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Bioactive compounds from *Haloarcula* sp. strain NOS1: Antimicrobial, anti-inflammatory and anti-prostatic carcinoma integrated with molecular docking approach

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Halophilic archaea are microorganisms that thrive in high-salinity environments and have potential applications in pharmaceuticals and industry due to their bioactive compounds. However, many haloarchaea bioactivities remain undiscovered. In this study, *Haloarcula* sp strain NOS1 was isolated, identified, and subjected to *in vitro* assessment of its antimicrobial, anti-inflammatory, and anti-prostate cancer properties. The ethyl acetate extract demonstrated significant antimicrobial activity against pathogenic microorganisms such as *Bacillus subtilis* ATCC 6633, *E. coli* ATCC 8739, *Candida albicans* ATCC 10221, *Pseudomonas aeruginosa* ATCC 90274, and *Staphylococcus aureus* ATCC 6538. Additionally, the bioactive compounds displayed dose-dependent inhibitory effects on blood hemolysis at varying doses, with percentages of 95.6%, 85.3%, 75.2%, 59.3%, and 46.5% at concentrations of 1000, 800, 600, 400, and 200 µl/mL, respectively. The cytotoxicity test revealed potential efficacy against the PC3 cell line, with an IC50 of 96.94% at concentrations varying between 62.5 µg/mL and 125 µg/mL. Furthermore, the bioactive compounds showed a notable ability to bind to prostate cancer proteins through a docking approach, with binding energy scores in the range of -5.6 to -6.6 kcal/mol. This suggests that these bioactive compounds could serve as promising therapeutic agents against pathogenic microorganisms and prostate cancer.

Keywords: *Haloarcula* sp strain NOS1, anti-prostatic carcinoma, Antimicrobial, anti-inflammatory, molecular docking

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INTRODUCTION

Cancer represents globally the leading cause of mortality and serves as a major barrier to enhancing life expectancy (Jin *et al*., 2023). Bladder and prostate cancers are urological malignancies that elevate incidence and mortality rates in individuals over 40 years of age (Aveta *et al*., 2023). Prostate cancers are related to acute inflammations as well as chronic inflammations and it is the second most prevalent cancer among men across the globe after lung cancer, accounting for the sixth most cause of cancer death (Wigner *et al*., 2021). Furthermore, high-fat diet (HFD), obesity, and microbial infections associated with prostate cancer (Maghsoudi *et al*., 2023). An innovative approach to cancer treatment entails the identification of novel drugs that specifically target malignant cells (De Angelis *et al*., 2021). Microorganisms are important sources of natural products, providing opportunities for the discovery of novel bioactive compounds with anticancer activity, associated with their short half-lives (Böhringer *et al*., 2017; Leinberger *et al*., 2021). Extremophiles offer a potential avenue for the discovery of novel bioactive compounds with medical applications, owing to their capacity to survive in extreme environments marked by high temperatures and low pH levels (Pfeifer *et al*., 2021; Giddings *et al*., 2022). Bioactive substances derived from extremophiles exhibit cytotoxic effects on the MCF-7 breast cancer cell line (Ibrahim *et al*., 2023).

Halophilic archaea have attracted attention from all scientists because of their unique traits, such as genetic versatility, low space needs for cultivation, and capacity to generate a wide range of bioactive compounds with unique qualities (Torregrosa-Crespo *et al*., 2017; Dutta & Bandopadhyay, 2022). Carotenoids obtained from different halophilic archaeal strains have demonstrated in vitro cytotoxic effects on HEPG2 carcinoma cell lines (Abbes *et al.,* 2013; Hou *et al*., 2018). It is still necessary to conduct more study to develop new anti-cancer chemicals from halophilic bacteria, even with current findings. So, the current study aimed to isolate haloarchaeal strains from North Sinai solar salterns and evaluated the antimicrobial properties of bioactive molecules derived from haloarchaea against different pathogens, beside *in-vitro* anticancer effects on prostate cell lines integrated with *in-silico* molecular docking analysis.

MATERIALS AND METHODS

Isolation of *Haloarcula* sp strain NOS1

Haloarcula sp strain NOS1 was isolated from salts obtained from Bardaweil lagoons in North Sinai with geographic coordinates of 31°02’25.8” N 33°21’19.6” E (Figure 1). Subsequent molecular characterization was conducted, and the sequence was submitted to GenBank under the accession number OM017152, as outlined by Elshafey *et al*. (2022). This strain was utilized in the present study.

Figure 1. Geographical representation of the Solar Saltern in North Sinai, Egypt.

Screening for antagonistic activity

A total of 30 halophilic archaeal strains were obtained as detailed by Elshafey *et al.* (2022), and these isolates were screened for their ability to antagonize each other (Quadri *et al*. 2016). Upon growth in appropriate broth media, the indicator strains were streaked on agar plates. The candidate strains were subsequently spot inoculated onto the agar surface containing the indicator strains and incubated for a duration of two weeks. The extent of the inhibition zone was subsequently determined.

Construction of phylogenetic trees

Phylogenetic analysis of *Haloarcula* sp strain NOS1 was conducted by aligning 16S rRNA sequences using Cluster W. The evolutionary tree was subsequently created utilizing neighbor-joining algorithms within the MEGA 11 software (Tamura *et al*., 2013).

Bioactive compounds from *Haloarcula* sp strain NOS1

The isolated strain NOS1 was cultured in a halophilic broth medium for ten days at 40°C to generate bioactive metabolites. The extraction was followed by ethyl acetate and separation via a separating funnel. The organic phases were collected, evaporated to dryness with a rotary evaporator, and then concentrated and stored at 4°C (Hamedo *et al*., 2023).

Analysis of bioactive compounds from *Haloarcula* sp. strain NOS1 using GC-MS

A direct capillary column TG–5 MS (30 m x 0.25 mm x 0.25 µm film thickness) was employed to analyze the chemical composition of bioactive compounds present in *Haloarcula* sp strain NOS1, utilizing a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA). The column oven temperature was initially set at 50°C, subsequently raised at a rate of 5°C/min to 230°C, maintained for 2 minutes, and then increased at a rate of 30°C/min to a final temperature of 290°C, held for an additional 2 minutes. The injector and MS transfer line temperatures were maintained at 250°C and 260°C, respectively. Helium served as the carrier gas at a steady flow rate of 1 ml/min. The solvent delay was 3 minutes, and 1 microliter diluted samples were automatically injected using an Autosampler AS1300 system linked to a gas chromatograph operating in split mode. EI mass spectra were measured at 70 eV ionization voltages within the m/z range of 40–1000 in full scan mode. The initial temperature of the ion source was set at 200°C. The identities of the components were established by comparing their retention times and mass spectra with data from the WILEY 09 and NIST 11 mass spectral databases (Baeshen *et al*., 2023).

Evaluation of antimicrobial activity

Bioactive compounds extracted from *Haloarcula* sp. strain NOS1 were tested for their antimicrobial activity against pathogenic strains as *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 90274), *Candida albicans* (ATCC 10221), and Mucor reinelloids that were obtained from Ain Shams Hospital. The inhibition zones were quantified utilizing the Agar-well Diffusion Method (Valgas *et al*., 2007; Hamedo *et al*., 2023). The pathogenic strains were cultured in suitable broth media and incubated at 37°C. A microbial inoculum (1 mL) was applied to the agar surface, followed by the creation of an inoculation well for the evaluation of bioactive compounds and gentamicin (30 µg/mL) as a control. The plates were then incubated, and the inhibition zone was evaluated.

Minimum inhibitory concentration (MIC) assessment

The microdilution technique was utilized to determine the minimum inhibitory concentration (MIC) for bioactive compounds derived from *Haloarcula* sp. strain NOS1. The MIC was determined through serial dilution using various concentrations of bioactive compounds (e.g., 0.06, 125, 250, 500, 1000 µg/mL) mixed with 10 mL of pathogenic strain suspension containing 5 × 10^^{6} CFU/mL. The mixture was then incubated with a control for 24-72 hours at 37°C. Visual turbidity of the tubes was observed before and after incubation to verify the MIC value, which is reached when no visible growth is present in the tubes (Andrews, 2001; Naz *et al.,* 2017).

Anti-inflammatory assay

Preparation of red blood cell suspension: All the blood was collected from healthy volunteers and placed in heparinized tubes. The tubes underwent centrifugation at 3000 r.p.m for 10 minutes. The red blood pellets were dissolved using normal saline. The volumes of the dissolved red pellets were measured and then reconstituted as a 40% v/v suspension using an isotonic buffer solution containing 10 mM sodium phosphate buffer at a pH of 7.4. The buffer solution was composed of 0.2 gm of NaH_2PO_4, 1.15 gm of Na_2HPO_4, and 9 gm of NaCL per liter of distilled water (Anosike *et al*., 2012).

Hypotonicity induced hemolysis: The hypotonic solution was allocated into duplicate pairs of tubes, with each tube containing varying doses of the extracts (100, 200, 400, 600, 800, and 1000 µg/ml, respectively). Each tube contained 5 ml of an isotonic solution. Control tubes contained 5 ml of vehicle (distilled water) and 5 ml of indomethacin at a concentration of 200 µg/ml. Following the addition of 0.1 ml of erythrocyte suspension, each tube was stirred gently. After a 1-hour incubation at room temperature, the mixtures underwent centrifugation for 3 minutes at 1300 r.p.m. The absorbance (OD) of hemoglobin in the supernatant was quantified at a wavelength of 540 nm utilizing a Spectronic spectrophotometer (Milton Roy). Hemolysis percentage was estimated by assuming that the hemolysis induced by distilled water represents 100% (Anosike *et al*., 2012). The percentage of hemolysis inhibition for the extract was assessed as follows:

% Inhibition of hemolysis= 1-((OD2-OD1)/(OD3-OD1)) *100

Where OD1 = Absorbance of the test sample in an isotonic solution, OD2 = Absorbance of the test sample in a hypotonic solution., OD3 = Absorbance of the control sample in a hypotonic solution.

Antiproliferation assay

Cell line: The PC3 cell line of prostate cancer was obtained from the Egyptian Vacsera Research Foundation. To maintain the cells, Dulbecco's Modified Eagle's Medium (DMEM) was supplemented with 10% (v/v) fetal bovine serum (FBS) or cell maintenance and incubated at 37°C with 5% CO_2. A 100 g/ml concentration of streptomycin and penicillin was used to prevent contamination.

Sample preparation: One milliliter of dimethyl sulfoxide (DMSO) is used to prepare the bioactive components of *Haloarcula* sp. strain NOS1, and then put into sterile containers. The samples were maintained at room temperature and shielded from light exposure. For cytotoxicity test, the extract volume was standardized to a maximum permissible DMSO concentration of 0.1% *(*Tolosa *et al*., 2015).

Evaluation of anticancer activity using the MTT assay

Bioactive compounds from *Haloarcula* sp. strain NOS1 were evaluated for their anticancer properties utilizing the3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) assay (Tolosa *et al*., 2015). To create a confluent monolayer, 96-well tissue culture plates were seeded with 1 X 10^5 cells/ml (100 ul/well) and incubated for 24 hours at 37°C. The growth medium was removed from the 96-well plates, and the cell monolayer was washed twice. Then the sample was diluted twice in RPMI medium and supplemented with 2% serum (maintenance

medium). Three wells served as controls, receiving only maintenance media, whereas the other wells were treated with each dilution (0.1 ml). The plate was subsequently incubated at 37°C and observed for indicators of toxicity, including disruption of the cell monolayer, cell rounding, shrinkage, or granulation. A 5 mg/ml MTT solution in PBS (BIO BASIC CANADA INC) was prepared, and 20 microliters of this solution were added to each well. The plate was Shaked at 150 r.p.m for 5 minutes. After incubation at 37°C and 5% CO_2, for 1-5 hr, the formazan, a metabolic product of MTT was solubilized in 200 microliters of DMSO. The optical density was measured at 560 nm and the association between optical density and cell viability ratio was investigated, and the IC50 value, which represents the 50% inhibitory concentration of the test chemicals, was calculated for the treated cell line. The cellular viability percentage was calculated using the formula.

Cell viability percentage = 100 (treatment optical density/control value)

Molecular docking analysis of *Haloarcula* sp. strain NOS1 bioactive compounds against prostatic carcinoma

Receptor protein, ligands preparation and docking analysis: The 3D structure of the Human Androgen Receptor (AR) antagonists, widely used for the treatment of prostate cancer (PC3), was retrieved from the Protein data bank (PDB ID: 2AX6). This structure had a crystal structure determined by X-ray diffraction with a resolution of 1.50 Å, and was prepared by eliminating water molecules, cofactor, and substrate. The active sites were determined using the symbol software. Additional preparations included the addition of Kollman charges and polar hydrogen using auto-dock Tools-1.5.7 (Trott and Olson 2010). The three-dimensional (3D) structure of the bioactive compounds extracted from *Haloarcula* sp strain NOS1 was retrieved from the PubChem database at http://www.pubchem.ncbi.nlm.nih.gov. And energy minimization was performed using Avogadro software (Hanwell *et al*., 2012). The docking analysis of the prepared ligands and protein was utilized by Auto-Dock Tools-1.5.7 Vina software and the interactions between the receptors (protein) and docked molecules were visualized using BIOVIA Discovery Studio 2021.

ADMET and pharmacokinetics profiles of *Haloarcula* sp. strain NOS1 bioactive compounds: Swiss-ADME, an online computational tool, is used to obtain ADMET and pharmacokinetics profiles (Daoud *et al*., 2021). The tool utilizes the SMILES formula to determine the physicochemical parameters, pharmacokinetic profile, and drug-likeness, with a prediction accuracy ranging from 72% to 94%.

Statistical analysis

We performed a one-way analysis of variance (ANOVA) utilizing Minitab to ascertain significant differences, presenting the data in terms of mean and standard deviation. We employed the multiple-range test to compare the means and confirm the detected differences, setting the least significant difference at a 0.05 significance level.

RESULTS

Isolation and characterization of *Haloarcula* sp strain NOS1

The *Haloarcula* sp. strain NOS1, isolated from a solar saltern, was recognized as a Gram-negative, non-spore-forming bacteria after incubation at 40°C for two weeks on agar plates with 20% salt. The colonies were translucent, smooth, and entire, with an orange-red, mucous appearance. During the active growth phase, the cells appeared as triangular flat disks, ranging from 1.0 to 3.0 µm in size (Figure 2a). Phylogenetic analysis indicated a 97.5% similarity with members of the *Haloarcula* genus (Figure 2b).

Antagonistic activity of a promising *Haloarcula* sp. strain NOS1

Haloarcula sp. strain NOS1 exhibited promising antagonistic activity against all other halo-archaeal strains are isolated from the solar salts, as demonstrated in the Supplementary Table.

Analysis of bioactive compounds from *Haloarcula* sp. strain NOS1 using GC-MS

The analysis of bioactive substances derived from *Haloarcula* sp. strain NOS1 revealed the existence of aliphatic and aromatic compounds. The most prevalent chemical was 1,2-benzene dicarboxylic acid, which made up roughly 35.1% of the total components. It was followed by 6-octadecenoic acid (16.52%), n-hexadecenoic acid (7.63%), stearic acid (3.9%), and linoleic acid ethyl ester (1.07%) (Table 1).

Antimicrobial activity

Figure 3 demonstrates that the bioactive components of *Haloarcula* sp. strain NOS1 showed a promising activity against human pathogenic strains than gentamicin. The results showed that the bioactive compounds were most effective against *Bacillus*

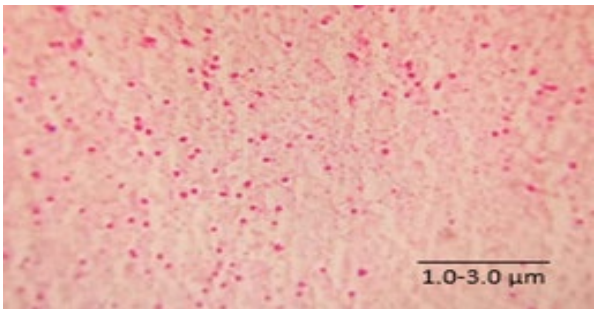


Figure 2a. Gram negative triangular flat disks shape.

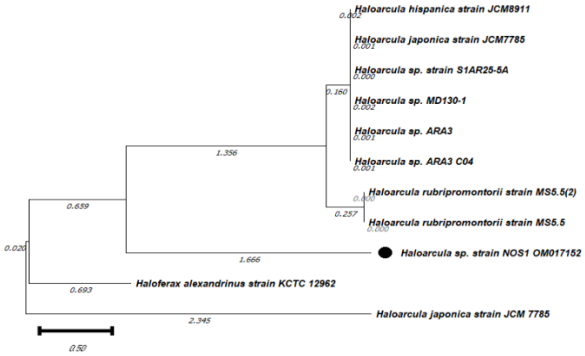


Figure 2b. Phylogenetic tree of *Haloarcula* sp strain NOS1 using the Neighbor-Joining method.

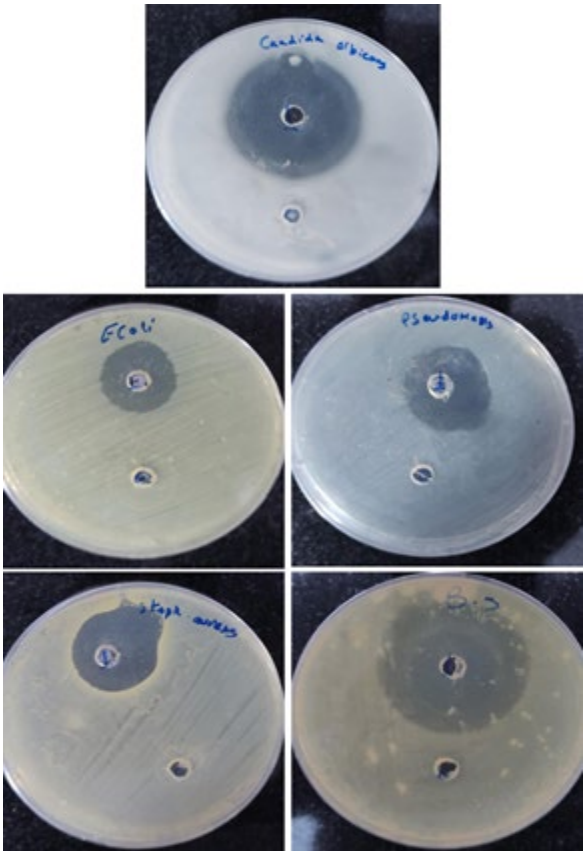


Figure 3. Antimicrobial activity of *Haloarcula* sp. strain NOS1 bioactive compounds against pathogenic microorganisms.

subtilis ATCC 6633, evidenced by a large inhibition zone of 34±0.85 mm. *E. coli* ATCC 8739 at 30±0.75 mm, *Candida albicans* ATCC 10221 at 29±0.72 mm, *Pseudomonas aeruginosa* ATCC 90274 at 28±0.70 mm, and *Staphylococcus aureus* ATCC 6538, which displayed less inhibition zone of 22±0.55 mm than control.

MIC of *Haloarcula* sp. strain NOS1 bioactive compounds

Figure 4 shows the susceptibility broth dilution test revealed the minimal inhibitory concentration (MIC) of bioactive compounds required to inhibit visible growth of microorganisms: 15.6 ± 0.39 µg/ml for *Bacillus subtilis* ATCC 6633, 62.5±1.5 µg/ml for *E. coli* ATCC 8739 and *Candida albicans* ATCC 10221, 125±3 µg/ml for *Pseudomonas aeruginosa* ATCC 90274, and 250 ± 6 µg/ml for *Staphylococcus aureus* ATCC 65.

Anti-inflammatory assay of *Haloarcula* sp. strain NOS1 bioactive compounds

Figure 5. illustrates the integrity of human red blood cell membranes (anti-inflammatory action) affected by bioactive compounds derived from *Haloarcula* sp. strain NOS1. The degree of protection exhibited a positive correlation with the quantities of the bioactive compounds. In comparison to control, the hemolytic inhibition at 1000, 800, 600, 400, and 200 µl/mL was 95.6%, 85.3%, 75.2%, 59.3%, and 46.5%, respectively. The findings show a dose-dependent suppression of hemolysis by the bioactive compounds obtained from *Haloarcula* sp. strain NOS1.

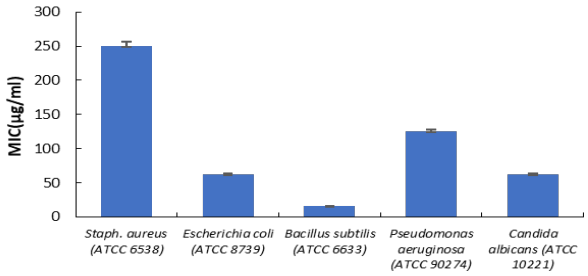


Figure 4. The Minimal Inhibitory Concentration (MIC) of *Haloarcula* sp. strain NOS1 bioactive compounds against pathogenic microorganisms.

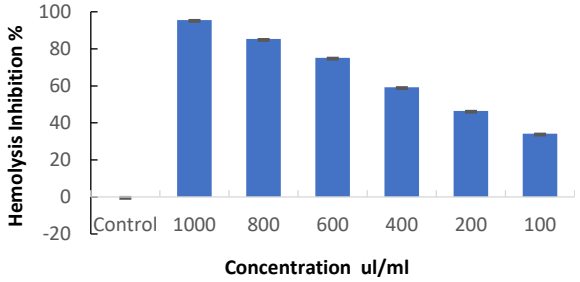


Figure 5. Anti-inflammatory assay of *Haloarcula* sp. strain NOS1 bioactive compounds.

Table 1. Bioactive compounds extracted from *Haloarcula* sp. strain NOS1 using GC-MS

No.	RT	Chemical name	Area %	Chemical formula	MW
1	23.43	Tetra decanoic acid	0.56	$C_{14}H_{28}O_2$	228
2	24.58	3-isobutylhexahydropyrrolo[1,2-a] pyrazine-1,4-dione	2.64	$C_{11}H_{18}N_2O_2$	210
3	24.88	Tetra decanoic acid, trimethylsilyl ester	0.67	$C_{17}H_{36}O_2Si$	300
4	26.17	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	0.58	$C_{17}H_{24}O_3$	276
5	26.31	4-Chloro-2-nitrobenzyl alcohol, n-pentyl ether	1.28	$C_{12}H_{16}ClNO_3$	257
6	26.5	N-Acetyl-l-arginine	0.82	$C_8H_{16}N_4O_3$	216
7	27.48	1,3,2-dioxaborinane, 2,4-diethyl -5-methyl-6-propyl	0.8	$C_{11}H_{23}BO_2$	198
8	27.86	n-Hexadecenoic acid	7.63	$C_{16}H_{32}O_2$	256
9	28.16	Pyrazofurin	1.38	$C_9H_{13}N_3O_6$	259
10	28.68	Hexadecenoic acid, trimethylsilyl ester	2.62	$C_{19}H_{40}O_2Si$	328
11	29.24	3,6-Diisobutyl-2,5-piperazinedione	0.63	$C_{12}H_{22}N_2O_2$	226
12	29.52	10-Octadecenoic acid, Methyl ester	0.31	$C_{19}H_{36}O_2$	296
13	30.09	1,2-Bis(2-hydroxyphenyl) ethylenediamine, N, N'-bis(2-butenylidene)-	0.5	$C_{22}H_{24}N_2O_2$	348
14	30.94	6-Octadecenoic acid	16.52	$C_{18}H_{34}O_2$	282
15	31.27	Stearic acid	3.9	$C_{18}H_{36}O_2$	284
16	31.57	3-ETHYL-3-HYDROXYANDROSTAN-17-ONE	1.42	$C_{21}H_{34}O_2$	318
17	32.11	Linoleic acid ethyl ester	1.07	$C_{20}H_{36}O_2$	308
18	34.09	17-Methoxyandrost-4-en-3-one o-methyl oxime	2.08	$C_{21}H_{33}NO_2$	331
19	34.5	Ergotamine	1.53	$C_{33}H_{35}N_5O_5$	581
20	36.65	Glycerol 1-palmitate	1.89	$C_{19}H_{38}O_4$	330
21	37.04	1,2-Benzenedicarboxylic	35.1	$C_{24}H_{38}O_4$	390
22	37.59	3-Ethyl-5-(2'-ethylbutyl) octadecane	0.48	$C_{26}H_{54}$	366

RT: retention time, MW: molecular weight

In vitro anticancer activity of *Haloarcula* sp. strain NOS1 bioactive compound prostatic carcinoma (PC3) cell line

Figures 6 and 7 show that ethyl acetate extracts of *Haloarcula* sp. strain NOS1 bioactive compounds exhibited significant antitumor efficacy against the PC3 cell line in a concentration-dependent manner and a lower concentration of 31.25 µg/mL of bioactive compounds demonstrated minimal toxicity to PC3 cells, whereas a higher concentration resulted in notable toxicity, with an IC50 value of 96.94% observed between 62.5 µg/mL and 125 µg/mL.

Molecular docking analysis of *Haloarcula* sp NOS1 bioactive compounds against prostatic carcinoma (Human Androgen Receptor)

According to the molecular docking analysis of bioactive compounds with the prostatic carcinoma Human Androgen Receptor (PC3) protein, the binding energy ranged from -5.6 to -6.6 kcal/mol (Table 2 and Figure 8). Hydrogen bonding is one of the chemical ligand-receptor interactions that can be investigated in Discovery Studio. Besides Van der Waals interactions between the ligand and receptor and Pi-stacking interactions are detected which indicate interactions between the aromatic rings of the ligand and receptor (Figure 8). These interactions aid in the development of promising treatment as well as insights into drug mechanisms. The pharmacological characteristics of compounds from *Haloarcula* sp. strain NOS1 were assessed using Lipinski's five rules. The results indicated that the five compounds selected for molecular docking exploration against the Human Androgen, responsible for prostatic cancer (PC3), were able to conform to Lipinski's rule. Analysis of the Swiss-ADME tool indicates that all examined substances have a high gastrointestinal absorption (GI) as predicted (Table 3). Based on these findings, it appears that this bioactive substance may be more effectively absorbed in the gastrointestinal tract and has potential as an oral medicine candidate.

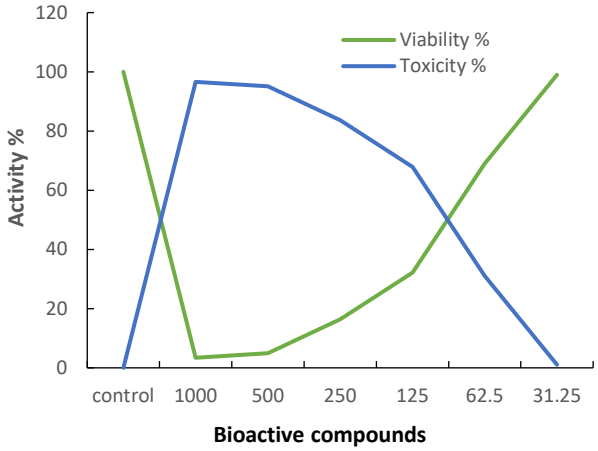


Figure 6. Cytotoxicity of *Haloarcula* sp. NOS1 bioactive compounds on PC3 cell line.

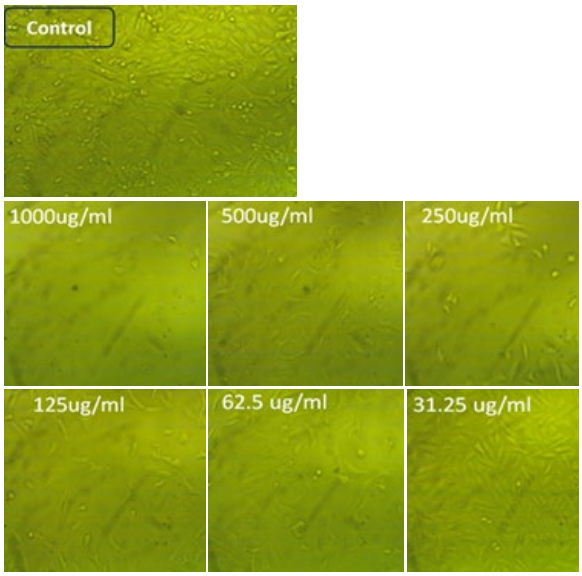
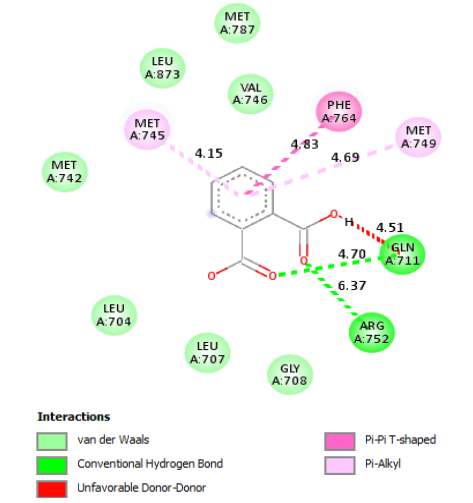
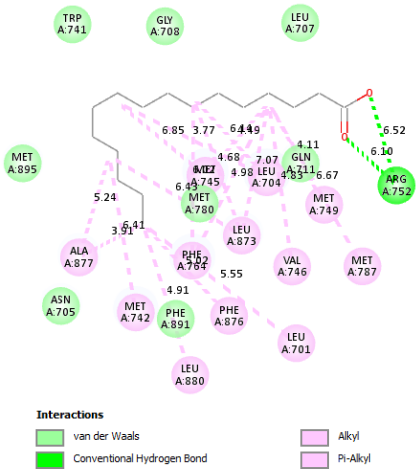


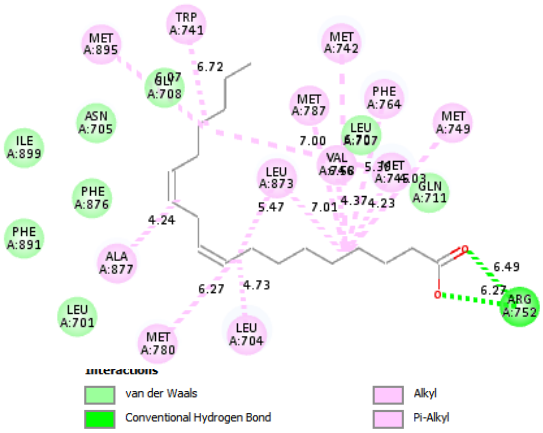
Figure 7. Effect of different concentration of *Haloarcula* sp. NOS1 bioactive compounds on PC3 cells.



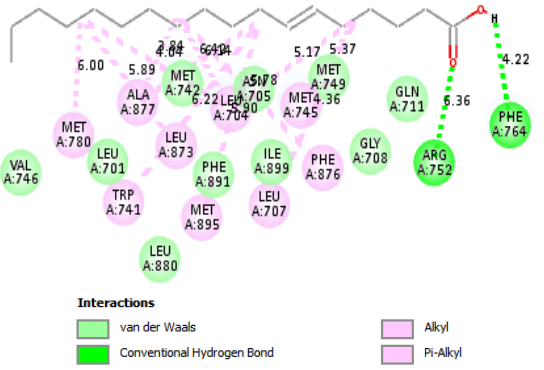
Human Androgen Receptor interaction with 1,2-benzendicarboxylic acid



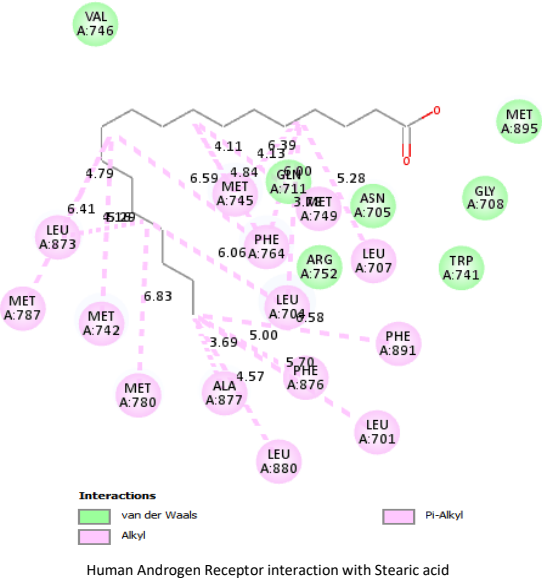
Human Androgen Receptor interaction with n-Hexadecenoic acid



Human Androgen Receptor interaction with Linoleic acid ethyl ester



Human Androgen Receptor interaction with 6-Octadecenoic acid



Human Androgen Receptor interaction with Stearic acid

Figure 8. Human Androgen Receptor interaction with *Haloarcula* sp. NOS1 bioactive compounds.

Table 2. The structures of the identified bioactive compounds with comparable binding activities with the target prostatic carcinoma (PC3) target protein.

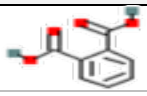
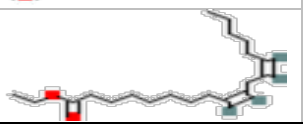
Bioactive compound name	Binding affinity (kcal/mol)	Structure
1,2-benzenedicarboxylic acid	-6.6	
6-Octadecenoic acid	-6.1	
n-Hexadecenoic acid	-6.3	
Stearic acid	-5.6	
Linoleic acid ethyl ester	-6.3	

Table 3. Predictions obtained using Swiss-ADME tool concerning the pharmacokinetics profile of investigated bioactive compounds.

Bioactive compounds	GI	BBB	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	LogKp (cm/s)
1,2-benzenedicarboxylic acid	High	-	-	-	-	-	-	-	-6.80
6-Octadecenoic acid	High	-	-	+	-	-	-	-	-2.60
n-Hexadecenoic acid	High	-	+	-	-	-	-	-	-2.77
Stearic acid	High	-	+	+	-	-	-	-	-2.19
Linoleic acid ethyl ester	High	-	+	+	-	+	-	-	2.97

GI: Absorption in the gastrointestinal tract, BBB: permeability of the blood-brain barrier, P-gp :P glycoprotein, CYP: human cytochrome P450, logKp : skin permeation coefficient.

DISCUSSION

Cancer remains one of the deadliest diseases. According to (Khalifa *et al*., 2019), 60% of anticancer drugs in use are derived from natural sources. More studies have emphasized the potential of marine microorganisms as a new source for producing secondary metabolites with significant pharmacological effects (Petersen *et al*., 2020; Karthikeyan *et al*., 2022). The rapid growth rate of these microorganisms facilitates the control of target substance production, which is advantageous for large-scale production (Karthikeyan *et al*., 2022). Archaea have been a subject of considerable interest among researchers, especially regarding their carotenoid content (Hou, 2018). Previous studies focused on halophilic archaea because of their ability to tolerate extreme environments (Abbes *et al*., 2013; Elshafey *et al*., 2022), resulting in the synthesis of distinctive molecules and metabolites not found in other organisms (Squillaci *et al*. 2017). To understand nutrient absorption in saline environments for haloarchaea the morphological analysis was conducted (Strakova *et al*., 2023). Conversely, the antagonistic activity of haloarchaea is essential in microbial ecology and its biotechnological applications. the present research was found that *Haloarcula* sp. strain NOS1 has the strongest antagonistic effect owing to the presence of antibiotic-like substances that prevent the growth of disease-causing microorganisms this concurs with previous studies on the antagonistic activity of halophilic microbes against human pathogenic bacteria (Santhaseelan *et al*., 2022). These compounds, also known as anti-inflammatory, have activities against inflammation and hemolysis as stated by Ramesh & Mathivanan (2013). Additionally, the stability of the lysosomal membrane of HRBCs is considered as the model for the stabilization of lysosomal membranes in inflammation, the release of biological mediators of inflammation, such as proteases and other hydrolytic enzymes, is prevented. This investigation showed that the inhibition of hemolysis is significant and supports the theory that *Haloarcula* sp. strain NOS1 may prevent the release of substances contributing to inflammation by stabilizing cell membranes (Singh *et al*., 2020). The bioactive compounds may bind to the phospholipid bilayer of the cell membrane, enhancing its integrity and preventing membrane disruption under stress (Bu *et al*., 2022). The demonstrated dose-dependent inhibition of hemolysis suggests that these compounds have potential as new anti-inflammatory drugs (Zhang *et al*., 2021).

Furthermore, the ethyl acetate extracts of *Haloarcula* sp. strain NOS1 has bioactive compounds which are capable of selectively killing cancer cells thus their therapeutic uses can be expanded further into the future as anticancer agents. Anticancer therapies available seek to induce programmed cell death within cancerous cells, and there are a host of natural substances that fall under this category. Judging from the degree of cytotoxicity obtained during the screening of the high coalitions these compounds will induce programmed cell death on PC3 cells, and apoptosis will follow (El-Seedi *et al*., 2022). Through two primary mechanisms: the intrinsic pathway and the extrinsic pathway the Programmed cell death occurs, both regulate pro-apoptotic and anti-apoptotic proteins (Suman *et al*., 2024). Bioactive compounds exhibit effectiveness against PC3 cell lines, underscoring the need to further their development as anticancer agents aimed at prostate cancer, a major global health concern (Gebru *et al*., 2021). Due to the extremophile origin of these compounds, their stability under harsh conditions and possibly different modes of action makes such compounds ideal candidates for use in drug delivery systems. The cytotoxic effects of *Haloarcula* sp. strain NOS1 are concentration-dependent-dose.

This suggests that compounds may preferentially target cancer cells and selectivity in cancer treatment is crucial as it reduces potential destruction of healthy tissues (Shilova *et al*., 202). The bioactive compounds identified in this study as (1,2-benzene dicarboxylic acid, 6-octadecenoic acid, n-hexadecenoic acid, stearic acid, and linoleic acid ethyl ester) have a high GI absorption, which is indicative of their good gastrointestinal absorption, which is a desirable property when creating oral formulations, according to the molecular docking prediction analysis. This property makes these compounds useful for the development of oral dosage forms which increase their therapeutical applicability (Patel &Patel 2022), and the compounds cannot penetrate through the BBB could be advantageous if the drug did not cross the BBB because the drug might be intended for peripheral tissues but not the CNS (Pardridge, 2020). Cytochrome P450 enzymes metabolize a large range of medications; if a chemical inhibits these enzymes, it may result in drug-drug interactions and impact the metabolism of other concomitant pharmaceuticals and because 6-octadecenoic acid , linoleic acid ethyl ester and stearic acid are known CYP1A2 enzyme inhibitors, they have a major impact on how certain antidepressants, antipsychotics, and theophylline are metabolized after being digested by this enzyme (Tan *et al*., 2024).

CONCOLUSION

The bioactive compounds derived from *Haloarcula* sp. strain NOS1 demonstrate notable anti-cancer activity against the PC3 cell line in a dose-dependent manner, indicating their potential as innovative therapeutic agents for the treatment of prostate cancer. Additional research is required to clarify the specific mechanisms of action, characterize the compounds, and assess their *in vivo* efficacy to maximize their therapeutic potential. The specific features of these compounds, which are produced by extremophiles, allow them to be used in a new method of targeting and treating cancer. *Haloarcula* sp. strain NOS1 and its bioactive compounds have favorable biokinetics and biopharmaceutics for therapeutic purposes such as higher oral bioavailability, different P-glycoprotein interacting substrates, CYP inhibitory action, differential skin permeability allowing various routes of administration from internal to external. The assessment of the drug-drug interaction potential and the CNS non-permeability of these agents is very important in the assessment of their suitability for further clinical development.

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Supplementary Table. Antagonistic activity of *Haloarcula* sp strain NOS1 against other haloarchaea strains

1.0.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1		-	+	+	-	-	-	-	-	-	-	+	-	+	+	+	-	+	+	-	-	-	-	+	-	-	+	-	-	-
2	-		+	+	+	+	-	-	+	-	-	+	-	-	+	-	-	-	-	+	+	-	+	-	+	-	-	+	-	+
3	-	-		-	-	-	-	-	-	-	+	-	-	+	+	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-
4	-	-	-		-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	+
5	-	-	-	+		+	-	-	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
6	+	-	+	+	-		-	-	-	-	-	+	-	-	-	-	+	-	+	-	+	-	-	-	-	-	+	-	-	-
7	-	-	-	+	-	-		-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-
8	-	-	-	+	-	-	-		-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+		+	+	+	+	+	+		-	-	+	-	+	-	-	-	-	+	-	-	+	+
10	+	+	+	+	+	-	+	+	-		+	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
11	-	+	+	+	-	-	-	-	-	-		-	-	+	+	+	-	-	-	-	-	-	+	+	-	+	-	-	-	-
12	-	-	+	+	-	-	-	-	-	-	+		+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	+
13	+	+	+	+	-	+	-	+	-	-	-	-		-	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-
14	+	+	+	-	-	+	-	+	-	-	+	+	-		-	+	+	-	-	-	-	-	+	-	-	-	+	+	+	-
15	+	+	+	-	-	-	+	+	-	-	-	+	+	-		+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
16	-	+	-	+	+	+	-	-	+	+	+	-	-	+	+		-	-	-	-	-	-	+	+	+	-	-	-	+	-
17	-	+	-	+	+	+	+	-	-	-	-	+	+	+	-	+		-	-	-	+	-	+	-	-	-	-	-	-	-
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21	-	+	+	-	+	+	-	+	+	+	-	-	+	+	-	+	-	-	-	-		+	-	-	-	-	-	-	-	-
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23	-	+	-	+	+	-	-	-	+	-	-	+	-	+	-	-	+	+	-	-	+	-		-	-	-	-	-	-	-
24	+	-	-	+	-	+	+	-	-	-	-	+	-	+	-	+	-	-	+	-	-	-	+		+	-	-	-	+	-
25	+	+	-	+	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-		+	-	-	-	-
26	-	+	-	-	+	-	+	-	-	-	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-		-	-	-	-
27	-	+	+	+	-	+	-	+	-	+	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-		+	-	-
28	-	-	-	+	+	-	+	-	-	-	+	+	-	-	+	-	-	+	-	+	-	-	+	-	-	-	-		+	-
29	+	+	-	+	+	-	+	+	-	-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	-	-	-	+		-
30	-	+	+	+	-	+	-	+	+	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	

Note: +Positive; - Negative.