https://doi.org/10.47552/ijam.v16i2.5464 ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 16 (2), 2025; 318-324

Anticancer effect of *Vitex negundo* Linn. (Verbenaceae) on MCF-7 Breast Cancer Cells and Its Antimicrobial Properties

Research Article

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Abstract

Breast cancer treatment presents significant challenges, with various therapeutic modalities often associated with side effects. Ongoing research is continuously striving to refine existing treatments and discover new, more targeted therapies that can minimize the burden of side effects while improving outcomes. Natural plant-derived compounds offer promising alternatives. *Vitex negundo* Linn. (VN) is a medicinal plant often used in ayurvedic medicine to treat a variety of ailments, some of which have been scientifically validated and it offers a potential natural alternative to conventional breast cancer treatments. In this context, the ethanolic extract of VN has demonstrated to have potent activity against the breast cancer cell line MCF-7. VN extracts expressed a dose-dependent cytotoxic effect against MCF-7, with increasing extract concentrations correlating to decreased cell viability. Notably, the extract selectively suppressed MCF-7 cell growth, with an inhibitory concentration of about 1000 µg/ml. Importantly, the extract appears to selectively target cancer cells, sparing normal, healthy cells. This selectivity is crucial for minimizing side effects often associated with conventional cancer treatments. VN serves as a potential indigenous antimicrobial agent that has been evaluated by the paper disc method to identify microorganisms susceptible to the ethanolic extract. The results based on the well-diffusion method confirmed the extracts of antimicrobial activity against sensitive microorganisms. Further research should explore its bioactive compounds, mechanisms of action, and in vivo efficacy to validate its clinical potential.

Keywords: *Vitex negundo Linn.,* Ethanol Extract, Cytotoxic, MCF-7, Anti-microbial.

Introduction

The population based cancer registries (PBCRs) measure breast cancer at 21.8% of all female cancer cases, which provides a starting point for research on the incidence of cancer in India across time and at different regions (1). Early detection, curative resection, and effective adjuvant or neoadjuvant therapy are the key factors that improve clinical outcomes in breast cancer, as the disease may not be reversed once it has advanced to an aggressive stage (2). Chemotherapy is still the mainstay for both advanced breast cancer (ABC) and early breast cancers (EBC), despite significant advancements in systemic medicines such as targeted therapy, hormone therapy, and immunotherapy. In EBC, adjuvant chemotherapy lowers the chance of recurrence and the death rate from breast cancer (3). In India, patients with breast cancer have a lower survival rate than in Western nations because of factors such as early beginning of the disease, late stage of the disease at presentation, delayed start of definitive management, and insufficient or fragmented treatment (4). While surgery, chemotherapy, and radiation therapy are among the typical therapeutic alternatives that have been suggested for the treatment of breast cancer, each of these treatments has significant disadvantages including tissue damage, fatigue, nausea (5). In this sense, the field of medicine, drug delivery, and anti-cancer applications has benefited greatly from the significant advancements made from herbal plants and their bioactive compounds (6).

In the realm of oncology, the use of herbal medicines as a supplemental or alternative treatment has gained widespread acceptance (7). As a result, several unique cytotoxic chemicals are identified from plants each year, offering up fresh avenues for the battle against cancer. Investigating naturally occurring

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molecules that could be valuable to the pharmaceutical industry is a focus for many researchers (8). Secondary metabolites from plants are still important for drug design because their core structures can be used as templates for the synthesis or semi-synthesis of novel substances that can be used to treat human diseases, even in the face of the growing popularity of synthetic products that have led to the existence of many drugs today (9). Furthermore, because of their high patient acceptance and tolerance, naturally occurring molecules continue to play a major part in the treatment of human illnesses, even though the safety and efficacy of synthesized medications are still up for debate (10). Phytochemicals are an adjuvant therapeutic option that may reduce the negative effects of radiation and continue to be an important source of drugs in the current anticancer drug research process. Numerous traditional anticancer medications, like camptothecin and paclitaxel, have been found and enhanced from phytochemicals. Therefore, studying phytochemicals helps develop new medications and therapy approaches that improve the prognosis and therapeutic results for patients with breast cancer (11).

VN is a hardy, diminutive tree or shrub belonging to the Verbenaceae family. Its natural range encompasses Sri Lanka, Madagascar, Afghanistan, Thailand, Malaysia, India, Pakistan, the Philippines, and Eastern Africa. It has been used for various medicinal purposes in the Ayurvedic and Unani medicines (12). Ayurveda has long recognized the therapeutic potential of VN and its leaf extract is historically used to treat inflammatory conditions, pain, and skin irritations. Emerging research indicates that this extract may offer additional benefits, including the management of diabetes and gout (13). Terpenoids, lignans, flavonoids, alkaloids, and glycosides were identified as the main chemical elements of VN in a previous phytochemical analysis. Similarly, seeds of VN have been reported to contain phenyl dihydronaphthalene-type lignan vitedoin A, a lignan alkaloid vitedoamine A, trinorlabdane type diterpene vitedoin B, 2α, 3α-dihroxyoleana-5, 12-dien-28-oic acid, and n-pentatriacontane (14). There is a wealth of information on the unique biological characteristics of extracts made from this plant. The methanol extracts of VN leaves have analgesic effects by dose-dependently suppressing the central nervous system (15).

Invitro studies on VN has been employed to examine the molecular mechanism underlying the prevention of human colon cancer (HCT-116) cell lines with the results indicating the suppression of cell cycle and promotes apoptosis of the colon cancer cells (16). The effects of VN on its identification of chrysoplenetin a bioactive constituent has served as a potent cytotoxic agent against pancreatic cancer cell line (PANC-1) (17). Corymbosin an another bioactive compound from VN has been mediated into the synthesis of zinc oxide nanoparticle to assess the anti-cancer activity and the possible mechanism to deliver the drugs to the target site (18). An isolated neolignan from VN purified vitexin compound 1 (VB1), has been reported to have promising clinical activity against a variety of cancers and promoted apoptosis through p53-dependent induction of p53 upregulated modulator of apoptosis (PUMA). It also caused mitochondrial dysfunction and Bax (Bcl-2-associated X protein) activation in colon cancer HCT-116 and LoVo cells. A p53, PUMA, or Bax deficiency prevented VB1-induced apoptosis and increased HCT-116 cell survival. Furthermore, in HCT-116 cells, VB1 and the chemotherapeutic medications 5-fluorouracil (5-FU) or NVP-BZE235 produced a synergistic antitumor effect through PUMA activation (19). Furthermore, the extract suppresses cell proliferation in a dose-and time-dependent manner (16). In a similar study the isolated fraction from VN leaves using column chromatography fractionation was analysed for its anti-proliferative effects on glioma cells and wound healing property (20). Based on the results, the cell cycle at the G1 phase, dramatically lowers glioma cell viability and proliferation, and inhibits cell migration in a dose-dependent way (21). In connection to these the potency of VN was evaluated in the current study against MCF-7 Breast cancer cell lines to determine the anticancer activity.

VN has demonstrated significant larvicidal activity against mosquito larvae, particularly those of Aedes aegypti and Anopheles stephensi. Flavonoid extracts derived from various parts of the plant have been identified as the primary bioactive compounds responsible for this effect (22). In addition to this it has been reported that VN has been found to be effective against harmful bacteria that cause diseases like cholera. Its extracts, especially from the leaves, have shown strong antibacterial properties in both laboratory tests and *in vivo* studies. This suggests that VN could be a potential natural remedy for bacterial infections (23). In correspondence to this it has been used traditionally in various cultures, including India and Malaysia, to combat malaria. Scientific research has also supported these traditional uses, with in vitro studies demonstrating the plant's anti-malarial properties (24). Based on these research findings, the antimicrobial potential of VN on the Salmonella species, Streptococcus species, Staphylococcus species and Escherichia coli was evaluated in the current study.

Material and Methods

Collection and Extract Preparation

VN was obtained in Chennai, Tamil Nadu, identified and verified by botanist from Presidency College. The collected plant leaves were gently cleaned in fresh water and allowed to dry in the shade at room temperature at dust free condition for 15 days. The shade dried leaves were crushed into finest powder using mortar and pestle and then powdered in a sterile environment to avoid contamination and then stored in an airtight container. 20 grams of VN powder was weighed, and mixed with 200 millilitres of ethanol, and heated to between 40 and 50ºC using a soxhlet apparatus. The resulting extract was filtered using a pure cotton cloth followed by further filtration with Whatmann No.1 filter paper. The filtered extract was kept at room temperature and a quantity of 2.29

grams was obtained at the end of the filtration process (25).

Invitro Extract Preparation

10 mg of VN was separately dissolved in 100 µl of DMSO and volume was made up with DMEM-HG supplemented with 10% inactivated Fetal Bovine Serum (FBS) to obtain a stock solution of 1 mg/10 µl concentration and sterilized by 0.22µ syringe filtration. Serial two-fold dilutions were prepared from this stock solution for further studies.

Cell line and Culture medium

Human Breast carcinoma cells, MCF-7 were obtained from National center for cell sciences (NCCS, Pune, India) and were cultured in DMEM-HG media supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in a humidified atmosphere of 5% CO^2 at 37^oC until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 well microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Cytotoxicity studies

The cell viability is assessed by MTT reduction assay. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using respective media viz., DMEM-HG containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension was added. After 24hrs, a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with medium and 100 µl of different concentrations of test substances (ranging from 1000 µg, 500 µg, 250 µg, 125 µg and 62.5 µg) were added. The plate was then incubated at 37°C for 72hrs in 5% CO^2 atmosphere (26). After 72hr of incubation, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3hrs at 37°C in 5% CO^2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The plates were protected from light throughout the procedure. The percentage growth inhibition was calculated using the standard formula and concentration of test substances, needed to inhibit the growth of the cell by 50% i.e., CTC50 values were generated from the dose-response curves. The inhibition was expressed as the percentage relative to cell control (27).`

Microbial Sample Collection

The microbial samples were sourced from Department of Medical Microbiology, Institute of Basic Medical Sciences, Chennai, Tamilnadu. The pathogens were isolated and cultured from clinical samples. The obtained pathogens were cultured in agar slant with optimal growth conditions for the maintenance of the culture. Agar slant culture provides a solid surface for microbial growth, while nutrient broth provides a liquid medium that supports the organisms' growth and development. The culture was maintained at 37ºC for 18 to 24 hours.

Identification of the Pathogens

Most presumed bacteria, including uncommon or rare ones, can be quickly identified using the Gram stain method. The bacteria were heat-fixed on the slide to stick the cells to the surface before applying crystal violet, a primary stain that turns all the bacteria purple, to the sample. In the bacterial cells, iodine is added to create a crystal violet-iodine complex (28). A broth culture of motile bacteria, approx. 18 h old; cavity slides, coverslip, petroleum jelly, soft agar medium was used for the Hanging drop method to detect the motility of the bacteria. Capsular staining was carried out to reveal the presence of the bacterial capsule and endospore staining was used to identify the presence of endospores in a bacterial sample. Biochemical tests such as Indole, Methyl red, Voges Proskauer, Citrate utilization test, Triple sugar iron test, Urease test, Catalase and Oxidase tests were carried out. The study samples were cultured in various growth media such as Deoxycolate citrate agar, Blood agar, Mannitol salt agar, Eosin methylene blue agar and Mueller-Hinton agar for growth and screening of bacteria.

Antimicrobial activity

VN antimicrobial activity was assessed using the Kirby-Bauer agar well diffusion method, which is a rather quick and simple way to measure antimicrobial activity. Gram-positive and gram-negative organisms were tested against the VN extract. The microbial suspension was cultured in nutrient broth medium, and the agar plates were covered with a mixture of test organisms. To allow for close sample contact, a tiny amount of sample is gently pushed over the nutrient agar plate that has been infected with microbial cells. Mueller-Hinton agar was swabbed with sterile cotton swabs containing an 18-hour-old broth culture of the matching bacterium and in each of these plates, a sterile cork borer was used to produce a 3 mm well. Plant extract was produced as a stock solution with concentrations of 20, 40, 60, 80, and 100 µg/ml. The plant extract was further dissolved as a stock in 1000 µg/ml. Then, various concentrations such as 20 µl, 40 µl, 60 µl, 80 µl and 100 µl/ml were used for antimicrobial study. For 18 to 24 hours, the plates were incubated at 37°C to look for pathogens. The zone of inhibition, or the area in which microbial growth is inhibited by the compound's antimicrobial effect, is measured to determine the plant extracts inhibitory effect on a specific microorganism. The diameter of the inhibition zone (mm) was measured and calculated by deducting the well diameter from the total inhibition zone diameter (29).

Results and Discussion

Vitex negundo Linn. Extraction

VN with potential medicinal properties, was subjected to a Soxhlet extraction process to isolate its bioactive compounds as shown in figure 1. This technique involves using ethanol solvent to repeatedly extract the desired compounds from the plant material (30). VN plant extract was prepared under laboratory conditions and extensively studied for its antimicrobial and anticancer activity. These studies aimed to identify the specific compounds responsible for the observed biological activities and to determine their mechanism of action. By understanding these underlying mechanisms, researchers can develop more effective and targeted therapies based on VN extracts (31).

Figure 1A depicts the finely grinded VN powder using mortar and pestle. 1B represents the preparation of ethanolic extract VN in soxhlet apparatus at 50°C in a sterile environment. 1C shows the filtration extract being dried out to obtain the final ethanolic extract of VN

Cytotoxic activity

The cytotoxic action of VN extracts against the MCF-7 breast cancer cell line exhibited a dose-dependent characteristic, with an increase in extract concentration was accompanied with a decrease in cell viability (Figure 2). It is noteworthy that this extract shown the ability to specifically suppress the growth of MCF-7 cancer cells, with an inhibitory concentration above 1000 µg/mL (Table 1). VN has been previously studied against human alveolar basal epithelial cells (A549 cells) in which the methanolic extract fraction and chloroform extract fraction had the greatest cytotoxicity against A549 cell lines among the four solvent extracts of *VN* stem, while the cytotoxicity of the petroleum ether and ethyl acetate extracts was lower. In correspondence to that the MCF7 cells responded in a similar pattern to the ethanolic extract of VN (32). The cell death rate in a dose dependent manner and lack of cell viability were the key features evaluated during the experimental period with VN. This is indicative in a similar study with U-937 pro-monocytic model cancer cell line with cell cycle arrest and decreased viability corresponding to the potency of VN at various concentrations of 7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/ml (32). The cytotoxicity of VN was examined in glioma cells, and the results were noteworthy in that they showed a dose-dependent inhibition of cell migration, an arrest of the cell cycle at the G1 phase, and a considerable decrease in glioma cell viability and proliferation. Strong antiproliferative drugs such as 7-hydroxy-3,4,5,6,8-pentamethoxyflavone, Virol-B, MomordinIa and Oryzalexin A are found through compound analysis. These results highlight VN's potential as a source of chemical with anticancer properties against glioma cells (21). Based on these various cell lines used in VN extract , it is understood that the ethanolic extract serves as a potential anticancer compound comprising the active inhibition of the proliferating cancer cells with decreased cell viability thereby ameliorating the breast cancer cell lines. Figure 3 represents the MCF7 breast cancer cell lines in control and VN treated groups. The results express a well-defined confluency in the control flask compared to the VN treated MCF7 cells with decreased cell viability and lack of adherence which is indicative of the antiproliferative activity at a concentration on 1000 µg/ml.

Table 1: Cytotoxic properties of the ethanolic leaf extract of VN on MCF-7 cells

Test Conc. (µg/ml)	Cytotoxicity (%)
62.5	19.29 ± 1.55
125	23.43 ± 0.65
250	27.94 ± 0.92
500	33.77 ± 4.24
1000	45.10 ± 5.05

Figure 2: Cytotoxic effect of VN with varying concentrations on MCF7 Cell lines

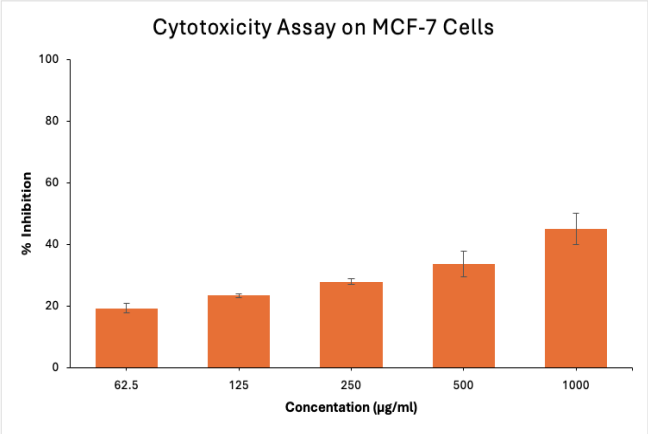

Figure 3: Morphological analysis of VN against the MCF7 breast cancer cell lines

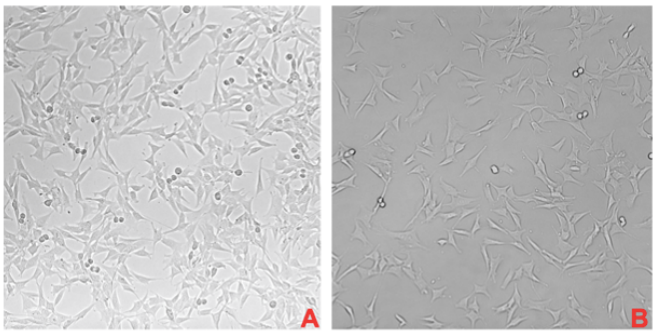

Figure 3A represents the control of MCF7 cell lines with profound cell viability and adherence. Figure 3B represents the VN treated MCF7 cells showing decreased confluency which may be due to the anti-cancer activity of VN at an IC50 concentration.

https://doi.org/10.47552/ijam.v16i2.5464 ISSN No: 0976-5921

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Bacterial isolation and identification

The results obtained from the gram staining provided the presence of two gram-positive and two gram-negative bacteria. The bacteria were characterized as gram-positive with the specific structural morphology of cocci in chain bacteria and gram-negative with rods in chain (33). Table 2 shows the identification, presence of capsule, motility and spore staining of the pathogens obtained from clinical specimens. The colony morphology of the bacterial pathogens was studied by streaking samples on Deoxycholate citrate agar (DCA), Blood agar, Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB) (34). Figure 4A represents the presence of *Salmonella* since most typical gut bacteria (pink-red colonies without black centres) ferment lactose but do not create H^2S (35). While *Shigella* spp. and *Salmonella* cannot ferment lactose, *Salmonella* can create H^2S, which is characterized by colourless colonies with or without black cores (36). According to the available data, colourless colonies with a black centre indicate the presence of *Salmonella* species in this current study (37). Figure 4B represents, on blood agar beta-haemolytic colonies show total lysis of the red blood cells around the colony, resulting in a clear zone. Some pathogens, such as Group A *Streptococcus* (GAS) and Group B *Streptococcus* (GBS), have this trait. The beta-haemolytic colonies expressed in the clinical sample under evaluation indicated the presence of *Streptococcus* species (38). The *Staphylococcus aureus* that ferments mannitol on MSA produces an acidic byproduct that turns the phenol red in the agar yellow. This is shown on the Mannitol Salt Agar by yellow colonies with yellow zones in figure 4C. The reaction of phenol red with the medium's pH gives rise to the colour of the colonies and the medium (39). Lactose is rapidly fermented by *Escherichia coli*, resulting in a high acid content (40). In figure 4D the green metallic sheen in EMB agar is caused by the reaction of the acid with the metachromatic qualities of methylene blue and eosin Y dyes. The green metallic sheen is also a result of E. coli moving around utilizing its flagella (41). Because EMB agar comprises the following combinations peptone lactose, dipotassium phosphate, eosin Y dye, methylene blue dye, and agar, it is intended to inhibit the development of Gram-positive bacteria (42). In connection to that Table 3 depicts the various biochemical tests employed to determine the microbial species.

Figure 4A shows the DCA culture of *Salmonella* species by the presence of black centered colorless colonies. 4B shows the blood agar growth medium containing the presence of beta hemolytic colonies indicating the presence of *Streptococcus* species. 4C depicts the MSA growth medium containing yellow colonies with yellow zone indicating the growth of *Staphylococcus* species. 4D denotes the EMB culture having dark blue or black with metallic green sheen indicating the presence of *Escherichia coli*.

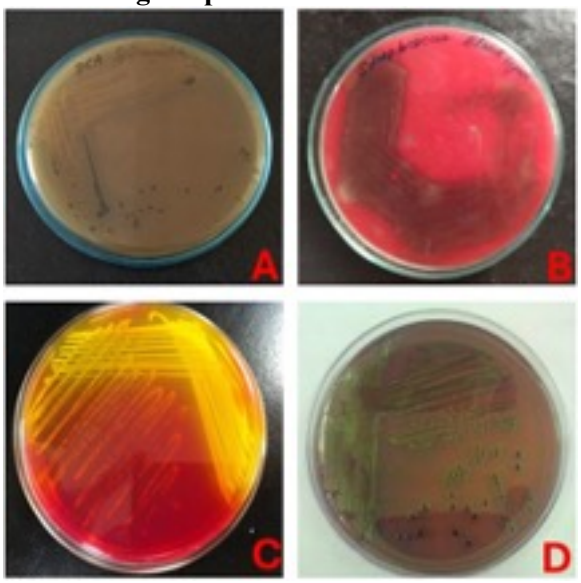

Table 2 : Identification of the microbial species by Gram staining, Capsule staining, Motility and Spore staining

S.No.	Test	Organisms			
		***Salmonella* species**	***Streptococcus* species**	***Staphylococcus* species**	***Escherichia coli***
1	Gram staining	Gram negative rods	Gram positive cocci	Gram positive cocci	Gram negative rods
2	Capsule staining	+	-	+	+
3	Motility	+	-	-	+
4	Spore staining	-	-	-	-

Table 3 : Biochemical analysis to determine the microbial species from the biological sample

S. No	Test	Organisms			
		***Salmonella* species**	***Streptococcus* species**	***Staphylococcus* species**	***Escherichia coli***
1	Catalase	+	-	+	+
2	Oxidase	-	+	-	-
3	Indole	-	-	-	-
4	Methyl red	+	-	-	+
5	Voges Proskauer	-	-	-	-
6	Citrate	+	+	+	-
7	Urease	-	-	+	-
8	TSI	-	A+/G+	-	A+/G+

Antimicrobial activity

Figure 5 illustrates the antibacterial activity of VN against *Salmonella* species (5A), *Streptococcus* species (5B), *Staphylococcus* species (5C), *Escherichia coli* (5D), Control (5E). The zone of inhibition was seen in all the bacterial species by VN expressing its antibacterial activity profoundly which may be due to its functional groups in the extract (43). Among the bacterial species, the ethanolic extract of VN possessed maximum zone oh inhibition against *Streptococcus* species (12mm). Our results show preferential activity against Gram-positive Streptococcus compared to other tested bacteria, suggesting specific mechanisms of action such as Disruption of cell wall biosynthesis, Interference with protein synthesis and Inhibition of quorum sensing pathways. The antibacterial activity of VN may be attributed to several different mechanisms with their key bioactive compounds flavonoids consisting of large group of polyphenolic compounds with a benzo-γ-pyrone structure and are ubiquitously present in these plants (44). Studies using water and methanol extracts demonstrate the antibacterial activity of VN leaf extracts against a variety of intestinal infections both *in vitro* and *in vivo*. Strong vibriocidal activity is shown by the methanolic extract of VN leaves in both vitro and in vivo settings, suggesting that it could be a useful alternative for treating cholera (45). Because of its numerous traditional use VN is a highly well-known medicinal plant. Nearly every part of plant is utilized in Ayurvedic and Unani therapy, and leaf extracts from VN have been shown to have pesticidal, antibacterial, and fungal properties (23). It appears that VN has the potential to be developed as an indigenous antibacterial agent due to its antimicrobial action against gram positive pathogens (46). Microorganisms susceptible to VN extracts were identified using the paper disc method, and the antibacterial activity of the extracts was confirmed on the sensitive microorganisms using the well-diffusion method (47). TLC studies showed that the ethanol extract contained flavonoids, terpenoids and chlorophyll derivatives (48). Presence of key bioactive compounds have been shown to exert antibacterial activities in many plant extracts such as myrtenal a compound of terpenoids present in cumin, lemon grass, mint is found to inhibit harmful pathogens in the intestine of experimental colon cancer bearing animals (49). In correspondence to that the functional groups in the VN extract delivers a strong antimicrobial activity against the pathogens in a dose dependent manner and amoxicillin was used a standard drug with a zone of inhibition of 15mm. Table 4 represents the zone of inhibition of VN against the pathogens with respective to concentration.

Figure 5A shows the zone of inhibition by VN against the *Salmonella* species at different concentrations. 5B depicts the maximum zone of inhibition In *Streptococcus* species. 5C shows the activity of VN against *Staphylococcus* species with prominent zone of inhibition. 5D exhibits the zone of inhibition in *Escherichia coli* at different concentration of VN. 5E is the control medium with no reaction towards the culture medium

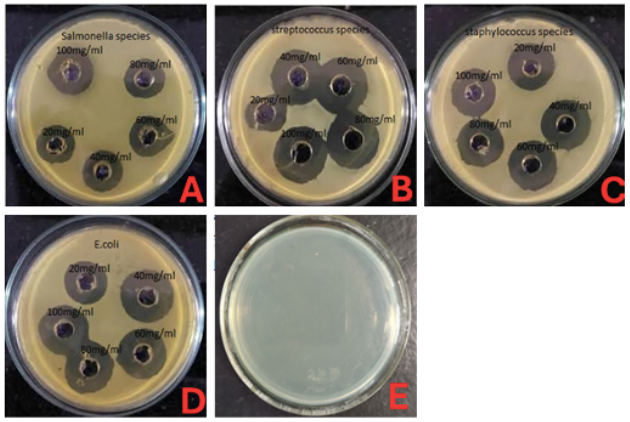

Table 4: Depicts the zone of inhibition of the ethanolic extract of VN against the pathogens in a dose dependent manner to exert the maximum potency of the plant extract

S. No	Organism used	Zone of inhibition of ethanolic extract of VN (mm)				
		Dilutions				
		20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml
1	*Salmonella* species	5	6	7	8	11
2	*Streptococcus* species	4	6	7	8	12
3	*Staphylococcus* species	5	5	6	6	8
4	*Escherichia coli*	5	6	5	6	7
5	*Amoxicillin*	6	7	10	12	15

Conclusion

In conclusion, breast cancer treatment continues to face significant challenges due to the substantial side effects associated with conventional therapeutic approaches, highlighting the urgent need for safer and more targeted alternatives. The ethanolic extract of *Vitex negundo* Linn. (VN) has emerged as a particularly promising candidate, demonstrating selective, dose-dependent cytotoxicity against MCF-7 breast cancer cells at concentrations around 1000 µg/mL while showing minimal effects on normal, healthy cells. This cancer cell-specific activity is especially valuable as it suggests potential for reduced systemic toxicity compared to standard chemotherapy regimens. Furthermore, the extract's demonstrated antimicrobial properties against sensitive microorganisms, as evidenced by well-diffusion assays, indicate additional therapeutic benefits that could prove valuable in managing treatment-related infections. The dual anticancer and antimicrobial activities position VN as a multifaceted natural therapeutic agent with significant clinical potential. However, to fully realize this potential, comprehensive follow-up studies are essential. Future research should prioritize the identification and isolation of the specific bioactive

compounds responsible for these effects, detailed investigation of the molecular mechanisms underlying its selective anticancer activity, and rigorous preclinical evaluation in appropriate animal models. Subsequent clinical trials would then be necessary to establish safety profiles, optimal dosing regimens, and potential synergistic effects with existing therapies. If these investigations yield positive results, VN-derived treatments could revolutionize breast cancer care by providing more tolerable, cost-effective therapeutic options that maintain efficacy while significantly reducing the debilitating side effects that currently compromise patients' quality of life.

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