Annals of the Entomological Society of America, XX(X), 2022, 1–11
https://doi.org/10.1093/aesa/saac025
Research

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Cyclic Depsipeptides and Linear Peptides With Cytotoxic and Antiphytopathogenic Activities From Symbiotic Bacteria of *Xenorhabdus* (Enterobacteriales: Morganellaceae) Genus

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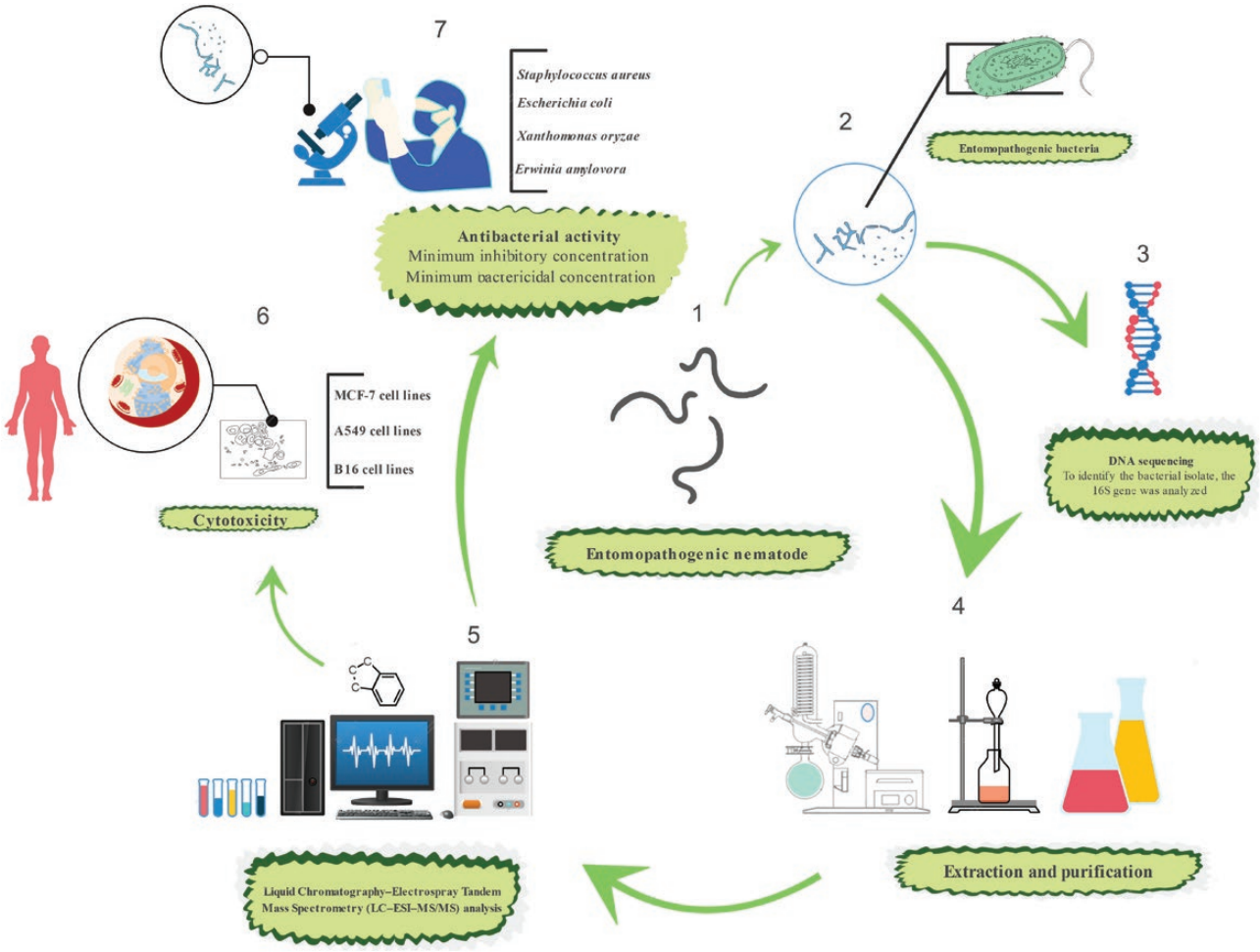
Subject Editor: Erin Scully

Received 25 July 2022; Editorial decision 30 October 2022

Abstract

On the basis of biological activities of the ethyl acetate extracts of four *Xenorhabdus* sp., including *Xenorhabdus nematophila* FUM 220, *Xenorhabdus nematophila* FUM 221, *Xenorhabdus bovienii* FUM 222, and *Xenorhabdus bovienii* FUM 223, *X. nematophila* FUM 220 was preferentially selected to track the isolation of responsible compounds. Chemical study on the ethyl acetate extract of *X. nematophila* isolate FUM220 which is derived from the native nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae), was evaluated, and eleven compounds, including xenocoumacin II (**1**), xenortide-396 (**2**), xenortide A (**3**), xenortide-410 (**4**), xenortide-449 (**5**), xenematide A 663 (**6**), rhabdopeptide-574 (**7**), rhabdopeptide-588 (**8**), rhabdopeptide-687 (**9**), rhabdopeptide-701 (**10**), and nematophin-273 (**11**) were characterized. In this experimental study, we surveyed the antitumoral potential of bacterial extract and bacterial metabolites to treat human breast cancer (MCF-7), human lung cancer (A549), and murine Tumor (B16) cell lines. We observed that all samples were cytotoxic, but bacterial extracts of *X. nematophila* FUM 220 and *X. bovienii* FUM 223 showed higher toxicity on mentioned cell lines. Potent cytotoxic activity was found for compounds 6 and 11 with IC_{50} of 6.2 μg/ml against human lung cancer A549 cell lines, too. These compounds showed moderated antibacterial activity against *Xanthomonas oryzae pv. oryzae* strain Xoo-IR42 (Xanthomonadales: Xanthomonadaceae) (MIC of 62.5 μg/ml) and *Staphylococcus aureus* strain 1112 (Bacillales: Staphylococcaceae) (MIC of 100 μg/ml). The bacterial extracts from *X. bovienii* FUM 222 showed strong inhibition of the growth of *S. aureus* strain 1112, by a minimal inhibitory concentration assay (MIC of 53.5 μg/ml). *Xenorhabdus* genera produce metabolites with potent cytotoxic and antibacterial activity. Single compounds can be isolated, identified, and commercialized, but various species or strains may change their anticancer or antimicrobial potential. The present study brings new clues regarding the qualified of *Xenorhabdus* as future peptide sources for supplying natural bioactive compounds and challenge multidrug-resistant bacteria, treat cancer, and plant diseases.

Graphical Abstract



Key words: secondary metabolite, *Xenorhabdus*, cell line, peptide, antimicrobial

Microorganisms, such as the fungi and bacteria use various strategies to compete for space and nutrients. In favorable competition, finite nutrients are rapidly consumed by certain microorganisms without direct interaction between competitors (Singh et al. 2015). The products of primary and secondary metabolism of these microorganisms usually show some forms of biological activity which are secreted into the media. Secondary metabolites have various and uncommon chemical structures, mostly low molecular mass show diverse activities (Solecka et al. 2012). Research on the biology of bacteria associated with entomopathogenic nematodes *Steinernema* spp (e.g., *Xenorhabdus* spp) and *Heterorhabditis* spp (*Photorhabdus* spp) (Enterobacteriales: Morganellaceae) led finding diverse active numerous novel compounds. In vitro cultures of *Xenorhabdus* revealed several antimicrobial products with antibiotic activity against a wide range of bacteria and fungi, including human pathogenic fungi and yeasts (Akhurst 1982, Webster et al. 2002, Dreyer et al. 2019, Racine and Gualtieri 2019). Approximately 7.5% of genome in *X. nematophila* encoding genes corresponded to secondary metabolism (Reimer 2014).

After entering the insect hemocoel, infective nematode juveniles (IJs) release the bacteria via mouth or anus. Entomopathogenic bacteria enter the reproduction phase and use hemolymph as a suitable culture medium. During this pathogenicity phase, bacteria produce a large number of secondary metabolites, including antimicrobial peptides, polyketides, proteases, and hydrolytic exoenzymes (Booysen et al. 2021). Secondary metabolites, as a broad-spectrum antimicrobial compound, play a key role to protect the host from other invasive microorganisms. The bacteria also produce a wide array of toxins, even in the absence of nematodes, that are lethal to the insect host when injected into the hemolymph of different insects (Yang et al. 2012).

Comparing *Xenorhabdus* and *Photorhabdus* due to their ability to produce secondary metabolites, more different active compounds are known from *Xenorhabdus* species. For example, nematophin has antifungal and antibacterial activity, including the human pathogenic clinical isolates is *S. aureus*. Cyclic depsipeptides like szentiamid produced by cultures of *Xenorhabdus szentirmaii*, showed activity against the malarial parasite *Plasmodium falciparum* (Haemosporida: Plasmodiidae) (Lengyel et al. 2005, Fodor et al. 2022). Nonribosomal peptide synthetases produce the majority of the secondary metabolites (NRPS) and fatty acid synthase (FAS)-like polyketide synthases (PKS) (Reimer 2014). This rich source of bioactive substances could promise a neglected approach toward isolation, characterization, and large-scale production of effective compounds for use in different areas, mainly health and therapy. Concerns about excessive use of antibiotics that make them resistant

Table 1. HR-ESI-MS data of all compounds described in this work

Compound	Detected mass	Calculated mass	Error (ppm)	Ion formula
Xenocoumacin II	407.2174 $[M + H]^+$	407.2177 $[M + H]^+$	0.6	$C_{21}H_{31}N_2O_6$ $[M + H]^+$
Xenortide-396	396.2640 $[M + H]^+$	396.2646 $[M + H]^+$	1.4	$C_{24}H_{35}N_3O_2$ $[M + H]^+$
Xenortide A	410.2815 $[M + H]^+$	410.2802 $[M + H]^+$	-3.2	$C_{25}H_{36}N_3O_2$ $[M + H]^+$
Rhabdopeptide-574	574.4307 $[M + H]^+$	574.4327 $[M + H]^+$	3.4	$C_{32}H_{56}N_5O_4$ $[M + H]^+$
Rhabdopeptide-588	588.4469 $[M + H]^+$	588.4483 $[M + H]^+$	2.4	$C_{33}H_{58}N_5O_4$ $[M + H]^+$
Rhabdopeptide-687	687.5142 $[M + H]^+$	687.5168 $[M + H]^+$	3.7	$C_{38}H_{67}N_6O_5$ $[M + H]^+$
Rhabdopeptide-701	701.5292 $[M + H]^+$	701.5324 $[M + H]^+$	4.6	$C_{39}H_{69}N_6O_5$ $[M + H]^+$

to a wide range of antibiotics and the need to explore new and cost-effective ways to supply antibiotics. A key concern about the global antibiotic resistance issue is the limited number of new drugs to overcome this threat because few numbers of new classes of antibiotics were approved in the last few decades.

Xenorhabdus bioactive compounds range from large and more complex antibiotics like bicornutin, xenoamicins, and various antimicrobial peptides (AMPs) to small compounds, such as xenocoumacins, xenortides, xenorhabdins, and indole derivatives. A group of AMPs is cyclic, like PAX lipopeptides, taxlllaids, xenobactin, and szentiamide, but other compounds are linear in structures including cabanillasin, rhabdopeptides, and bacteriocins (Houard et al. 2013). So far, two compounds, namely xenorhabdicin and xenocin structurally known as bacteriocins are identified from *X. nematophila* (Dreyer et al., 2019).

The detection and presentation of antibiotics revolutionized human therapy and plant protection. Notwithstanding the stunning results, different problems have occurred thereinafter (Fodor et al. 2012). Bacterial resistance to antibiotics has become a global problem worth thinking about. *Escherichia coli* (Enterobacterales: Enterobacteriaceae) and *S.aureus* are key components of antibiotic-resistant bacteria that develop novel resistance mechanisms to the emergence and spread of disease (Vozik et al. 2015). A comprehensive strategy is required to combat these infections, which includes antimicrobial supervision and the expansion of new antimicrobial agents effective on multi-resistant gram-positive and gram-negative pathogens (Fodor et al. 2012). Bacterial infections are among the major reasons for severely limiting the commercial productivity of fruit trees worldwide. Bacterial infections are among the major reason for severely limiting the commercial productivity of fruit trees all over the world. Fire blight is one of the most dangerous diseases of plants in Rosaceae family results from infection by gram-negative bacterium *Erwinia amylovora* (Enterobacterales: Erwiniaceae) that affects many crops in agriculture and horticulture, especially apple and pear trees (Böszörményi et al. 2009, Vozik et al. 2015). There are various natural isolates of streptomycin-resistant *E. amylovora* strains, around the world. Thus, the control program of *E. amylovora* on apple and pear trees is currently lacking (Vozik et al. 2015). According to the items mentioned herein, new antimicrobial agents with various modes of action are required which vindicate the researcher's efforts toward achieving new sources (Fodor et al. 2012).

This work is designed to study on characterization and structural determination of secondary metabolites from four strains of *Xenorhabdus* and evaluate the potency of bioactive compounds on human cancer cells and phytopathogenic bacteria. The findings of this study are expected to provide evidence to support the hypothesis that different species of *Xenorhabdus* can be a major source of compounds with anticancer and antibacterial properties. Besides, transaction activities of bioactive compounds from *Xenorhabdus* on plant prokaryotes will increase our knowledge about this potential compound to be considered in future plant protection programs.

Methods

Strain and Culture Conditions

Four *Xenorhabdus* strains, including two strains of *X. nematophila* (FUM 220 and FUM 221), and two strains of *X. bovienii* (FUM 222 and FUM 223), isolated from *Steinernema carpocapsae*, and *Steinernema feltiae* were used here.

Xenorhabdus strains were routinely cultivated on NBTA (Nutrient agar + 0.0025% bromothymol blue + 0.004% triphenyl tetrazolium chloride). The fresh liquid culture was started from single blue colonies as phase I which was found in abundance on nutrient broth media and incubated at 30°C for 48 hr (Kumar et al. 2018).

DNA Extraction, PCR, and DNA Sequencing

To extract genomic DNA of bacteria, a single clone of pure culture was cultured into 5 ml of NB medium and kept on the horizontal shaker at 28°C for 48 hr. To obtain bacterial cell mass, the bacterial suspension was transferred into a microtube and centrifuged for 1 min at 8,000 rpm. The bacterial genome was extracted using the Trizol reagent (Sigma) according to the manufacturer's instructions. To identify the bacterial isolate, the 16S gene was analyzed. A primer sent of the 16S rDNA gene fragment containing 27F (Forward primer 5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (Reverse primer 5′-TACGGCTACCTTGTTACGA-3′) were used for amplifying this gene (Heuer et al. 1997). After PCR reaction, the amplicons quality and quantity were checked on 1% agarose gel stained by a green-viewer (SYBR). Sequencing of PCR products was done using Macrogen Company (Korea).

The sequence peaks were checked for any error, and consensus sequences were created and used for molecular characterization via nBLAST and the reconstruction of a phylogenetic tree.

Alignment and Phylogenetic Analyses

To construct the phylogenetic tree, several verified sequences of 16S gene from the genus *Xenorhabdus* were downloaded from the Genbank and aligned with 16S sequences of the isolates. Sequences were aligned using MEGA-X software, and the phylogenetic tree was reconstructed using Neighborhood matching (NJ) (Saitou and Nei 1987). To evaluate tree topology, 10,000 bootstrap clones were performed and bootstrap values with more than 70% were considered nodes (Felsenstein 1985). 16S sequence of *Photorhabdus*

luminescens subsp. *kleinii* strain KMD37 (HM072284) was used as outgroup.

Bacteria Phenotypic Characterization

Phenotypic characterization was carried out using methods that are approved for *Xenorhabdus*. (Akhurst 1986, Peel et al. 1999, Kazmierczak et al. 2016).

Extraction and Purification

After 72 hr incubation, the XAD-16 beads from 18 L of cultures were extracted with 20 liter of EtOAc (3 times). The EtOAc fraction was filtered, and the solvent was removed using a rotary evaporator from Heidolph Instruments (Germany) at 45°C to afford a dark orange extract (1.20 g). The extract was subjected to gel chromatography on Sephadex LH-20 (2.5 × 100 cm) and eluted with MeOH to give 15 fractions (Fr. 1–15) with the aid of TLC analysis. The fractions were further purified by semipreparative HPLC using an Onyx Monolithic C18 (Phenomenex, Torrance, CA) (100 mm × 10 mm) with a gradient elution of MeOH and H_2O (20–100%) as an eluent including 0.1% TFA at a flow rate 5 ml/min within 16 min to yield 27 compounds (**Xh-1–27**).

Liquid Chromatography–Electrospray Tandem Mass Spectrometry (LC–ESI–MS/MS) Analysis

The isolated compounds, along with the crude EtOAc extract were analyzed using the UltiMate 3000 system (Thermo Fisher) coupled to an Impact II qTof mass spectrometer (Bruker). Compounds were eluted on an ACQUITY UPLC BEH C_{18} column (130 Å, 2.1 × 100 mm, 1.7 µm) using a MeCN/0.1% formic acid in H2O (5:95 → 95:5% over 15 min, flow rate 0.4 ml/min). A scan range of 100–2,000 m/z in positive ionization mode was performed to detect the compounds. Source settings: capillary voltage 4,500 V, nebulizer gas pressure (nitrogen) 3 bar, ion source temperature 200°C, the dry gas flow of 8 ml/min (Tobias et al. 2017).

Bacterial Strains and Culture Media

Bacterial extracts and isolated compounds were tested against four strains of antibiotic-resistant bacteria, including *S. aureus* 1112, *E. coli* 1330, and two species of plant-pathogenic bacteria *Xanthomonas oryzae* pv. *oryzae* strain Xoo-IR42 and *E. amylovora* strain EaUMG3. Bacterial strains were cultured overnight at 37°C on Mueller Hinton agar (MHA).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC values were determined according to previous studies (Shakeri et al. 2018). Briefly, various concentrations of bacterial extract (1.71–0.02 mg/ml) and isolated compounds (0.1–0.0156 mg/ml) were prepared by 2-fold serial dilution of the sample. Then, 200 µl of each sample concentration in a sterile 96-well microtitre plate was inoculated with 20 µl of a 10^6 CFU/ml overnight bacterial culture. Cell density was determined using a Neubauer hemocytometer under light microscope after staining with trypan blue. Negative and positive controls were used for each tested strain. After overnight incubation at 37°C, 20 µl of 2, 3, 5-triphenyltetrazolium chloride (TTC, 5 mg/ml) was added to each well as a colorimetric indicator of bacterial growth and incubated for 30 min at 37°C. MIC was determined as the lowest concentration of the sample that showed no color change, while MBC was the lowest concentration without any bacterial growth represented MBC.

Cytotoxicity

Cell Culture

MCF-7 (human breast cancer), A549 (human lung cancer), and Murine Tumor (B16) were purchased from NCBI (National Cell Bank of Iran). Cell lines were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (Gibco, Invitrogen, Paisely) and penicillin (100 units/ml) and streptomycin (100 µg/ml) for the desired growth in a humidified incubator at 37°C in an atmosphere of 5% CO_2.

AlamarBlue Assay

The cytotoxicity of samples was assessed using an AlamarBlue (BioSource Invitrogen, Paisely, UK) proliferation assay (van Haaften et al. 2011), with slight modification. Cells were plated on 96-well microplates at a density of 1×10^4. After 24 hr, cells were treated with the samples at different concentrations. After 48 hr, the incubated cells were treated with AlamarBlue reagent (10% of tissue culture volume). After 1–4 hr the absorbance was measured at 600 nm using a Biotech Plate Reader (BioTek Instruments, Winooski, VT). Doxorubicin was used as a positive control, and viability of cells was obtained via comparison of absorbance of negative control wells with treated wells.

Statistical Analysis

Cell line viability was control-corrected (Abbott 1925), and square-root transformed when required to meet assumptions of normality and homogeneity of variances. In this experiment, control-corrected viability was subjected to multifactorial analysis of variance (ANOVA) and means separation by Tukey test (SAS Institute 2002–2003). Cell viability data were subjected to three-way ANOVA and a completely randomized factorial design followed by Tukey's multiple-comparison test ($P < 0.05$). Five replications and two repetitions were used to conduct the tests.

Results

Identification of Bacterium

BLAST analysis was performed using the 16S rDNA sequence of *Xenorhabdus* isolate. A reconstructed phylogenetic tree whose branch length is 0.23100367 has been shown. Next to the branch, the bootstrap test shows the percentage of the replication tree in which the relevant taxa are clustered (10,000 replications) (Felsenstein 1985). The tree is drawn in proportions using the length of the branches in the same units as the evolutionary distance used to derive the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. This analysis involved 32 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 1,107 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018).

Bacterial Detection Results Using Biochemical Testing

Bacteria isolated from *S. carpocapsae* nematode were cultured on three different culture media, and the bacterial colony color was

recorded after forty-eight hours. The color of the bacterial colony on NBTA and NA media was blue and ivory, respectively. The results of the motility test showed the motility of the isolated bacteria. The subject bacteria also grew upon exposure to antibiotics. When the bacteria were injected into the wax moth larvae, the carcasses turned into typical injections by *Xenorhabdus* species. The test results for catalase, lipase, and recitinase were negative, positive, and positive for this bacterium, respectively. These results indicated that the isolated bacteria were strains of species *X. nematophila* (Fig. 1). In the case of two bacteria strains isolated from *S. feltiae*, distance analysis and phylogenetic tree confirmed the bacterial identity as *X. bovienii*.

Bioactive Compounds

A preliminary metabolite fingerprint of the *X. nematophila* extract was obtained by LC-ESI-MS/MS analysis (Figs. 2 and 4). Compounds identified as xenocoumacin II (**1**), xenortide-396 (**2**), xenortide A (**3**), xenortide-410 (**4**), xenortide-449 (**5**), xenematide A 663 (**6**), rhabdopeptide-574 (**7**), rhabdopeptide-588 (**8**), rhabdopeptide-687 (**9**), rhabdopeptide-701 (**10**), and nematophin-273 (**11**) (Figs. 3) (Table 1). Of these, only three compounds (**4**, **6**, and **11**) had sufficient amounts to perform the relevant biological tests. The structures of compounds **1-11** were known and have been previously reported (Reimer 2014). The results of the biological activities of the isolated compounds and bacterial extracts are shown in Tables 2 and 3.

Diagrams of Cytotoxicity of Ethyl Acetate Extracts and Metabolites on Cell Lines, MCF-7, A549, and B16 Using AlamarBlue Tests

Figure 5 are diagrams from the AlamarBlue test, drawn separately for each cell lines.

Effect of Concentration

Control-corrected viability was significantly affected by three cell lines ($F = 345.77$; $P < 0.0001$; df = 2, 539), samples ($F = 954.63$; $P < 0.0001$; df = 5, 539), and samples concentration ($F = 1761.08$; $P < 0.0001$; df = 2, 539), there were significant interactions for cell lines * samples ($F = 189.04$; $P < 0.0001$; df = 10, 539), cell lines * samples concentration ($F = 4.47$; $P < 0.0015$; df = 4, 539), samples * samples concentration ($F = 61.98$; $P < 0.0001$; df = 10, 539), and cell lines * samples * samples concentration ($F = 8.21$; $P < 0.0001$; df = 20, 539). When data were analyzed with cell lines * samples * samples concentration combinations as treatments, the highest survival was related to B16 cell lines treated with xenortide-410 sample at concentrations of 1.56 and 3.125 µg/ml, A549 cell lines treated with *X. bovienii* FUM 222 sample at concentrations of 1.56 µg/ml. and

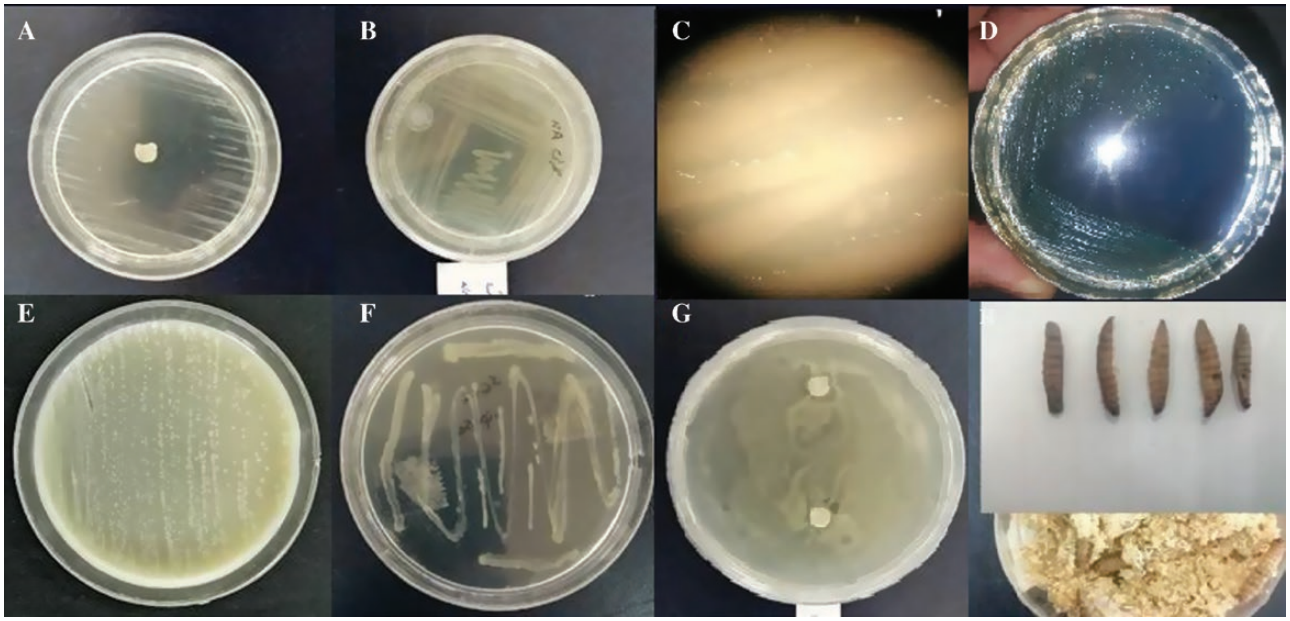


Fig. 1. Biochemical tests; A, Antibiotic and control; B, C, Bacterial colony on NBTA; D, Dye absorption on NA; E, Lesitinase; F, Lipase; G, Motility; H, Mortality, and control.

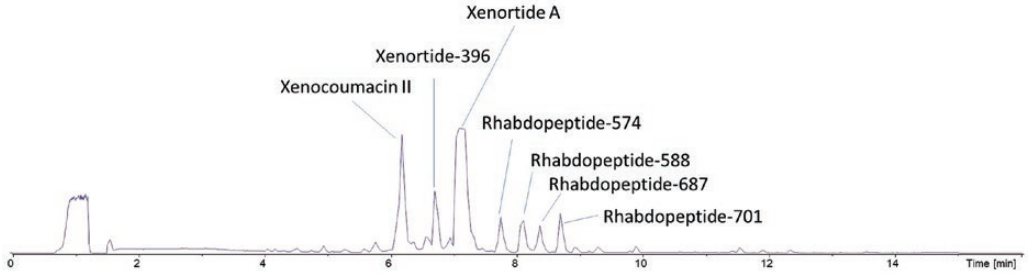


Fig. 2. LC-MS profile of *X. nematophila* EtOAc extract.

Table 2. IC_{50} results in MCF-7, A549, and B16 cell line. *X. bovienii* FUM 222 (A), *X. nematophila* FUM 221 (B), *X. nematophila* FUM 220 (C), *X. bovienii* FUM 223 (D), Xenortide-410 (E), Xenematide & nematophin (F), Doxorubicin (G)

Sample	Sample content (mg)	IC_{50} (µg/ml)		
		MCF-7	A-549	B-16
A	130	20.8 ± 0.33	16.9 ± 0.08	2.6 ± 0.08
B	130	25.6 ± 1.59	6.3 ± 0.04	12.5 ± 0.22
C	1200	6.6 ± 0.13	4.3 ± 0.06	3.7 ± 0.20
D	140	6.7 ± 0.16	2.4 ± 0.07	2.6 ± 0.12
E	2.4	40.9 ± 1.55	11.2 ± 0.56	54.6 ± 2.09
F	3.6	15.9 ± 0.71	6.2 ± 0.16	11.3 ± 0.24
G	–	0.0747	0.0134	0.01957

Table 3. Mean determination of MBC and MIC concentrations of bacterial extracts and metabolites (µg/ml). *X. bovienii* FUM 222 (A), *X. nematophila* FUM 221 (B), *X. nematophila* FUM 220 (C), *X. bovienii* FUM 223 (D), Xenortide-410 (E), Xenematide & nematophin (F), Tetracycline (G), Gentamicin (H)

Sample	Sample content (mg)	*S. aureus*		*E. coli*		*X. oryzae*		*E. amylovora*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A	130	53.5	1,714	1714	–	107	1,714	53.5	1,714
B	130	857	1,714	1714	1714	53.5	1,714	53.5	1,714
C	1,200	107	1,714	1714	1714	53.5	1,714	53.5	1,714
D	140	107	1,714	1714	1714	53.5	1,714	53.5	1,714
E	2.4	–	–	–	–	–	–	–	–
F	3.6	100	–	–	–	62.5	–	125	–
G	–	180	360	–	–	–	–	–	–
H	–	–	–	5	10	–	–	–	–

MCF-7 cell lines treated with xenortide-410 and *X. nematophila* FUM 221 samples at a concentration of 1.56 µg/ml. Some of the lowest viability was related to B16 cell lines treated with *X. bovienii* FUM 222 and *X. bovienii* FUM 223 samples at a concentration of 6.25 µg/ml, MCF-7 cell lines treated with *X. nematophila* FUM 220 sample at concentrations of 6.25 µg/ml, B16 cell lines treated with *X. bovienii* FUM 222 sample at a concentration of 3.125 µg/ml and MCF-7 cell lines treated with *X. bovienii* FUM 223 sample at concentrations of 6.25 µg/ml.

Results of IC_{50} Calculation Using PRISM Software

The results obtained in the present work show that sample (A) caused cell death on B16 cell lines (IC_{50} = 2.64 µg/ml) and was less cytotoxic to MCF-7 and A549 cells. Sample (B) was shown to be active on B16 and MCF-7 cancer cells with low toxicity compared with A549 cell lines. Among all the samples, sample (E) had the lowest effect on B16 (IC_{50} = 54.61 µg/ml) and MCF-7 (IC_{50} = 40.9 µg/ml) cell lines.

Results of Antibacterial Activity

The inhibitory effect of samples on these antibiotic-resistant bacteria showed the MIC and MBC ranging from 1714 to 53.5 mg/ml. *X. oryzae* and *E. amylovora* were equally sensitive to antimicrobial compounds of samples (B), (C), and (D) (MIC = 53.5 µg/ml and MBC = 1714 µg/ml). *S. aureus* was sensitive to the antimicrobial compounds of various samples except for sample (B). All samples had low activity against gentamicin-resistant human pathogenic *E. coli*. Sample (F) had a moderate growth inhibitory effect on *S. aureus*, *X. oryzae*, and *E. amylovora* studied. No bacterial strains were susceptible to sample (E) (see Table 3).

Discussion

The current word aimed to address the potency of secondary metabolites (SM) from four strains of *Xenorhabdus* on human cell targets as well phytopathogenic prokaryotes. To our knowledge this project provides first data for SM of symbiotic bacteria of *Xenorhabdus* on human cancer cell lines. It was demonstrated that 1.56–6.25 µg/ml of each sample could decrease the proliferation of MCF-7, A549, and B16 in a dose-dependent. Analysis of data with cell lines * sample * composition of sample concentration as a treatment, showed that sample (A) and then sample (D) at concentrations 2 and 3 can have good effects on the survival rate of B16 cancer cell line. Samples (C) and (D) at concentrations 3 can have good effects on the survival rate of MCF-7 cancer cell line. It is noteworthy that sample (F) at concentration 3 can have moderate effects on the survival rate of MCF-7 cancer cell line. Also, the tested samples had more favorable effects on B16 and MCF-7 compared to A549.

Our data seem to indicate that there is a large difference between MCF-7 and A549 in the expression of the multidrug resistance protein, which induces more resistance in the A549 cell lines. MRP1 (multidrug resistance-associated protein) and LRP (pulmonary resistance-associated protein) were mainly elevated in A549 cells. The review of study results showed that MCF-7 cells were more sensitive than A549 cells to DOX treatment. Moreover, the analysis of

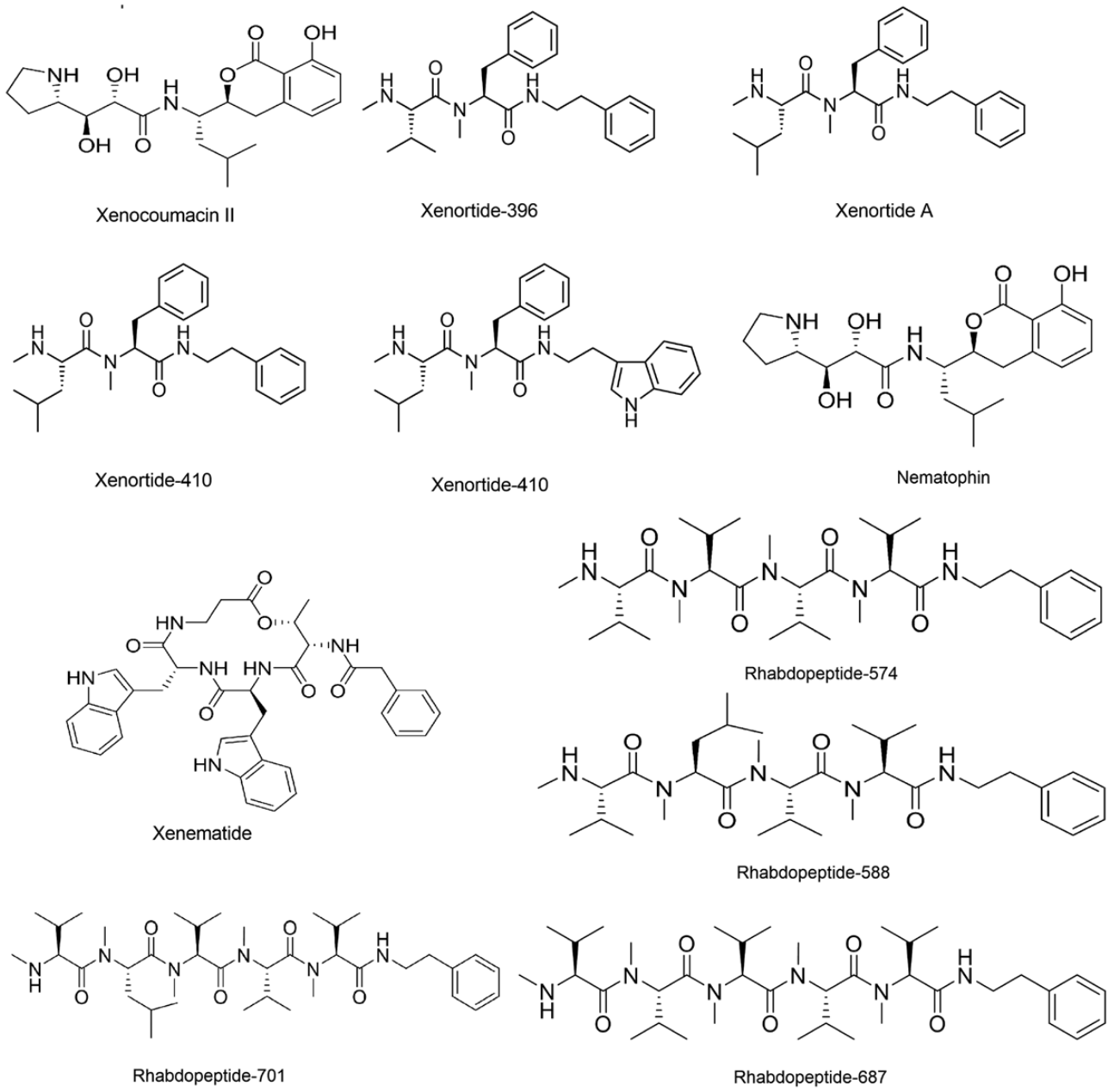


Fig. 3. Structures involved in this study.

P-gp (P-glycoprotein) and MRP expression did not show significant differences between the two cell lines while a high expression of LRP was detected in A549 cells (Meschini et al. 2002). In a number of previous studies, the low level of LRP expression in MCF-7 cells was reported (Kickhoefer et al. 1998). Considering that LRP is a human main vault protein (MVP) that plays a role in the drug resistance of cancer cells in a cell-type-dependent manner (Chen et al. 2016), the absence of this protein has probably caused the B16 cell line to be more sensitive to the tested compounds. An overview of the results of research shows that increasing the expression of antioxidant enzymes that increase the production of oxygen free radicals is an effective way to treat cancer. For example, genistein, an anticancer isoflavonoid, increases the expression of antioxidant enzymes. In the prostate cancer cell line, which inhibits the production of oxygen free radicals, it inhibited these cells (Bilir et al. 2017). Decreased expression of antioxidant enzymes has also been shown to be involved in breast cancer (Kasapović et al. 2008). The phthalic acid produced by *Photorhabdus temperata* M1021 has antioxidant activity (Ullah et al. 2014). JS-38 (mitothiolore) is a synthetic version of a metabolite isolated from *Xenorhabdus* sp. which exhibits distinct antitumor effects without tissue or species characteristics. Given this, it could possibly be useful in treating human clinical cancers. Although the main mechanism of antitumor effect is not known, the results show that the main factor inhibiting tumor growth is the induction of apoptosis (Min-Yu et al. 2014). Sample (C) showed the strongest cytotoxic activity against the MCF-7 cell line. Also note that samples (E) and (F) are also very active, but less effective than the sample (C). The reason for this, is the presence of more metabolites in the bacterial extract that causes a synergistic effect. Based on these results, it can be inferred that *X. nematophila* FUM220 (IC_{50} = 6.6 µg/ml) has a higher effect on MCF-7 cell lines compared to the *X. nematophila* FUM221 (IC_{50} = 25.6 µg/ml). Comparing the

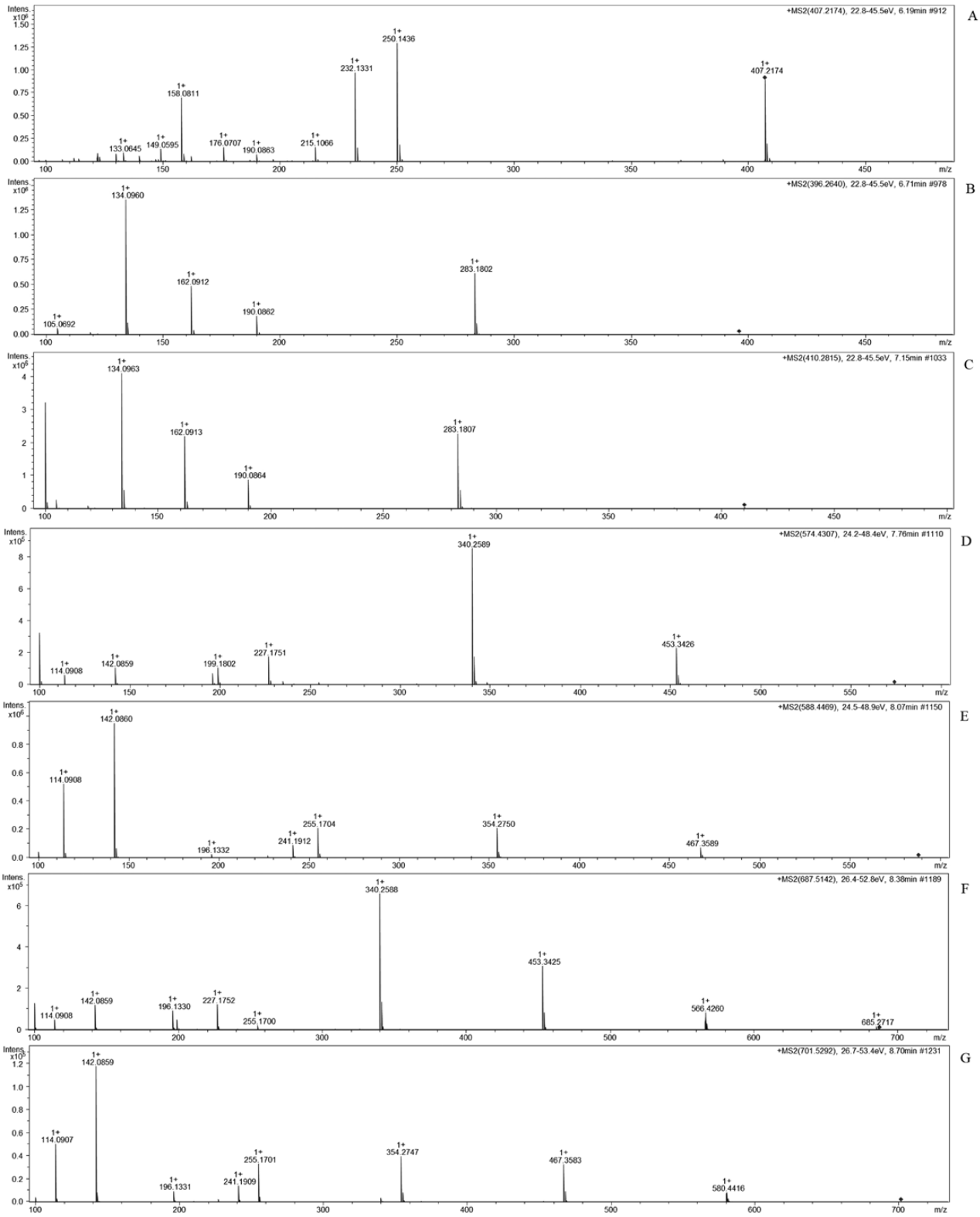


Fig. 4. HR-ESI-MS of xenortide-396 (A), Xenortide A (B), Xenocoumacin II (C), Rhabdopeptide-701 (D), Rhabdopeptide-687 (E), Rhabdopeptide-588 (F), Rhabdopeptide-574 (G).

effect of samples (E) and (F) with sample (C), it was found that there were more compounds in the extract of *X. nematophila* FUM 220 that can be effective on the MCF-7 cell lines. As shown in Table 2, sample (D) with IC_{50} of 6.7 µg/ml has the same effect as sample (C). This may be due to the presence of metabolites with similar structures in two mentioned bacterial extracts. About this cytotoxicity test, the effect of sample (F) was higher than the sample (E). This is probably due to the synergistic effect of xenematide and nematophin

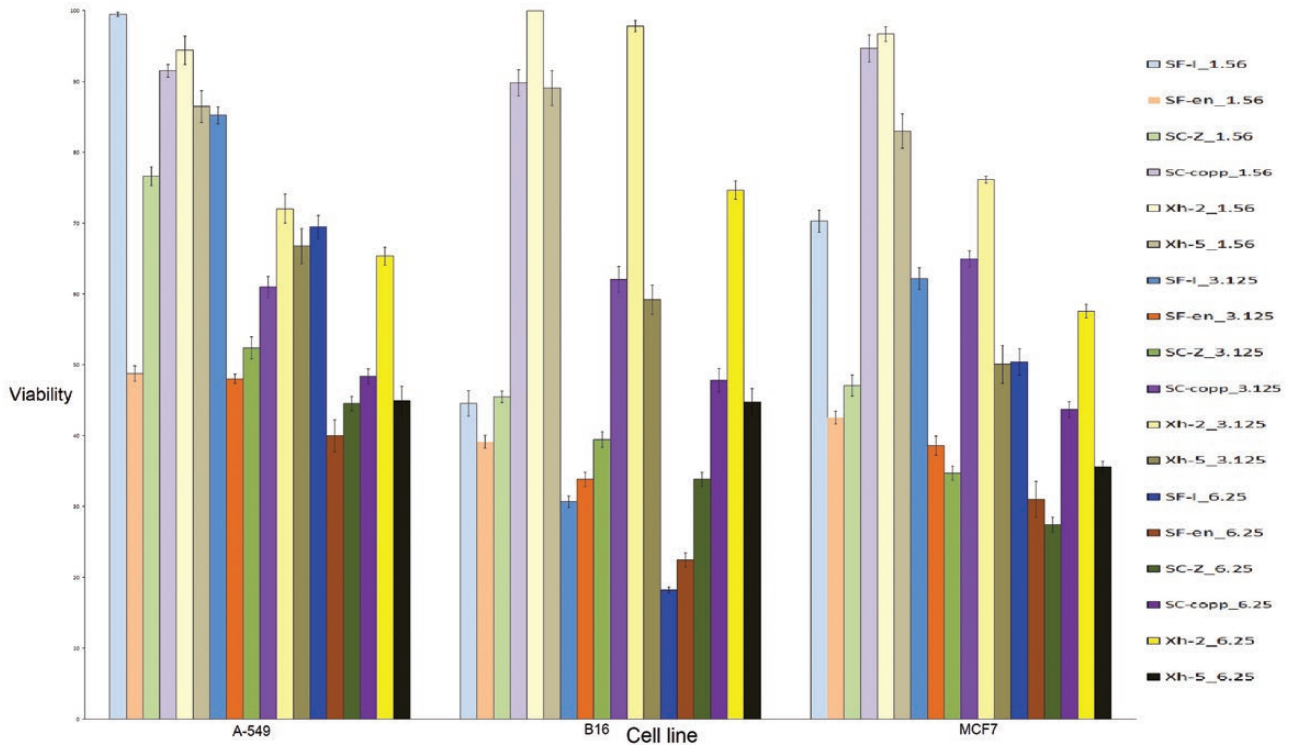


Fig. 5. Effect of cell lines on viability (±SE) exposed to three concentrations (1.56, 3.125, and 6.25µg/ml) of *X. bovienii* FUM 222, *X. nematophila* FUM 221, *X. nematophila* FUM 220, *X. bovienii* FUM 223, Xenortide-410, Xenematide & nematophin. Different letters indicate significant differences between interactive treatment (three-way ANOVA and Fisher's protected LSD at $P < 0.05$).

in the sample (F). Among bacterial extracts, sample (B) had the least effect on the MCF-7 cell line. Based on the above, it can be guessed that the extract of *X. nematophila* FUM220 contains compounds that have a good effect on human breast cancer.

Considering the cytotoxicity of A549, the effect of the sample (D) with IC_{50} of 2.4 µg/ml was much higher than the sample (A) with IC_{50} of 16.9 µg/ml. Similar to our results, extracellular metabolites of *Xenorhabdus stockiae* (Enterobacteriales: Morganellaceae) were previously shown to have high cytotoxicity against the same cell lines, A549, with IC_{50} of 8 µg/ml (Chandrakasan et al. 2017). Besides, samples (A), (C), and (D) have a similar effect on B16 cell lines.The results demonstrated that, among the bacterial extracts, potent cytotoxicity was found for samples (D) (IC_{50} = 2.6 µg/ml), and (A) (IC_{50} = 2.6 µg/ml). By comparing the cytotoxicity effect of sample (A) on three cell lines, we found that this compound has a lower effect on MCF-7 (IC_{50} = 20.8 µg/ml) and A549 (IC_{50} = 16.9 µg/ml) than B16 cell lines (IC_{50} = 2.6 µg/ml). Sample (E) had the least effect against MCF-7 (IC_{50} = 40.9 µg/ml), A549 (IC_{50} = 11.2 µg/ml), and B16 (IC_{50} = 54.6 µg/ml), respectively. So it can be assumed that compounds with less effect on mouse cancer cells are probably less effective on human cancer cells as well. A comparison of IC_{50} results of samples (C) and (D) against the three mentioned cells lines indicated that the extracts of these bacteria had similar effects. The results demonstrated that among the bacterial extracts, potent cytotoxicity was found for sample (C) and sample (D) against MCF-7 (IC_{50} = 6.6 and 6.7 µg/ml), A549 (IC_{50} = 4.3 and 2.4 µg/ml), and B16 (IC_{50} = 3.7 and 2.6 µg/ml), respectively. The IC_{50} results on MCF-7, A549, and B16 cell lines demonstrated that the effect of sample (F) was higher than sample (E). This is probably due to the synergistic effect of xenematide and nematophin in the mentioned sample. Among bacterial extracts, sample (B) had the least effect on the MCF-7 cell line. Comparing the IC_{50} results shows that the MCF-7 cell lines are highly tolerant. Comparison of IC_{50} results of MCF-7; A549 and B16 cell lines showed that the effect of sample (C) was higher than sample (B). This can be very important for the isolation and identification of secondary metabolites of native bacteria in the future. IC_{50} results of MCF-7, A549, and B16 cell lines showed that the effect of sample (D) is greater than sample (A). This indicated that the commercial strain of *X. bovienii* has more valuable compounds for isolation and identification than the noncommercial strain. From *Xenorhabdus* source, another metabolite, xenoamicin A, indicated activity against mammalian L6 cells (IC_{50} = 68.5 µg/ml) (De Mandal et al. 2021). According to the studies performed on the *Xenorhabdus* genus, it has been determined that the extract of this bacterium can be toxic to MCF-7, A549, and B16 cell lines in terms of its compounds of xenorhabdins, xenoroxides, puromycin, etc. These data confirm the hypothesis that *Xenorabdus* species and strains could be a rich source for diverse bioactive against human cell lines. In the case of antimicrobial activities, all extracts showed moderate activity against *E. amylovora* with MIC of 53.5 µg/ml. All samples except A (MIC = 107 µg/ml) showed moderate activity against *X. oryzae* with a MIC of 53.5 µg/ml, therefore, it can be said that *X. oryzae* has less susceptibility to sample (A) than *E. amylovora*. Although sample (C) had an equal effect on both plant pathogens, the compound (sample F) isolated from the same bacterial extract, doesn't had the same effect on *X. oryzae* (MIC = 62.5 µg/ml) and *E. amylovora* (MIC = 125 µg/ml). Bacterium *X. oryzae* showed greater sensitivity to sample (F) than *E. amylovora*. According to Table 3, most samples had moderate effects on two gram-negative phytopathogenic bacteria, but their effects on gram-negative human pathogen were very weak (MIC = 1714 µg/ml). Meanwhile, the highest antimicrobial activity against *S. aureus* was observed for samples (A), (C), (D), and (F) with MIC of 53.5, 107, 107, and 100 µg/ml, respectively.

By a glance over Table 3, we can detect much stronger effects for xenematide and nematophin (sample F) than tetracycline, so it can be assumed that further exploration on these compounds could provide more information to find an alternative compound of tetracycline. However, sample (C) showed no activity. Xenematide was previously shown high antibacterial activity against *Bacillus subtilis* (Bacillales: Bacillaceae), *E. amylovora, Pseudomonas fluorescens* (Pseudomonadales: Pseudomonadaceae), *Pseudomonas syringae,* and *Ralstonia solanacearum* (Burkholderiales: Burkholderiaceae) with MIC value of 10 µg/ml. Among other derivatives, xenematides B, F, and G, previously isolated from *X. budapestensis*. Among these, xenematide F exhibited moderate activity against *Pseudomonas aeruginosa* (MIC = 32 µg/ml), while higher activity was detected for xenematide G with MIC of 16 µg/ml against *B. subtilis* (Xi et al. 2019). We also isolated xenematide, our results for xenematide and nematophin (sample F) showed lower antibacterial activity against *E. amylovora* with MIC of 125 µg/ml could have corresponded to inter-strain variability. Besides, xenocoumacin II also displayed moderate antiphytopathogenic activity against *Xanthomonas campestris*, *R. solanacearum*, and *P. syringae* (MIC = 40 µg/ml) and antibacterial activity against *B. subtilis* (MIC = 20 µg/ml) (Lang et al. 2008). Endosymbiont *X. stockiae* extracts were previously shown antibacterial activity against *S. aureus* strain PB36 with MIC value of 3,900 µg/ml and *Acinetobacter baumannii* strain AB322 (Pseudomonadales: Moraxellaceae) with MIC value of 7,810 µg/ml (Muangpat et al. 2020). Xenorxide, previously isolated from *X. bovienii* strain A21, and nematophin, from *X. nematophilus* strain BC1, exhibited moderate activity against *E. coli* ATCC25922 (MIC= >100 µg/ml). Xenorxide has potent activity against *S. aureus* ATCC 29213 (MIC = 6 µg/ml) while higher activity was detected for nematophin with MIC of 0.75 µg/ml against the same bacterial strain of *S. aureus*. In addition, xenorxide also has displayed potent antibacterial activity against *S. aureus* 0012, and *S. aureus* 0017 (MIC = 3 µg/m^{l}), while higher antibacterial activity was detected for nematophin against *S. aureus* 0012 (MIC of 1.50 µg/ml) and *S. aureus* 0017 (MIC of 0.75 µg/ml) (Webster et al. 2002). Nematophin was shown effective against *S. aureus* (MIC = 0.125 µg/ml) and methicillin-resistant *S. aureus* (MRSA) (MIC = 1.5 µg/ml) (De Mandal et al. 2021). Our results for nematophin and xenematide showing higher MIC could correspond to inter-strain variability.

A comparison of the results of antibacterial activity and cytotoxicity well shows that samples (D) and (C) have similar and very close effects; it is assumed that these two bacterial species produce compounds with similar structures. To our knowledge, this is the first work on comparison of bioactive potencies among several strains/ species of *Xenorhabdus*. Regarding restricted finding on cytotoxicity of bioactive compounds of *Xenorhabdus*, our information which provided data for three compounds, 4, 6, and 11 on three cancer cell lines could be significant in future. Some results of the antibacterial activity tests showed some difference with few available ones. This may be due to differences in species or experimental strain, secreted compounds, culture conditions, or research methods.

However, the results of this study suggest that symbiotic bacteria of the genus *Xenorhabdus* associated with pathogenic nematodes may be an excellent source of bioactive compounds for combating multidrug-resistant bacteria and treating cancer and plant diseases. It clearly shows that there is. In addition, broader research into the isolation, identification, and characterization of these secondary metabolites may lead to the discovery of new potential anticancer and antibacterial agents. This study highlights the worthy of *Xenorhabdus* as future peptide sources for the supply of natural bioactive compounds. Anyway, more studies are required to optimize the conditions for large-scale production and commercialization of these compounds as drug alternatives. The finding of the work support that endosymbionts of EPNs represent a new and rich source of SM that should be explored in the future.

Conclusion

In summary, the cytotoxic and antibacterial effects of ethyl acetate extract and pure substances isolated from different strains of *Xenorhabdus* bacteria were successfully evaluated. Anticancer activity of the tested samples demonstrated a tremendous dose-dependent inhibitory activity against all three cell lines MCF-7, A549, and B16. Also, the antibiotic ability of the tested samples to inhibit the growth of human and plant pathogenic bacteria makes this research a good basis for conducting reliable clinical and field tests. The overall results suggest that different strains of *Xenorhabdus* bacteria produces compounds with a wide range of biological activities and, they can provide new bioactive substances as a potential system in the field of medicine as well plant protection methods.

Funding

This study was funded by the Ferdowsi University of Mashhad (p3/48171) and grant of Iran National Science Foundation (INSF) for project 97024982.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Akhurst, R. 1982. Antibiotic activity of *Xenorhabdus* spp., bacteria symbiotically associated with insect pathogenic nematodes of the families Heterorhabditidae and Steinernematidae. Microbiology. 128: 3061–3065.

Akhurst, R. J. 1986. *Xenorhabdus nematophilus* subsp. *poinarii*: its interaction with insect pathogenic nematodes. Syst. Appl. Microbiol. 8: 142–147.

Bilir, B., N. V. Sharma, J. Lee, B. Hammarstrom, A. Svindland, O. Kucuk, and C. S. Moreno. 2017. Effects of genistein supplementation on genome-wide DNA methylation and gene expression in patients with localized prostate cancer. Int. J. Oncol. 51: 223–234.

Booysen, E., M. Rautenbach, M. A. Stander, and L. M. Dicks. 2021. Profiling the production of antimicrobial secondary metabolites by *Xenorhabdus khoisanae* J194 under different culturing conditions. Front. Chem. 9: 115.

Böszörményi, E., T.Érsek, , A. Fodor, A. Fodor, L. S.Földes, , M. Hevesi, and A.Kormány, . 2009. Isolation and activity of *Xenorhabdus* antimicrobial compounds against the plant pathogens *Erwinia amylovora* and *Phytophthora nicotianae*. J. Appl. Microbiol. 107: 746–759.

Chandrakasan, G., P. Seetharaman, S. Gnanasekar, M. Kadarkarai, and S. Sivaperumal. 2017. *Xenorhabdus stockiae* KT835471-mediated feasible biosynthesis of metal nanoparticles for their antibacterial and cytotoxic activities. Artif. Cells Nanomed. Biotechnol. 45: 1675–1684.

Chen, Y. -L., T. -Y. Yang, C. -L. Wu, K. -C. Chen, S. -L. Hsu, and C. -M. Hsueh. 2016. Mechanisms underlying lung resistance-related protein (LRP)-mediated doxorubicin resistance of non-small cell lung cancer cells. Chin. J. Physiol. 59: 331–347.

De Mandal, S., A. K. Panda, C. Murugan, X. Xu, N. S. Kumar, and F. Jin. 2021. Antimicrobial peptides: novel source and biological function with a special focus on entomopathogenic nematode/bacterium symbiotic complex. Front. Microbiol. 12, 555022.

Dreyer, J., M. Rautenbach, E. Booysen, A. Van Staden, S. Deane, and L. Dicks. 2019. *Xenorhabdus khoisanae* SB10 produces Lys-rich PAX lipopeptides and a Xenocoumacin in its antimicrobial complex. BMC Microbiol. 19: 1–11.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evol. 39: 783–791.

Fodor, A., I. Varga, M. Hevesi, A. Máthé-Fodor, J. Racsko, and J. A. Hogan. 2012. Novel anti-microbial peptides of *Xenorhabdus* origin against multidrug resistant plant pathogens, pp. 147–196. *In* Biochemistry, genetics and molecular biology – a search for antibacterial agents. InTech.

Fodor, A., M. Gualtieri, M. Zeller, E. Tarasco, M. G. Klein, A. M. Fodor, and G. M. Furgani. 2022. Type strains of entomopathogenic nematode-symbiotic bacterium species, *Xenorhabdus szentirmaii* (EMC) and *X. budapestensis* (EMA), are exceptional sources of non-ribosomal templated, large-target-spectral, thermotolerant-antimicrobial peptides (by both), and iodinin (by EMC). Pathogens. 11: 342.

van Haaften, C., C. C. Duke, A. M. Weerheim, N. P. Smit, P. M. van Haard, F. Darroudi, and B. J. Trimbos. 2011. Potent cytotoxic effects of *Calomeria amaranthoides* on ovarian cancers. J. Exp. Clin. Cancer Res. 30: 1–6.

Heuer, H., M. Krsek, P. Baker, K. Smalla, and E. Wellington. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Appl. Environ. Microbiol. 63: 3233–3241.

Houard, J., A. Aumelas, T.Noël, , A. Givaudan, V. Fitton-Ouhabi, P. Villain-Guillot, and M. Gualtieri. 2013. Cabanillasin, a new antifungal metabolite, produced by entomopathogenic *Xenorhabdus cabanillasii* JM26. J. Antibiot. (Tokyo) 66: 617–620.

Kasapović, J., S. Pejić, A. Todorović, V. Stojiljković, and S. B. Pajović. 2008. Antioxidant status and lipid peroxidation in the blood of breast cancer patients of different ages. Cell. Biochem. Funct. 26: 723–730.

Kazmierczak, K. M., D. J. Biedenbach, M. Hackel, S. Rabine, B. L. de Jonge, S. K. Bouchillon, and P. A. Bradford. 2016. Global dissemination of bla KPC into bacterial species beyond *Klebsiella pneumoniae* and in vitro susceptibility to ceftazidime-avibactam and aztreonam-avibactam. J. Antimicrob. Chemother. 60: 4490–4500.

Kickhoefer, V. A., K. S. Rajavel, G. L. Scheffer, W. S. Dalton, R. J. Scheper, and L. H. Rome. 1998. Vaults are up-regulated in multidrug-resistant cancer cell lines. J. Biol. Chem. 273: 8971–8974.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111–120.

Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35: 1547–1549.

Lang, G., T. Kalvelage, A. Peters, J. Wiese, and J. F. Imhoff. 2008. Linear and cyclic peptides from the entomopathogenic bacterium *Xenorhabdus nematophilus*. J. Nat. Prod. 71: 1074–1077.

Lengyel, K., E. Lang, A. Fodor, E.Szállás, , P. Schumann, and E. Stackebrandt. 2005. Description of four novel species of *Xenorhabdus*, family Enterobacteriaceae: *Xenorhabdus budapestensis* sp. nov., *Xenorhabdus ehlersii* sp. nov., *Xenorhabdus innexi* sp. nov., and *Xenorhabdus szentirmaii* sp. nov. Syst. Appl. Microbiol. 28: 115–122.

Meschini, S., M. Marra, A. Calcabrini, E. Monti, M. Gariboldi, E. Dolfini, and G. Arancia. 2002. Role of the lung resistance-related protein (LRP) in the drug sensitivity of cultured tumor cells. Toxicol. In Vitro. 16: 389–398.

Min-Yu, L., X. Lin, C. Geng-Hui, W. Yong-Xiang, W. -X. Xiong, L. Fei, and Z. Zhang. 2014. Oral JS-38, a metabolite from *Xenorhabdus* sp., has both anti-tumor activity and the ability to elevate peripheral neutrophils. Chin. J. Nat. Med. 12: 768–776.

Muangpat, P., M. Suwannaroj, T. Yimthin, C. Fukruksa, S. Sitthisak, N. Chantratita, and A. Thanwisai. 2020. Antibacterial activity of *Xenorhabdus* and *Photorhabdus* isolated from entomopathogenic nematodes against antibiotic-resistant bacteria. PLoS One. 15: e0234129.

Peel, M. M., D. A. Alfredson, J. G. Gerrard, J. M. Davis, J. M. Robson, R. J. McDougall, and R. J. Akhurst. 1999. Isolation, identification, and molecular characterization of strains of *Photorhabdus luminescens* from infected humans in Australia. J. Clin. Microbiol. 37: 3647–3653.

Racine, E., and M. Gualtieri. 2019. From worms to drug candidate: the story of odilorhabdins, a new class of antimicrobial agents. Front. Microbiol. 10: 2893.

Reimer, D. (2014). Identification and characterization of selected secondary metabolite biosynthetic pathways from *Xenorhabdus nematophila*. Dissertation, Frankfurt am Main, Johann Wolfgang Goethe-Universität, 2013.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

SAS Institute. 2002–2003. SAS 9.1 for windows. SAS Institute, Cary, NC.

Shakeri, A., V. Soheili, M. Karimi, S. A. Hosseininia, and B. S. Fazly Bazzaz. 2018. Biological activities of three natural plant pigments and their health benefits. J. Food Chem. Toxicol. 12: 356–361.

Singh, S., D. Orr, E. Divinagracia, J. McGraw, K. Dorff, and S. Forst. 2015. Role of secondary metabolites in establishment of the mutualistic partnership between *Xenorhabdus nematophila* and the entomopathogenic nematode *Steinernema carpocapsae*. Appl. Environ. Microbiol. 81: 754–764.

Solecka, J., J. Zajko, M. Postek, and A. Rajnisz. 2012. Biologically active secondary metabolites from Actinomycetes. Open. Life. Sci. 7: 373–390.

Tobias, N. J., H. Wolff, B. Djahanschiri, F. Grundmann, M. Kronenwerth, Y. -M. Shi, and S. J. Pidot. 2017. Natural product diversity associated with the nematode symbionts *Photorhabdus* and *Xenorhabdus*. Nature microbiology, 2: 1676–1685.

Ullah, I., A. L. Khan, L. Ali, A. R. Khan, M. Waqas, I. -J. Lee, and J. -H. Shin. 2014. An insecticidal compound produced by an insect-pathogenic bacterium suppresses host defenses through phenoloxidase inhibition. Molecules. 19: 20913–20928.

Vozik, D., K. Belafi-Bako, M. Hevesi, E. Boeszoermenyi, and A. Fodor. 2015. Effectiveness of a peptide-rich fraction from *Xenorhabdus budapestensis* culture against fire blight disease on apple blossoms. Not. Bot. Horti Agrobot. Cluj Napoca. 43: 547–553.

Webster, J. M., G. Chen, K. Hu, and J. Li. 2002. Bacterial metabolites. Entomopathog. Nematol. 99: 114.

Xi, X., X. Lu, X. Zhang, Y. Bi, X. Li, and Z. Yu. 2019. Two novel cyclic depsipeptides Xenematides F and G from the entomopathogenic bacterium *Xenorhabdus budapestensis*. J. Antibiot. (Tokyo) 72: 736–743.

Yang, J., H. -M. Zeng, H. -F. Lin, X. -F. Yang, Z. Liu, L. -H. Guo, and D. -W. Qiu. 2012. An insecticidal protein from *Xenorhabdus budapestensis* that results in prophenoloxidase activation in the wax moth, *Galleria mellonella*. J. Invertebr. Pathol. 110: 60–67.