ORIGINAL ARTICLE

Apoptotic and Cytotoxic Effect of Nisin on Triple-Negative Breast Cancer Cell Line, MDA-MB-231: One Health-Oriented Strategy for Cancer Control

Andrewslin Jani[1], T. A. Hema[1], Chanthini Kanagaraj Muthu-Pandian[2]

[1]Department of Microbiology, Malankara Catholic College, Manonmaniam Sundaranar University, Kanyakumari, Tamil Nadu, India, [2]Department of Pharmacology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India.

Abstract

Background: As one of the most prevailing cancer types, the hostile nature of breast cancer presents itself with significant treatment challenges besides becoming resilient toward conventional therapeutic strategies, demanding an urgent need for a better treatment option. **Objectives:** Hence, the study explores the anticancer potential of nisin, a bacteriocin, synthesized by *Lactococcus lactis*. **Materials and Methods:** *L. lactis*, prebiotic sourced from curd, was exposed to triple negative breast cancer (TNBC) cell line, MDA-MB-231, and its cytotoxic and apoptotic effects were evaluated. Nisin was isolated from *L. lactis* strain LB11 and confirmed by spectrophotometric assay (220 nm) through comparison with standard. Nisin was then carried forward for cytotoxic assay. **Results:** This revealed its ability to endorse a dose-dependent toxicity on TNBC cells (IC50 24.959 µg/mL) that were affirmed by the morphological changes observed in treated cells such as shrinkage and loss of spindle shape, attributed to membrane damage instigated by exposure to nisin. This was also further supported by fluorescence imaging studies that showed treated cells with both chromatin condensation and nuclear fragmentation, confirming early as well as late apoptosis. Negative effect of nisin on mitochondrial membrane potential of treated cancer cells confirmed by progressive loss of fluorescence, indicative of intrinsic apoptotic pathway, along with caspase activation. **Conclusion:** These results hence affirm the capabilities of nisin to invoke cytotoxicity and apoptosis in TNBC cells, to be used as a prospective therapeutic agent for breast cancer, aligning with sustainable development goal of good health and well-being. However, additional studies are required to establish its suitability as an alternative to conventional therapies.

Key words: Cell shrinkage, good health and well-being, lantibiotic, mitochondrial membrane potential loss, nuclear fragmentation

INTRODUCTION

Breast cancer has been diagnosed as the most leading cancer-causing mortality in women, with current projections leading to more than 60% of death rate by 2050.[1] Besides the availability of versatile treatment procedures that include surgery and therapies such as chemo, radio, and endocrine, recurrent occurrence of the disease often happens at the rate of about 30% due to drug resistance or incomplete tumor removal. This also occurs due to dose-limiting toxicities accompanied by myelosuppression, fatigue, cardiotoxicity besides impaired life quality.[2] Survivors are also diagnosed with an enhanced rate of co-morbidities, including cardiovascular

Address for correspondence:
Dr. T. A. Hema, Department of Microbiology, Malankara Catholic College, Manonmaniam Sundaranar University, Mariagiri - 629 153, Tamil Nadu, India.
E-mail: hemaraj15@gmail.com

Chanthini Kanagaraj Muthu-Pandian, Department of Pharmacology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai- 602105, Tamil Nadu, India.
E-mail: chanthinik.smc@saveetha.com

Received: 22-11-2025
Revised: 22-12-2025
Accepted: 31-12-2025

diseases, osteoporosis, metabolic syndromes as well as several psychological distress, that leads to enhanced health-care burdens, constraining further therapeutic options.[3] These consequences hence highlight the need for other therapeutic options that are not only effective but also safe, to serve as next-gen therapeutic agents that promise to offer enhanced sensitivity besides an amplified patient tolerability.[4]

Several biological compounds, including melittin, lactaptin, TP4, and several peptides from plants such as buckwheat or mung beans, have been proved with potentials of anti-cancer properties against breast cancer in several *in vitro* preclinical studies.[5] As reported, these biomolecules are stated to exert anticancer properties by more than one mode of action that includes the triggering of mitochondrial cytochrome c release along with activating caspase, besides the induction of checkpoints for arresting cell-cycle of cancer cells.[6] These cumulative actions then pave the way for the initiation of selective apoptosis in cancer cells, thereby affirming their use as adjuncts that could overcome the development of resistance.

Among the biological sources of anticancer compounds, lantibiotics, especially those from lactic acid bacteria, widely used in food fermentation, are recently gaining attention as widely sought therapeutic agents as anticancer compounds due to their potential cytotoxic besides immunomodulatory effects.[7] Besides having proven their bioactive potentials beyond antimicrobial effects, this group of bacteria, already a part of food fermentation, is being recognized for its safety profile as well.[8] Plantaricin, gassericin, and bactofencin are some of the most effective anticancer peptides among those synthesized by these lactic acid bacteria, reported to exert both anti-proliferative as well as pro-apoptotic activities as evidenced in innumerable cancer models.[9,10] In addition to these compounds, a lantibiotic peptide produced by *Lactococcus lactis*, nisin – a prospective anticancer compound – has been recognized for its explicitly characterized mechanism of causing membrane pore formation and induction of apoptosis, besides being familiarized as "safe" due to their food-grade status.[11]

These combined abilities of nisin, along with their efficacy to enhance the performance of chemotherapeutic agents in addition to overcome resistance developed in cancer cells to conventional treatment therapies, make it relatively more effective than other lantibiotic peptides.[12,13] Nisin has been reported with potentials to bind with the lipids of plasma membrane of cancer cells, disrupting their integrity, forming pores, consequently elevating intracellular calcium, which subsequently triggers apoptotic pathways. This, in turn, modifies Bcl-2 family proteins, that are reported to enhance drug uptake, thereby evading resistance mechanisms of cancer cells, transforming them to a susceptible state.[14] However, elaborative preclinical and clinical characterization are lacking opening new epochs for studies that require mechanistic affirmations of nisin's anticancer potentials.

This study hypothesizes that nisin elicits dose-dependent cytotoxicity and cell death in breast cancer cells through membrane perturbation and signaling changes, justified by its established bioactivity and the urgent need for safer alternatives amid escalating global burden and treatment failures; preclinical validation is vital for repurposing this accessible agent. Breast cancer cell lines will be treated with nisin, followed by viability (MTT), morphological (microscopy), and apoptosis (flow cytometry, caspase) assays using standard protocols.

MATERIALS AND METHODS

Isolation and identification of nisin-producing bacteria

Curd samples were serially diluted tenfold and 100 µL aliquot part were spread on MRS agar (37°C for 48 h) to enable lactic acid bacterial growth. Colonies with typical lactic acid bacterial morphology were picked and provisionally characterized using routine phenotypic assays, including Gram staining, catalase testing, and sugar fermentation profiles, and were then confirmed as nisin-producing *L. lactis* subsp. lactis by 16S rRNA gene polymerase chain reaction, sequencing, BLAST comparison with NCBI databases, and phylogenetic analysis in MEGA X with neighbor-joining and 1000-replicate bootstrapping.[15,16]

Cell culture (MDA-MB-231) and maintenance

The cell line MDAMB231 was maintained in Dulbecco's Modified Eagle Medium containing fetal bovine serum and 1% antibiotic mixture (37°C;5% CO_2), with addition of trypsinethylenediaminetetraacetic acid (0.25%) once they reached 80–90% confluence to keep them in the logarithmic growth phase for experiments and to ensure reproducible cell density and viability across replicates.[17]

Nisin isolation

An overnight culture of *L. lactis* Lb11 was inoculated at 2% (v/v) into MRS broth, followed by incubation at 30°C for 48 h under aerobic, agitated settings (150 rpm, initial pH 5.5). After incubation, the culture pH was adjusted to 4.0 and the broth was centrifuged at 8000 × g for 10 min at 4°C to obtain a cell-free supernatant, which was then concentrated by ultrafiltration and analyzed alongside a commercial nisin standard by measuring absorbance at 220 nm in a spectrophotometer.[18,19]

Cell viability assessment (MTT assay)

For cytotoxicity testing (MTT assay), nisin (10–200 µg/mL) and MDAMB231 cells (5×10^4 cells per well) were co-seeded, followed by the addition of MTT solution (0.5 mg/mL) and

recording absorbance for 24, 48, or 72 h at 570 nm to calculate IC_{50} values from dose–response curves.[20] Microscopic examination of treated cultures was carried out using an inverted microscope to document morphological aberrations.[20]

Apoptosis detection

Apoptotic cell death was further evaluated through Acridine Orange/Ethidium Bromide (AO/EB) dual staining following a published protocol, with MDAMB231 cells grown and treated with nisin (20 and 30 µg/mL), then harvested, washed, stained, and examined immediately under a fluorescence microscope (Zoe fluorescent cell imager, BioRad) to distinguish viable, early apoptotic, late apoptotic, and necrotic populations.[21]

Mitochondrial membrane potential (MMP) assay

Cells were exposed to 10 µM rhodamine 123 at 37°C for 30 min in the dark to permit mitochondrial accumulation of the dye, then washed twice with phosphate-buffered saline to remove unbound dye and examined immediately using a fluorescence microscope (Zoe fluorescent cell imager, BioRad). A reduction in green fluorescence signal was interpreted as a loss of MMP.[22]

Statistical analysis

Quantitative results are presented as mean ± standard deviation, based on three independent experiments. One-way analysis of variance was used to determine group differences with $P < 0.05$ considered statistically significant.

RESULTS

Isolation and identification of nisin-producing bacteria

Serial dilution and plating of curd samples on MRS agar yielded presumptive *L. lactis* colonies confirmed as *L. lactis* subsp. *lactis* (strain LB11) through phenotypic tests (Gram-positive cocci, catalase-negative, lactose fermentation) and 16S rRNA sequencing, submitted to NCBI, PX499742 (99.8% identity to NCBI reference, phylogenetic tree through MEGA11 Maximum Likelihood analysis, Tamura-Nei model).

Nisin isolation and quantification

Presence of Nisin in cell-free supernatant was confirmed by absorbance peak at 220 nm matching commercial standard, yielding 50 µg/mL [Figure 1].

Cell cytotoxicity assay

Nisin treatment reduced MDA-MB-231 viability dose-dependently: 78.60 ± 4.46% (10 µg/mL), 66.61 ± 5.65% (20 µg/mL), 37.05 ± 5.33% (30 µg/mL), 23.55 ± 2.70% (40 µg/mL), 17.52 ± 2.19% (50 µg/mL) vs. control (100%), with inhibition rates 21.39–82.48% [Table 1].

IC_{50} was 24.959 ± 1.686 µg/mL after 24 h [Figure 2]. Microscopy revealed apoptosis hallmarks (shrinkage, blebbing, and detachment) at 20 and 30 µg/mL [Figure 3].

Apoptosis detection

Staining demonstrated dose-dependent programmed cell death in nisin-treated MDA-MB-231 cells: control cells showed uniform green fluorescence indicating viability; 20 µg/mL treatment induced yellow fluorescence with chromatin condensation marking early apoptosis; 30 µg/mL yielded orange fluorescence with nuclear fragmentation, signifying late apoptosis [Figure 4].

Under control conditions, the cells displayed intense green fluorescence, indicating healthy mitochondrial activity. In contrast, cells exposed to nisin showed a progressive loss of fluorescence, reflecting a reduction in MMP, and this decline occurred in a dose-dependent manner [Figure 5].

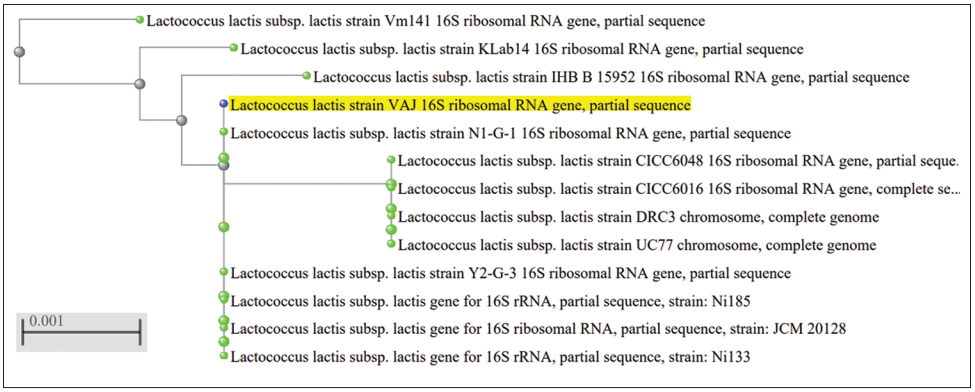

Figure 1: Phylogenetic tree of *Lactococcus lactis* subsp. *lactis* LB11. The scale bar represents 0.001 substitutions per site

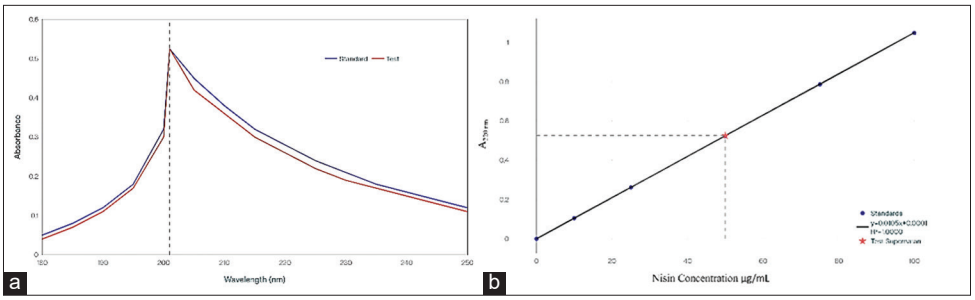

Figure 2: (a) Absorption spectrum of purified nisin from *Lactococcus lactis* LB11 (red line) overlaid with commercial nisin standard (blue line), showing characteristic peptide backbone peak at λ_{max} = 220 nm; (b) Calibration curve for nisin quantification using standard solutions (10–100 µg/mL) at 220 nm (y = ×0.0105 + 0.0001, R^2 = 1.0000) with test supernatant interpolation confirming ~50 µg/mL yield

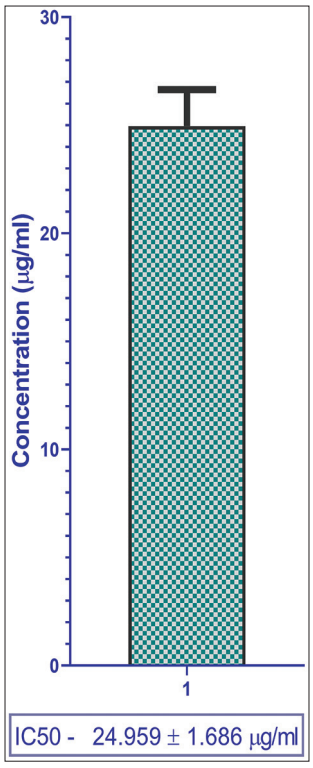

Figure 3: IC_{50} of nisin

Table 1: Dose-dependent effects of crude nisin on cancer cell viability besides inhibition after 24 h treatment (MTT assay, *n*=3, mean±SD)

Concentration (µg/mL)	Viability (%)	Inhibition (%)
Control	100	0
10	78.60±4.46	21.39
20	66.61±5.65	33.39
30	37.05±5.33	62.95
40	23.55±2.70	76.45
50	17.52±2.19	82.48

DISCUSSION

The increasing incidence of breast cancer is attributed to emerging resistance against chemotherapies that also lack bio-safety coupled with the existence of minimal biological treatment options.[23] In view to tackle this emerging problem, the current study isolated nisin-producing *L. lactis* LB11, isolated from curd, and evaluated its anticancer potential on the triple-negative breast cancer (TNBC) cell line. The study focused on elaborating the mechanistic action of nisin by evaluating its ability to evoke cytotoxicity, apoptosis as well as its potential to inhibit MMP of treated cancer cells.

High nisin-producing strain, LB11 isolated from curd, was identified as *L. lactis* subsp. *lactis* (NCBI: PX499742, 99.8% identity) by pre-growing it on selective MRS media. *L. lactis* nis$^+$ are reported to vigorously yield nisin under lactic-acid selective environments facilitated by the activation of nis gene cluster.[24] Isolated nisin (~50 µg/mL) was then confirmed by the characteristic spectrum peak at 220 nm, by comparing with the standard. This was associated with the presence of thioether bridges that correspondingly match with that of nisin previously isolated from *L. lactis* strains characterized post fermentation.[25] A similar spectral signature at 220 nm by nisin was previously reported in published literature.[19,26]

A dose-dependent reduction in the viability of nisin-treated cancer cells was observed in our study, with an IC_{50} value of 24.959 µg/mL. The ability of nisin to reduce cell viability could be attributed to their cell membrane permeabilizing potential, by specifically interacting with membrane lipids, causing pore formation, triggering ion influx-efflux, severely affecting their metabolic functioning.[27,28] It was also evident from the microscopic images of treated cells that displayed severely altered morphology, such as cell contraction and blebbing. This eventually causes cell death by activating extrinsic apoptosis cascades.[29] Likely consequences of cancer cell's membrane permeabilization effects leading to cancer cell death of breast cancer MCF-7 cell line were reported in a previous study that corroborates with the ability of nisin to selectively bind with membrane lipids of cancer cells and invoking cytotoxicity as well as cell death.[30]

Further, fluorescence imaging of AO/EB-stained treated cancer cells was observed with yellow and orange colors,

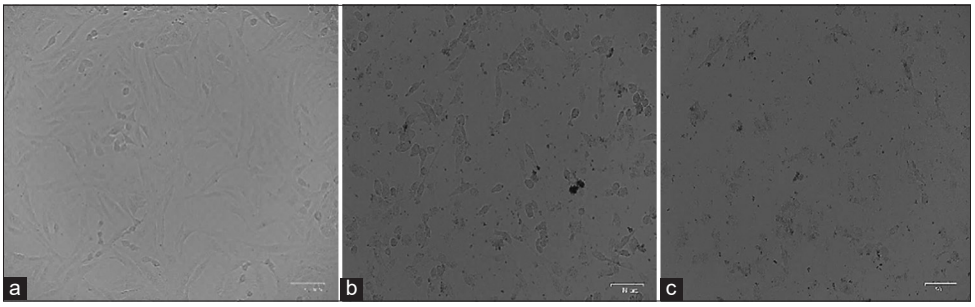

Figure 4: Morphological changes in nisin-treated MDA-MB-231 cells (a) control; nisin treated - (b) 20 µg/mL; (c) 30 µg/mL

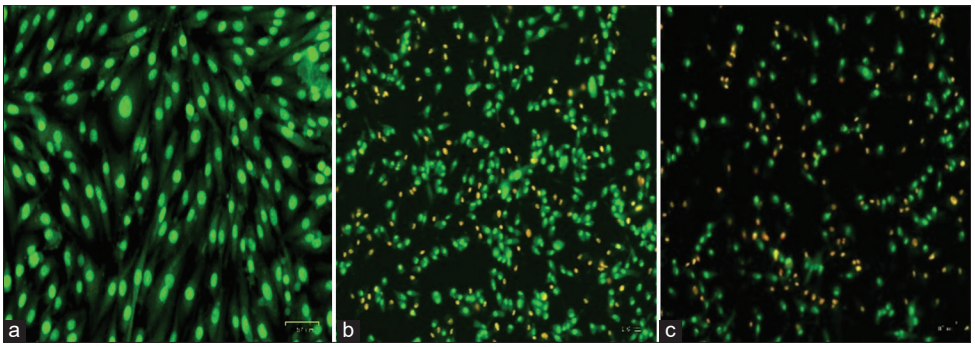

Figure 5: Apoptotic effect of nisin-treated MDA-MB-231 cancer cells (a) Control; nisin treated - (b) 20 µg/mL; (c) 30 µg/mL

indicating the incidence of apoptosis from early to later stages marked by chromatin condensation as well as nuclear fragmentation, respectively. This was in accordance with the results of Mohammadi *et al*. who reported the reduction of viable green light-emitting TNBC cancer cells, and increasing yellow and orange light-emitting cells with increased dose of nisin.[31] Another study also corroborated with our results of chromatin condensation and nuclear fragmentation as sign if early and late apoptosis in cancer cells, induced by nisin, which was found to trigger cell death (>95%) through CHAC1-mediated pathways.[32]

This cascade of reactions correlates with a dose-dependent loss of MMP in treated cancer cells. The ability of nisin to forfeit MMP is regarded as a response of the intrinsic apoptosis pathway activation, which triggers cytosolic cytochrome c release. This further activates caspases, downstream, thereby promoting cell death through Bax-like pro-apoptotic Bcl-2 family protein modulations.[32] This pro-apoptotic pathway activation by nisin in treated breast cancer cells by enhancing reactive oxygen species synthesis along with causing mitochondrial dysfunction aligns with previously reported studies.[27,30]

CONCLUSION

The findings of the current research work reveal the cytotoxic and apoptotic potentials of nisin isolated from food-grade lactic acid bacteria *L. lactis* LB11 in a dose-dependent manner. Supported by morphological and biochemical evidences, the study implies the selective binding of nisin to cancer cell lipids, thereby triggering set of cascade reactions that activate both intrinsic and extrinsic apoptotic pathways, collectively leading to a decreased cell viability, also accompanied by cells exhibiting reduced MMPs, further endorsing its favorability as novel anticancer therapeutic agent that is not only efficient but also safe aligning with sustainable goal of good health and well-being.

REFERENCES

1. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, *et al*. Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast 2022;66:15-23.
2. Prabakaran B, Viswanathan A, Brahma N, Vimal S, Manigandan T. BCL-2 expression with respect to its prognosis in amongst breast carcinoma. TPM Test Psychom Methodol Appl Psychol 2025;32:1315-8.
3. Bodai BI, Tuso P. Breast cancer survivorship: A comprehensive review of long-term medical issues and lifestyle recommendations. Perm J 2015;19:48-79.
4. Tinoco G, Warsch S, Glück S, Avancha K, Montero AJ. Treating breast cancer in the 21st century: Emerging biological therapies. J Cancer 2013;4:117-32.
5. Marcela GM, Eden CS, Rosalva ME. Bioactive peptides from legumes as anticancer therapeutic agents. Int J Cancer Clin Res 2017;4:81.
6. Peng K, Zheng Y, Xia W, Mao ZW. Organometallic anti-tumor agents: Targeting from biomolecules to dynamic bioprocesses. Chem Soc Rev 2023;52:2790-832.
7. Niamah AK, Al-Sahlany ST, Verma DK, Shukla RM, Patel AR, Tripathy S, *et al*. Emerging lactic acid bacteria

bacteriocins as anti-cancer and anti-tumor agents for human health. Heliyon 2024;10:e37054.
8. Kaur S, Kaur S. Bacteriocins as potential anticancer agents. Front Pharmacol 2015;6:272.
9. Ismael M, Huang M, Zhong Q. The bacteriocins produced by lactic acid bacteria and the promising applications in promoting gastrointestinal health. Foods 2024;13:3887.
10. Maghsood F, Johari B, Rohani M, Madanchi H, Saltanatpour Z, Kadivar M. Anti-proliferative and anti-metastatic potential of high molecular weight secretory molecules from probiotic *Lactobacillus reuteri* cell-free supernatant against human colon cancer stem-like cells. Int J Pept Res Ther 2020;26:2619-31.
11. Kamarajan P, Hayami T, Matte B, Liu Y, Danciu T, Ramamoorthy A, *et al*. Nisin ZP, a bacteriocin and food preservative, inhibits head and neck cancer tumorigenesis and prolongs survival. PLoS One 2015;10:e0131008.
12. Thoda C, Touraki M. Probiotic-derived bioactive compounds in colorectal cancer treatment. Microorganisms 2023;11:1898.
13. Bajuri UK, Ramasamy K, Lim SM. Anticancer and anti-angiogenic potentials of probiotics and their bioactive metabolites against colorectal cancer and its tumour microenvironment: A narrative review. Sci Lett 2025;19:141-74.
14. Azmi MF, Al Khateeb A, Froemming GA, Ab Rahim S, Omar E. Nisin as a potential anticancer agent. Sci Eng Health Stud 2021;15:21010007.
15. Mahdy SA, Kalaichelvan PT. Isolation and characterization of nisin-like peptide produced by *Lactococcus lactis* isolated from Indian curd. Eng Tech J 2013;31:298-309.
16. Sudarsan N, Hemas, Mahendran V, Muthu-Pandian CK. LC-MS profiling of *Terminalia chebula* seed reveals mechanistic insights supporting activity against multidrug-resistant diabetic foot ulcer isolate. Fitoterapia 2025;188:107002.
17. Vishnupriya N, Jayaraman S, Veeraraghavan VP. Camptothecin anti-cancer activity against breast cancer cells (MDA MB-231) targeting the gene expression of wnt/beta-catenin pathway - an *in silico* and *in vitro* approach. Texila Int J Public Health 2025. DOI: 10.21522/TIJPH.2013.SE.25.01.Art017
18. Forestier A, Belguesmia Y, Krier F, Drider D, Dhulster P, Firdaous L. Recovery of nisin from culture supernatants of *Lactococcus lactis* by ultrafiltration: Flux properties and separation efficiency. Food Bioprod Process 2022;136:196-210.
19. Choi HJ, Cheigh CI, Kim SB, Pyun YR. Production of a nisin-like bacteriocin by *Lactococcus lactis* subsp. Lactis A164 isolated from kimchi. J Appl Microbiol 2000;88:563-71.
20. Kaavyaunni, Antony S, Chanthini KM. Biogenically synthesized *Moringa oleifera* leaf extract-derived silver nanoparticles exhibit potent antimicrobial and anticancer effects via oxidative stress and apoptotic pathways in AGS gastric cancer cells. Biocatal Agric Biotechnol 2025;67:103674.
21. Prithiksha N, Priyadharshini R. *In vitro* molecular mechanisms of anticancer activity of stevioside in human osteosarcoma cell Lines (sarcoma osteogenic). Contemp Clin Dent 2024;15:198-201.
22. Sudhakaran G, Velayutham M, Aljarba NH, Al-Hazani TM, Arokiyaraj S, Guru A, *et al*. Nimbin (N1) and analog N3 from the neem seeds suppress the migration of osteosarcoma MG-63 cells and arrest the cells in a quiescent state mediated via activation of the caspase-modulated apoptotic pathway. Mol Biol Rep 2023;50:7357-69.
23. Vasudevan S, Kannan K, Raghavan AV, Sulochana S. Analyzing tumor budding scores in invasive breast carcinoma: A tertiary care center study in South India. J Pharm Bioallied Sci 2024;16 Suppl 2:S1850-3.
24. Saraiva MA, Birri DJ, Brede DA, Baracat-Pereira MC, Queiroz MV, Nes IF, *et al*. Nisin Z production by wild strains of *Lactococcus lactis* isolated from Brazilian (Italian type) fermented sausage. Int J Microbiol 2020;2020:9309628.
25. Zhang J, Caiyin Q, Feng W, Zhao X, Qiao B, Zhao G, *et al*. Enhance nisin yield via improving acid-tolerant capability of *Lactococcus lactis*. Sci Rep 2016;6:27973.
26. Sun Z, Zhong J, Liang X, Liu J, Chen X, Huan L. Novel mechanism for nisin resistance via proteolytic degradation of nisin by the nisin resistance protein NSR. Antimicrob Agents Chemother 2009;53:1964-73.
27. Sadri H, Aghaei M, Akbari V. Nisin induces apoptosis in cervical cancer cells via reactive oxygen species generation and mitochondrial membrane potential changes. Biochem Cell Biol 2022;100:136-41.
28. Wang Y, Wang Y, Sun T, Xu J. Bacteriocins in cancer treatment: Mechanisms and clinical potentials. Biomolecules 2024;14:831.
29. Kumari AM, Latha CB, Padmanabhan RA, Vijayakumar N, Ramachandran R. Antibacterial peptide nisin activates p53 gene expression and triggers apoptosis in human colon cancer cells. Pept Sci 2025;117:e24379.
30. Chernyshova DN, Tyulin AA, Ostroumova OS, Efimova SS. Discovery of the potentiator of the pore-forming ability of lantibiotic nisin: Perspectives for anticancer therapy. Membranes (Basel) 2022;12:1166.
31. Mohammadi A, Kazemipour N, Ghorbankhani GA, Morovati S, Hashempour Sadeghian M. Glycated nisin enhances Nisin's cytotoxic effects on breast cancer cells. Sci Rep 2024;14:17808.
32. Czabotar PE, Garcia-Saez AJ. Mechanisms of BCL-2 family proteins in mitochondrial apoptosis. Nat Rev Mol Cell Biol 2023;24:732-48.

Source of Support: Nil. **Conflicts of Interest:** None declared.