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Vitex negundo L. leaf extract suppresses the cell cycle and promotes apoptosis in colon cancer cells

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Colon cancer has become a frequent malignancy worldwide. *Vitex negundo* (*V. negundo* L.), is an herb used in traditional medicine to treat many kinds of diseases. This study investigated the molecular mechanism involved in colon cancer prevention using human colon cancer (HCT-116) cell lines with methanolic leaf extract of *V. negundo* L. DPPH and NOX radical scavenging properties, cell cycle analyses, Annexin V-FITC/PI, MTT assay, and qRT-PCR were utilized to determine *Vitex negundo's* impact on antioxidant properties, cytotoxicity, cell cycle and apoptosis in HCT-116 cells. Our results revealed that *V. negundo* L. exhibited strong DPPH and nitric oxide radical scavenging properties in a dose-related manner. Additionally, the extract inhibits cell growth in a way that is dependent on both time and dose. The IC_{50} was found to be 54 µg/mL. Annexin V-FITC/PI confirmed apoptosis induction. Furthermore, the cell cycle studies demonstrated that the arrest occurred during the G2/M phase. The essential appliance of G2/M arrest is believed to be related to CDK1, survivin and cyclin B1 gene downregulation, as confirmed by qRT-PCR. In conclusion, our findings show that *V. negundo* L. extract has excellent antioxidant properties, suppresses the cell cycle, and promotes apoptosis.

Keywords: Antioxidant activity, Colorectal cancer, HCT116-colon cancer cells, *Vitex negundo* L.

The second most common type is Colorectal cancer (CRC), with an estimated 3.2 million new cancer cases expected by 2040[1]. CRC is the 2nd most common cause of death from cancer in the United States and the ninth leading cause in India[2]. In underdeveloped countries, about ten percent of CRC deaths occur. The carcinogenic effects of numerous treatments are assumed to be raising the occurrence rates among people. In this issue, there is currently no specific medicine available to treat CRC more effectively[3,4]. Even with the significant advances made in the development of medicines for the treatment of cancer, much work needed. Because of their antioxidant, pro-apoptotic, anti-metastatic, and anti-proliferative qualities, a number of natural substances have demonstrated the ability to modify cellular signaling pathways. Over two-thirds of human malignancies may be preventable *via* lifestyle and dietary changes[5]. Moreover, various food nutrients are rich in vitamins, minerals, fiber, flavonoids, and phenolic compounds assist in promoting health and cure a variety of disorders[6]. Furthermore, phenolic compounds have anticancer and antioxidant properties that could be exploited to build natural anticancer medicines for treating CRC.

The large, fragrant shrub *Vitex negundo* L., sometimes called the Chinese chaste tree, grows in large quantities in China, India and other south asian countries. In traditional medicine, it is frequently used, especially in Southeast Asia[7]. Antimicrobial, anti-inflammatory, anticancer, antioxidant, and antiulcer activities can be found in almost every portion of this plant[8]. According to phytochemical research, the plant contains 30% volatile oil, 10% flavonoids and phenolic chemicals, 8% glycosides, and other ash content[9].

The antioxidant, antibacterial, and anticancer properties of *V. negundo* L. leaf extracts have been widely researched using *in vitro* studies[10]. Badgujar *et al*. described anti-cancer and antioxidant activity using human cancer cell lines, these findings

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indicating a possible action against oxidative damage due to its radical scavenging activity of DPPH, which suggests the ability to inhibit cancer cell lines[11]. Studies using *V. negundo* L. leaf extract revealed potent antioxidant and anti-inflammatory effects. Several small compounds extracted from *V. negundo* L. induced apoptosis and act as anti-cancer, anti-bacterial, and antidiabetic actions[12]. Evn-50, a chemical derived from *V. negundo* L. seeds, inhibits cell proliferation and has anti-cancer action in pancreatic, colon, ovarian, and breast cancer cell lines[13]. In MAPK and AKT signaling pathways, it also inhibits ERK1/2. The lignin compounds VB1 and VB2 from Evn-50 could stop cancer cells from entering the G2/M phase and promote apoptosis, revealing a unique mechanism of lignin anti-cancer action[14,15]. Phytochemicals use a variety of ways to slow or prevent the spread of cancer, including reduced oxidative stress, decreased cell proliferation, induced programmed cell death, inhibited angiogenic activity, and cell cycle arrest. Studies revealed that many phytochemicals are in the pipeline as possible anticancer medicines[16]. When creating anticancer medications, blocking one or more proteins or processes contributing to cancer development is necessary[17].

Even though a lot of research has been done on *V. negundo's* pharmacological properties, no studies have been shown to check its anti-cancer properties. Our previous studies identified 12 major compounds by GC-MS analysis. These phytocompounds including, 1,4-DI-O-Benzylidene-DL-ribitol,Dibenz [a,c]cycloheptan-7-amine,1,2,3-trimethoxy,4-(3-methylanilino)-4-oxobutanoic acid, Ethyl (E,2S)-2-[3-[tert-butyl(dimethyl)silyl] oxypropyl]-2-cyano-7-phenylhept-3-enoate, 9-[4,5-dihydroxy-6-(hydroxymethyl) oxan-2-yl]-1H-purin-6-one, alpha-Ethynyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenemethanol,4-Tert-Octylphenol, 3-hydroxy-5-methyl-2-propan-2-ylcyclohexa-2,5-diene-1,4-dione, Ethyl4-(3,7,12-trihydroxy-10,13dimethyl2,3,4,5,6,7,8,9,11,12, 14,15,16,17tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate, (E)-5-(5,5,8a-trimethyl-2-methylidene3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl)-3-methylpent-2-enoicacid and 2-(4a-methyl-8-methylidene1,2,3,4, 5,6,7,8a-octahydronaphthalen-2-yl)propan-2-ol. All these compounds showed anti-cancer properties based on our molecular docking studies[18]. We assume that these compounds synergistically could inhibit colon cancer cell (HCT116) proliferation, indicating that this is a promising therapeutic agent for CRC treatment.

Materials and Methods

Chemicals and Reagents

Dimethyl sulfoxide (95%), Methanol (99.8%), Dulbecco's Phosphate buffer saline (Dulbecco's PBS), Foetal Bovine Serum (FBS), Apoptosis Detection Kit (KTA0002), 0.25% Trypsin-EDTA solution-HiMedia, MTT reagent, Annexin V-AbFlourTM 488, Propidium Iodide (PI), and 1x PBS - HiMedia were obtained from MP Biomedicals, Germany. 70% ethanol, PI/RNase staining solution, BD Biosciences, 2,2-diphenyl-1-picrylhydrazyn (DPPH), Trizol reagent was procured from Sigma (St Louis, USA).

Extract preparation

The leaves of *V. negundo* L. were taken from the University of Agriculture Sciences, GKVK, Bangalore, Karnataka, India. A taxonomist from the University of Agricultural Sciences, GKVK, Bangalore, identified and certified the plant species. For *V. negundo* L., a voucher specimen with catalog number 4853 was submitted to the same department's herbarium. The methanolic extract was prepared using a rota evaporator. We used 2g of methanol extract dissolved at 10 mg/mL in sterile MilliQ water for the biological studies[19].

In vitro assays

The extract was subjected to qualitative phytochemical screening for tannins, alkaloids, phenols, terpenoids, glycosides, flavonoids, and saponins, as described previously[19]. Total polyphenols, flavonoids, and tannin content were also analyzed, as described earlier[20]. Using the once-established standardized procedures, a leaf extract from *V. negundo* L. was demonstrated to have a radical-scavenging effect[21]. By calculating the percentage of inhibition and measuring the drop in absorbance at 517 nm, it was possible to assess the ability to scavenge the stable free radical DPPH. For determining the amount of nitric oxide (NOx) produced by sodium nitroprusside, the Griess reaction was utilized[22]. The inhibition rate was determined through determining the absorbance of the chromophore produced by diazotizing nitrite with sulphanilamide and then combining with naphthalene diamine at 546 nm.

Cell culture

All commonly used drugs and chemicals (analytical grade) were bought from Himedia, Mumbai, India, and Sigma Chemical Co., St Louis, USA. The HCT116 cell lines were cultivated in

DMEM with High Glucose supplemented with 10% (*v/v*) FBS after being acquired from the National Centre for Cell Sciences (NCCS), Pune. Cisplatin was used as a reference drug for growth inhibition studies[23].

Cytotoxicity assay

Cytotoxicity assay was completed as defined[24,25]. HCT-116 cells were trypsinized and pelleted down at 300 x g in a 5 mL centrifuge tube grown in T-25 flasks. 10,000 cells were added to the original number per 200 µL of media, and then the plate had been incubated at 37°C with 5% CO_2 for a duration of 24 h. The remaining medium had been aspirated after a 24-h period. Individual wells were filled with 200 µL of various test drug concentrations (12.5, 25, 50, 100, 250 µg/mL). The media that contained drugs was aspirational when the plate was withdrawn from the incubator, and 10% after adding MTT reagent to each well until the final concentration reached 0.5 mg/mL, the mixture was incubated for three h at 37°C with 5% CO_2 in the atmosphere. To solubilize the formazan produced, the plate was carefully stirred in a shaker with 100 µL of solubilization solution (DMSO). The absorbance between 570 and 630 nm was measured using a microplate reader. The background and blank were subtracted to find the percentage growth inhibition. Utilizing the dose-response curve, the calculation the quantity of extract needed to 50% (IC_{50}) inhibit cell growth. To determine the percentage of cell viability, the following equation was used:

$$\text{Cell viability (\%)} = \frac{\text{Mean OD of the Sample}}{\text{Mean OD of the Blank}} \text{ x } 100$$

Apoptosis assay

Apoptosis assay was achieved as labeled earlier[26]. Based on the cell viability assay results, the most efficient concentration of the methanolic extract was used to analyze apoptosis in the HCT-116 cell line. Briefly, HCT-116 cells after being cultivated on a 6-well plate at a density of 1.5 x 10^5 cells/mL, the cells were cultured in a CO_2 incubator for a full day at 37°C, and then washed with 1 mL 1X PBS. Cells were incubated for 12-16 h with 54 µg/mL extract or 50 µg/mL cisplatin. One well had been left untreated as a negative control. The 1X PBS was replaced with 200 µL of trypsin-EDTA solution and cultured for three to four min at 37°C, followed by centrifugation for five min at 25°C at 300 x g. The centrifugation step was repeated twice with 1X PBS to wash cells. Finally, the cells were suspended at a concentration of 1 x 10^6 cells/mL in 1X Binding Buffer. To 100 µL of the cell suspension (1 x 10^5 cells), 5 µL of AbFlour 488 Annexin V was added and incubated at room temperature (25°C) for 15 min in the dark, then by 2 µL of propidium iodide and 400 µL of 1X binding buffer was added to respectivetubes. After gentle vortexing, cells underwent flow cytometry (Beckman Coulter, USA).

Cell cycle analysis

In a 6-well plate, HCT-116 cells were cultivated at a density of 1.5 x 10^5 cells/mL. The following day, the cells were cleaned with 1 mL of 1X PBS and then cells were treated with 54 µg/mL leaf extract and 50 µg/mL cisplatin, followed by incubation for 24 h in 2 mL culture media. one of the wells remained untreated as a negative control. Cells were stable in 70% cold ethanol and vortexed to avoid clumps. Cells were washed twice with 1xPBS, followed by 400 µL of PI-RNase solution, mixed thoroughly, and maintained for five to ten min at room temperature. They were then subjected to flow cytometry analysis as described previously[27].

qRT-PCR

Total RNA was extracted using a Trizol reagent from both treated and untreated HCT-116 cells in accordance with the manufacturer's instructions. The creation of cDNA required one microgram of total RNA. SsoFast EvaGreen Supermix (Bio-Rad, CA, USA) was used for qRT-PCR, and each reaction was processed using a Qiagen Rotor-Gene 6000 real-time PCR apparatus. The reference gene in this case was 18S ribosomal RNA (18S rRNA). Table 3 contains a list of the primers utilized in the target gene amplification. The fold change was computed using the $2^{-\Delta\Delta Ct}$ technique.

Statistical Analysis

To examine the data, SPSS version 2 was used (Chicago, IL, USA) and one-way ANOVA. All statistical comparisons were regarded as statistically important ($P< 0.05$) among groups, and the data is shown as the mean ± standard deviation of three separate studies.

Results

Qualitative examination revealed the occurrence of saponins, terpenes, glycosides, alkaloids, and reducing sugars found in methanolic extracts of *V. negundo* L. leaf extracts, as shown in (Table 1).

Total phenolics, flavonoids, and tannins were detected in substantial levels in *V. negundo* L. leaf extracts, as exposed in (Fig. 1). With increasing concentration (5-25 µg/mL), the percentage of DPPH and nitric oxide radical scavenging activities increased (Fig. 1A). The maximum scavenging activity was recorded at 25 µg/mL (Fig. 1B & C). Butylated hydroxytoluene (BHT) was used as a positive control. (++) medium measure favorable response was received for the extract's chemical group. (+++) The positively charged group in the extract received a favorable response in larger quantity. (−) The organic group in the extract yielded a negative response.

Increasing quantities of *V. negundo* L. extract were applied to HCT-116 cells in order to evaluate the extract's impact on the growth of cancer cells. (0, 12.5, 25, 50, 100, and 250 µg/mL) for 24 h, and the images were presented in (Fig. 2A). The dosage reaction curve was utilized to obtain the IC_{50} values. The outcomes demonstrated that the *V. negundo* L. extract reduced cell viability as shown in (Fig. 2B), with an IC_{50} of 54 µg/mL in the HCT-116 cell line. Consequently, the concentration of 54 µg/mL of *V. negundo* L. extract was selected for further investigation.

The extract's proapoptotic activity was evaluated using an Annexin V-FITC/PI double labeling evaluation on HCT-116 cell lines treated with 54 µg/mL of *V. negundo* L. extract for 24 h. The rate of Annexin V-positive cells showed that applying the *V. negundo* L. extract to cell lines significantly increased the quantity of apoptotic cells, as presented in (Fig. 3). Compared to the control, the rates of early and late apoptosis in HCT-116 treated cells were 35.4 and 16.6 percent. However, the treated HCT-116 cells exhibited significantly greater apoptotic rates, suggesting early-stage apoptosis.

The impact of the *V. negundo* L. extract on the advancement of the cell cycle was evaluated using flow cytometry in HCT-116 cells. Compared to its controls, the *V. negundo* L. extract induced cell cycle arrest in G2/M in HTC-116 cells and the data presented in (Fig. 4). HTC-116 cells showed a significant rise in the sub-G1 population, comprised of G0/G1 cells with damaged DNA. This shows cancer cell division suppression and a G2/M cell cycle arrest and the data was presented in (Table 2). Cisplatin-treated cells also showed a G2/M stage arrest, confirming the induction of apoptosis after 24 h.

HCT-116 cells treated with *V.negundo* L. and Cisplatin drug. Flow cytometry was utilized to evaluate the measurement of cells distributed in various cell cycle phases. The information was displayed as the mean ± SD from three individual studies, as explained in the methods. A $P< 0.05$ is reflection about as a statistically significant.

Table 1 — Qualitative analysis of *V. negundo* L. leaf extract

Constituents	Test	Methanol
Terpenes	Liebermann-Buchard	+++
Flavonoids	Ferric chloride test	++
Saponins	Frothing	++
Glycosides	Brown-ring	+++
Alkaloids	Dragendorff's test	+++
Tannins	lead acetate test	+
Phenolic compounds	Shinoda test	+++
Reducing sugars	Fehling	++
Carbohydrates	Naphthol	++
Amino acids	Ninhydrin	--

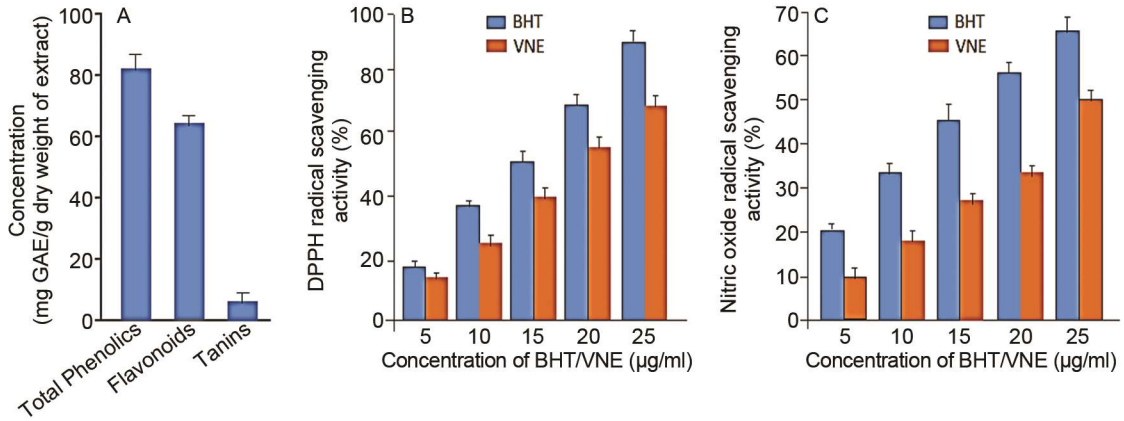


Fig. 1 — (A) Total phenolics, flavonoids, and tannin content in *V. negundo* L. extract (VNE) were determined; and (B & C) represent nitric oxide and $DPPH^+$ radical scavenging properties of *V. negundo* L. extract. The data is from three independent experiments explained in the methods. The data is shown as mean ± SD. A $P< 0.05$ is considered as a statistically significant. BHT: Butylated hydroxy toluene

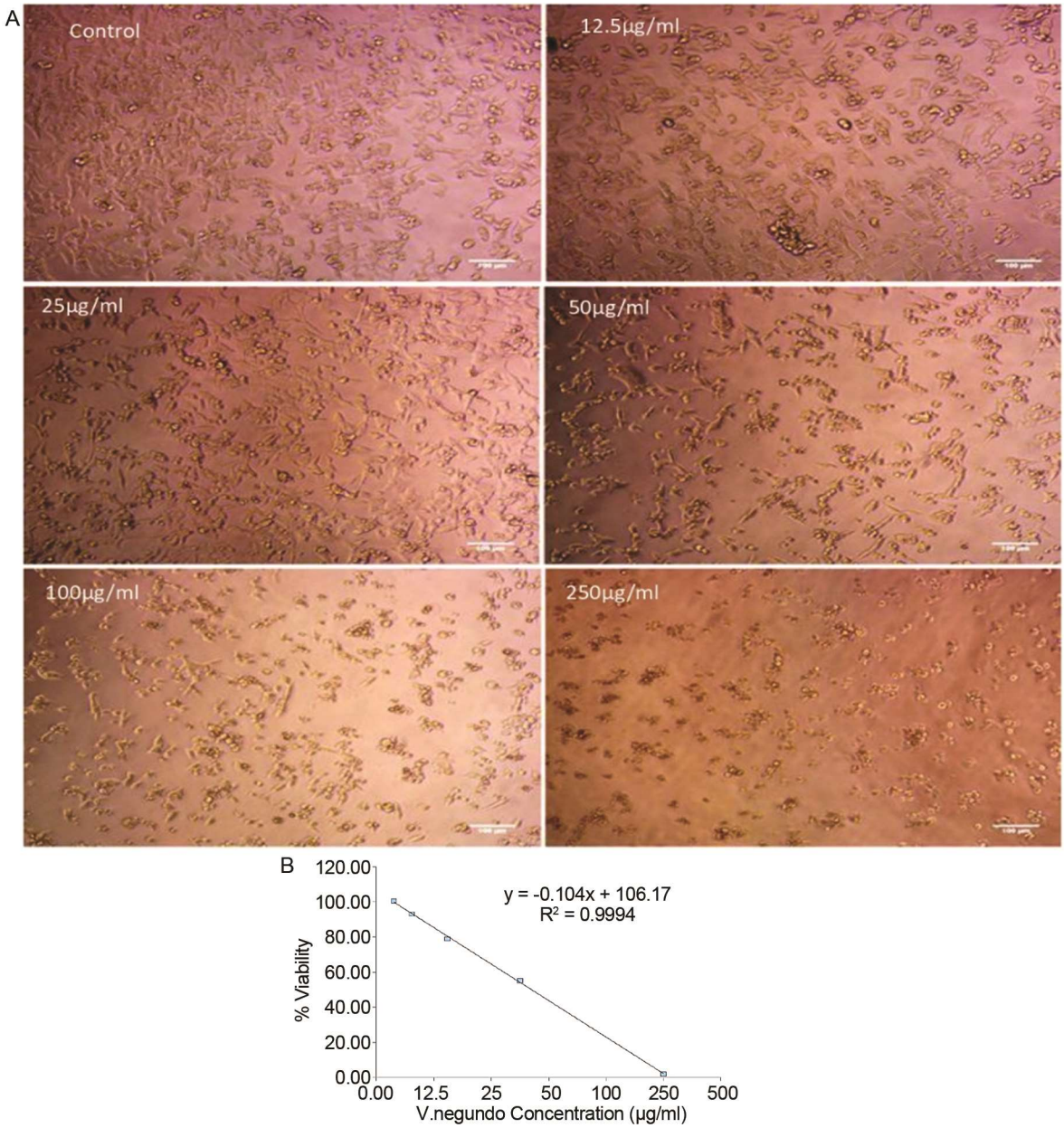
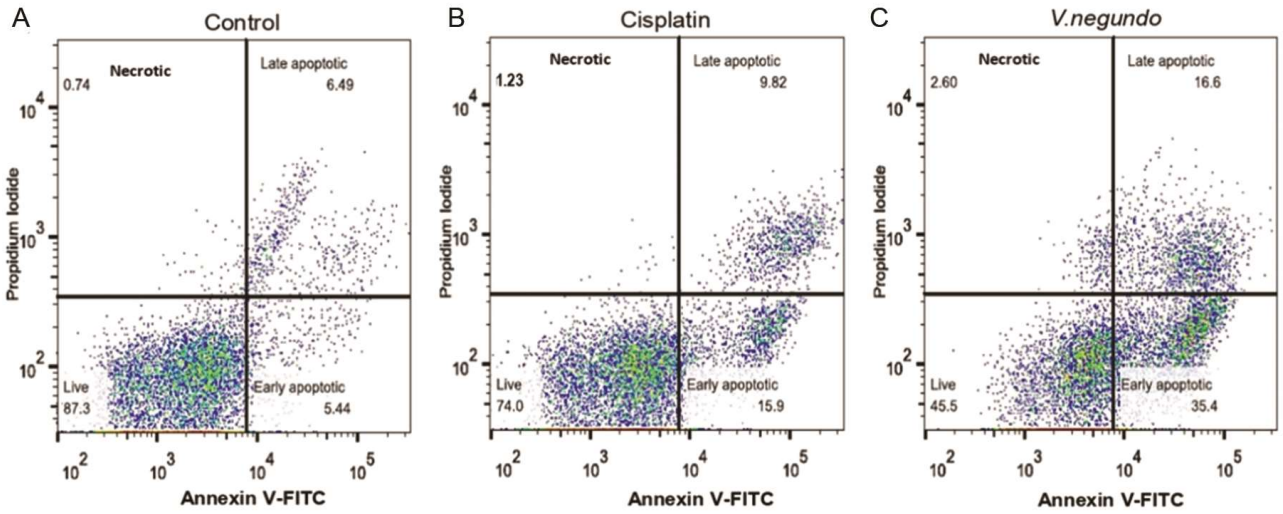


Fig. 2 — Cytotoxicity effect of *V. negundo* L. methanolic leaf extract in HCT-116 cell lines. (A) images of the untreated and treated cells for 24 h in different *V. negundo* L. concentrations; and (B) Influence of *V. negundo* L. leaf extract on cell viability. The data is from three independent experiments that are detailed in the section on techniques, the data is displayed as mean ± SD. A $P< 0.05$ is considered as a statistically significant

qRT-PCR was used to check the expression of several genes controlling the evaluation of cell death (Survivin) and G2/M transition (Cyclin B and Cdk1) for the exploration of the molecular pathways behind apoptotic activation and G2/M arrest. Cyclin B, survivin and Cdk1 gene expression levels were considerably reduced in the *V. negundo* L. extract-treated HTC-116 cell lines compared to control counterparts as shown in (Fig. 5 and Table 3).

Discussion

Colon cancer is becoming more common and severe all over the world, gene alterations and microsatellite instability cause the appearance of colorectal cancer[28]. Cancer is now treated using immunotherapy, surgery, chemotherapy, radiotherapy, and anticancer drugs, all of which have been demonstrated to be inefficient due to side effects such as drug resistance, recurrence, and impact on non-



Sample	% of Cells			
	Live	Necrotic	Early Apoptotic	Late Apoptotic
Control	87.3 ± 5.3	0.74 ± 0.05	5.44 ± 0.2	6.49 ± 0.3
Cisplatin Treated	74 ± 7.6	1.23 ± 0.03	15.9 ± 1.3	9.82 ± 0.4
V.Negundo -Treated	45.5 ± 6.1	2.6 ± 0.08	35.4 ± 2.2	16.6 ± 1.5

Fig. 3 — Using flow cytometry, the amount of necrosis and apoptosis was evaluated in the HCT-116 cell line utilizing Annexin V/PI staining; 54 µg/mL of *V. negundo* L. leaf extract was administered to the treatment groups, while the control group received no treatment at all. The data from three separate independent experiments was displayed as mean ± SD described in the methods section. Considered statistically significant is a p-value less than $P< 0.05$

targeted cells, among other toxicity concerns[29]. People seeking alternative medicines to treat cancers more effectively without side effects. One such alternative medicine is herbal medicine, which is rich in phytochemicals. Phytochemicals are less toxic and more accessible to extract than synthetic drugs, making them more effective anticancer therapies[30]. Many phytochemical components are found in *V. negundo* L. and they exhibit anticancer capabilities[31,32].

Phytochemicals like flavonoids, phenolic compounds, steroids, and terpenoids in *V .negundo* leaf extracts have been well documented[33,34]. Moreover, significant amounts of total phenolics, flavonoids, and tannins have been identified. The extract was found to include both phenolic and non-phenolic components by GC-MS analysis[18,35]. Molecular docking studies were performed to test the inhibitory activity of these compounds against WNT-signalling proteins (APC, AXIN, β-catenin, GSK-3β and DSH),which play major role in colorectal cancer progression. Our in silico studies revealed that six compounds were exhibited strong inhibitory activity[18]. However, synergetic effects of these compounds under *in vitro* or *in vivo* conditions were not investigated. Hence, the present investigation aimed to understand underlying molecular mechanisms and their synergetic effect using HCT116 cellline. Increased oxidative stress is triggered by free radicals or reactive oxygen species (ROS), such as superoxide, hydroxy ethyl, and hydroxy radicals. All these radicals have a high probability of fast reactions with proteins and lipids, leading to the peroxidation of lipids and proteins. Scavenging of free radicals may be one of the significant mechanisms of protection in the current investigation, as evidenced by the dose-dependent *in vitro* scavenging of DPPH and nitric oxide radicals by *V. negundo* L. leaf extract. Phenolic chemicals like flavonoids play significant role in oxidative stress prevention. These compounds demonstrated antioxidant action by chelating metal ions or the presence of functioning hydroxyl groups scavenges free radicals. Metal ion chelation may be critical in preventing free ion production, which affects target biomolecules.

The various bioactive substances that are present in plants may be responsible for their activity and account for the differences in the anticancer efficacy

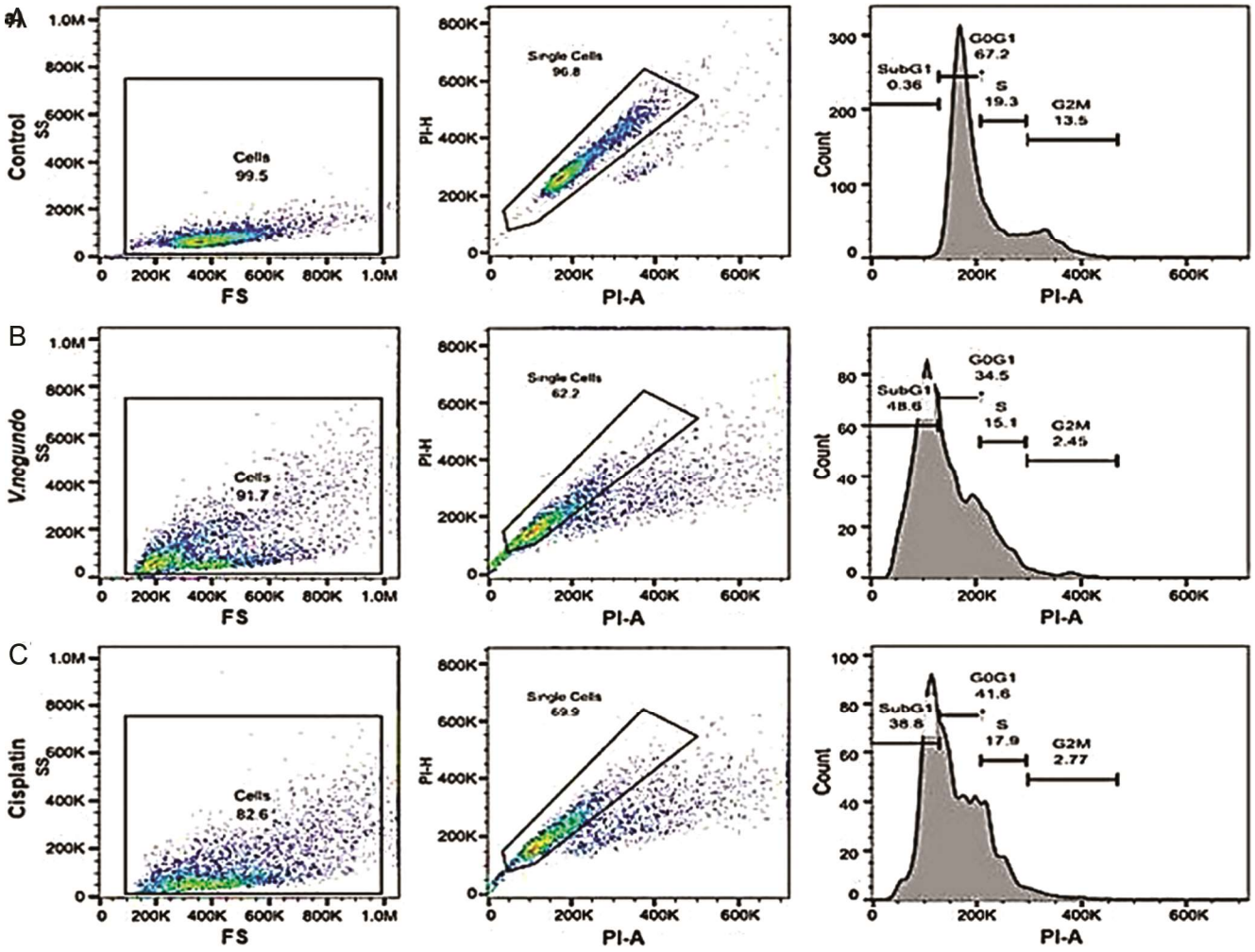


Fig. 4 —Effect of *V. negundo* (54 µg/mL) and Cisplatin (50 µg/mL) on cell cycle analysis in HCT-116 cancer cells. Assess the cell-cycle distribution utilizing flow cytometry following propidium iodide staining of the cells. Three separate experiments' equivalent of data were combined and given as mean ± SD in the methods section. A $P< 0.05$ is regarded as having statistical significance

Table 2 — Effect of *V. negundo* L. on cell cycle in HCT-116 cancer cells

S. No.	Sample Name	% of cells in different phases of the cell cycle			
		SubG1	G0/G1	S	G2/M
1	Control	0.36 ± 0.02	67.2 ± 5.1	19.3 ± 1.5	13.5 ± 1.8
2	Cisplatin treated	38.8 ± 2.7	41.6 ± 4.4	17.9 ± 2.4	2.77 ± 0.3
2	*V. negundo* L. treated	48.6 ± 4.2	34.5 ± 4.6	15.1 ± 3.2	2.45 ± 0.3

of medicinal plants. The cytotoxicity of *V. negundo* L. leaf extract on colon cancer cells and the precise mechanism of action were not investigated. Many anticancer medicines produced from plants have been beneficial in treating various malignancies[36,18]. Several molecular reactions and interactions happen between flavonoids, phenolic compounds, enzymes, and proteins, making them toxic to cells or slowing cell proliferation. Several studies have found that the cytotoxicity and anticancer activities of natural plants are attributable to the presence of flavonoid compounds, which play an essential role in chemoprevention by influencing signal transduction and cell angiogenesis[38]. The MTT assay shows that cells treated with *V. negundo* L. extract misplace their capacity to growth in a time and dose-dependent manner, which could be due to cell death promotion. Based on the determined IC_{50} with the highest inhibitory effect, a dose of 54 µg/mL for 24 h was selected for further examination. Polyphenolic

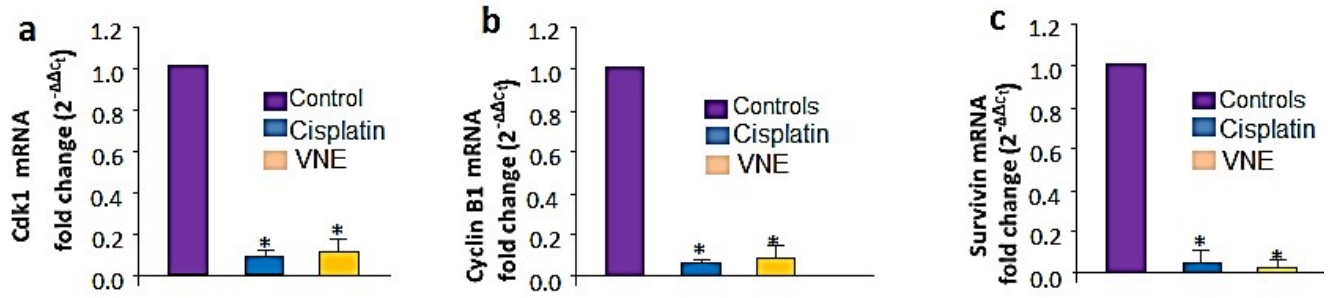


Fig. 5 —Effects of the *V. negundo* L. HCT-116 cancer cell lines' expressions of Cdk1, Cyclin B1, and Survivin are examined in an extract. For a duration of 24 h, cells were subjected to either the extract at 54 µg/mL or the vehicle (0.1% DMSO). The Cyclin B1, Cdk1, and Survivin gene expression relative fold regulation by qRT-PCR, **P*< 0.01. (VNE) *V. negundo* L. leaf extract

Table 3 — List of primers used for the quantitative real-time PCR

Genes	Forward (5′-3′)	Reverse (5′-3′)
Survivin	5′ACCGCATCTCTACATTCAAG3′	5′CAAGTCTGGCTCGTTCTC3′
CDK1	5′CAGACTAGAAAGTGAAGAGGAAGG3′	5′ACTGACCAGGAGGGATAGAA3′
Cyclin B1	5′AAGAGCTTTAAACTTTGGTCTGGG3′	5′GTTTGTAAGTCCTTGATTTACCATG3′
18S rRNA	5′TCAGATACCGTCGTAGTTCCG3′	5′CAGCTTTGCAACCATACTCCC3′

chemicals play a significant role in cancer prevention. Previous research found that methanolic extracts of *V. negundo* L. contained more levels of polyphenolic chemicals[39]. The observed cytotoxicity could be attributed to polyphenolic chemicals found in *V. negundo* L. extract.

The Annexin V-FITC/PI test was utilized for determining if cell death mediated by *V. negundo* L. extract was caused by apoptotic activity. This approach detects early apoptosis by monitoring the phospholipid serine translocation from the cell membrane's inner to outer layer[40]. The concentration of Annexin V-FITC, which binds to the transmitted phosphatidylserine, was measured in order to determine the quantity of apoptotic cells. To distinguish between viable, early apoptotic, late apoptotic, and necrotic cells, propidium iodide was used. Flow cytometry examination of HCT-116 cells demonstrated that treatment with *V. negundo* L. extract shifted the cell population toward apoptosis. The HCT-116 cells were treated for 24 h with 54 µg/mL of *V. negundo* L. extract. The late and early apoptotic rates were substantially higher than the controls. These findings show that *V. negundo* L. extract can activate apoptotic signaling proteins. Previous research has also shown that extracts, rather than individual chemicals, synergize apoptosis[42]. A positive control, cisplatinwas used at 50 µg/mL and it was shownsignificant increase in apoptotic activity. When the quantity of early apoptotic cells increases, so does the quantity of PI-negative and Annexin V-positive cells with AbFlour 488. Conversely, when the quantity of late apoptotic/necrotic cells increases, so do the numbers of PI-positive and Annexin V-positive cells with AbFlour 488. The extract's substantial impact of proapoptotic agents on HCT-116 cells could be attributed to its high amount of phenolic compounds, which have several biological features relating to their molecular formula, such as antioxidant capacity.

The cell cycle analysis was used to understand the molecular processes underpinning the proapoptotic and cytotoxic effects observed. Studies on the cell cycle revealed that the extract can stop HCT-116 cell lines' cell cycle at the G2/M phase. The cell cycle is mainly regulated by checkpoints, which ensure that cells advance correctly through the cell cycle. When DNA is broken, the cell cannot enter mitosis because of the G2/M checkpoint[43]. As a result of this, the expression of the three genes, Cdk1 based on their involvement in cell cycle arrest at the G2/M phase, cyclin B1 and survivin were studied. Apoptosis suppression, promotion of cell proliferation, and angiogenesis are all linked to survivin up-regulation in the G2/M phase of the cell cycle. Additionally, during the premitotic phase, survivin binds to spindle microtubules, enabling tumor cells to evade identification during the G2/M phase of the cell cycle. Cyclin B1 has been linked to carcinogenesis and the advancement of cancer. It is crucial to the cell cycle's

migration from the G2 to the M phase. Moreover, the Cdk1/cyclin B1 complex controls the G2 to M phase transition after interacting with the cyclin-dependent kinase 1 (Cdk1), cyclin B1, originally Cdc2[44]. The results of the qRT-PCR confirmed the significant downregulation of the extract's proapoptotic activity, and the influence of the *V. negundo* L. extract on the expression of the mRNAs for cyclin B, Cdk1, and survivin accounts for the observed cell cycle arrest.

Conclusion

Our findings show that the presence of phytochemicals in *V. negundo* L. extract exhibits effective cytotoxic action on HCT-116 cells. One of the processes by which herbal medicines demonstrate increased antioxidant activity is most likely their ability to scavenge free radicals. Furthermore, the phytocompounds synergistically caused apoptosis, inhibited the G2/M stage of the cell cycle, and promoted cell death. Finally, our data suggest that *V. negundo* L. could be used to treat colon cancer as a possible therapeutic drug.

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Conflict of interest

All authors declare no conflicts of interest.

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