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Antimicrobial, anti-inflammatory, anticancer and antiviral activity of bioactive compounds from *Pseudomonas aeruginosa* isolated from Mediterranean Sea, Alexandria, Egypt

Abdelraheem N. Mohamed[1], Abdel Kareem S.H. Mohamed[2], Asmaa M. Zahran[3], Ahmed Mohamed Gad[4] and Alsayed E. Mekky[*5]

[1] *South Egypt Cancer Institute, Assiut University, Assiut 71111, Egypt.*
[2]*Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Assiut 71111, Egypt.*
[3]*Department of Clinical Pathology, South Egypt Cancer Institute, Assiut University, Assiut 71111, Egypt.*
[4] *National institute of Oceanography and Fisheries (NIOF), Qayed Bay, Anfoushy, Alexandria 21556, Egypt.*
[5]*Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Nasr City 11884, Cairo, Egypt.*

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ABSTRACT

In this study, four seawater and sediment samples were collected, Marine *Pseudomonas aeruginosa* (K3) was isolated from Alexandria, Egypt, and identified by using 16S rRNA gene sequencing and biochemical analyses. Optimization was carried out with various parameters to determine the optimum conditions to produce bio active compounds. *Pseudomonas aeruginosa* k3 is known for producing bioactive compounds that are effective against a variety of pathogenic microorganisms, including *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6633), *Klebsiella pneumonia* (ATCC 13883), *Enterococcus faecalis* (ATCC29212), *Vibrio fluvialis* (ATCC33809), and *Pseudomonas aeruginosa* (ATCC9027). GC-MS chromatography was used to identify the bioactive components. Additionally, we investigated the antiviral (HAV and CoxB4), anti-inflammatory (HRBC hemolysis and membrane stabilization), and anticancer (HepG2, Vero, and MCF-7) activity.

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Introduction

According to Bharathi et al. (2021), the ocean covers over 70% of the earth's atmosphere and is a habitat to an enormous variety of ecosystems on aquatic, terrestrial, and chemicals. In contrast to molecules originating from the terrestrial environment, various novel compounds are unique in their structural, functional, and metabolic features have been synthesized by marine species as a result of different marine physicochemical parameters (Bharathi, et al. 2021 and Mohammed et al., 2024).

They are also a Centre for novel bioactive substances with incredible potential in the biomedical field (Balan et al. 2016 and Radwan et al 2024).

Prokaryotes are prevalent in marine habitats, with a population of 103 bacteria per microliter on the ocean's surface and a number of 1×1029 in oceans (Azam & Malfatti 2007, Bao, Li et al. 2018, Bar-On and Milo 2019). According to Bao et al. (2018), they are also essential for marine food chains, biogeochemical cycles, and global climate change. According to Bao et al. (2018), they are also essential for marine food chains, biogeochemical cycles, and global climate change.

***Corresponding author Email address*: alsayedessamy@azhar.edu.eg (Alsayed E. Mekky)**

The marine environment is rich in natural resources. There is an increasing demand for medications that can be produced with the active substance found in marine sources. Bioactive compounds originating from marine sources (flora & fauna) have been used historically and currently to treat a wide range of diseases, and they serve as interesting compounds in both their (Dhinakaran et al. 2012) native and modified synthetic forms (Dhinakaran et al. 2012). *P. aeruginosa* is a common, Gram-negative bacillus that may be found on plant and animal tissues as well as in a variety of environments, including soil marshes and seawater. It's prevalent in both community and hospital sites because of its capacity for minimum nutrition and physical condition tolerance (Mushtaq et al. 2020 and Ramadan et al 2024).

In essence, it might be considered an opportunistic bacterium. Several past studies have reported the isolation of *Pseudomonas* species from the marine environment (Isnansetyo et al. 2009).Up to 3,800 microbial metabolites that are biologically active have been identified in bacteria. 795 bioactive compounds are produced by Pseudomonas, of which 610 are antibiotics and 185 have bioactive characteristics other than antibiotic action (Barakat et al. 2015).

The vast majority of biological activity originated in the seas. Numerous novel substances having photo protective, anti-microtubule, anti-proliferative, anti-tumor, cytotoxic, and antifouling characteristics are produced annually by marine organisms. Recently, there have been several studies of the isolation of novel microorganisms from the marine environment (Hassan et al. 2016 and Aly et al 2024).

Marine microorganisms are an abundant source of novel biomolecules due to their amazing physiological and metabolic properties. Fungi, bacteria, and cyanobacteria are isolated from different marine sources generate a variety of bioactive compounds with antimicrobial actions against a wide range of diseases, which can be used instead of conventional treatments. Furthermore, physiologically active compounds with varying degrees of activity, such as antibacterial, anti-cancer, anti-microtubule, and antifouling features, have been extracted from marine sources (Abdel-Monem et al. 2013 a, b; Hassan et al. 2017).

The aim of the current study was to isolate and characterize marine bacteria from sea water and sediments in Alexandria's coastal region that are capable of producing bioactive substances such as antimicrobial, anticancer, antiviral, and anti-inflammatory.

Materials and Methods

Sampling and isolation of strain

Four samples were taken in the winter and summer of 2023 from various locations in Egypt along the Mediterranean Sea. At about 8 meters' depth, seawater samples were collected in polypropylene bottles. After that, the sample was kept at 4 °C in a refrigerator. 1 ml of each sample was dissolved in 9 ml sterile seawater (10^{-1} dilution) of sterile seawater, and the mixture was vigorously stirred for a minimum of one minute. Aseptically, 1 mL of the 10^{-1} dilution was added to a new tube that held 9 mL of sterile seawater (10^{-2} dilution) and well mixed. To get the dilutions 10^{-3}, 10^{-4}, and 10^{-5}, this dilution procedure was repeated.

100 µL from each sample was inoculated and streaked onto sterile seawater nutrient agar (g/l): (beef extract 3 g, peptone 5 g, agar 20 g, 1000 ml seawater, PH adjusted 7.0–7.2) (Difco laboratories) Petri plates using a glass stick. Plates were incubated at 30 °C for 48 h. Well-isolated colonies were circled on the back of the plate and numbered. Cells of selected colonies were re-streaked onto a nutrient agar plate and incubated at 30 °C for 24–48 h. Each colony was isolated based on morphological appearance and subculture twice to ensure purity. The pure culture was preserved by freezing in 20% glycerol (v/v) at -70°C for long-term storage.

Screening of isolates for antimicrobial activity

The process of obtaining pure isolates involved cultivating each isolate separately in 500 mL Erlenmeyer flasks using broth seawater nutrient medium. The samples were then incubated at 30°C for 24 hours at a rate of 150 r/min in a shaker incubator. Finally, the samples were centrifuged for 15 minutes at 4°C at 10,000 rpm. Using eight pathogen strains, the antibacterial activity of pure isolates was assessed using the well diffusion agar technique on nutrient agar (NA). These include *Vibrio fluvialis* (ATCC33809), *Klebsiella pneumoniae* (ATCC-13883), Escherichia coli (*ATCC-8739), *Pseudomonas aeruginosa* (ATCC-9027), *Staphylococcus aureus* (ATCC-25923), *Enterococcus faecalis* (ATCC29212), and *Bacillus subtilis* (ATCC6633), these clinical isolates were obtained from Marine Microbiology Department, National Institute of Oceanography and Fisheries, Alexandria, Egypt.

Moreover, the active marine isolates obtained were subjected to secondary screening using the supernatant of the growth cultures under shaking conditions and tested by the agar diffusion method against the previously mentioned pathogenic strains. In a petri dish, 100 µL of test microorganisms were incubated, spreading on the surface dish with a glass stick. A well was made in the plates with a sterile core borer (9 mm). The supernatant

(100 µL) was introduced into the well, and plates were incubated at 30 °C for 24 hrs. Microbial growth was determined by measuring the diameter of the zone of inhibition. This process was carried out in (Botany and microbiology department, faculty of Science, Al-Azhar University branch Assuit).

Morphological and biochemical characteristics

The most potent marine isolate with antibacterial activity against the selected pathogenic strain was mentioned. The isolated strain was identified biochemically and morphologically (by motility, shape, gram staining, and spore staining)**.**

Optimum conditions for antimicrobial production

The marine isolate that was active looked into what happened when the incubation temperatures, initial pH, agitation, and incubation times were changed. The optimum parameters for fermentation were established.

Molecular identification and phylogenetic analysis

Genomic bacterial DNA was isolated and purified from the bacterial cells using the Wizard® Genomic DNA Purification Kit according to the manufacturer's instructions (Promega Corporation, Madison, WI, USA). The purified DNA was used to amplify the 16S rRNA gene by the polymerase chain reaction (PCR) technique using universal primers as follows: (F28:5′-AGAGTTTGATCCTGGCTCAG-3′) and (R933:5′-CTTGTCCGGGCCCCCGTCAAT-3). The PCR reaction was programmed on a SimpliAmp Thermal Cycler (Applied BiosystemsTM, Waltham, MA, USA).

The reaction was optimized on the following conditions: PCR conditions consisted of an initial denaturation at 95 ◦C for 5 min (30 cycles): each cycle consisted of denaturation at 94 ◦C for 30 s, annealing at 55 ◦C for 45 s, extension at 72 ◦C for 1.5 min, and a final extension at 72 ◦C for 10 min. The PCR product was purified using a GenEluteTM PCR clean-up kit according to the manufacturer's instructions (Sigma-Aldrich, Burlington, MA, United States). This process was carried out at National Research Centre, Next, samples were sequenced by the sequencing service of ACGT-Germany using ABI 3730xL System (Applied Biosystems™, Waltham, Ma, USA). The sequences were compared with the 16S rRNA gene sequences in the GenBank database using the BLAST online search tool (NCBI, Bethesda, MD, USA). A phylogenetic tree was constructed using MegaX software (Saitou, Nei et al. 1987) to determine the evolutionary relationship of the obtained isolate sequence with sequences from other related bacteria. The obtained nucleotide sequence was further submitted to the GenBank database to obtain an accession number. This process carried out at Sigma Company in Egypt

Gas chromatography mass spectrometry (GC-MS) analysis

The chemical composition of samples was performed using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 35 C and then increased by 3°C/min to 200°C; held for 3 min.; increased to the final temperature of 280°C by 3°C/min; and hold for 10 min. The injector and MS transfer line temperatures were kept at 250 and 260 °C, respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min, and diluted samples of 1 µl were injected automatically using the auto-sampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of the WILEY 09 and NIST 11 mass spectral databases.

In vitro biological assays

Anticancer activity using MTT Protocol

Using the MTT assay technique, various doses of crude extract were examined for their anticancer and cell proliferation properties against HepG2, Vero, and MCF-7 cancer cells (human breast carcinoma). All the cells were purchased from cell culture units at Vacsera Co., Egypt.

Anti-inflammatory assay

Studies on the anti-inflammatory effects of various crude extracts *in vitro* anti-inflammatory effectiveness have been assessed by the human red blood corpuscles membrane stabilization procedure. The specimens used in this experiment were dissolved in a hypotonic solution of distilled water. The graded dosages of the specimen (100-1000 µg/ml) in a hypotonic solution (5 ml) were put in duplicate pairs (per dose) within the centrifuge tubes. Additionally, duplicate pairs for every level of the centrifuge tubes were filled with isotonic solution (5.0 ml) containing graded levels of the specimen (100–1000 µg/ml). Five milliliters each of the distilled water and indomethacin at 200 µg/ml were found in the control tubes. Every tube was filled with 0.1 ml of erythrocyte suspension, which was well mixed. The solutions had been spun at 1300 rpm for

three minutes after being left for one hour at the ambient temperature (36 °C). The supernatant's hemoglobin concentration was estimated to be absorbed at 538 nm (Anosike et al. 2012)

Evaluation of cytotoxic effect towards vero cells and Antiviral Activity using MTT Protocol using MTT Protocol

The cytotoxicity and maximal non-toxic level of crude extract towards vero cells were determined according to (Fouda et al. 2022). Then, vero cells were placed in 96-well micro-titer plates at a density of 104 cells/well with 200 µL growth media to examine the impact of crude extract on the reduction of the HAV and CoxB4 virus infection capacity. The cells were then left adherent for the entire night at 36°C in 5% CO2. For one hour, a viral suspension was incubated at ambient temperature with non-lethal doses of crude extract (1:1, v/v). Following incubation, one well containing Vero cells received 100 µL of the viral/sample solution, whereas the remaining three wells, which held only Vero cells as well as development media, were regarded as non-infected cells (control). The plates were shaken for six minutes at 160 rpm, and then they were incubated for twenty-four hours at 36°C with 6% CO2 to enable the virus to multiply. Using the formazan crystal absorbance measurements employed in the MTT solution as specified for the cytotoxicity experiment, the cell viability of both affected and non-infected Vero cells was assessed. The variation in measurements across the optical densities of infectious and unaffected cellular viabilities was used to calculate the HAV and CoxB4 virus infection capacity (Fouda et al., 2022). All the viruses and cells were purchased from cell culture units at Vacsera Co., Egypt.

Statistical analysis

Data are represented as means. The SPSS version 28 software SPSS Inc., Chicago, IL, USA) was used to evaluate the statistics where $p < 0.05$ refers to a dramatic change.

Results

Isolation and morphological and biochemical Characteristics

d. Included were first, second, third, and non-coding codon positions. All gaps and absent data were eliminated from each location. Ultimately, the dataset comprised a total of 919 locations. Tamura et al. (2007) performed evolutionary analyses using MEGA5 (Figure 2). The partial sequence of the 16S ribosomal RNA gene from Pseudomonas aeruginosa strain Mekky003 has been officially registered in the GenBank database under accession number PP838309.

Table 1 Morphological characters and biochemical tests of the *Pseudomonas aeruginosa* (K30).

Characteristics	*Pseudomonas aeruginosa* (K30)
Gram stain	Negative
Shape	Rod
Motility	Motile
Oxidase test	Positive
Catalase test	Positive
Acid fast stain	Negative
Glucose	Non-fermented
Maltose	Non-fermented
Lactose	Non-fermented
Sucrose	Non-fermented
Indole production	Negative
Citrate utilization	Positive
Urease	Negative
Nitrate reduction	Positive
Gelatin hydrolysis	Negative

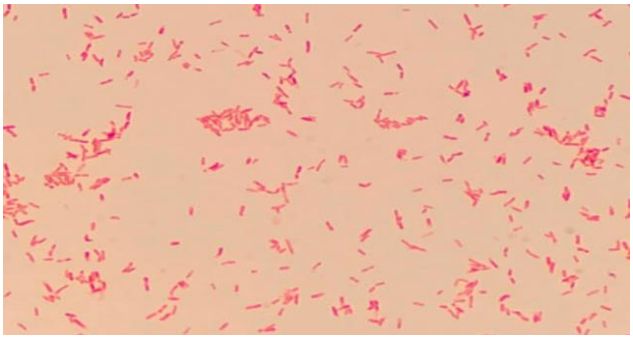

Fig 1. Gram stain of K30 (*Pseudomonas aeruginosa*).

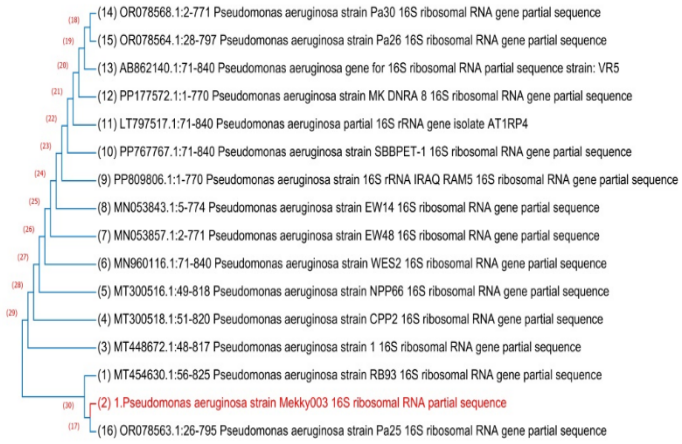

Fig 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

By using GC-MS, a total of 17 chemicals were found in the non-polar fraction of *P. aeruginosa* (Figures 3 and 4, Table 2).

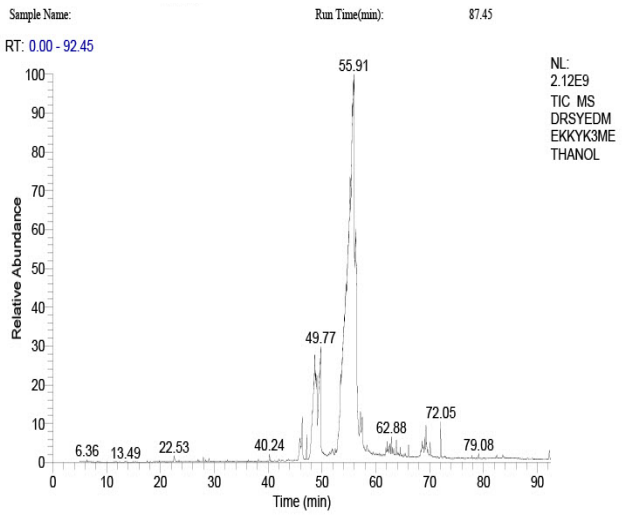

Fig 3. GC/MS analysis of the fraction of *P. aeruginosa*.

The major components were 1-Nonadecene, Tetradecane, 2,6,10-trimethyl-, 1-Heptacosanol, (3S,8aS)-3-(4-isobutylhexahydropyrrole-o [1,2 a pyrazine-1,4 dione, Pyrrolo (1,4-dione) hexahydro-3-2-methylpropyl 1,2apyrazine, Phenazine-1-carbixylic acid and Acridine-4-carboxylic acid.

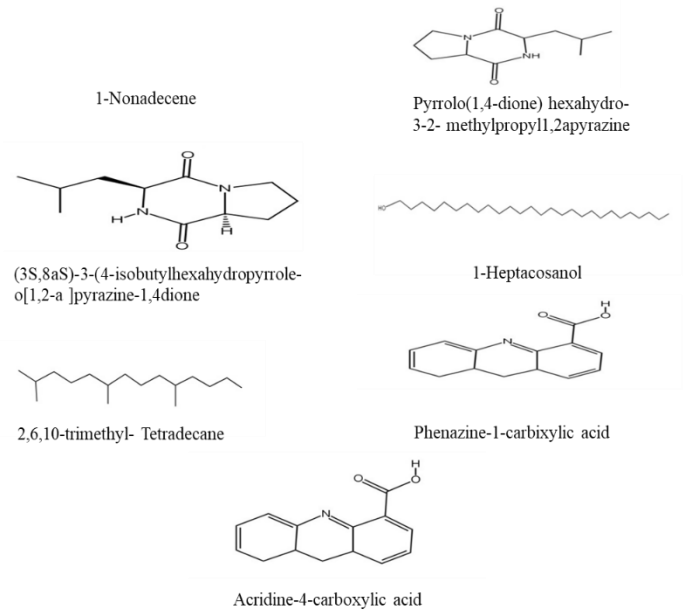

Fig 4. Chemical structure of selected metabolites identified from *P. aeruginosa* extract.

Antimicrobial activity

The following twelve bacterial isolates from marine sediments were tested for antimicrobial activity against pathogenic test strains: *Vibrio fluvialis* (ATCC33809), *Pseudomonas aeruginosa* (ATCC 902), *Bacillus subtilis* (ATCC6633), *Escherichia coli* (ATCC 8739), *Klebsiella pneumonia* (ATCC 13883), and *Enterococcus faecalis* (ATCC 29212). It has been molecularly identified as *Pseudomonas aeruginosa* (even though it is in the GENBANK NCBI *Pseudomonas aeruginosa* strain Mekky003 16S ribosomal RNA partial sequence K 30 with accession No. PP838309), and the most promising isolate, named K 30, exhibited a lot of antimicrobial activity against pathogenic test strains (Table 1, Figure 2).

Bioactive compounds activity against pathogenic bacteria

Figure (5) shows the antimicrobial activity of the K30 (P. aeruginosa) against different pathogenic reference strains. Data is expressed as the mean ± SD. P value was significant if < 0.05. Different letters refer to significant differences.

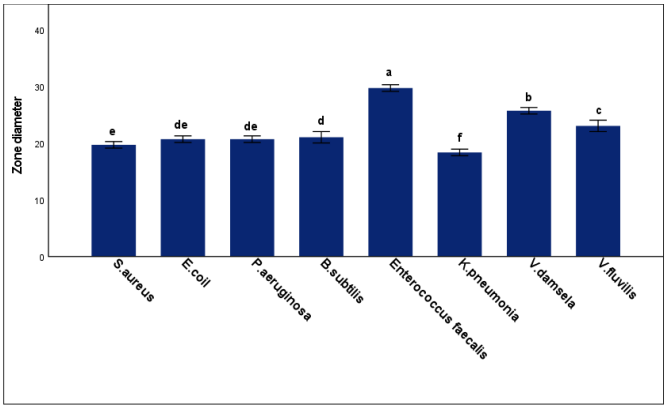

Fig 5. Zone diameter of bioactive compound activity of the K30 (*P. aeruginosa*) against different pathogenic reference strains.

Effect of initial fermentation medium pH values of K30

The maximum antimicrobial activity was detected at pH7 as expressed in figure 6.

Effect of different incubation temperatures of the K30

It could be noticed that with the 30 mm inhibitory zones, temperatures of 30 °C produced the greatest antibacterial productivity. The inhibitory zone showed a gradual decrease and reached 9 mm when the temperature was elevated to 50°C as shown in figure 7.

Table 2 GC-MS data of the non-polar fraction of *P. aeruginosa*

	R T	Compounds	Formula	Mwt.	Area%	Advantages	References
1	6.36	Styrene	C_8H_8	104	0.09	Plastic, latex paints coating, rubbing and polyesters	Zhang et al. (2022)
2	22.53	1-Nonadecene	$C_{19}H_{38}$	266	0.39	Antimicrobial, anti-inflammatory and	Amankwah et al. (2022)
3	19.76	Dodcance	$C_{12}H_{26}$	170	0.08	Antifungal Production of engine and jet fuels	
4	23.44	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240	0.09	Antifungal, antibacterial and nematicidial	Arabo et al. (2022)
5	26.95	1 Heptacosanol	$C_{27}H_{56}O$	396	0.14	Antifungal, antimicrobial, antiviral, and antioxidant	Amankwah et al. 2022)
6	28.41	Tetradecane	$C_{14}H_{30}$	198	0.11	Organic solvent	
7	28.99	(3S,8aS)-3-(4-isobutylhexahydropyrrole-o[1,2-a]pyrazine-1,4-dione	$C_{11}H_{18}N_2O_2$	210	0.20	Anti-inflammatory	Rupesh et al. (2012)
8	38.17	Cyclobutane, 1,2-diphenyl-	$C_{16}H_{16}$	208	0.13		
9	46.36	hexahydro-3-2-Methylpropyl 1, 2apyrazine	$C_{16}H_{32}O_2$	210	3.04	Antimicrobial and anti-inflammatory	Amankwah et al. (2022)
10	48.19	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.18	Fatty acids	
11	49.27	Phenazine-1-carbixylic acid	$C_{13}H_7N_2O_2$	223	0.06	Antimicrobial and Anticancer	Kama et al. (2012)
12	51.42	9-Octadecadienoic acid (Z)-	$C_{18}H_{34}O_2$	282	0.2	Fatty acids	
13	51.97	Methyl9,9-dideutro-Octadecanoate	$C_{19}H_{36}D_2O_2$	300	0.26	Fatty acids	
14	58.34	Phenazine-1-carboxyamide	$C_{13}H_9N_3O$	223	0.44	Bio control	
15	63.07	Acridine-4-carboxylic acid	$C_{14}H_9NO_2$	238	0.38	Antimicrobial and anticancer	Zheng et al. (2022)

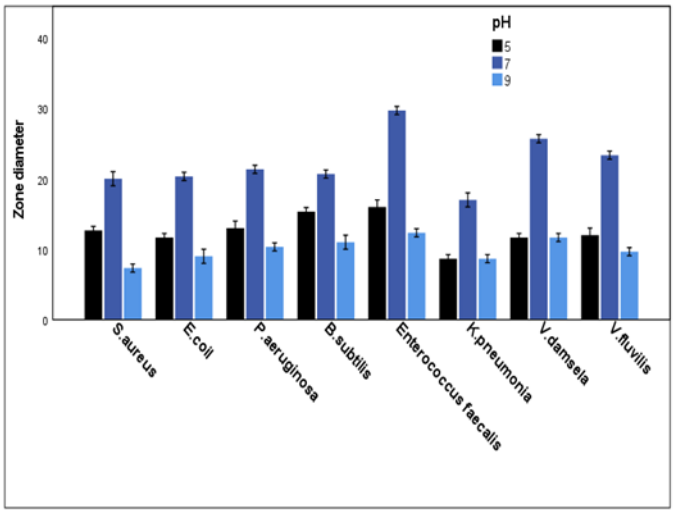

Fig 6. Bioactive compound activity in different pH of the K30 (*P. aeruginosa*).

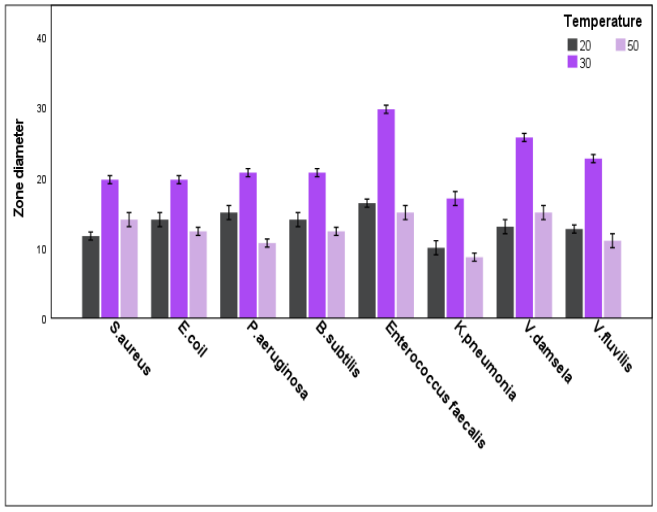

Fig 7. Bioactive compound activity at different temperatures of *P. aeruginosa*.

Effect of different agitation speeds and incubation period on the biological activity of the K30 isolate

A maximal inhibition zone of 30 ml was reported at 180 r.p.m. Even after reducing the speed of agitation to 100RPM, the antibacterial activity increased as shown in figure (8).

Figure 9 illustrates the bioactive compound activity associated with the incubation periods of the K30 isolate. The graph demonstrates the variation in bioactive compound production over time, highlighting distinct peaks and trends corresponding to different incubation durations. The results indicate that the K30 isolate exhibits optimal bioactive compound activity at specific incubation periods, suggesting a time-dependent synthesis of these compounds. This temporal variation in activity could be

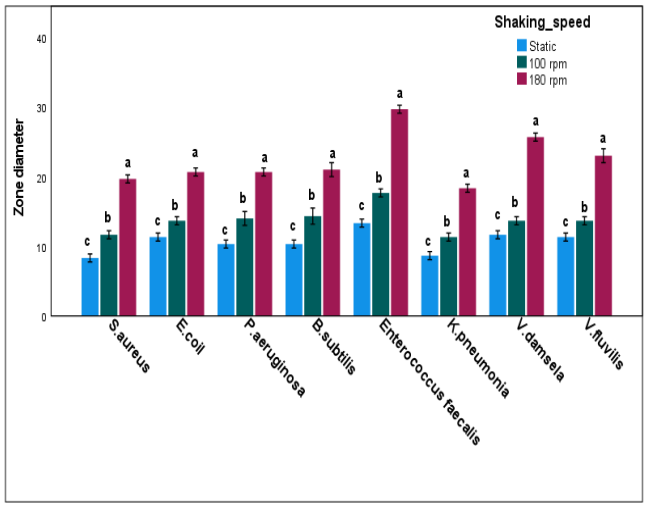

Fig 8. Bioactive compound activity in different agitation speed of the K30 (P. *aeruginosa*).

attributed to the metabolic dynamics of the isolate, where certain incubation times favor the production of bioactive metabolites.

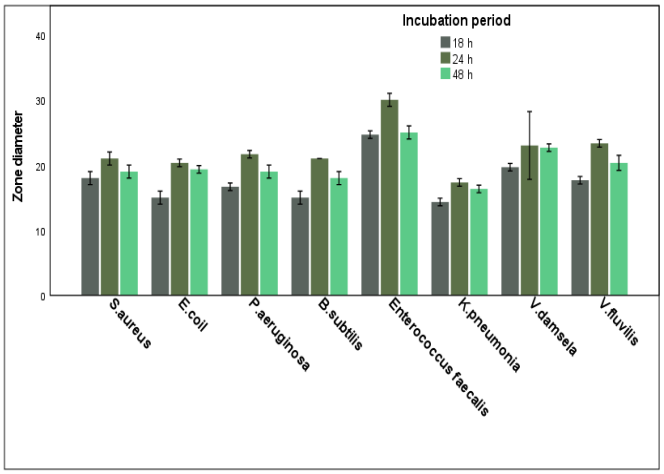

Fig 9. Bioactive compound activity related to incubation periods of the K30 isolate.

In vitro biological activities of K30

Anticancer activity using MTT protocol

For cancer MCF7 cell lines, the highest concentrations of the produced crude extract at 1000 and 500 µg/mL resulted in the lowest cell viability. The results indicate that, when the concentration of crude extract decreases, cell viability increases. The IC50 value for the comprehensive extract derived from *P. aeruginosa* when subjected to breast cancer cell lines (MCF7 cell lines) was calculated at several concentrations using the MTT protocol. It showed that

the IC50 value of the total extract extracted from *P. aeruginosa* when tested against breast cancer cell lines (MCF7) was determined at various doses. An IC50 value of 198.48µg/mL was detected.

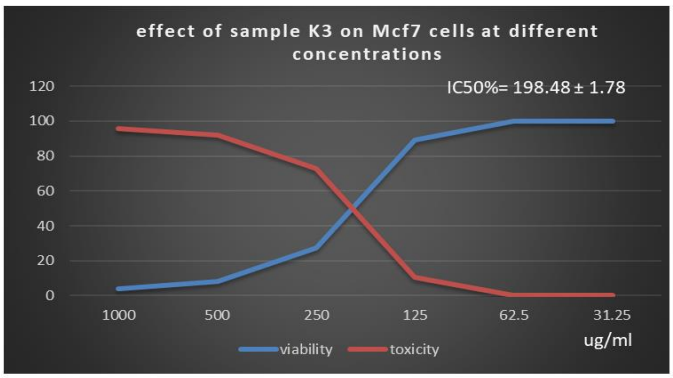

Fig 10. Effect of *P. aeruginosa* crude extract on MCF7 cells at different concentrations.

For cancer MCF7 cell lines, the highest concentrations of the produced crude extract at 1000 and 500 µg/mL resulted in the lowest cell viability. The results indicate that, when the concentration of crude extract decreases, cell viability increases. The IC_{50} value for the comprehensive extract derived from *P. aeruginosa* when subjected to breast cancer cell lines (MCF7 cell lines) was calculated at several concentrations using the MTT protocol. It showed that the IC_{50} value of 198.48 ±1.78 µg/mL.

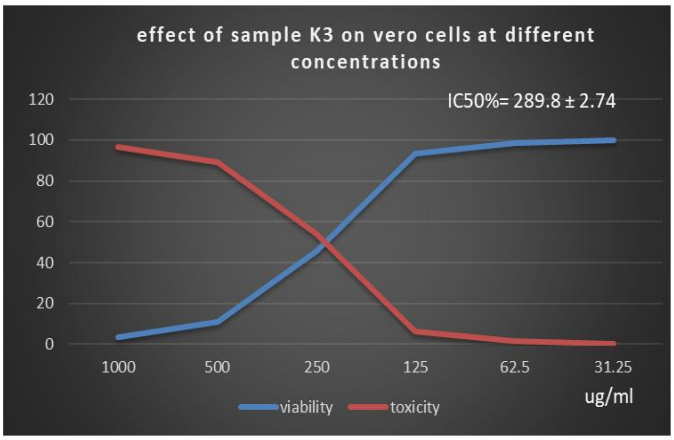

Fig 11. Effect of *P. aeruginosa* crude extract on Vero cells at different concentrations.

Using the MTT assay, the IC50 value of the total extract extracted from *P. aeruginosa* when tested against liver cancer cell lines (HepG2 cells) was determined at various doses. An IC50 value of 289.8 ± 2.74µg/mL was detected. Using the MTT assay, the IC_{50} value of the total extract extracted from *P. aeruginosa* when tested against liver cancer cell lines (HepG2 cells) was determined at various doses. An IC50 value of 122.21± 0.73 µg/mL was detected.

Anti-inflammatory assay

It could be noticed that upon using descending levels of total extract extracted from *P. aurogonisa*, the hemolysis inhibition percentage decreased compared to indomethacin as the standard. The IC_{50} value of the total extract extracted from *P. aurogonisa* after it was stabilized by membrane and hemolysis with HRBC. Compared to Stander Indomethacin, which was used as a reference, it had an IC50 value of 16.5 µg/mL. However, when it was tested for HRBC hemolytic and membrane stabilization, the crude extract showed very little anti-inflammatory efficacy. IC50 = 5.73 µg/ml.

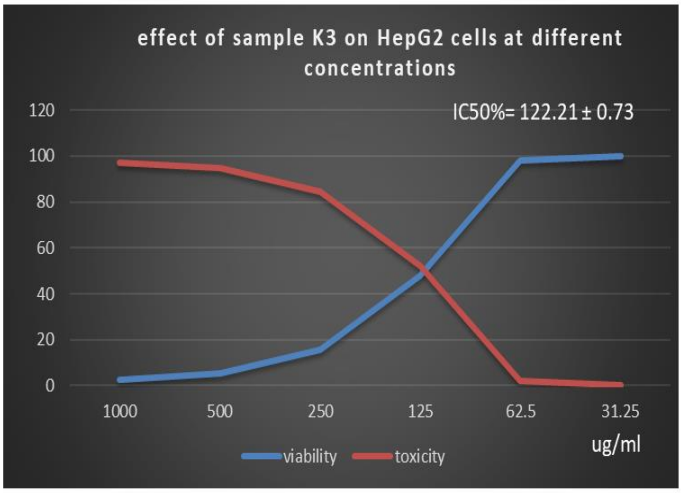

Fig 12. Effect of *P. aeruginosa* crude extract on HepG2 cells at different concentrations.

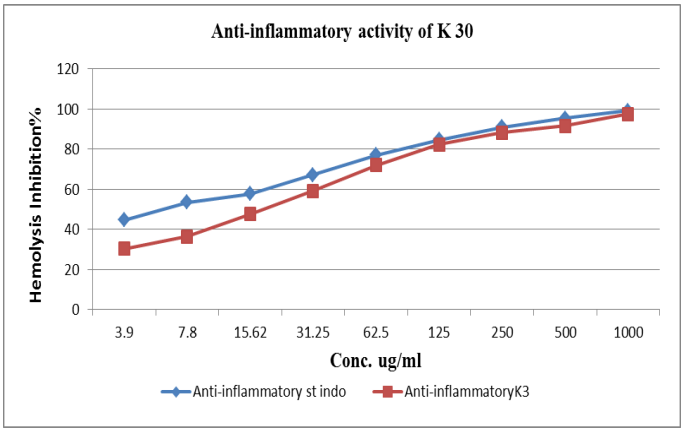

Fig 13. Anti-inflammatory activity of *P. aeruginosa* crude extract and positive control.

Cytotoxicity and Antiviral Activity using MTT Protocol

The total extract from *P. aeruginosa* was investigate d for toxicity on Vero normal cells before its antiviral activity investigated. The sample should have a potent antiviral effect on the viruses without having an appreciably negative impact *P. aeruginosa* total extract demonstrated clear cytotoxicity on treated Vero cells in the 1000–125 µg/mL range. Its cytotoxic level that inhibits 50% of cells (CC50) was measured and found to be 289.8 ± 2.74 µg/mL, indicating minimal cytotoxicity in *P. aeruginosa* total extract. While the maximum non-toxic level of total extract extracted from *P. aeruginosa* was at 31.25 µg/mL. applied to test their antiviral activity. The antiviral effect (%)total extract extracted from *P. aeruginosa* towards HAV and CoxB4 viruses were increased upon applying ascending levels of total extract extracted from *P. aeruginosa* is depicted in (Figure 14 and Figure 15).

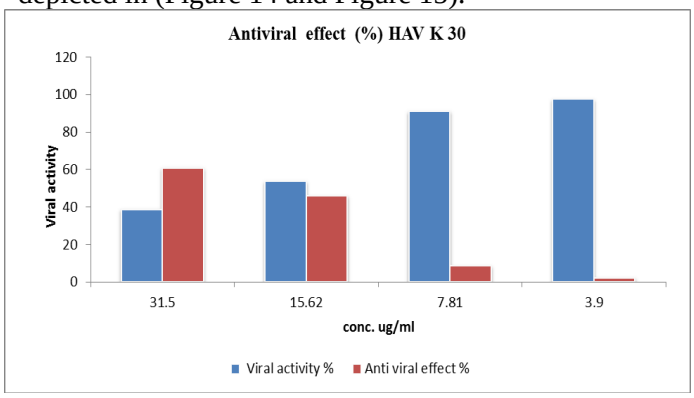

Fig 14. Antiviral effect (%) HAV virus upon using various levels of *P. aeruginosa* crude extract.

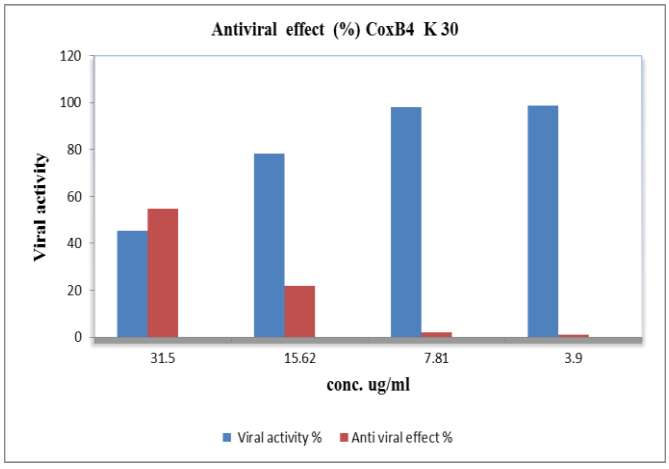

Fig 15. Antiviral effect (%) CoxB4 virus upon using various levels of *P. aeruginosa* crude extract.

Discussion

Recent reviews have shown that marine-derived microbes are a valuable source of these natural compounds. As a result, the current study was carried out to identify marine microorganisms from Egypt's Mediterranean coastline that show antimicrobial activity against pathogenic test strains. One of the best isolates was then characterized both phenotypically and Geno typically, and an environmental optimization study was completed (AEDMS et al. 2011). Twelve marine isolates in all were obtained during our current study, and the most effective antibacterial activity against several pathogenic test strains was the main consideration for our selection. The isolate was recognized as *P. aeruginosa* (by 16SrRNA gene sequencing) based on morphology, biochemical analysis, and gene sequencing. This marine bacterium was identified as P. aeruginosa when it was isolated from Mediterranean seawater. Diffusion abilities were evaluated, and *P. aeruginosa* was found to be the most promising strain.

Pseudomonadaceae is a family of bacteria that is widely distributed in the environment, including fresh water, marine, and soil environments. *P. aeruginosa* is a member of this family. Its catabolic adaptability and medicinal importance have attracted significant interest (Barakat et al. 2015).

The optimization study carried out in this study has shown that *P. aeruginosa* have the ability to inhibit various pathogenic test microorganisms at 150 rpm to a maximum of 30 mm. An early study by Loptanev et al. (1973) showed that *Bacillus polymyxa* produces more alkaline protease at pH 7.0 when aeration speed is increased from 75 to 200 rpm. *Bacillus* cereus strain 146 had maximal protease activity at 170 rpm agitation speed after 48 hours of incubation, according to Kumar and Takagi's 1999 publication. Beg et al. (2003) came to the conclusion that this speed improved the aeration of the culture medium, which may result in an adequate amount of dissolved oxygen in the media. Additionally, bacteria will take up more nutrients (AEDMS et al. 2011).

Many cellular processes, including the regulation of secondary metabolite production, are affected by changes in the external pH (Solé et al., 1997). Through initial pH (5, 7, and 9) adjustments, the effects of pH have been studied. The original pH of 7.0 produced the greatest inhibition zone. Chang et al. (1991) and Yousaf (1997) reported similar results, indicating that an initial

pH of 7.0 produced the greatest bacitracin production from *P. aeruginosa*. (AEDMS et al. 2011).

The temperature where antimicrobial biosynthesis proceeded was shown to be of great importance. The results showed that the zone of inhibition increased as temperature rose, reaching a maximum value of 30 mm at temperatures between 30°C and 40°C. It was demonstrated that the inhibition zone reached 30 mm after 24 hours, at which the maximal antimicrobial production was observed. According to Awais et al. (2008), during 48 and 72 hours, *B. plumilus* provided the greatest inhibition against *S. aureus*. (AEDMS et al. 2011).

Regarding metabolomics profiling, the nonpolar fraction's GC-MS analysis showed a significant prevalence of a specific group of chemicals (Mekky et al 2024). A notable presence of these compounds is seen in Table 17, most of which have been found here for the first time from P. aeruginosa. Among them is 2apyrazine (MW: 210, MF: C16H32O2, area% 3.04), also known as hexahydro-3-2-Methylpropyl 1. Its characteristics include a wide spectrum of antibacterial and anti-inflammatory properties (Amankwah et al., 2022). Area percentage of cytidine-4-carboxylic acid (MW: 238, MF: C14H9NO2) is 0.38. Previous research has confirmed the antimicrobial and anticancer effects of this substance (Zheng et al., 2022).

1-Heptacosanol (MW: 396, MF: C27H56O, area%0.14) Previous research has demonstrated the antifungal, antimicrobial, antiviral, and antioxidant properties of this compound. Investigated was the ethyl acetate extract from *P. aeruginosa's in vitro* biological effectiveness for anti-inflammatory, antiviral, and anticancer activities.

Different concentrations (1000–0 µg/mL) showed diminishing rates of inhibition depending on the experimental work, suggesting increased anti-inflammatory action. According to the results of the present investigation, the crude extract of K3 significantly reduced inflammation. The obtained results reveal an IC50 value of 16.5 µg/mL for the anti-inflammatory activity assessed through the HRBC hemolytic and membrane stabilization method.

One of the most significant challenges to human health worldwide that still has to be introduced is the spread of cancer. Recently, several active substances most notably, nanoparticles have demonstrated notable effectiveness against a variety of cancer cells, raising the prospect of employing them in cancer treatment or as delivery systems or ways to administer anti-cancer medications (Ibrahim et al. 2022 and Mekky et al 2023). The MTT test method is highly sensitive, accurate, colorimetric, and able to measure live cell numbers after an infusion of active substances in order to evaluate biochemical cell processes (Ghasemi et al. 2021 and Elhalik et al 2024). Thus, using the MTT analysis approach, the efficacy of the crude extract of K3 against three cancer cell lines was examined in the present work.

The IC50 value for the same extract against breast cancer cell lines (MCF7), evaluated using the MTT assay, was determined to be 198.48 ± 1.78 µg/mL. The IC50 value for the same extract against Vero, evaluated using the MTT assay, was determined to be 289.8 ± 2.74 µg/mL. (Vero), evaluated using the MTT assay, was determined to be 289.8 ± 2.74 µg/mL. The IC50 value for the same extract against HepG2, evaluated using the MTT assay, was determined to be 122.21 ± 0.73 µg/mL.

People all around the world are affected by the problem of viral disease and numerous, sometimes deadly illnesses caused by different viruses. Developing and renewing infections caused by viruses are the source of infectious outbreaks in human societies. Despite sometimes significant progress in the development of antiviral medications, they are not able to completely prevent all viral diseases. Furthermore, drug-resistant viruses are frequently found, and there are currently relatively few antiviral alternatives available for treating viral infections (Gaikwad et al. 2013 and Alshawwa et al 2022).

In this study, using the MTT assay, the crude extract of K3 showed a promising antiviral impact toward HAV and Cox-B4. According to their research, the viral activity of HAV and Cox-B4 was increased, and the antiviral effect was declined with the decreasing concentration of crude extract K3.

Conclusion

Microorganisms in the marine environment are abundant and have the potential to produce bioactive substances. The current study aimed to find marine bacteria as an ecofriendly source of bioactive substances for a wide variety of applications. According to the current approach's findings, marine *P. aeurogonisa* produces bioactive substances that may be used as antimicrobials and could potentially have application in the future in medication as antiviral, anticancer, and anti-inflammatory drugs. In the future investigation, the extracted bioactive substances' complete identification will be observed.

Conflict of interest

The authors declare that they have no conflict of interest.

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