Original Article

Anti-proliferative Activity of *Vitex negundo* Leaf Extracts on PA1 Human Ovarian Cancer Cell Lines

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ABSTRACT

In Ayurveda, *Vitex negundo* (VN) is used as a drug to manage pain, inflammation, and problems related to polycystic ovary disease and the menstrual cycle. The bioactive compounds isolated from this plant exhibit anti-inflammatory, anti-oxidant, anti-cancer, and microbicidal properties. The shrub VN is known for its role in the modulation of cellular events like apoptosis and cell cycle. There is still a scarcity of data in the literature on the cytotoxic activity of VN extracts on ovarian cancer. Therefore, in the present study, the phytochemical composition, anti-oxidant, and anti-cancer activities of leaf extracts were evaluated. The chloroform and methanol fractions exhibited higher phenolic content (161.04 ± 0.02 mg/g GAE and 152.56 ± 0.05 mg/g GAE, respectively) than those of other fractions. The aqueous and petroleum ether fractions exhibited higher flavonoid content (215.27 ± 0.28 mg/g QE and 111.82 ± 0.05 mg/g QE, respectively). The acetone and methanol extracts showed significant anti-oxidant capacities. Both leaf extracts of VN inhibited PA1 cancer cell growth in a dose-dependent manner with IC_{50} values of 88.01 ± 3.14 and 112.30 ± 1.93 µg/ml, respectively, as compared to the standard drug Doxorubicin with IC_{50} value 2.91 µg/ml (P<0.05, One-way ANOVA). The gas chromatography-mass spectroscopy (GC-MS) analysis allowed us to identify twenty-five bioactive compounds in acetone extract and twenty-two in methanol extract. Therefore, further studies should focus on the isolation of novel compounds that are more effective and less toxic, and that constitute interesting substitutes for the development of anti-cancer drugs.

Keywords: *Vitex negundo* (VN), Ovarian cancer, Anti-proliferative, Anti-oxidant activities, GC-MS analysis

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1. Introduction

Vitex negundo Linn. (VN) belongs to the family *Verbenaceae*. It is an aromatic shrub distributed throughout India. It is a traditional medicine in Sri Lanka, Nepal, Pakistan, China, the Philippines, Indonesia, and Bangladesh (1). In the Ayurvedic system of medicine, it is used to manage pain, inflammation, and problems related to polycystic ovary disease and the menstrual cycle. Different species of *Vitex* vary in chemical composition, thus producing various phytochemicals that are extracted from leaves, seeds, and roots in the form of volatile oils. The phytoconstituents in VN are flavonoids, lignans, steroids, various polyphenolic compounds, terpenoids, glycosidic iridoids, and alkaloids. Moreover, VN is known for its role in the modulation of cellular events like apoptosis, cell cycle, sperm motility, polycystic ovary disease, and problems related to the menstrual cycle (2). Ovarian carcinoma is the leading cause of all fatal diseases among women. The cancer statistics (2020) showed that the estimated number of new cases is 313,959, with deaths around 207,252 (3). Despite developments in treatment for ovarian carcinoma, survival rates continued to be poor because of chemoresistance and lack of biomarkers to detect the disease at an early stage (4, 5). Hence, ovarian cancer is often diagnosed at an advanced stage, with most new cases spreading beyond the primary site. The mortality rate of ovarian cancer has declined by nearly 30% since the mid-1970s due to reductions in the incidence rate and improvements in treatment strategies. Despite some patients surviving beyond five years after diagnosis, the absence of specific early symptoms and effective early detection strategies remain significant challenges (6). Various aspects influence the disease progression, such as genetic and epigenetic factors. Approximately 10-15% of familial ovarian cancers result from the breast cancer gene mutations BRACA1 and BRACA2. The mutations and loss of tumor protein 53 (TP53) function are found in 60-80% of familial and sporadic cases of this disease (7). These oncogenes will turn on various signaling pathways that lead to pathogenicity (8, 9). The present study aimed to explore the *in vitro* anti-oxidant and anti-proliferative activities of leaf extracts of VN against human ovarian cancer cell lines.

2. Materials and Methods

2.1 Collection and Preparation of Plant Material

Fresh leaves of VN (Voucher specimen no.: KUD/BT/PS/MH/02) were collected from the Botanical Garden of Karnatak University Dharwad, Karnataka, India (Figure 1). The plant was identified and authenticated at the Department of Botany, Karnatak University Dharwad, Karnataka, India. The leaves were washed, shade-dried, and homogenized to a coarse powder with a mechanical grinder. The dried samples were pulverized to powder and preserved in an air-tight container for further study. The extraction of dried leaf powder (50 gm) of VN was carried out with solvents (500 ml) of increasing polarity, starting from petroleum ether (VNP), Chloroform (VNC), Acetone (VNA), Methanol (VNM), and distilled water (VNW) in the Soxhlet apparatus for 8-10 h. The solvents were evaporated in a rotary evaporator under reduced pressure, and the concentrated fraction was dried. The resultant crude yield was weighed, and the extracts were preserved in moisture-free conditions for further use.

2.2 Phytochemical Analysis

Preliminary phytochemical screening was carried out for all extracts of VN following the protocol of Deepti et al. (10). The crude extracts were tested for the presence of phytochemicals, such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpenoids.

2.3 Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteau method was followed to determine the total phenol content as previously described by Singleton et al. (1999) with minor modifications (11). In addition, gallic acid was used as the standard. The phenolic content was reported as micrograms per milligram gallic acid equivalent (GAE). The TPC in plant extract was determined by extrapolating the absorption of unknown samples on a standard calibration curve obtained with gallic acid.

2.4 Determination of Total Flavonoid Content (TFC)

The quantification of the total flavonoids present in the leaf extracts of VN was carried out as per Phuyal et al. (2020) with slight modifications (12). The standard curve was prepared using quercetin as standard. The TFC was reported in micrograms per milligram quercetin equivalent (QE). The TFC was determined by extrapolating the absorption of unknown samples on a standard calibration curve obtained with quercetin.

2.5 Determination of Anti-Oxidant Activities

2.5.1 DPPH Free Radical- scavenging Assay

The radical scavenging activity of VN leaf extracts was determined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical as a reagent, according to the method of Rice-Evans et al. (1997). The DPPH radical solution (1 ml) in methanol (60 µM) was mixed with 100-500 µl of sample solution in methanol. The mixture was incubated for 30 min in the dark at room temperature, and the absorbance was measured at 517 nm using a UV-visible spectrophotometer (13). In addition, ascorbic acid was

Figure 1. Photograph of *Vitex negundo*

used as a standard reference. This activity was calculated using the following equation:

$$Percentage\ of\ inhibition = [(Ac - At)/Ac] \times 100$$

Where Ac denotes the absorbance of the control reaction, and At is the absorbance of the test sample.

2.5.2 Phospho-molybdenum (PM) Assay

The total anti-oxidant activity was estimated by the PM assay using the standard procedure of Prieto et al. (1999). Various leaf extracts of VN at different concentrations ranging from 100 to 500 µl were added to each test tube containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. Afterward, these test tubes were incubated at 95°C for 90 min. After incubation, they were normalized to room temperature, and the absorbance of the reaction mixture was measured at 695 nm (14). Ascorbic acid was also used as a standard reference.

2.5.3 Ferric Reducing Anti-oxidant Power (FRAP) Assay

Ferric ions' reducing power was measured according to Oyaizu's method (1986). Various leaf extracts of VN in different concentrations ranging from 50 to 300 µl were mixed with 2.5 ml (0.2 M) Phosphate buffer (pH 7.4), and 2.5 ml (1% w/v) Potassium ferricyanide and the mixture was incubated at 50°C for 30 min. After incubation, 2.5 ml (10% w/v) Trichloroacetic acid and 0.5 ml (0.1% w/v) Ferric chloride were added to the mixture and incubated for 10 min (15). The absorbance was measured at 700 nm using a UV-visible spectrophotometer. It is noteworthy that ascorbic acid was used as a reference standard.

2.6 Determination of Cell Viability by MTT Assay

2.6.1 Cell Culture

The PA1 human ovarian cancer cell lines were procured from the National Centre for Cell Science (NCCS), Pune, India. The cells were subcultured in Dulbecco`s modified eagle medium (DMEM) with low glucose, supplemented with 10% fetal bovine serum and antimycotic 100X solution in tissue culture flasks.

2.6.2 MTT Assay

The cells were seeded in a 96-well flat-bottom microplate and maintained at 37ºC in 95% humidity and 5% CO_2 overnight. Various concentrations (200, 100, 50, 25, 12.5, 6.25 µl/ml) of samples were treated. The cells were incubated for another 48 hours. The wells were washed twice with Phosphate buffered saline (PBS), and 20 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) staining solution was added to each well and then the plate was incubated at 37ºC. After 4 hours, 100 µl of Dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. Then, the absorbance was recorded using a microplate reader at 570 nm (16). The following treatment groups were set up for the study:

Negative control: cells + media

Positive control: cells + Doxorubicin

Test groups: cells + methanol extract of VN leaves and cells + acetone extract of VN leaves

2.7 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The GC-MS analysis was performed on a combined GC-MS instrument (Agilent 5977 MSD) at SAIF-IIT Madras. Around 1 µl sample aliquot was injected into the column using a Split mode with the temperature set at 260°C. The GC program was initiated by a column temperature set at 75°C for 0.5 min., increased to 180°C at a 5°C/min rate, and held for 5 min. The mass spectrometer was operated with the mass source at 250°C. Helium was used as the carrier gas (1.00 ml/min).

2.8 Statistical Analysis

All experiments were performed in triplicates (n=3). All the variables were analysed using Microsoft Excel and later interpreted by IBM SPSS software Inc. Version 20.0 for test of significance by One way ANOVA. p – value <0.05 is considered statistically significant.

3. Results

3.1 Phytochemical Analysis

The phytochemical analysis of crude extracts of VN revealed the presence of secondary metabolites synthesized in the plant that include phytochemicals, such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpenoids (Table 1).

3.2 Total Phenolic Content

The results indicated a significant level of phenolic compounds in various leaf extracts of VN. The chloroform extract exhibited a higher presence of phenolic content than that of other extracts, calculated from the calibration curve (R^2=0.9601), which was equal to 161.04 ± 0.02 mg/g GAE. The TPC of methanol, aqueous, petroleum ether, and acetone were 152.56 ± 0.05, 129.46 ± 0.03, 90.42 ± 0.04, and 85.07 ± 0.08 mg/g GAE respectively.

3.3 Total Flavonoid Content

The aqueous fraction indicated a higher presence of flavonoid content as 215.27 ± 0.28 mg/g QE, calculated from the calibration curve (R^2=0.9954). The TFC of petroleum ether, methanol, acetone, and chloroform extracts were 111.82 ± 0.05, 108.55 ± 0.20, 115.03 ± 0.02, and 103.70 ± 0.05 mg/g QE, respectively.

3.4 Anti-oxidant Activities

The acetone and methanol fractions exhibited the highest anti-oxidant capacity in DPPH, FRAP, and PM assays. Various concentrations of all the extracts were subjected to DPPH assay. Maximum inhibition was observed in the acetone and methanol fractions. There was a dose-dependent increase in the percentage of inhibition for all the concentrations (Table 2). Accordingly, the order of scavenging activity of various fractions was found to be VNA>VNM>VNC>VNP>VNW. The IC_{50} values were ascorbic acid (39.05), petroleum ether (558.38), chloroform (532.11), acetone (659.82), methanol (806.96) and aqueous (1031.10). In the PM assay, chloroform and acetone fractions exhibited higher absorbance than that of other fractions (Figure 2). The total anti-oxidant capacity of all five extracts was found to decrease in the order VNC>VNP>VNM>VNW>VNA. In the FRAP assay, methanol and acetone extracts showed higher absorbance compared to other extracts. It should be noted that aqueous extract showed lower absorbance, when compared with other extracts. The reducing power of all five extracts was found to decrease in the order VNM>VNC>VNP>VNW>VNA, presented in figure 3. In addition, significant differences were observed among the samples.

3.5 Cell Viability Assay

The acetone and methanol fractions of VN inhibited PA1 cancer cell growth in a dose-dependent manner with IC_{50} values of 88.01 ± 3.14 and 112.30 ± 1.93 µg/ml, respectively, as compared to the standard drug Doxorubicin with 2.91 ± 0.03 µg/ml and a negative control (P<0.05, One-way ANOVA). In the untreated group, the cells were intact and attached and exhibited spindle-shaped morphology. In treated samples, the cells were in a round-shaped morphology, and some showed attached cells (Figures 6 and 7). The results revealed changes at the morphological level where the cancerous cells underwent shrinkage and death, leading to apoptosis in PA1 human ovarian cancer cell lines.

3.6 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The GC-MS chromatogram of 25 peaks of acetone extract is indicated in figure 4. Moreover, the GC-MS chromatogram of 22 peaks of the compounds detected is shown in figure 5. Components of the acetone and methanol extracts were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in the National Institute of Standard and Technology (NIST) library database (library version: MS computer library).

4. Discussion

The natural products isolated from medicinal plants have played a key role in the cancer treatments. Earlier studies on traditional medicine and recent achievements with naturally occurring anti-cancer agents encourage researchers to identify and study more potential phytoconstituents for treating cancers (17). An increasing amount of clinical research supports the practice of integrative oncology and the implementation of integrative gynecological oncology models along with conventional cancer treatments. Further research in this regard needs to create clinical guidelines for integrative oncology interventions for the treatment of ovarian cancer (18). Various risk factors, such as genetic, demographic, hormonal, reproductive, gynecologic, and lifestyle issues, are associated with ovarian cancer. Diet, nutrition, lifestyle, and physical activity have been studied as possible predisposing factors (19). Therefore, recent research has focused more on multifactorial approaches to cancer treatments, including the development of new drugs isolated from plant sources that are non-toxic to healthy cells and effective against cancer cells. In the present study, polar fractions, such as chloroform and petroleum ether extracts, showed better activities in the PM assay. In the FRAP assay, methanol and chloroform fractions exhibited higher absorbance than that of other extracts. However, in the DPPH radical scavenging assay, acetone and methanol fractions exhibited a higher percentage of inhibition compared to other extracts. A study by Tiwari and Tripathi (2007) concluded that the polar fractions of VN exhibit potent anti-oxidant activities (20). In another study by Meng et al. (2021), anti-oxidant and cytotoxic activities of *in vitro* propagated VN

Table 1: Preliminary phytochemical screening of *Vitex negundo* leaf extracts

Constituent	Test	Petroleum ether	Chloroform	Acetone	Methanol	Aqueous
Alkaloids	Mayer's Wagner's	- -	- +	+	- +	- -
Flavonoids	$FeCl_3$ test Lead acetate	+ +	+ -	+ +	+ +	+ +
Glycosides	Keller-Killani Bromine water	- -	- -	- -	+ +	+ -
Phenols	Lead acetate	+	+	+	+	+
Saponins	Foam test	-	-	-	-	+
Tannins	$FeCl_3$ test	-	-	+	+	-
Steroids		+	+	-	-	+
Terpenoids	Salkowski's	+	+	+	+	-

'+' indicates the presence and '-' indicates the absence of phyto-constituents

Table 2: Determination of percentage inhibition of DPPH radical scavenging activity of VN extracts

Concentration (mg/ml)	Percentage of inhibition					
	Ascorbic acid	Petroleum ether	Chloroform	Acetone	Methanol	Aqueous
100	79.80±0.06	07.15±0.07	22.47±0.08	75.34±0.01	72.18±0.03	20.70±0.05
200	82.14±0.03	16.17±0.03	28.07±0.02	78.34±0.04	74.48±0.07	21.81±0.04
300	83.20±0.08	23.41±0.05	30.02±0.02	79.51±0.05	77.37±0.02	26.43±0.07
400	84.69±0.08	31.55±0.05	32.12±0.03	81.69±0.04	80.88±0.07	29.94±0.05
500	86.82±0.06	39.46±0.07	33.98±0.04	83.16±0.05	81.34±0.02	31.24±0.07

Values are mean ± SE, n = 3. Results were analysed using Microsoft Excel.

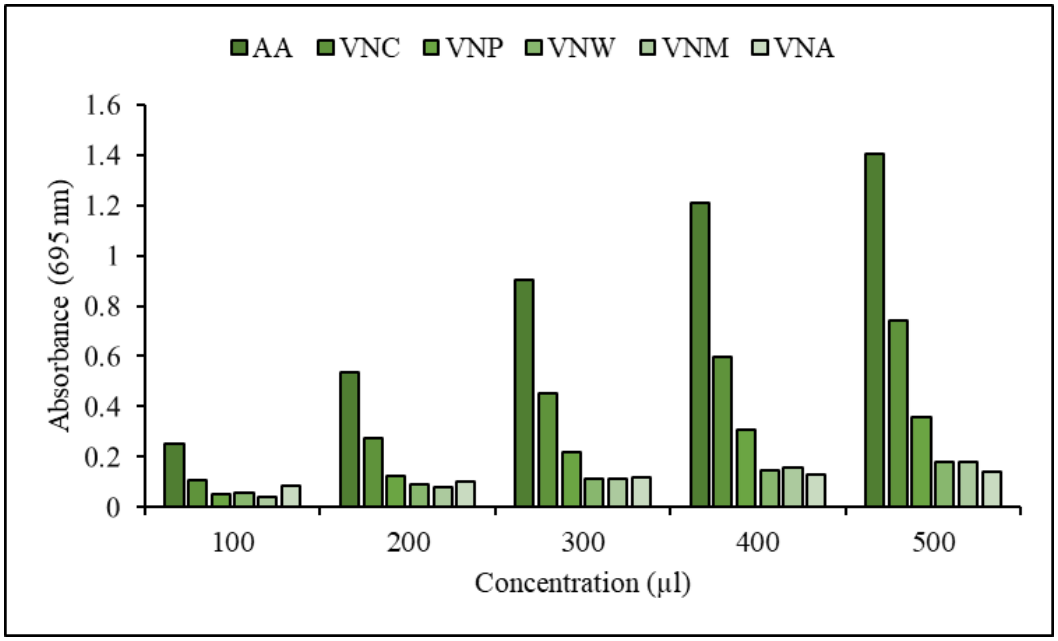

Figure 2. Phospho-molybdenum assay of *Vitex negundo* leaf extracts. Ascorbic acid is used as a standard.

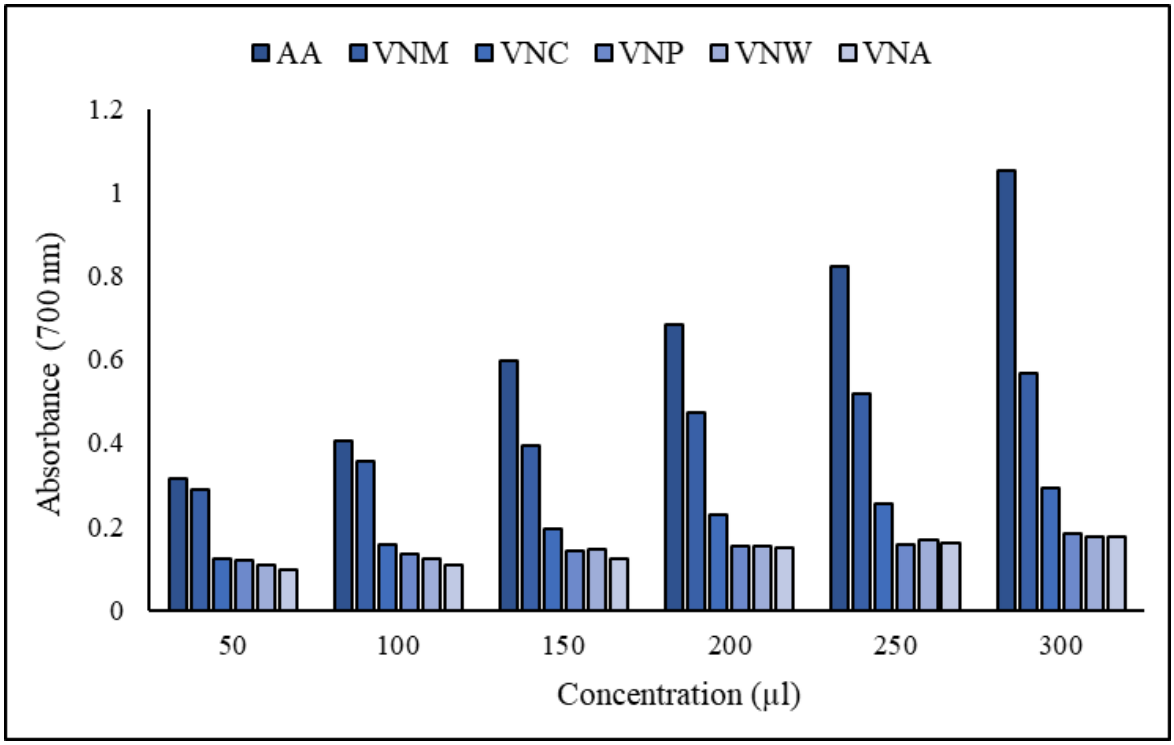

Figure 3. Ferric Reducing Antioxidant Power (FRAP) assay of *Vitex negundo* leaf extracts. Ascorbic acid is used as standard.

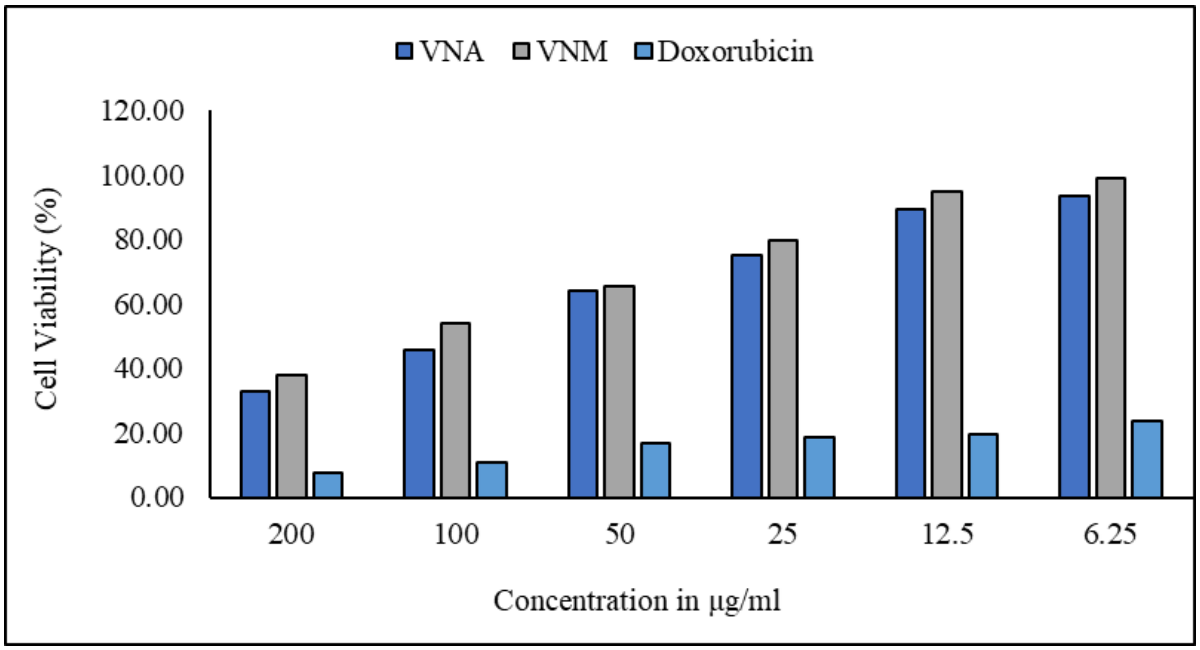

Figure 6. Percentage Cell viability of *Vitex negundo* extracts (acetone, methanol) and Doxorubicin drug on PA1 Ovarian cancer cell lines ($p<0.05$, One-Way ANOVA).

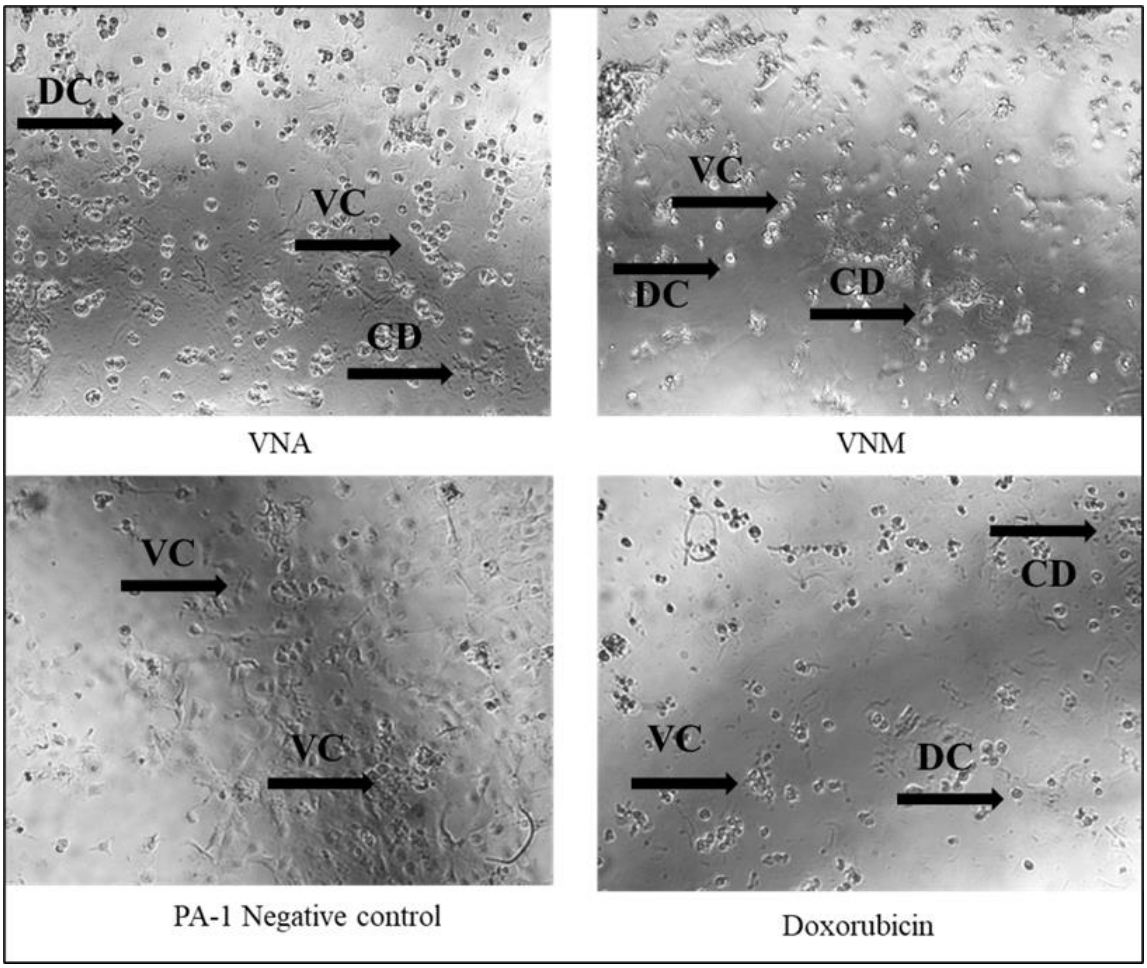

Figure 7. Morphological changes of PA1 Ovarian Cancer Cell lines, negative control, Doxorubicin and VN plant extracts (acetone, methanol) for 48 hours VC- Viable cells, DC- Dead cells, CD- Cell debris

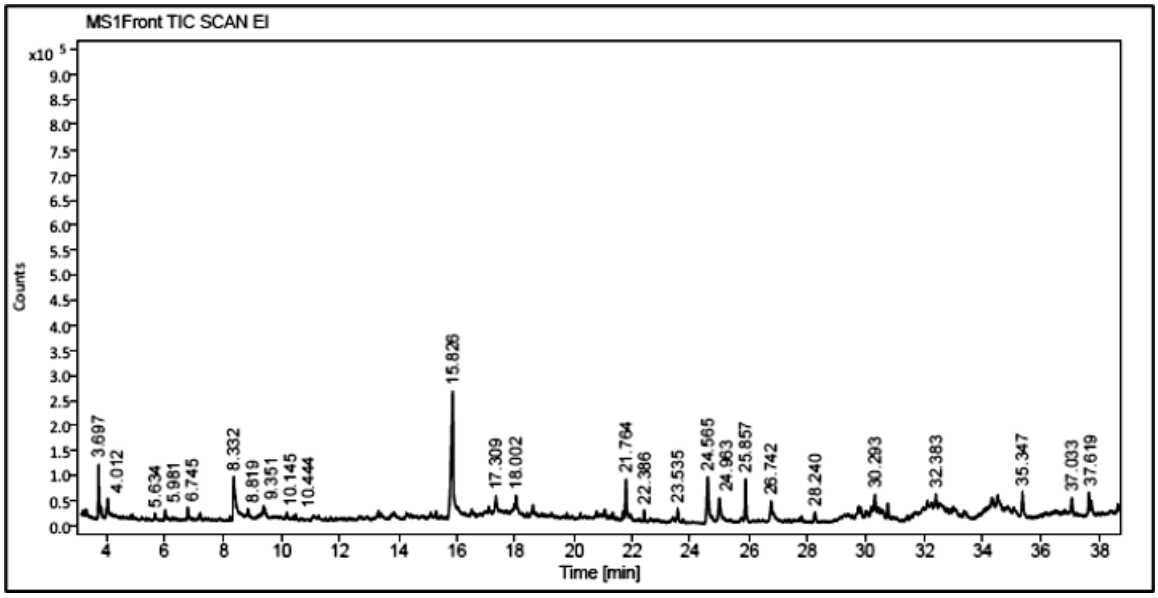

Figure 4. GC-MS Chromatogram of acetone extract of *Vitex negundo* showing 25 bioactive compounds.

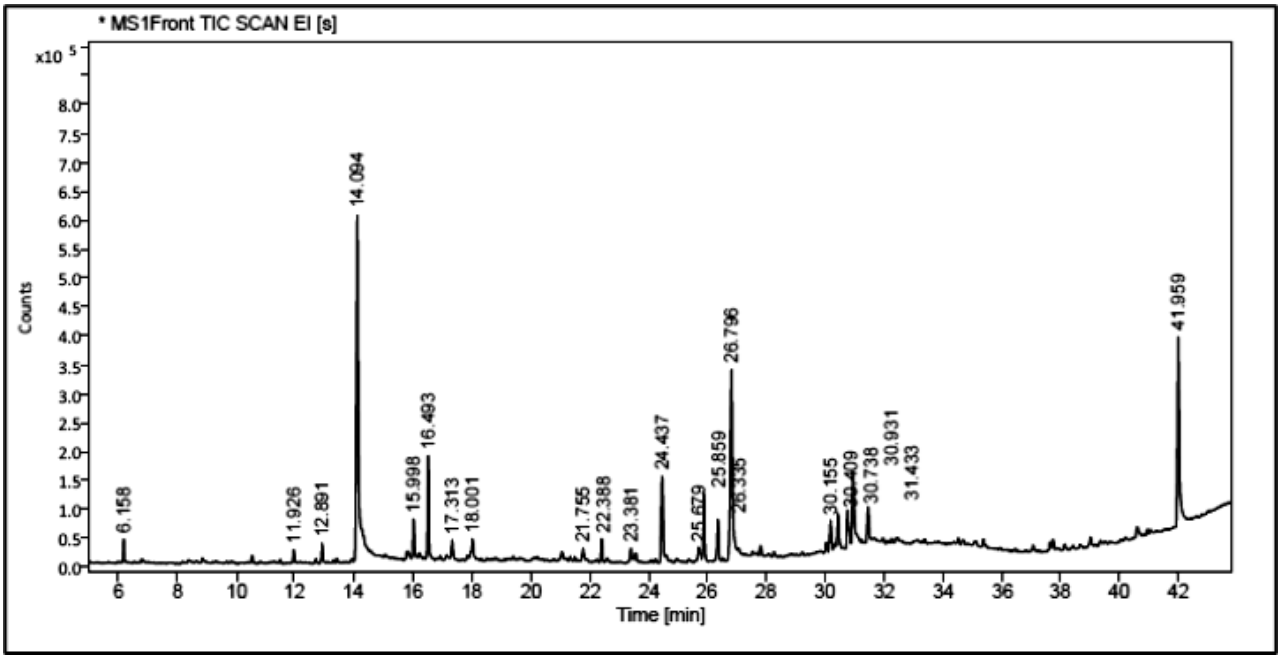

Figure 5. GC-MS Chromatogram of methanol extract of *Vitex negundo* showing 22 bioactive compounds.

demonstrated significant inhibition of breast cancer cell lines (SUM159 and MCF7) (21). In previous studies, ethanolic and aqueous extracts of VN exhibited anti-tumor effects against Dalton's Ascitic Lymphoma (DAL) in Swiss Albino mice. The 5-fluorouracil was the standard drug used (22). Liu et al. (2018) showed that VB1 (vitexin compound 1), a phytoconstituent purified from VN, inhibited the growth of melanoma cells and arrested the cell cycle in the G2/M phase; in contrast, the effects were not observed significantly in the normal cells (23). In another study, an ethanolic extract of VN-loaded gold nanoparticles (VN- AuNPs) was developed that suppressed the growth of human gastric cancer cell lines (24). Recently, Van Vo et al. (2022) reported that the following purified compounds, namely artemetin, Vitexicarpin, and penduletin, were examined for anti-proliferative activity against HepG2 and MCF-7 cell lines by a cell viability assay (25). The outcomes suggested that these compounds had significant *in vitro* anti-cancer activity against human breast and liver cancer cell lines. Qiu et al. (2017) stated that phytoconstituents, such as iridoid glucoside, phenol glucoside, and nine other known compounds, were isolated from this plant (26). Two compounds showed inhibitory effects on Nitric oxide production in RAW 264.7 macrophages (Abelson leukaemia virus-transformed cell line derived from BALB/c mice). The liquid chromatography-mass spectrometry data by Meena et al. (2022) indicated similarities in the ethanolic fractions of VN root and small branches (27). Similarly, GC-MS data demonstrated several volatile constituents in VN extracts. The GC- MS analysis of VNA showed the presence of significant peaks. The first peak obtained was related to Glycerine, which has a geroprotector activity. The next peaks were relevant to Cyclohexanamine, N-3-butenyl-N-methyl- (peak 3), Methyl 6-oxoheptanoate (peak 9), and Vitexifolin D (peak 24), which are believed to possess anti-cancer activity. The second peak is also known as Chlorobutanol, which is used as a pulp sedative after dentinal treatment of superficial or deep caries. Peak 20 is related to n-Hexadecanoic acid and is reported to have anti-androgenic, anti-oxidant, anti-inflammatory, haemolytic, pesticide, nematicide, mosquito larvicide, and 5-α reductase inhibitor activities (28). Moreover, Isoambreinolide (peak 22) exhibits anti-tubercular activity (Figure 4) (Table 3). The GC- MS analysis of VNM showed the presence of significant peaks. The first peak was related to Undecane, which is a naturally occurring alkane hydrocarbon and is reported to have anti-inflammatory, anti-allergic and immune-suppressant effects. 2,4-Di-tert-butylphenol (peak 5) and Karanjin (peak 6) are known to exhibit anti-oxidant, anti-hyperglycaemic, and anti-cancer activities (29). Phytol (peak 18) is used as a Schistosomicide drug. Moreover, anti-nociceptive, anti-oxidant, anti-inflammatory, and anti-allergic effects are observed (30). It is noteworthy that octadecanoic acid had bactericidal activity (Figure 5) (Table 4). The anti-proliferative potential of acetone and methanol fractions of VN significantly inhibited PA1 cancer cell growth in a dose-dependent manner compared to the standard drug Doxorubicin. These fractions have effective anti-oxidant and anti-cancer capacities. The results provide new scientific evidence for the use of medicinal plants for the treatment of cancer. The GC-MS analysis revealed the presence of 25 bioactive compounds in the leaf acetone fraction and 22 compounds in the leaf methanol fraction of VN based on the peak area, retention time, and mass-to-charge ratio. The present study confirmed the presence of various phytoconstituents with significant biological activities, which validates the use of VN as a potential source to develop more effective and less toxic drugs.

Table 3 GC-MS analysis of *Vitex negundo* leaf extract (Acetone extract)

Peak	Name	Retention time	Area %
1	Glycerine	3.70	3.51
2	Cyclohexanecarboxylic acid, 2-hydroxy-, ethyl ester	4.012	2.57
3	Cyclohexanamine, N-3-butenyl-N-methyl-	5.638	0.42
4	Methyl 11-oxo-9-undecenoate	5.981	0.84
5	DL-Arabinose	6.744	1.28
6	Catechol	8.332	8.16
7	3-Cyclohexene-1-propanal	8.819	1.84
8	Acetic acid, 2,2'-[oxybis(2,1-ethanediyloxy)]bis-	9.351	2.75
9	Methyl 6-oxoheptanoate	10.145	1.9
10	(4R,S)-4-(2-Butyl)-cis-bicyclo[4.3.0]-2-nonen-8-one	10.445	0.37
11	Benzoic acid, 4-hydroxy-	15.827	16.89
12	9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester, (Z,Z,Z)-	17.308	3.58
13	Tetradecane, 2,6,10-trimethyl-	18.002	4.29
14	9-Thiabicyclo[3.3.1]non-7-en-2-ol	21.765	4.19
15	Methoxyacetic acid, 2-tetradecyl ester	22.384	0.87
16	2-Pentadecanone, 6,10,14-trimethyl	23.535	3.68
17	Spiro[3,5-dioxatricyclo[6.3.0.0(2,7)]undecan-6-one-4,2'-cyclohexane], 9,11-dihydroxy-1'-isopropyl-2,4'-dimethyl-	24.566	7.39
18	Cyclohexanone, 2-(2-nitro-2-propenyl)-	24.966	2.74
19	Hexadecanoic acid, methyl ester	25.86	4.36
20	n-Hexadecanoic acid	26.741	3.01
21	(1R,4aS,6R,8aS)-8a,9,9-Trimethyl-1,2,4a,5,6,7,8,8a-octahydro-1,6-methanonaphthalen-1-ol	28.242	1.1
22	Isoambreinolide	30.292	11.49
23	Radiatin	32.386	2.85
24	Vitexifolin D	35.349	2.65
25	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1- en-2-yl) octahydronaphthalen-1(2H)-one	37.031	4.12

Table 4: GC-MS analysis of *Vitex negundo* leaf extract (Methanol extract)

Peak	Name	Retention time	Area %
1	Undecane	6.158	0.95
2	Tricyclo[4.2.2.0(1,5)]decan-7-ol	11.926	0.66
3	6-Methyl-cyclodec-5-enol	12.891	1.00
4	4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-	14.094	28.50
5	2,4-Di-tert-butylphenol	15.998	2.12
6	Karanjin	16.493	5.26
7	Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl-	17.313	0.98
8	Octadecane, 6-methyl-	18.001	1.81
9	2-Oxa-7-thiatricyclo[4.4.0.0(3,8)]decane	21.755	0.69
10	Tetradecane, 2,6,10-trimethyl-	22.388	0.18
11	Neophytadiene	23.381	1.17
12	7-Chloro-4-methoxy-3-methylquinoline	24.437	4.94
13	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	25.679	1.18
14	Hexadecanoic acid, methyl ester	25.859	4.00
15	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	26.335	2.29
16	n-Hexadecanoic acid	26.796	14.73
17	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl]cyclopropyl]methyl]-, methyl ester	30.155	1.86
18	Phytol	30.409	2.66
19	Heptadecanoic acid, 16-methyl-, methyl ester	30.738	2.84
20	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	30.931	5.91
21	Octadecanoic acid	31.433	2.04
22	Nalbuphine	41.959	13.23

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Authors' Contribution

Study concept and design: P.S.S. and M.B.H.
Identification the plant sample: J.M.
Analysis and interpretation of data: P.S.S.
Cell line studies: V.M.K.
Drafting of the manuscript: P.S.S
Critical revision of the manuscript: M.B.H.

Ethics
We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest
No potential conflict of interest was reported by the author(s).

Data Availability
All data generated or analysed during this study are included in this published article.

References

1. Vishwanathan AS, Basavaraju R. A review on *Vitex negundo* L.: A medicinally important plant. Eur J Biol Sci. 2010;3(1):30-42.
2. Gill BS, Mehra R, Navgeet G, Kumar S. *Vitex negundo* and its medicinal value. Mol. Biol. Rep. 2018;45(6):2925-34.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49.
4. Atallah GA, Abd. Aziz NH, Teik CK, Shafiee MN, Kampan NC. New predictive biomarkers for ovarian cancer. Diagnostics. 2021;11(3):465.

5. Siegel R, Naishadham D, Jemal A. Cancer statistics for Hispanics/ Latinos, 2012. CA Cancer J Clin. 2012;62(5):283-298.
6. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A, Siegel RL. Ovarian cancer statistics, 2018. CA Cancer J Clin. 2018;68(4):284-296.
7. Bast Jr RC, Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. Nat. Rev. Cancer. 2009;9(6):415-428.
8. Wang X, Wang E, Kavanagh JJ, Freedman RS. Ovarian cancer, the coagulation pathway, and inflammation. J. Transl. Med. 2005; 3:1-20.
9. Holmes CE, Levis JE, Ornstein DL. Activated platelets enhance ovarian cancer cell invasion in a cellular model of metastasis. Clin. Exp. Metastasis. 2009; 26:653-661.
10. Deepti K, Umadevi P, Vijayalakshmi G. Antimicrobial activity and phytochemical analysis of *Morinda tinctoria* Roxb. leaf extracts. Asian Pac. J. Trop. Biomed. 2012; 2(3):S1440-1442.
11. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin- ciocalteu reagent. Methods Enzymol. 1999; 299:152–178.
12. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. Sci. World J. 2020; 23-25.
13. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997;2(4):152-159.
14. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phospho-molybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 1999; 269(2): 337–341.
15. Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. Diet. 1986;44(6):307-15.
16. Kumbar VM, Muddapur UM, Bhat KG, Shwetha HR, Kugaji MS, Peram MR, Dindawar S. Cancer stem cell traits in tumor spheres derived from primary laryngeal carcinoma cell lines. Contemp. Clin. Dent. 2021; 12(3): 247.
17. Shoeb M. Anticancer agents from medicinal plants. Bangladesh J. Pharmacol. 2006 1(2): 35–41.
18. Ben-Arye E, Lavie O, Heyl W, Ramondetta L, Berman T, Samuels N. Integrative Medicine for Ovarian Cancer. Curr. Oncol. Rep. 2023; 1-10.
19. Hada M, Edin ML, Hartge P, Lih FB, Wentzensen N, Zeldin DC, Trabert B. Prediagnostic Serum Levels of Fatty Acid Metabolites and Risk of Ovarian Cancer in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial Serum Fatty Acid Metabolites and Ovarian Cancer Risk. Cancer Epidemiol Biomarkers Prev. 2019; 28(1): 189-197.
20. Tiwari OP, Tripathi YB. Antioxidant properties of different fractions of *Vitex negundo* Linn. Food Chem. 2007; 100(3): 1170-76.
21. Meng X, Mu F, Zhang Z, Wu X, Gao T, Zhao F, Xue J, Duan Y, Zhang H. Cytotoxic, clonal fidelity, and antioxidant evaluation of in vitro propagated *Vitex negundo* var. cannabifolia. Plant Cell Tissue Organ Cult. 2021; 146: 473-82.
22. Dewade DR, Christina AJ, Chidambaranathan N, Bhajipale NS, Tekade NP. Antitumor activity of *Vitex negundo* Linn. against Dalton's Ascitic Lymphoma. Int. J. Pharmtech Res. 2010; 2(2):1101-4.
23. Liu N, Wang KS, Qi M, Zhou YJ, Zeng GY, Tao J, Zhou JD, Zhang JL, Chen X, Peng C. Vitexin compound 1, a novel extraction from a Chinese herb, suppresses melanoma cell growth through DNA damage by increasing ROS levels. J. Exp. Clin. Cancer Res. 2018; 37:1-6.
24. Yun Z, Chinnathambi A, Alharbi SA, Jin Z. Biosynthesis of gold nanoparticles using *Vitex negundo* and evaluation of pro-apoptotic effect on human gastric cancer cell lines. J. Photochem. Photobiol. B, Biol. 2020; 203:111749.
25. Van Vo G, Nguyen TP, Nguyen HT, Nguyen TT. In silico and in vitro studies on the anti-cancer activity of artemetin, vitexicarpin and penduletin compounds from *Vitex negundo*. SPJ. 2022; 30(9):1301-14.
26. Qiu C, Tong L, Yuan T, Wang F, Zhao F, Chen L. Constituents from Vitex negundo var. heterophylla and their inhibition of nitric oxide production. J. Nat. Med. 2017; 71: 292-298.
27. Meena AK, Perumal A, Kumar N, Singh R, Ilavarasan R, Srikanth N, Dhiman KS. Studies on physicochemical, phytochemicals, chromatographic profiling & estimation and in-silico study of Negundoside in roots & small branches of *Vitex Negundo* plant. Phytomed. Plus. 2022; 2(1): 100205.
28. Chirumamilla P, Dharavath SB, Taduri S. GC–MS profiling and antibacterial activity of *Solanum khasianum* leaf and root extracts. BNRC. 2022; 46(1): 127.
29. Singh A, Bhatt G, Gujre N, Mitra S, Swaminathan R, Limaye AM, Rangan L. Karanjin. Phytochemistry. 2021; 183: 112641.
30. de Moraes J, de Oliveira RN, Costa JP, Junior AL, de Sousa DP, Freitas RM, Allegretti SM, Pinto PL. Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease Schistosomiasis mansoni. PLOS Negl. Trop. Dis. 2014; 8(1): e2617.