above 2 demonstrates selectivity against cancer cells, while the value less 2 indicates toxicity to both cancer and normal cells. Several *in vitro* anti-tumor studies have relied on the SI to conclude that a given compound has selective anticancer activity [29–31]. We found that the SI of the *H. salina* extract varied from 2.2 to 4.5, revealing a selective effect of the extract towards cancer cells (Table 1). On the other hand, the activity of the *H. salina* extract against different human cancer cell lines (HepG2, A549, HCT, and MCF7) may be due to the presence of compounds such as; ethyl iso-allocholate, 9-(Z)octadecenamide (Z)-, oleic acid, palmitic acid, α-amyrin, valeric acid, tetramethyl-2-hexadecen, and cyclopentaneundecanoic acid (Table 3 and Fig. 3) in the extract, which may have cancer preventive and anticancer activity [32–44]. We thus conclude that the *H. salina* extract has the potential for exploitation in the discovery of novel bioactive compounds as potential anticancer agents.

Antimicrobial activities of the *H. salina* extract

The *H. salina* crude extract showed activity against three tested Gram-negative bacterial species, while it was effective only on two of the tested Gram-positive bacteria (Table 2). Among the tested bacteria, *S. lutea* was most sensitive to the extract, followed *by E. coli*, *S. typhi*, *K. pneumonia*, and *B. cereus* with the inhibition zones of 25, 17, 15, 12, and 8 mm, respectively. The antimicrobial activities of the *H. salina* extract were comparable to those induced by control antibiotics: amoxicillin, amikacin, and ciprofloxacin (Table 2). Moreover, there were no significant differences in the activity of the extract and the antibiotic Amikacin against *S. lutea*, *E. coli*, and *K. pneumonia*. Also, there were no significant differences in the activity of the extract and the antibiotic Ciprofloxacin against*S. lutea*. The activity of the *H. salina* extract against *S. lutea*, *E. coli*, *S. typhi*, *K. pneumonia*, and *B. cereus* may be due to the presence of compounds like; ethyl iso-allocholate, 9-(Z) octadecenamide (Z)-, palmitic acid, α-amyrin, valeric acid, tetramethyl-2-hexadecen, and cyclopentaneundecanoic acid (Table 3 and Fig. 3) in the extract, which may have antimicrobial activity

Table 2 Antimicrobial activity of the *H. salina* extract and three antibiotics against 8 pathogenic bacterial strains (inhibition zone expressed in mm). N/A-not active. Data are presented as mean±SD, *n*=3

Extract	*S. lutea*	*E. coli*	*K. pneumonia*	*S. typhi*	*S. aureus*	*B. cereus*	*B. subtilis*	*E. fecalis*
H. salina crude extract	$25±1.2^{ef}$	$17±0.9^{j}$	$12±0.6^{kl}$	$15±0.5^{k}$	N/A	$8±0.6^{a}$	N/A	N/A
Amoxicillin (25 μg/ml)	$35±2.2^{b}$	N/A	N/A	$35±1.2^{b}$	$8±0.3^{a}$	$45±1.9^{m}$	N/A	$28±2^{d}$
Amikacin (30 μg/ml)	$25±1.7^{ef}$	$18±0.6^{ij}$	$14±1.6^{k}$	$27±2.1^{de}$	$20±0.9^{hi}$	$25±1.2^{ef}$	$20±1.7^{hi}$	$12±0.^{3l}$
Ciprofloxacin (5 μg/ml)	$27±0.8^{de}$	$30±1.2^{c}$	$21±1.7^{g}$	$35±1.2^{b}$	$18±0.9^{b}$	$35±1.9^{j}$	$25±1.7^{ef}$	$24±1.2^{fg}$
Negative control	0	0	0	0	0	0	0	0
ANOVA (*P*-value)								
H. salina Vs. Amoxicillin	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
H. salina Vs. Amikacin	<0.0.15	<0.0.45	<0.0.09	<0.0001	<0.0001	<0.0001	-	<0.0001
H. salina Vs. Ciprofloxacin	<0.1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a-l]: Different letters indicate statistically significant differences groups ($P<0.05$, Tukey's test).

Table 3 Bioactive compounds of the *H. salina* extract detected by GC-MS

Identified compound	Rt	Area %	M.F.	M.wt.	Reported medically relevant activities	References
Cyclopentaneundecanoic acid, methyl ester	3.505	1.67	$C_{17}H_{32}O_2$	268.4	Antimicrobial, anti-inflammatory, cancer preventive, hepatoprotective	[36,43]
Valeric acid, 4-methyl-	5.399	3.72	$C_6H_{12}O_2$	116.16	Antimicrobial, anticancer	[41,42]
2-Piperidinone	11.71	4.19	C_5H_9NO	99.13	Unknown	
Benzenepropanoic acid 1-methylethyl ester	16.053	2.88	$C_{12}H_{16}O_2$	192.2542	Unknown	
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	28.762	1.81	$C_{20}H_{40}O$	296.5	Antimicrobial, anti-inflammatory, anti-cancer, diuretic	[43,44]
Palmitic acid	30.278	11.65	$C_{16}H_{32}O_2$	256.42	Antioxidant, antimicrobial, anticancer	[37,38]
Oleic acid	34.003	12.3	$C_{18}H_{34}O_2$	282.4614	Anti-inflammatory, cancer preventive	[36]
Oleic acid amide	34.341	4.96	$C_{18}H_{35}NO$	281.5	Antiallergic activity, anti-inflammatory	[46,47]
9-(Z)Octadecenamide, (Z)-	37.454	20.6	$C_{18}H_{35}NO$	281.4766	Antimicrobial, anticancer	[34,35]
α-Amyrin	43.788	10.73	$C_{30}H_{50}O$	426.7	Antimicrobial, anticancer	[39,40]
Ethyl-3,7,12-trihydroxycholan-24-oate (Ethyl iso-allocholate)	49.293	23.1	$C_{26}H_{44}O_5$	436	Antimicrobial, anticancer	[32,33]

[32–44]. Therefore, the *H. salina* extract crude extract could be a promising candidate as a natural source of antimicrobial agents.

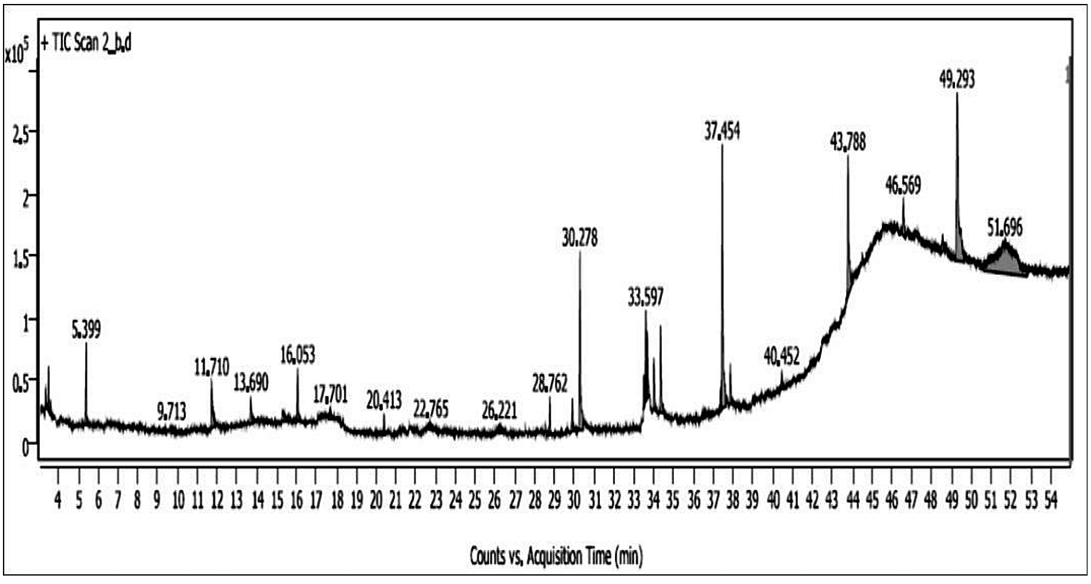
Chemical characterization and the medical activities of *H. salina* bioactive compounds

GC-MS analysis of the *H. salina* extract revealed about 11 compounds of potential medical interest that might be responsible for anticancer and antimicrobial activites (Table 3 and Fig. 3). Among the bioactive compounds, ethyl iso-allocholate occurred with the highest peak area of 23.1%. This compound was effective against *P. mirabilis*, *E. coli*, *S. aureus*, and *P. aeruginosa* bacteria [32,45]. Some *in vitro* and *in vivo* anticancer studies have also suggested that this compound induced caspase-dependent apoptosis in lung cancer cells (A549) and reduced tumor growth, moreover it was being safe in normal tissues [33].

9-(Z)octadecenamide, (Z)- occurring with a high peak area of about 20.6% has previously been found among other bioactive compounds in a crude extract of *Citrus aurantifolia* leaves and showed strong activity against some pathogenic bacteria [34]. Additionally, the *Halomonas* sp. BS4 extract containing 9-(Z) octadecenamide, (Z)- effectively controlled some pathogenic bacteria and fungi as well as epithelial carcinoma cells [35].

Among other interesting compounds we identify in *H. salina*, cyclopentaneundecanoic acid, methyl ester and 2-hexadecen-1-ol, 3,7,11,15-tetramethyl occurred with the lowest peaks of about 1.6 and 1.8%, respectively. Promising medically relevant activities have been previously attributed to these compounds, such as antimicrobial, anti-inflammatory, and cancer preventive [36,43]. Some compounds we identified in

Figure 3



Chromatogram peaks of GC-MS analysis of the *H. salina* extract.

H. salina, such as benzenepropanoicacid 1-methyl ethyl ester or 2-piperidinone are presently unclear for relevant medical activites, and future research is required to analyze if these compounds could be responsible to the anticancer and antimicrobial activities of the *H. salina* extract.

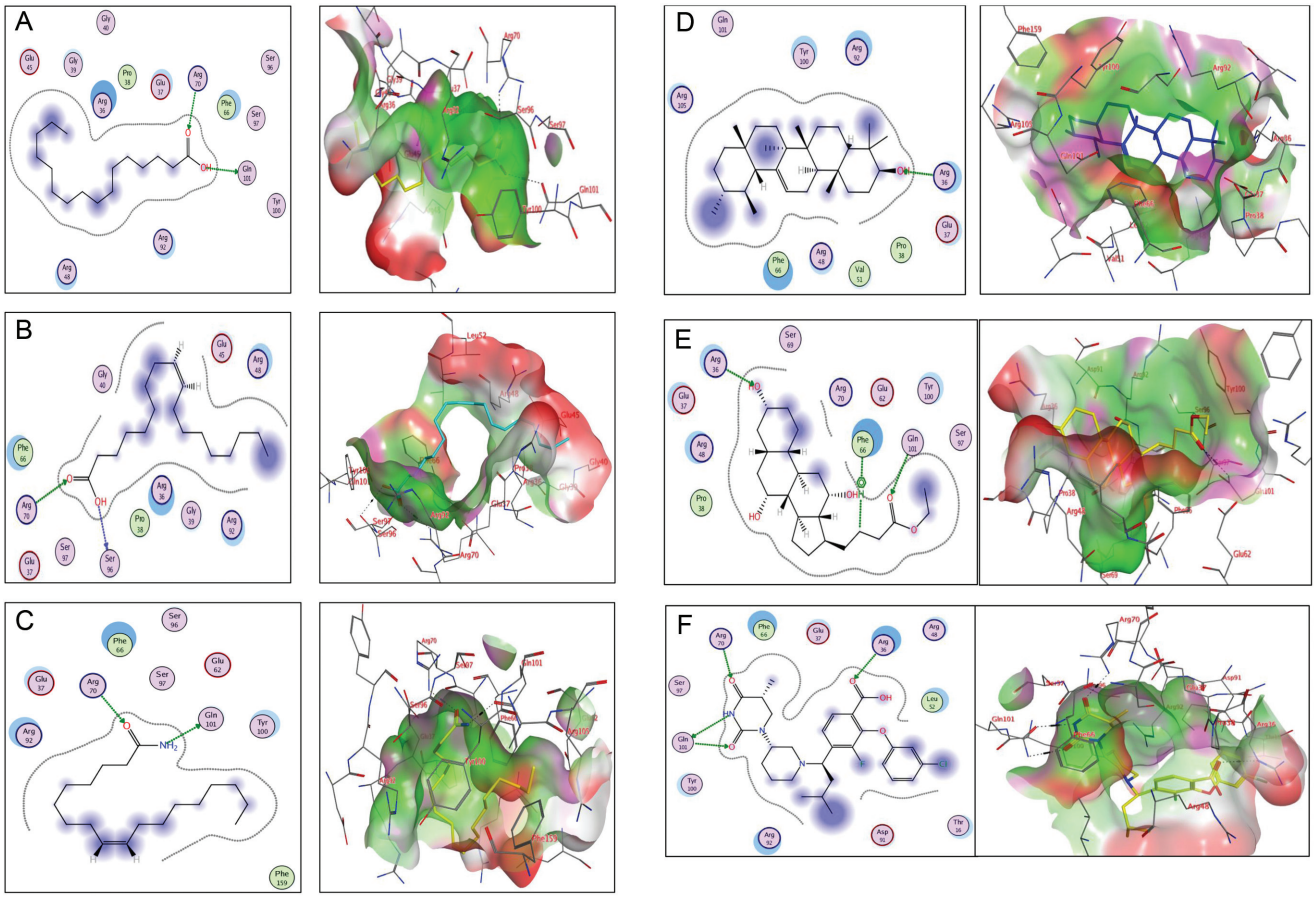
Docking inside the active site of thymidylate kinase (PDB: 4QGG)

Docking simulation of compounds (6,7,9-11) inside the active site of TMK was performed on 4QGG code downloaded from protein data bank. The co-crystallized ligand was first re-docked for validation revealing docking score value=-12.002 kcal/mol with a root-mean-square deviation (RMSD) of 1.68 Å. Three carbonyl groups were able to form three hydrogen bonds with the most important residues for binding in the active site Arg36, Arg70 and Gln101, in addition to another hydrogen bond between NH group and Gln101. Compounds under investigation displayed binding energies ranging from -6.172 to -10.239 kcal/mol comparing with the ligand having energy score=-10.741 kcal/mol. By examining the binding modes of the tested compounds, it was found that the C=O groups of compounds 6, 7, 8 were able to form hydrogen bonds with Arg70 the most conserved amino acid in the binding site and resembling the mode of binding with the legend. Additionally, compound 6 contributed via two hydrogen bonds with Gln101. Furthermore compound 11 revealing the highest score was fitted in the active site via two hydrogen bonds with Arg36 and Gln101 besides arene-H interaction with Phe66 (Fig. 4, Table 4).

Conclusion

The *H. salina* extract offers the potential for further exploitation in the discovery of novel anticancer compounds. It is also promising as a source of alternative bioactive molecules that can be used as alternative to antibiotics, especially against Gram-negative bacteria. Also, molecular docking revealed that nearly all the test compounds were able to bind with the binding site via two or three binding interactions resembling that of the ligand, besides having nearly the same mode of interactions. Compound **11** was able to fit inside the binding site

Figure 4



2D (left side) and 3D (right side) images of the proposed binding interactions of compounds 6 (A), 7(B), 9 (C), 10 (D), 11 (E) and ligand (F) into the active site of TMK.

Table 4 The docking scores and interacting residues of compounds (6,7,9-11) inside the binding site of TMK

Compound no.	Docking score (kcal/mol)	Interacting residues
6:Palmitic acid	–8.716	*Arg70, Gln101*
7:Oleic Acid	–8.853	*Arg70, Ser96*
9: 9-Octadecenamide, (Z)-	–9.233	Arg70, Gln101
10:α-Amyrin	–6.172	Arg36
11: Ethyl iso-allocholate	–10.239	Arg36, Phe66, Gln101
Ligand	–10.741	Arg36, Arg70, Gln101

via two hydrogen bonds with Arg36 and Gln101, in addition to arene-H interaction with Phe66, having high score=-10.239 Kcal/mol. Our work lays the ground to future investigations aiming at purifying the bioactives from *H. salina* and assessing their individual activities against pathogenic bacteria and cancer cells.

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Data availability Statement: The data presented in this study are fully available in the main text of this article.

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Conflicts of interest
The authors declare no competing interests. The authors certify that they do not have any other participating organization, entities, or persons in the intellectual property rights in the current study subject and materials discussed in this manuscript.

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