Innovations

"Chemical Composition, Antimicrobial and Anticancer Activities of Methanol Leaf Extract of Naringi Crenulata (Roxb.) Nicolson (Rutaceae): A Promising Source of Plant-Based Therapeutics"

V. Surya Ramani[1], P. Rama Rao[2], Ch. Murali Krishna[3], Y.T. Rajesh Babu[4], S. B. Padal[5]

Corresponding Author: **Dr. Y. T. Rajesh Babu**

Abstract: *The study investigates the chemical composition, antimicrobial, and anticancer activities of the methanol leaf extract of Naringicrenulata (Roxb.) Nicolson (Rutaceae). Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified eight major bioactive compounds, with 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester being the most abundant (60.79%). The antimicrobial potential was evaluated against Gram-positive and Gram-negative bacteria using the cup plate agar diffusion method. The methanol extract exhibited significant antibacterial activity, with the highest inhibition zones observed against Bacillus subtilis(22.6 ± 0.57 mm) and Staphylococcus aureus(19.3 ± 0.57 mm). The anticancer efficacy of the extract was tested against MDA-MB-231 (breast cancer) and Hep 3B (liver cancer) cell lines using the MTT assay. The extract displayed potent anticancer activity against MDA-MB-231, with an IC_{50} value of 31.5 ± 1.42 µg/ml, whereas Hep 3B exhibited moderate sensitivity (IC_{50} = 194.1±2.28 µg/ml). These results highlight N. crenulata as a promising source of bioactive compounds with antimicrobial and anticancer potential, warranting further exploration for pharmaceutical applications.*

Keywords: *Anticancer, GC-MS, Cytotoxicity, Phytochemicals, Breast Cancer, Liver Cancer*

1. Introduction

The incidence of cancer-related deaths increased by 17% between 2005 and 2015, emphasizing the urgent need for expanded research into developing innovative anticancer drugs alongside existing treatments (Mabberley, 1997). Cancer remains the second leading cause of death worldwide, accounting for approximately 9.6 million fatalities in 2018. Globally, one in every six deaths is attributed to cancer, with around 70% of these deaths occurring in low- and middle-income countries

(Morton, 1987). Projections indicate that by 2030, there could be 26 million new cancer diagnoses and 17 million deaths caused by the disease (Mabberley, 2008).

Cancer is a complex, multi-stage process characterized by uncontrolled cell growth. These abnormal cells often invade and destroy healthy tissue, disrupting normal bodily functions. Various factors, such as tobacco use, chemical exposure, poor dietary habits, and environmental influences, contribute to cancer development. Conventional cancer treatments often have significant side effects on healthy tissues, and concerns have been raised about the resistance of tumors to existing therapeutic drugs. Consequently, there is an urgent need for more effective treatment strategies to combat this disease.

Plant extracts serve as a promising source for the discovery and development of anticancer drugs. Plants produce a diverse array of biologically active compounds, including secondary metabolites such as alkaloids, flavonoids, phenolics, and carotenoids, which have shown potential in cancer therapy (Randrianariveloet al., 2015; Ahmed et al., 2013). Since ancient times, plant-based medicines have played a crucial role in treating various ailments and have been integral to traditional systems of medicine, such as Ayurveda, Unani, and Siddha (Singh &Usha, 2010).
Naringicrenulata, commonly known as the lime berry, is a member of the Rutaceae family. Previously classified under the genus Glycosmis as Glycosmiscrenulata, it has since been reclassified into the genus Naringi (Mabberley, 1997; Morton, 1987; Mabberley, 2008). This woody shrub or small tree is characterized by its thorny branches and can grow up to 6 meters in height. The plant features pinnate leaves with a citrus aroma, small white flowers, and round, reddish-orange fruits approximately 1 cm in diameter, which are known for their tangy flavor. Its seeds are primarily dispersed by birds and other frugivorous animals, making it an integral part of its ecosystem (Randrianarivelo et al., 2015).
Phytochemical studies have revealed that N. crenulata contains a variety of bioactive compounds, including alkaloids, flavonoids, coumarins, and terpenoids(Ahmed et al., 2013; Singh &Usha, 2010; Kirtikar & Basu, 1918; Chopra et al., 1956). These compounds contribute to its diverse pharmacological activities. Extracts from N. crenulata have exhibited significant antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer properties (Gupta &Kesari, 2008; Kumar &Pandey, 2013; Sharma & Sharma, 2012; Khan & Ali, 2010; Jena & Gupta, 2011). These bioactive constituents underscore the plant's therapeutic potential and its widespread use in traditional medicine, particularly in Ayurveda and folk medicinal practices across South and Southeast Asia (Warrier et al., 1995; Nadkarni, 1976; Sharma et al., 2001).

2. Materials and Methods

2.1. Plant material collection and extraction of phytochemicals

Naringi crenulata leaves were collected from Konam village (17˚57'58.28" N; 82˚50'44.01" E, 319 m elevation), Chodavaram Mandal, Alluri Sitaramaraju District, Andhra Pradesh, India. The fresh leaves, were thoroughly washed, then shade-dried, mechanically pulverized, and stored. The phytochemicals were extracted using the soxhlet extraction method. 30 g of dry powder was extracted with 300 mL of methanol as a solvent. The extracts were concentrated in a vacuum rotary evaporator at 60°C. The crude extract obtained after thorough drying was utilized to evaluate potential anti-mitotic, anti-proliferative, and anti-cancer effects.

2.2. GC-MS analysis

The Agilent Technologies GCMS (GC-8890, GC/MS 5977 MSD) equipped with a (DB-WAX) & HP-5 MS UI column (30 m x 250 μm x 0.25 μm) was used to analyze the extracts. Split injection mode with a 1 μL of injection volume (15:1 split ratio), 18 mL/min split flow, and a 3 mL/min purge flow; Oven temperature ranges from 75°C to 360°C and column temperature ranges from 60°C to 325°C. As the carrier gas, 99.999% helium gas was used at a constant flow rate of 1.2 mL/min, and mass spectra were recorded at 70ev. The GC-MS lasts for a total of 53.5 minutes. Each of these chemicals was recognized and described by analyzing the mass fragmentation patterns and retention indices in the Spectral Library and Database (licensed NIST 2017 Library; software: Open Lab CDS 2.5 version) (Stein et al., 1994).

2.3. Microbial strains

The Microbial Type Culture Collection (MTCC), Chandigarh, provided the microbes used in this analysis. The microorganisms are used to test for antimicrobial activities, categorizing them into Gram-positive and Gram-negative bacteria. For Gram-positive bacteria, the strains include Bacillus subtilis (MTCC 28) and, Staphylococcus aureus (MTCC 96), all of which were cultured in Muller-Hinton medium at 37 °C for 24 hours with Streptomycin serving as the positive control. For Gram-negative bacteria, the strains tested were E. coli (MTCC 476) and Pseudomonas fluorescens (MTCC 664), also grown under the same conditions and with Streptomycin as the positive control. This setup provides a standardized approach to evaluating the antimicrobial efficacy of substances against these specific bacterial strains.

Antimicrobial activity screening

The cup plate agar diffusion method was used to evaluate the antimicrobial activity of extracts (Hossain et al., 2024). For the bacterial strains nutritional agar medium was employed to inoculate the plates with overnight cultures of the test organism. Four 5 mm cups were punched on each of the plates at intervals of equal length

using sterile cork borers, and each cup was filled with 50 µL of each concentration of the plant extract (i.e., 10 mg, 5 mg, and 2.5 mg). As a positive control, streptomycin (2 mg) was used and incubated for 24 hours at 37°C. The inhibitory zones' diameters were measured during the studies, which were done in triplicate.

2.3. Anti-cancer activity

2.3.1. Cell culture

MDAMB231 is a highly aggressive triple-negative breast cancer cell line was grown in minimal essential medium (MEM) alpha, together with a human liver cancer cell line (Hep3B). Cells were kept at 37°C in a 5% CO_2 humidified incubator. Cells were passaged at 80% confluency, with medium refreshed every 2-3 days (Maqsood et al., 2018).

2.4.2. Cell culture, maintenance of cell lines, growth medium, and treatment conditions

MDAMB231(Breast carcinoma) and Hep3B (Liver carcinoma) were collected from ATCC. Cell lines were maintained in Dulbecco's modified eagle medium (DMEM) with FBS (10%), Foetal Calf Serum (FCS) (2–10%), penicillin (100 units/ml) and streptomycin (100 µg/ml) at 37˚C with 5% CO_2. Cell culture was performed using standard procedures in a laminar airflow chamber.

2.4.3. MTT Assay

The cytotoxicity of the given samples on the MDAMB231 and Hep3B cell lines was assessed using the MTT test (Van et al., 2011). The cells (10000 cells/well) were grown in 96-well plates for 24 hours in DMEM media with 10% FBS and 1% antibiotic solution at 37°C with 5% CO_2. Cells were treated the next day with formulations at concentrations ranging from 1-1000 µg/ml (made in an incomplete medium). After 24 hours of incubation, MTT Solution (250µg/ml) was added to the cell culture and incubated for 2 hours. After the experiment, the culture supernatant was collected, and the cell layer matrix was dissolved in 100 µl of Dimethyl Sulfoxide (DMSO). The results were read at 540 and 660 nm using an Elisa plate reader (iMark, Biorad, USA).

3. Result and Discussion

3.1. GC-MS evaluation

According to the GC-MS spectrum, the methanol leaf extract of Naringi crenulata contained a variety of compounds identified at different retention times (Rt). The mass spectrometry analysis provides insights into the structure and composition of these compounds. The fragmentation of larger molecules into smaller ones produces distinct peaks with specific m/z ratios (Figure 1), which serve as unique fingerprints for each compound. A total of eight distinct chemical compounds were identified in

the extract, each with varying retention times, molecular weights, and relative abundances (Table 1).

The most abundant compound was 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (Rt: 40.212, Area: 60.79%), an ester with a molecular weight of 390.6 g/mol, making it the major constituent of the extract. Caryophyllene (Rt: 12.812, Area: 11.28%), a sesquiterpene with a molecular weight of 204.35 g/mol, also contributed significantly to the chemical profile. Another notable compound was Oxirane, dodecyl (Rt: 29.162, Area: 10.27%), a compound with a molecular weight of 242.4 g/mol, further emphasizing the richness of the extract.

Other compounds, such as Quizalofop-P-ethyl (Rt: 39.916, Area: 6.87%) and Scopoletin (Rt: 20.145, Area: 3.67%), showed moderate relative abundances. Minor components, including 4,4'-Thiodianiline (Rt: 16.613, Area: 1.31%) and 1,6-Heptadiene, 2,5-dimethyl (Rt: 22.104, Area: 1.66%), were present in smaller amounts, potentially contributing to the synergistic effects of the extract.The diversity of compounds, including sesquiterpenes, esters, and coumarins, highlights the complex phytochemical composition of the sample. While the most abundant compounds, such as 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, likely play a primary role in the biological activity of the extract, the less abundant components, such as 4,4'-Thiodianiline and 1,6-Heptadiene, 2,5-dimethyl, may enhance its overall therapeutic potential through complementary interactions.

Table 1: Compounds identified in the GC-MS analysis of the methanol leaf extract of Naringi crinulata

S. No	Rt time	Detected Compounds	CAS Number	Molecular Formula	Molecular Weight g/mol	Area (%)
1	5.255	1-Butanol, 3-methyl-, formate	110-45-2	$C_6H_{12}O_2$	116.1583	4.14
2	12.812	Caryophyllene	87-44-5	• $C_{15}H_{24}$	204.35	11.28
3	16.613	4,4'-Thiodianiline	139-65-1	$C_{12}H_{12}N_2S$	216.3	1.31
4	20.145	Scopoletin	92-61-5	$C_{10}H_8O_4$	192.17	3.67
5	22.104	1,6-Heptadiene, 2,5-dimethyl	68701-90-6	C_9H_{16}	124.22	1.66

6	29.162	Oxirane, dodecyl	3234-28-4	$C_{15}H_{30}O_2$	242.4	10.27
7	39.916	Quizalofop-P-ethyl	100646-51-3	$C_{19}H_{17}ClN_2O_4$	372.8	6.87
8	40.212	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	137-89-3	$C_{24}H_{38}O_4$	390.6	60.79

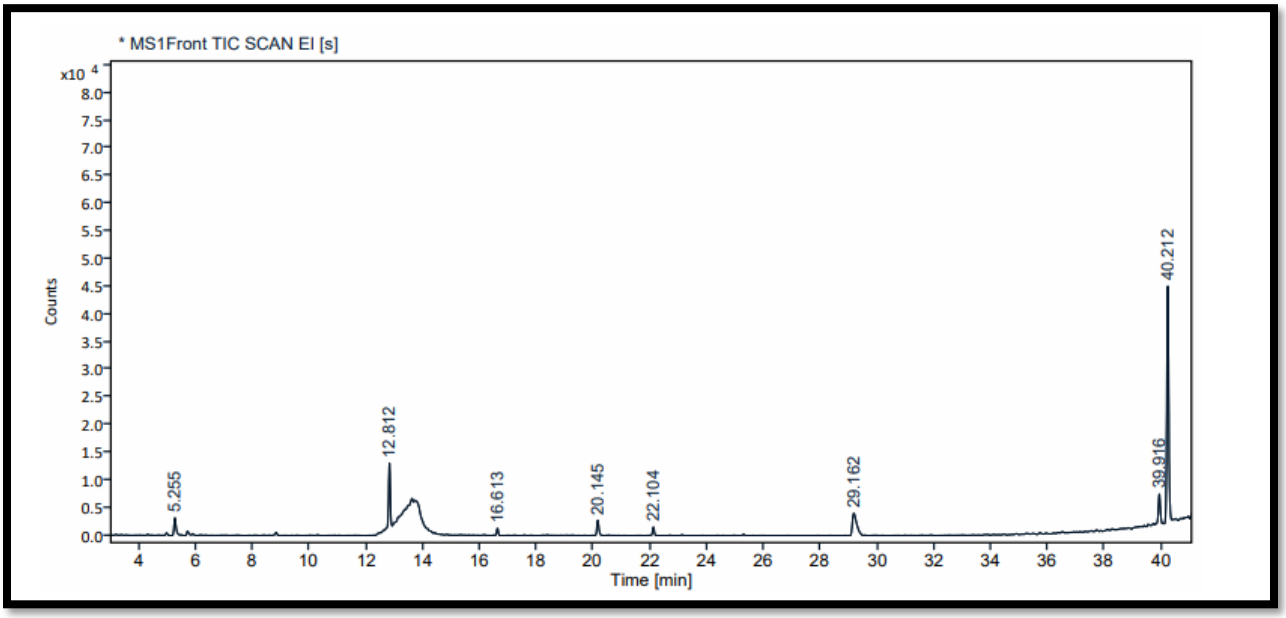

Figure 1: GC-MS chromatogram of the methanol leaf extract of Naringi crinulata

Antimicrobial activity

The results from the antimicrobial activity tests on Naringi crenulata leaf extracts against different bacterial strains are summarized in Table 2. Zones of inhibition were measured in millimeters for methanol (Me), petroleum ether (PE), and aqueous (Aq) extracts at concentrations of 10 mg, 5 mg, and 2.5 mg, with streptomycin (+ Ve) serving as the positive control. The methanol extract exhibited significant antibacterial activity against all tested bacteria, with the highest zones of inhibition observed against Bacillus subtilis(22.6 ± 0.57 mm at 10 mg) and Staphylococcus aureus(19.3 ± 0.57 mm at 10 mg). The activity decreased at lower concentrations, with notable inhibition zones still present against B. subtilis (14.3 ± 0.57 mm at 5 mg) and S. aureus (17 ± 1 mm at 5 mg). Moderate inhibition was also observed against E. coli (16 ± 1 mm at 10 mg) and Pseudomonas fluorescens (10 ± 1 mm at 10 mg).

The petroleum ether extract showed limited antibacterial activity, with zones of inhibition observed only against B. subtilis (15.3 ± 0.57 mm at 10 mg) and S. aureus (11 ± 1 mm at 5 mg). No inhibition was observed against E. coli orP. fluorescens. The

aqueous extract, on the other hand, did not display any inhibitory activity against any of the tested bacterial strains, regardless of concentration.

In comparison, the positive control (streptomycin) showed significantly larger inhibition zones, ranging from 26.2 ± 1.23 mm to 32.6 ± 1.52 mm, highlighting the lower efficacy of the plant extracts. Among the tested extracts, the methanol extract demonstrated the highest antibacterial activity, particularly against Gram-positive bacteria (B. subtilisand S. aureus), suggesting that the methanol extract contains potent bioactive compounds with antibacterial properties. The petroleum ether extract displayed mild activity, while the aqueous extract showed no antibacterial effect.

Table 2: Antibacterial activity of different extracts of Narimgi crinulata

Extract	**Gram -ve bacteria**						**Gram +ve bacteria**					
	P. fluorescens			**E. coli**			**Bacillus subtilis**			**Staphylococcus aureus**		
	10mg	5 mg	2.5mg	10mg	5 mg	2.5mg	10mg	5 mg	2.5mg	10mg	5 mg	2.5mg
Me	10 ±1	8.3± 0.57	7.3± 0.57	16 ±1	14.3 ±0.57	7.3±0.57	22.6 ±0.57	14.3± 0.57	11± 1	19.3± 0.57	17±1	12.3 ±0.57
PE				-	-	-	15.3 ±0.57	11±1	-	-	-	-
Aq	-	-	-	-	-	-	-	-	-	-	-	-
+ Ve	26.2 ± 1.23			28.3± 0.57			31 ± 1			32.6 ± 1.52		

Data showing zone of inhibition in mm. Aq: aqueous extract, Me: methanol extract, PE: Petroleum ether extract. Values represent mean ± standard deviations; "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive.

3.3. Anticancer activity

Naringi crenulata leaf methanol extracts were tested for cytotoxicity on the human breast cancer (MDAMB231) and Liver cancer (Hep3B) cell lines. Cell viability was measured at various doses (25, 50, 100, 250 and 500μg/mL). Results were reported.

3.3.1. Anti-Cancer Activities on breast cancer cell line MDAMB231

The anticancer activity of the methanolic extract of Naringi crenulata leaves was assessed at varying concentrations, showing dose-dependent effects on cell viability and cytotoxicity in the breast cancer cell line MDA-MB-231 (Figure 2). At the highest

tested concentration (500 µg/ml), the extract exhibited significant anticancer potential, with a mean cell viability of 8.77 ± 2.11% and cytotoxicity of 91.23%, indicating strong induction of cancer cell death. At 250 µg/ml, the extract demonstrated a similar level of potency, with 8.93 ± 2.43% cell viability and 91.07% cytotoxicity.

Moderate cytotoxicity was observed at 100 µg/ml, where the extract maintained 9.41 ± 0.89% cell viability and 90.59% cytotoxicity. At 50 µg/ml, the extract showed reduced activity with 33.17 ± 5.35% cell viability and 66.83% cytotoxicity. At 25 µg/ml, the cytotoxicity further decreased, maintaining 73.68 ± 3.50% cell viability and only 26.32% cytotoxicity.The negative control exhibited 100.00 ± 1.71% cell viability, confirming the absence of cytotoxic effects, while the positive control demonstrated a cell viability of 52.79 ± 4.96%, validating its expected anticancer activity.

These results indicate that the methanolic extract of Naringi crenulata leaves has potent anticancer activity at higher concentrations (500 µg/ml and 250 µg/ml), with a sharp decline in effectiveness at lower concentrations (50 µg/ml and 25 µg/ml). The findings suggest the potential use of this extract as a selective cytotoxic agent in cancer treatment, especially at higher concentrations.

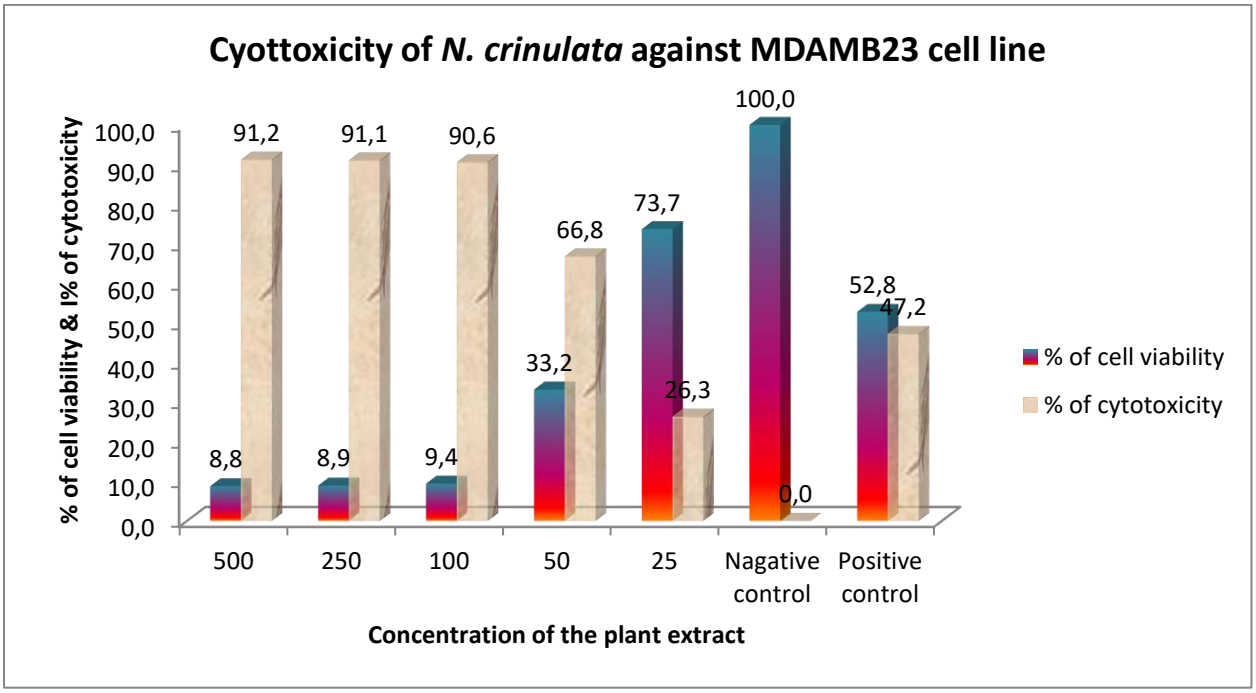

Figure 2: Methanol extract's cell viability and cytotoxicity percentage on breast cancer cell line MDAMB231

The cytotoxic activity of the methanol extract of Naringi crenulata against the breast cancer cell line MDA-MB-231 was evaluated, and the IC50 value was determined to be 31.5 ± 1.42 µg/ml (Figure 3). The dose-dependent effect of the extract is depicted

in the IC_{50} graph, where increasing concentrations result in a significant decline in cell viability. At the IC_{50} point, approximately 50% of the cancer cells were inhibited, indicating a strong cytotoxic effect of the methanol extract.

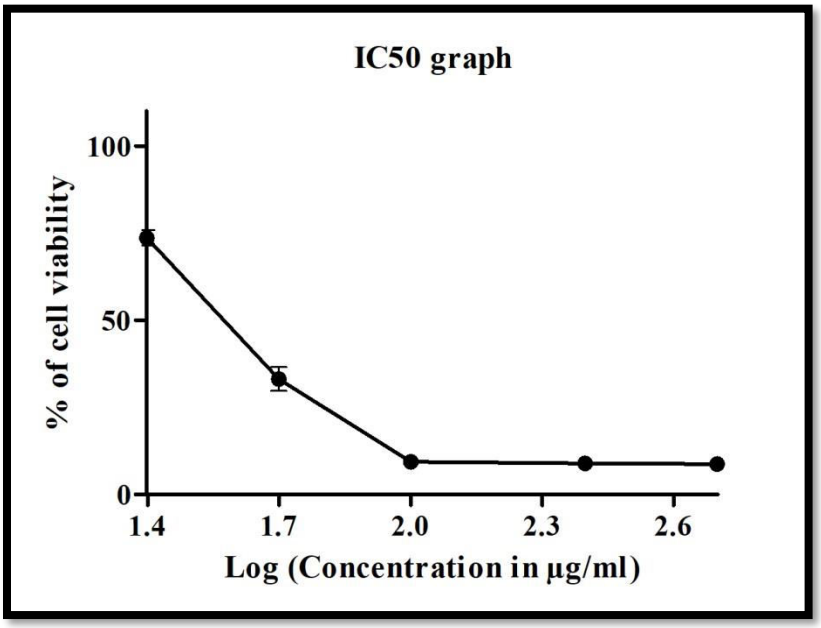

Figure 3: IC_{50} Values of cytotoxicity of methanol extracts of N. crinulataon breast cancer cell line MDAMB231

3.3.2. Anti-cancer activity against Human Liver cancer cell line (Hep 3 B)

The anticancer activity of the methanolic extracts of Naringi crenulata leaves showed a concentration-dependent effect on cell viability and cytotoxicity (Figure 4). At the highest concentration (500 µg/ml), the sample exhibited strong anticancer activity, with a cell viability of 5.79 ± 1.79% and cytotoxicity of 94.21%, indicating significant cell death. At 250 µg/ml, moderate cytotoxicity was observed, with 38.98 ± 2.60% cell viability and 61.02% cytotoxicity.

At 100 µg/ml, the extract exhibited reduced cytotoxicity, maintaining 78.78 ± 4.10% cell viability and 21.22% cytotoxicity. At lower concentrations (50 µg/ml and 25 µg/ml), cytotoxicity was negligible, with cell viability exceeding 99%, similar to the negative control. The positive control demonstrated a cell viability of 47.61 ± 3.95% and cytotoxicity of 52.39%, confirming the experimental validity.

These findings suggest that the methanol extract of N. crenulata has potent anticancer effects against the Hep 3B liver cancer cell line, particularly at higher concentrations, and is largely non-toxic at lower concentrations. This highlights its potential as a selective cytotoxic agent for liver cancer treatment.

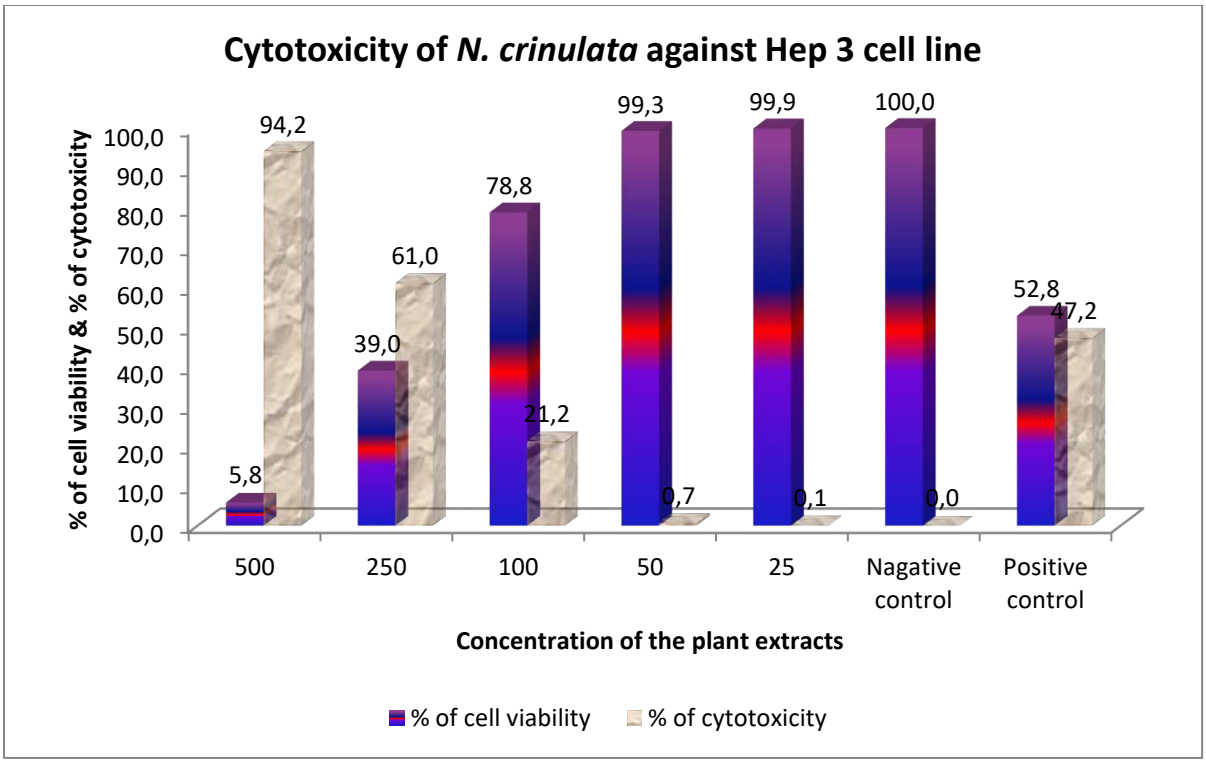

Figure 4: Methanol extract's cell viability and cytotoxicity percentage on liver cancer cell line Hep 3B

The methanol extract of Naringi crenulata was tested for its cytotoxic activity against the liver cancer cell line Hep 3B. The IC50 value was determined to be 194.1±2.28µg/ml (Figure 5), indicating that the extract exhibits weak cytotoxicity at higher concentrations. The IC50 graph demonstrates a dose-dependent decrease in cell viability with increasing concentrations of the extract. However, the extract required a relatively high concentration to achieve 50% inhibition of cell viability, suggesting limited anticancer potential against Hep 3B cells.

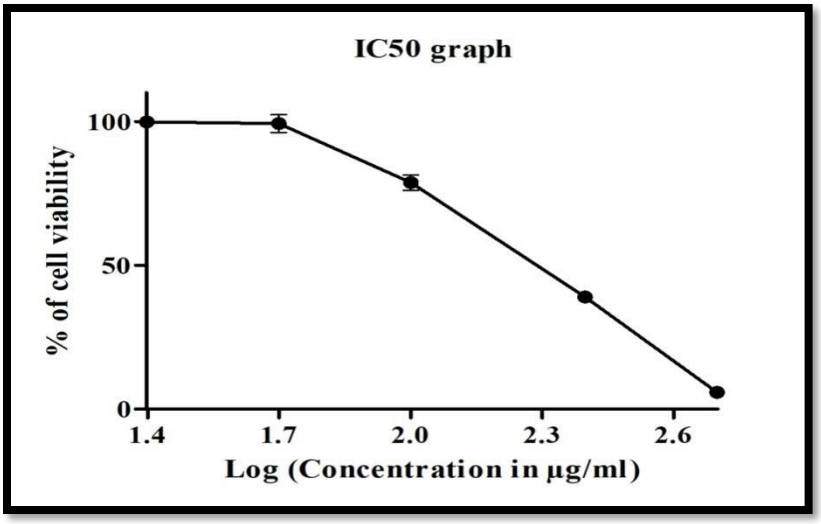

Figure 5: IC_{50} Values of cytotoxicity of methanol extracts of N. crinulataon liver cancer cell line Hep 3B.

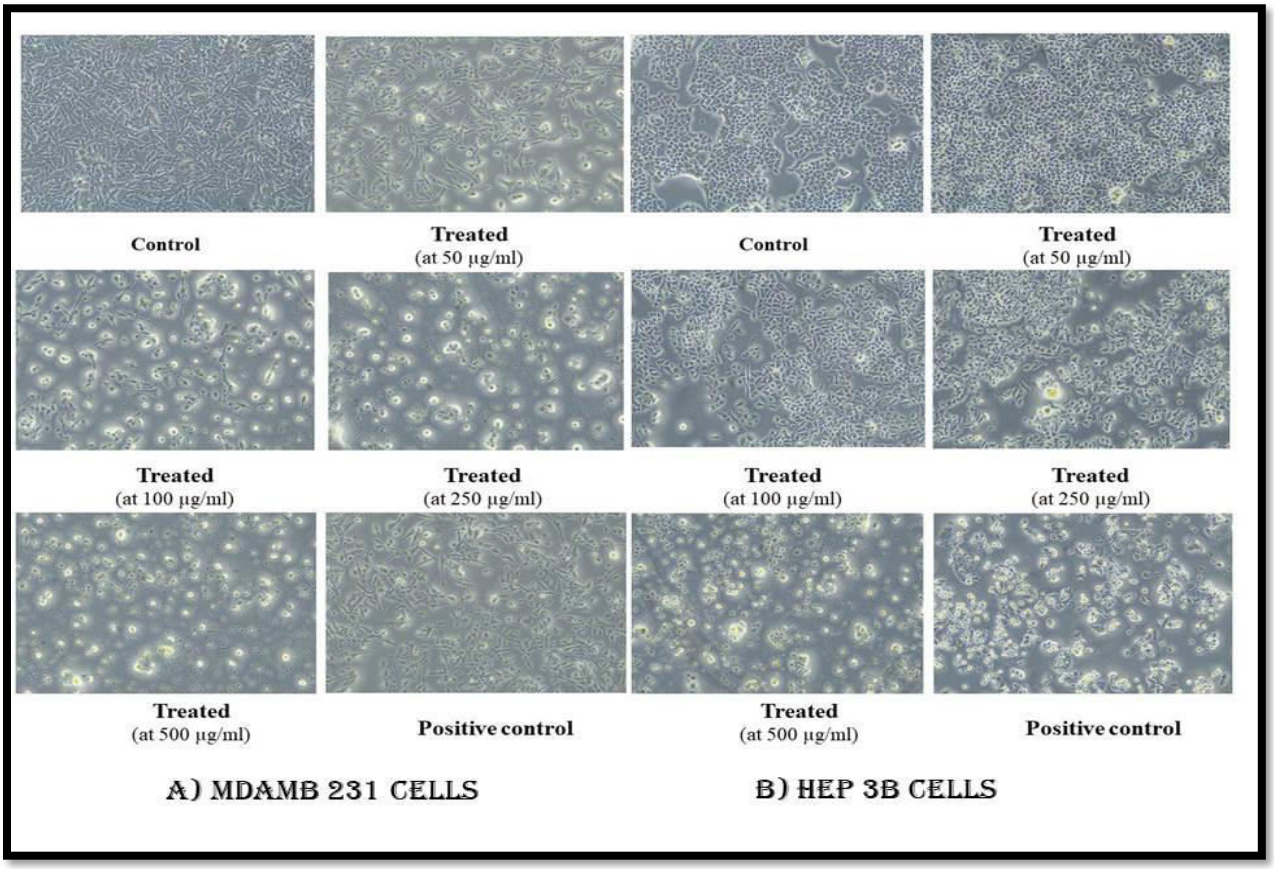

Figure 6: The phase contrast image indicates considerable morphological alterations in cancer cells treated with N. crinulata methanol leaf extract. A) MDAMB231 cell line and B) Hep 3B cell line

In figure 6 MDA-MB-231 cells (A), morphological changes were noticeable at higher concentrations, particularly at 500 µg/ml, where cells appeared rounded, shrunken, and detached, indicating significant cytotoxicity. At lower concentrations (50–250 µg/ml), only minor alterations were observed, and the cells largely retained their normal elongated structure, resembling the control group. In the Hep 3B cells (B), a similar concentration-dependent effect was observed. At 500 µg/ml, the cells exhibited pronounced morphological changes, including rounding, shrinking, and detachment from the surface, suggesting strong cytotoxic activity. However, at lower concentrations (50–250 µg/ml), the cells remained mostly intact and maintained their typical structure, similar to the control group.These results indicate that Naringi crenulata methanol leaf extract induces dose-dependent cytotoxic effects, with significant morphological alterations at higher concentrations. However, its cytotoxicity appears to be limited at lower doses, suggesting only moderate anticancer potential against both breast and liver cancer cell lines.

4. Discussion

In a study by Suresh et al. (2014), the methanolic extract of Naringi crenulata callus was analyzed using GC-MS, leading to the identification of several bioactive compounds. Notably, the analysis revealed the presence of estragole, methyl cis-9-octadecenoate, and alpha-tocopherol (vitamin E), among others. The study suggested that these compounds could contribute to the plant's potential anti-inflammatory, anticancer, and immunomodulatory activities. Another investigation by Suresh et al. (2014) focused on the ethanol extract of Naringi crenulata leaves, utilizing GC-MS to determine its phytochemical constituents. The analysis identified various compounds, including fatty acids, esters, and terpenoids, which are known for their biological activities. The study highlighted the potential therapeutic applications of these compounds, aligning with the traditional medicinal uses of the plant**.** Sarada et al. (2011) performed a GC-MS analysis on the ethanolic extracts of Naringi crenulata leaves and bark. Their investigation identified 17 compounds in the leaves and 23 compounds in the bark. Notable constituents in the leaves included caryophyllene (12.22%), while the bark contained significant amounts of octane, 3,5-dimethyl- (24.96%), 2-dimethylsilyloxytridecane (11.54%), and l-octanol, 3,7-dimethyl- (10.14%). Further research by Suresh et al. (2014) examined the leaves and bark of Naringi crenulata through GC-MS analysis. This study identified a range of bioactive components, such as methyl phenylacetate, hexadecane, and estragole. The findings provided a comprehensive chemical profile of the plant, supporting its traditional use in various disorders and suggesting avenues for future pharmacological studies.Rajesh et al. (2019) conducted a GC-MS analysis of Naringi crenulata callus culture, identifying 27 bioactive compounds, with key constituents

including hexadecanoic acid, methyl ester (17.24%), octadecanoic acid (14.58%), phytol (12.75%), β-sitosterol (10.91%), and squalene (9.83%).These compounds are known for their diverse pharmacological properties, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities.

Latha et al. (2005) investigated the antimicrobial activity of Naringi crenulata ethanolic leaf extract, revealing the presence of alkaloids, tannins, phenols, and carbohydrates. The extract exhibited significant antibacterial effects, with inhibition zones of 16.4 ± 0.8 mm for Bacillus subtilisand 14.7 ± 1.2 mm for Klebsiella pneumoniae. Antifungal activity was also noted, with zones of 18.2 ± 1.1 mm for Aspergillus niger and 15.6 ± 0.9 mm for Mucor species. These results suggest Naringi crenulata as a potential source of natural antimicrobial agents, encouraging further research on its bioactive compounds.In another study by Chinna thambi et al. (2022), synthesized silver nanoparticles (AgNPs) using methanolic leaf extracts of N. crenulata and evaluated their antibacterial activity against multidrug-resistant bacteria. The results showed significant inhibition zones, with Staphylococcus aureus exhibiting the highest susceptibility (22.4 ± 0.7 mm) followed by Escherichia coli (21.8 ± 0.6 mm), Klebsiella pneumoniae (20.3 ± 0.5 mm), Streptococcus pyogenes (19.6 ± 0.4 mm), and Vibrio cholerae (18.2 ± 0.5 mm). These findings suggest that nanoparticle formulations enhance the antimicrobial efficacy of N. crenulata. Additionally, Sarada et al. (2012) investigated the pharmacognostic and phytochemical properties of N. crenulata stem extracts, reporting a total phenolic content of 82.6 ± 1.2 mg GAE/g extract and total flavonoid content of 45.3 ± 0.8 mg QE/g extract. The antimicrobial activity was assessed against various bacterial strains, showing inhibition zones of 17.1 ± 0.4 mm for Staphylococcus aureus, 16.5 ± 0.5 mm for Escherichia coli, and 14.8 ± 0.6 mm for Bacillus subtilis. The high phenolic and flavonoid content suggests that these compounds contribute significantly to the antibacterial properties of N. crenulata.

Vishnu et al. (2017) evaluated the anticancer activity of ethanolic extracts from N. crenulata and Toddaliaasiatica against cervical cancer (CASKI) cell lines. The study employed an MTT assay to determine cell viability, revealing that the N. crenulata extract exhibited significant cytotoxic effects. At a concentration of 100 µg/mL, the extract reduced cancer cell viability to 38.7%, compared to the untreated control. The half-maximal inhibitory concentration (IC50) was determined to be 52.6 µg/mL, indicating potent anticancer activity.Further supporting these findings, Manjula and Norman (2017) conducted a review on the medicinal potential of N. crenulata, emphasizing its traditional use for various ailments, including cancer. Interviews with traditional healers revealed that decoctions prepared from the plant were commonly used to treat tumors and inflammatory conditions. Though this review did

not provide direct numerical data, it highlighted the plant's therapeutic relevance and the need for scientific validation of its anticancer properties. Another study by Mekap et al. (2016), the antidiabetic properties of Naringi crenulata were evaluated using ethanol extracts of the plant's leaves and bark. While the primary focus was on antidiabetic activity, the study also reported preliminary findings regarding the plant's anticancer potential. In vitro cytotoxic assays revealed that the ethanol extracts exhibited significant cytotoxic activity, with cell viability reductions exceeding 40% at concentrations above 50 µg/mL. These results suggest that various parts of N. crenulata may contain bioactive compounds contributing to its anticancer effects.

Conclusion

The findings of this study confirm that the methanol leaf extract of Naringi crenulata possesses significant antimicrobial and anticancer properties. GC-MS analysis identified several bioactive compounds, with 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester being the most abundant. The methanol extract demonstrated potent antibacterial activity, particularly against Gram-positive bacteria, suggesting its potential as a natural antimicrobial agent. Additionally, the extract exhibited strong cytotoxic effects against breast cancer cells, while moderate anticancer activity was observed against liver cancer cells. These results highlight the potential of N. crenulata as a promising candidate for anticancer and antimicrobial drug development. Further studies should focus on isolating the active compounds and evaluating their mechanisms of action for clinical applications.

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Author Address:
[1]Research scholar, AU- TDR hub, Andhra University, Visakhapatnam, Andhra Pradesh, India
[2]Lecturer, Government Degree College, Thogaram, Andhra Pradesh, India
[3]Lecturer, Government Degree College for Women, Araku vally, Andhra Pradesh, India
[4]Lecturer, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India
[5]Professor, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India

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