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Some Cytogenetic Potentials of Vitex negundo Methanolic Extract on MCF-7 Cell Line

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ABSTRACT

Breast cancer has become a common malignancy in most parts of the world. *Vitex negundo* has been used as a traditional remedy for many diseases. This study investigated the molecular mechanism of breast cancer prevention using breast cancer cell line MCF-7. In this study, an overview of the widely used *Vitex negundo* plant and its alcoholic, Gas chromatography-mass spectrometry (GC-MS) was used to assess the extract, cell cycle assay and apoptosis by high content screening assay. in GC-MS. The biologically active phytochemical components of the alcoholic extract of the plant were examined. A GC-MS study showed that 27 chemical substances were present. Analysis of the MCF-7 cell life cycle revealed that the plant's alcoholic extract stopped the cell cycle in a concentration-dependent way. Three concentrations—200, 100, and 50 g/ml—were employed. In the highest concentration the cancer cell life cycle was inhibited at sub-G2, whose value increased from 0.56 to 76.8. Thus, the extract prevented the cells from entering the G2 phase, whose value in untreated cells was 22.1 and became 3.51 after treatment with the alcoholic extract. Apoptosis by High-content screening provided an overview of the effects of the alcoholic extract on the cell by measuring a number of parameters. Different concentrations of the extract were tested (200,100,50 and 25 µg/ml) and the results showed that the highest concentration compared to each viable cell count's untreated cells (VCC) (2222.5±38.8, 3411±162.6), nuclear intensity (NI)(577±11.3, 435±21.2), membrane permeability (CMP)(138.5±26.1 ,95±7.0), mitochondrial membrane permeability (MMP)(361±12.0, 249±33.9), and cytochrome c (Cc)(673.5±10.6, 494±19.7).

Keywords: *Vitex negundo, Cytogenetic, Apoptosis, Alcoholic extract.*

1. INTRODUCTION

Cancer is one of the foremost common maladies within the world and is the moment driving cause of passing after cardiovascular and heart illnesses in Iraq. Breast cancer is one of the foremost common sorts of cancer among ladies and speaks to the most noteworthy rate of dangerous tumors until 2018. It is the most common type, with an incidence of 54% [1].

Breast cancer is the foremost common sort of cancer among women and is the foremost common cause of passing in both creating and created nations.. Multiple treatments are used to prevent the development of this type of disease, but the death rate is still high, as it represents 22.9% of all types of cancer in women [2].

Advance has been made in creating techniques for treating cancer, but cancer is still the driving cause of passing worldwide [3]. In the United States, breast cancer is also the most common cancer among women, and each year about 32% of cancer cases are breast cancer [4]. *Vitex negundo* is a large aromatic tree called the Chinese chaste tree. It grows in large quantities in China, India and South Asia. It is, utilized in conventional pharmaceutical, particularly in South and East Asia, because of the antioxidant and anti-ulcer activities in every part of this plant [5]. This plant has gained importance due to its content of many phytochemicals that extricated from the clears out, seeds and roots within the frame of unstable oils. The plant components in it are flavonoids, lignans, steroids, polyphenolic compounds, terpenoids, glycosidic iridoids, and alkaloids [6]. Human cancer cell lines were used to test the efficacy of *V. negundo* plant extracts and found to have anticancer, antioxidant and antimicrobial effects [7]. Many compounds extracted from this plant were tested and found to be effective in stimulating programmed cell death anti-cancer and anti-diabetic, the lignan EVn-50, prevents the proliferation of cancer cells in breast, ovarian, pancreatic and colon cancer lines [8]. The lignin compounds VB1 and VB2 of EVn-50 can prevent cancer cells from entering the G2 phase and thus promote apoptosis, revealing a unique mechanism of lignin's anticancer action [9]. Phytochemicals use several pathways to reduce the growth of cancer cells and prevent their spread. These pathways include reducing oxidative stress, stimulating programmed cell death, reducing cell proliferation, inhibiting vascular activity, and stopping the cell cycle [10].

2. MATERIAL AND METHODOLOGY

1) **Sample collection and diagnosis**
2) **Preparation of the aqueous and alcoholic extract**
3) **Gas Chromatography-mass spectrometry (GC-MS)**

Methods:-

1) Sample collection and diagnosis

Vitex negundo was collected from the local market in August 2024 and classified by Dr. Ibrahim S. Al-Jubouri, College of Pharmacy, Al- Mustansiriyah University.

2) Preparation of the aqueous and alcoholic extract

After drying, the leaves of the plant were pulverized, and 150 g of sample was loaded in thimble and extracted in 250 ml of distilled water for 4hours for aqueous extract, and methanol 80% for alcoholic extract using a Soxhlet apparatus. Then extracted successively by hot soxhlet extraction method. The extract solution was placed in the dishes at 37°C and incubated in the incubator [11]. The dried plant extract were stored inside sterilized tube in refrigerator:

3) Gas Chromatography-mass spectrometry (GC-MS)

Investigation was performed utilizing MSD and GC-MS QP 2020. Within the GC, a 30 ml x 0.25 D $ 0.25 m Rxi-5 Sil MS coordinates silica capillary column was utilized. The instrument temperature was at first set to 70 C° and kept up for 5 min. The oven temperature was increased to 300 C0 at the end of the experiment. Mass spectra of the components in the samples were acquired using a detector operating in scan mode between 40 and 650 z/m. The MS took 5 min to start and 51 min to complete, with a solvent break time of about 5 min [12].

3. EXPERIMENTAL WORK

Cell cycle assay

Breast cancer cell line MCF-7 from the College of Malacca, Staff of Medication, Office of Pharmacolggy, Middle for Investigate in Novel Therapeutics, Malaysia, was utilized The cell cycle of the MCF-7 cancer cell line was inspected utilizing the Cycle Test TM Also DNA Reagent pack.

a) Kit Contents

- Buffer Solution (3 vials, 50 ml each). Utilize this solution to gather or solidify cell suspensions.
- Solution A (10 ml): Utilize his solution for enzymatic separation to cut strong tissues and analyze cell layers and structures and store the solution at room temperature 20 C^0- 25C^0 (prepared to utilize).
- Solution B (8 ml): Utilize to inhibit trypsin movement and process and analyze RNA. Store the solution at 20C^0-25C^0 (prepared to utilize).
- Solution C (8 ml): This solution is for DNA binding at final concentrations of not less than 125 µg/ml. Store the solution away from light at a temperature between 2°C – 8°C.

b) Cell Preparation

Cells were seeded at a thickness of 5 × 10^5 cells per well in 12-well plates. The plates were brooded at 37 C^0 and 5% CO_2 for 24 h. After hatching, the medium was evacuated and the cells were treated with the substances at concentrations of 200, 100 and 50 µg/ml for 24 h to look at the cell life cycle. The taking after steps were followed to get the cell suspension:

- The culture medium was removed and the cells were washed utilizing Phosphate buffer arrangement.
- 2 to 3 ml of trypsin/horsein solution was included to the cell. The vessel was rearranged to cover the monolayer totally with tender blending. Incubated at 37C^0 for 1 to 2 minutes, until the cells were withdrawn from the vessel.
- RPMI culture medium (15-20 ml) was included as the cells were withdrawn from the conjugation surface interior the culture medium using a pipette.
- The cell suspension was put within the tube numbered 17 × 100 mm.
- The cells were centrifuged for 5 minutes at room temperature.
- The filtrate was isolated from the accelerate and 1 ml of Buffer arrangement was included.
- The cells were centrifuged for 5 minutes at room temperature.

- The past two steps were rehashed. After centrifugation, the filtrate was isolated and the accelerate was re-suspended in 1 ml of buffer arrangement.
- Cells were tallied employing a hemocytometer and the cell concentration was balanced to 1.0 x 106 cells/ml with buffer arrangement to be prepared for recoloring and examination utilizing Flowcytometry.

c) Staining protocol

- Centrifuge the cell suspension at 400 rpm for 5 min at room temperature (20°C - 25°C).
- Expel the filtrate and include 250 µl of arrangement A (trypsin arrangement) to each tube. Blend the tube tenderly by tapping it by hand.
- Brood the blend for 10 min at room temperature.
- Include 200 µl of arrangement B to all tubes with delicate shaking by tapping the tube by hand.
- Hatch for 10 min at room temperature.
- Include 200 µl of cold arrangement C (2°C-8ºC) (P| recolor arrangement) to each tube with tender shaking.
- Brood the blend for 10 min within the fridge (2°C-8ºC).
- At last, the mixture was sifted employing a 50 um nylon work into a unused sterile tube for investigation utilizing Flowcytometry.

Apoptosis by High Content Screening (HCS) assay

- Cytotoxicity test was performed to degree five parameters of alcoholic extricate *in vitro*.
- The parameters utilized for cytotoxicity test were:
- Practical cell check (VCC), Add up to Atomic Concentrated (TNI), Cell Film Penetrability (CMP), Mitochondrial Film Potential (MMP) and Cytochrome c discharge. The parameters were measured utilizing Tall Substance Screening analyzer (Thermo Logical), Cluster Filter XTi. Therapeutic Items and Home grown Medications Inquire about Center / Division of pharmacy / Staff of Pharmaceutical / College of Malaksa

a) Kit content

- Primary Antibody of Cytochrome c (75 µL).
- Dye Light™ 649 Conjugated Goat Anti-Mouse IgG (75 uL).
- Potential Color of Mitochondrial Layer (1 Pack).
- Color of Porousness (25 HL).
- Hoechst color (30 L)
- Wash Buffer (10)
- Permeabilization Buffer (10X Dulbecco's PBS with 1% Triton® X-100) (100ml)
- Blocking Buffer (10X) (85 mL)
- Tinny Plate Seal Gathering (7 Packs).

b) Cell preparation

When the cancer cells shaped a streaming monolayer within the culture medium, the culture medium was depleted and the cell layer was washed twice with PBS. The cells were collected after including 2-3 ml of trypsin-EDTA arrangement and incubated at 37°C for a number of minutes until the cells withdrawn. The cells were weakened to 7.5×10^4 cells/ml^{-1} in RPMI medium. 100 ul of the cell suspension was included to each well of a 96-well plate. The cells were brooded overnight at 37C° in 5% CO_2. At that point, the cells were treated with 25, 50, 100, 200 µg/ml of the plant alcoholic extract and incubated for 24 h at 37C0 and 5% CO_2.

c) High Content Screening Protocol (HCS)

- After incibation, cell staining solution (50 pl) was added to each well. Cells were incubated at 37 °C for 30 min.
- Gently with draw the culture medium and staining solution and add 100 ul of fixation solution, incubate for 20 min at room temperature.
- Gently aspirate the fixation solution and add 100 ul of wash solution X1.

- Remove the wash solution and add 1X permeabilization buffer. Incubate for 10 min at room temperature.
- Remove the permeabilization buffer and wash the plate twice with 100 ul of wash buffer X1.
- Withdraw the wash solution and add 100 ul of 1X blocking buffer and incubate for 15 min at room temperature.
- Blocking buffer was omitted and 50 ul of primary antibody solution was added. The plate was incubated for 60 min protected from light at room temperature.
- Primary antibody solution was removed and the plate was washed three times with 100 Ml 1X wash buffer.
- Wash buffer was removed and 50 ul of secondary antibody/stain solution was added Incubated for 60 min at room temperature.
- Antibody/stain solution was removed and washed three times with 100 ul IX wash buffer.
- 100 ul 1X wash buffer was added.

Statistical Analysis

Factual investigations of the information were calculated utilizing Chart and Crystal form 6 Chart Cushion, CA, Program Inc, and La Jolla) where statistical data and arithmetic differences were expressed mean ± standard error. Also, ANOVA and Duncan analysis were used, and significant differences were identified statistically and morally ($p \leq 0.05$).

4. RESULT AND DISCUSSION

Identification of the alcoholic extract of Vitex negundo leaves by GC-mass analysis

The biologically active phytochemicals of the alcoholic extract were identified using GC-MS, a direct and rapid analysis technique for identifying compounds, characterized by its sensitivity and specificity. The investigation of the compounds was carried out for the alcoholic extricate of Vitex negundo takes off. The instrument utilized to character the dynamic compounds appeared in Table (1) and Figure (1) is based on the top zone, maintenance time, atomic weight, atomic equation and structural form of the compounds with biological activity for the plant. 27 chemical compounds have been identified in the *Vitex negundo* plant.

Pentanamide, Methyl formate, Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl) , Eucalyptol, 2-Nonene (E), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, trans-Ocimenol, 4-Octen-3-one, Terpinyl formate, 1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E) , Phthalic acid, Benzoic acid, p-tert-butyl, tau.-Cadinol, Tetradecanoic acid, 4-Pyrrolidin-1-yl-benzene-1,3-diol, p-Toluic acid, TMS derivative, n-Hexadecanoic acid, Phenol, 2-(1,1-dimethylethyl)-4-(1,1,3,3-tetramethylbutyl) , Myrcenyl acetate, Epilupeol; 20(29)-Lupen-3alpha-ol, acetate (isomer 1), Lupeol, 7-Hexadecyn-1-ol, Octadecanoic acid, 1H-Indene, 2-[(Dimethylamino)methylidene]cycloheptan-1-one,2,4-Di-t-butyladamantane-2,4-diol , 2-Heptanone.

These compounds, their chemical formulas, their structural formulas, as well as other criteria such as retention time, molecular weight, and peak area are shown in Table (1) as follows:

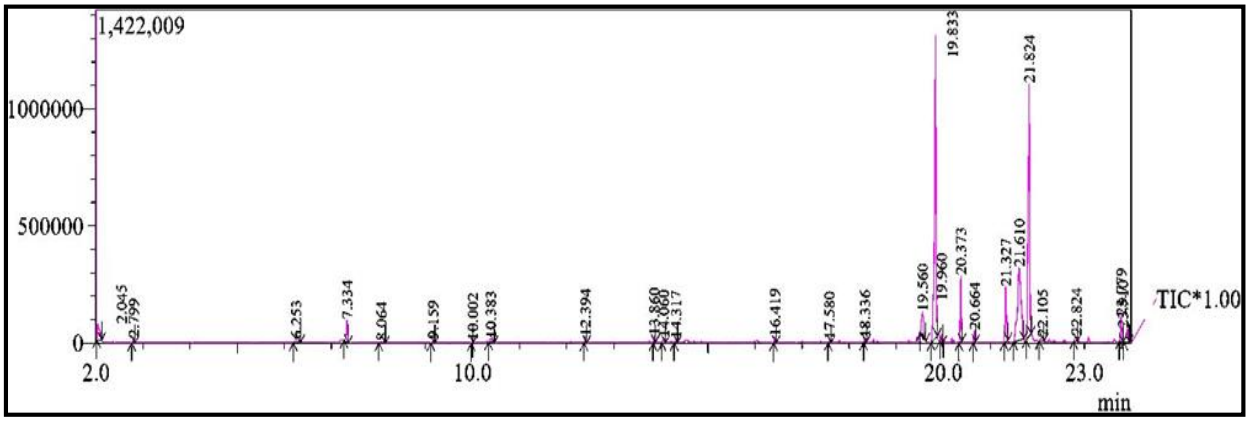


Fig.No.1: GC-mass Chromatogram of *Vitex negundo*.

Table.No.1: Main chemical components of methanolic extract of *Vitex Negundo* leaves.

Peak Area	Molecular Weight	Molecular Formula	Compound Name	Retention Time	Peak NO.
%2.18	101	$C_5H_{11}NO$	Pentanamide	2.045	1
0.40%	91	$C_3H_9NO_2$	-2Amino-1,3-propanediol	2.799	2
0.87%	136	$C_{10}H_{16}$	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl(	6.253	3
1.83%	154	$C_{10}H_{18}O$	Eucalyptol	7.334	4
0.29%	126	C_9H_{18}	-Nonene (E)2	8.064	5
0.29%	144	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	9.159	6
0.11%	154	$C_{10}H_{18}O$	trans-Ocimenol	10.002	7
0.37%	126	$C_8H_{14}O$	-4:Octen-3-one	10.383	8
0.39%	182	$C_{11}H_{18}O_2$	Terpinyl formate	12.394	9
0.46%	204	$C_{15}H_{24}$	-1,3,6,10:Dodecatetraene, 3,7,11-trimethyl-, (Z,E)	13.860	10
0.44%	262	$C_{15}H_{18}O_4$	Phthalic acid	14.060	11
0.37%	178	$C_{11}H_{14}O_2$	Benzoic acid	14.317	12
0.43%	222	$C_{15}H_{26}O$	.tau.-Cadinol	16.419	13
0.20%	228	$C_{14}H_{28}O_2$	Tetradecanoic acid	17.580	14
0.24%	179	$C_{10}H_{13}NO_2$	-4Pyrrolidin-1-yl-	18.336	15
3.12%	208	$C_{11}H_{16}O_2Si$	p-Toluic acid	19.560	16
29.55%	256	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	19.833	17
0.53%	262	$C_{18}H_{30}O$	Phenol	19.960	18
4.90%	332	$C_{22}H_{36}O_2$	5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate	20.373	19
0.93%	468	$C_{32}H_{52}O_2$	Epilupeol	20.664	20
4.67%	440	$C_{31}H_{52}O$	Lupeol	21.327	21
16.63%	238	$C_{16}H_{30}O$	-7Hexadecyn-1-ol	21.610	22
25.67%	284	$C_{18}H_{36}O_2$	Octadecanoic acid	21.824	23
0.55%	208	$C_{15}H_{28}$	1H-Indene	22.105	24
0.61%	167	$C_{10}H_{17}NO$	)]-2Dimethylamino)methylidene]cycloheptan-1-one	22.824	25
2.33%	280	$C_{18}H_{32}O_2$	-2,4Di-t-butyladamantane-2,4-dio	23.779	26
1.63%	224	$C_{13}H_{20}O_3$	2-Heptanone	23.910	27

The table indicates that hexadecanoid acid has the highest peak area, followed by octadecanoic acid, then to 7-Hexadecyn-1-ol , In a previous study, it was reported that octadecadienoic corrosive, hexadecanoic corrosive and methyl ester are the most components within the methanolic extricate of the clears out. Octadecadenoic corrosive constituted 21.93%. This plant can be used to treat many diseases and is an important source for developing treatments and research [13]. Octadecadenoic

acid induces the death of breast cancer mesenchymal stem cells by decreasing the expression of C-MYc. [14]. Hexadecanoic acid, also known as palmitic acid, has picked up consideration for its helpful properties.

Pharmacological considers have appeared that this corrosive shows noteworthy anti-inflammatory, antioxidant, and immunomodulatory impacts. In later a long time, hexadecanoic corrosive has risen as an anticancer operator with demonstrated adequacy against different malignancies counting gastric cancer, liver cancer, cervical cancer, breast cancer, and colorectal cancer. Its antitumor impacts incorporate actuating apoptosis in cancer cells, repressing cancer cell expansion, stifling metastasis and attack, upgrading affectability to chemotherapy, and progressing safe work. The most anticancer instrument of palmitic corrosive (Dad) includes actuating apoptosis through the mitochondrial pathway, encouraged by upgrading the generation of intracellular receptive oxygen species (ROS)

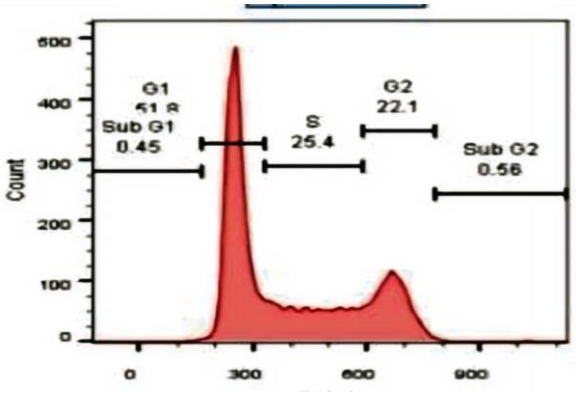
[15]. The nearness of Torunol Acetate, which could be a subsidiary of terpenoid compounds.

Terpenoids are one of the foremost critical metabolites in Vitex and chemically, they contain five carbon isoprene units, shaping monoterpenes (five carbon iotas), hemiterpenes (C5), sesquiterpenes (fifteen carbon iotas), diterpenes (twenty carbon iotas), and triterpenes (thirty carbon particles).. Pharmacologically, this class of active compounds has shown antioxidant, antimicrobial, anti-inflammatory, and antitumor properties [16].

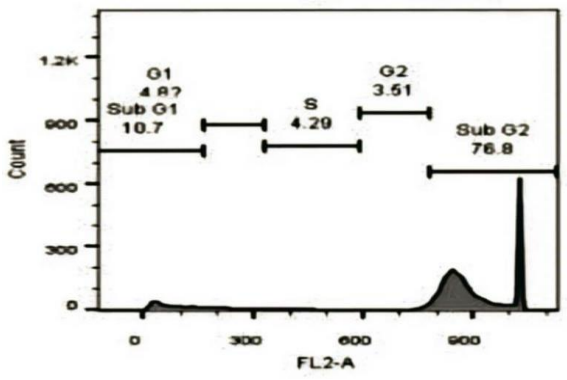
Cell cycle assay on MCF-7:

The impact of the alcoholic extricate of V. negund on breast cancer cell line MCF-7 was assessed utilizing flow cytometry measuring cells distributed in different stages of the cell cycle for 24 h and compared to their controls.

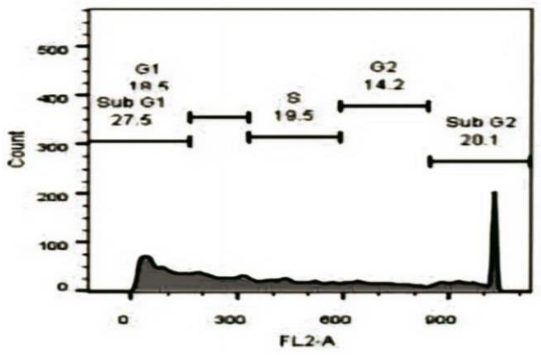
The relative distribution of cells in each of the Gap (G1), synthesis (S) and Gap (G2) phases is shown in Table (2) and Figure (2) after treating them with the alcoholic extract of *V. negundo* for 24 hours. At the lowest concentration of 50, the result was that the G1 value decreased from 51.8 to 18.5 at the while sub G1 value, which increased from 0.45 to 27.5. Likewise, the G2 value decreased from 22.1 to 14.2 at the expense of G2, whose value increased from 0.56 to 20. While at the concentration of 100, the extract was more effective. The G1 and S values decreased significantly as compared with control value, increased from 22.1 to 60.4. There was also an increase in the values of both sub G1 and sub G2. The increase in these values indicates that the cell is preparing to stop and enter the programmed cell death stage. At the concentration of 200, the extract was more effective and the highest value was sub G2, which reached 76.8 at the expense of G1, S and G2. The percentage of MCF-7 cells programmed to apoptosis depended on the concentration of the extract, increasing with increasing concentration as compared to the control group.



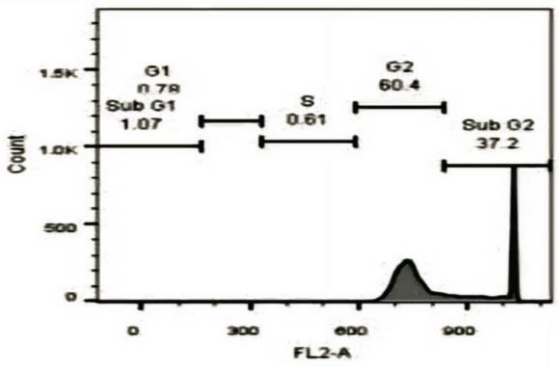
Control



Treatment 200 µg/ml



Treatment 50 µg/ml



Treatment 100 µg/ml

Fig.No.2: Alcoholic extricate of *Vitex negundo* demonstrate the rate of cells totally different stages of cell cycle.

Table.No.2: Relative distribution of cells in different stages of MCF-7 cell cycle treatment with V.negundo alcoholic extract.

Concentration (µg/mL)	Mean distribution % ± SD				
	Sub G_1	G1	S	Sub G_2	G2
Untreated	0.45±0.01	51.8±8.36	25.5±4.31	0.56±0.02	22.1±1.78
200	10.7±1.02[a]	4.8±1.12[a]	4.2±1.05[a]	76.8±8.32[c]	3.5±1.02[a]
100	1.7+0.98[a]	0.78+0.14	0.61+0.16[c]	37.2±3.54[c]	60.4+5.70[d]
50	27.5+2.32[b]	18.5+2.21[c]	19.5+3.13[b]	20.1+1.99[b]	14.2+3.12[b]

The anticancer effects of the alcoholic extract are based on its ability to inhibit the activity of MCF-7 as well as the hepatocellular carcinoma lineage by arresting the cell cycle within the G2 stage. This capture was related with expanded papsphorylation of histone 3 at ser15 and Cdk1 phosphorylation at Tyr15, in addition to diminish within the expression of Cdc25c and the expression of cyclin B1. This activity is attributed to the pure lignan VB1 and Evn-50, which is a mixture of lignan compounds present in the alcoholic extract of *V. negundo* [17].

Apoptosis by High Content Screening assay:

High-content assay for monitoring programmed cell death this assay is a predictive assay as it monitors different and free toxicities within the same cell. It covers a wide range of impacts conjointly gives a quantitative degree of numerous data related to harmfulness. In this manner, the cytotoxicity of the alcoholic extricate of the plant was assessed in MCF-7 cells after 24 hours utilizing distinctive concentrations of the extricate. In this examination, the alcoholic extract of the plant was used to detect changes in MCF-7 viable cell count (VCC), nuclear intensity (NI), membrane permeability (CMP), mitochondrial membrane permeability (MMP), and cytochrome c (Cc).

Table.No. 3: Cytotoxic effect of alcoholic extract on MCF-7 cells on multicellular parameters HCS.

Conc. (µg/ml)	HCS Paramter (mean±SD)				
	VCC	TNI	CMP	MMP	CC
	mean ±SD	mean ±SD	mean ±SD	mean ±SD	mean ±SD
untreated	3411±162.6[a]	435±21.2[a]	95±7.0[a]	249±33.9[a]	494±19.7[a]
200	2222.5±38.8[b]	577±11.3[b]	138.5±26.1[b]	361.5±12.0[b]	673.5±10.6
100	2838±299.8[c]	481±5.6[c]	125.5±34.6[b]	281.5±12.0[a]	542.5±7.7[c]
50	3501±35.3[a]	444±8.4[a]	92.5±10.6	275±19.7[a]	511.5±4.9[a]
25	3447±41.0[a]	434.5±6.3[a]	85.5±20.5	264±8.4[a]	512.5±3.5[a]

VCC: Viable Cell Count; TNI: Total Nuclear Intensity; MMP: Mitochondrial Membrane Potential; CMP: Cell Membrane Permeability; Cc: Cytochrome C. indicate significant at $p \leq 0.05$.

Viable Cell Count (VCC)

Results in Table (3) and Figure (3) that the practical check of MCF-7 cells was altogether diminished when treated with 200 and 100 µg/ mL (2222.5±38.8, 2838±299.8) separately as compared with untreated cells (3411±162.6), whereas no critical contrasts of MCF-7 cells in VCC when treated with 50 and 25 µg /mL (3501±35.3, 3447±41.0) respectively vs. untreated cell.

This result was a solid bolster for the cytotoxic potential of the alcoholic extricate on the MCF-7 cell line, as the decrease in cell number was dose-dependent and the foremost critical diminishment showed up at concentrations of 200 and 100 g/ ml. In a study, cytotoxicity was demonstrated for a liver cancer cell line, and this was related with a critical increment in lactate dehydrogenase (LDH) discharge in HepG2 cells. Moreover, caspase-3 actuation proposes that the watched cytotoxicity was intervened by the natural apoptotic pathway [18].

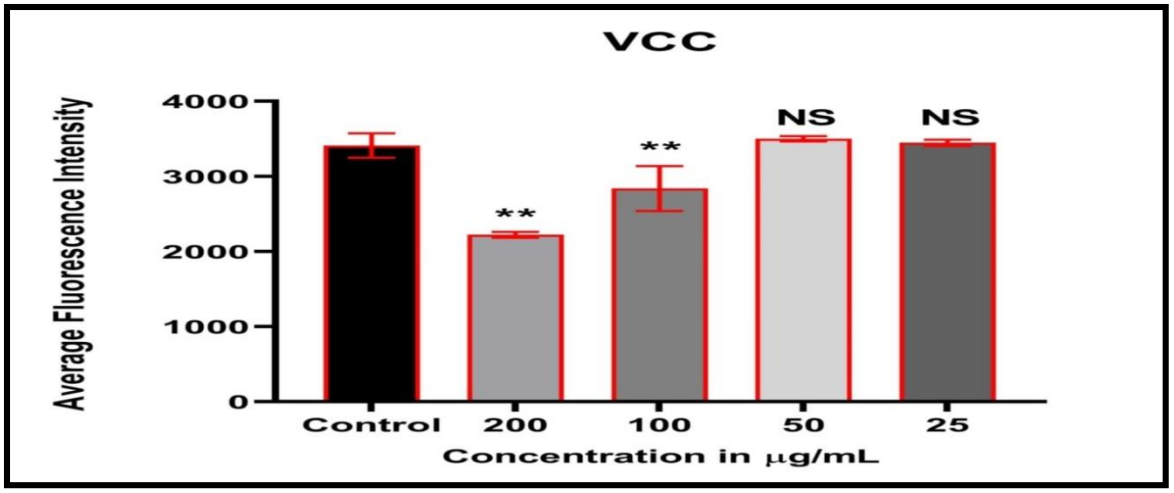


Fig.No. 3: Viable cell count of MCF-7 cells after 24 hr of exposure to the alcoholic extract. at 37ºC p ≤ 0.05, NS: Non-Significant n= 3.

Total Nuclear Intensity (TNI)

Treatment of MCF-7 cells with the alcoholic extricate at a concentration of 200 g/ mL driven to anincrease within the measure of the atomic thickness (577±11.3) contrast were critical gnd at a concentration of 100 g/mL (481±5.6) as appeared within the Table (3) and Figure (4) which is ascribed to atomic swelling recolor with Hoechst recolor as the cores show up to be for the most part condensed in reaction to treatment with the extricate compared to cells without treatment (435±21.2).

Flavonoid, contained in alcoholic extricates of the plant have anticancer exercises. These exercises may be related to inhibition,of cell expansion and grip, incitement of cell separation and attack, capture of the cancer cell cycle, and modified cell passing (19). *In vivo* studies show that flavonoids prevent cancer through their effects on the molecular stages of initiation, promotion, and progression [20]. To realize cancer-preventing or helpful impacts. Based on these comes about, flavonoids can be created as natural cancer-preventing chemotherapeutic agents [21].

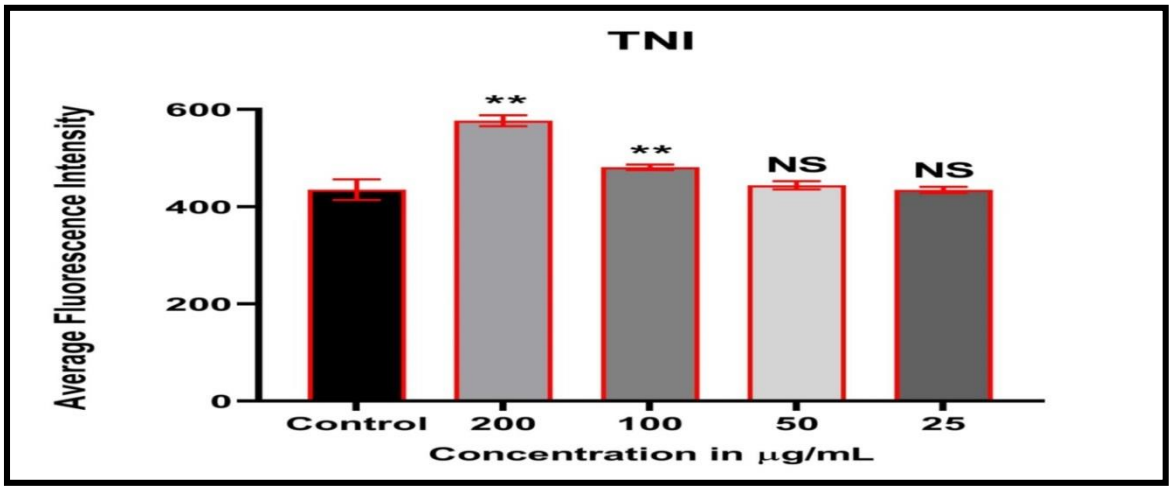


Fig.No. 4: Total nuclear density of MCF-7 cells after 24 hr of exposure to the alcoholic extract. at 37ºC p ≤ 0.05, NS: Non-Significant n= 3.

Cell Membrane Permeability(CMP)

Cell membrane permeability is associated with programmed cell death and cytotoxicity. A common phenotypic include of cytotoxicity is damage to the integrity of the cell membrane [22]. As shown in Table (3) Fig. (5). The permeability of the MCF **7**-cell membrane was affected at concentrations of 200 and 100 µg/ml (138.5± 26.1and 125.5±34.6). The differences were clear, but at the 50 and 25 µg/ml concentrations (85.5±20.5 and 92.5±10.6) when compared to the untreated groups (95 ±7.0).

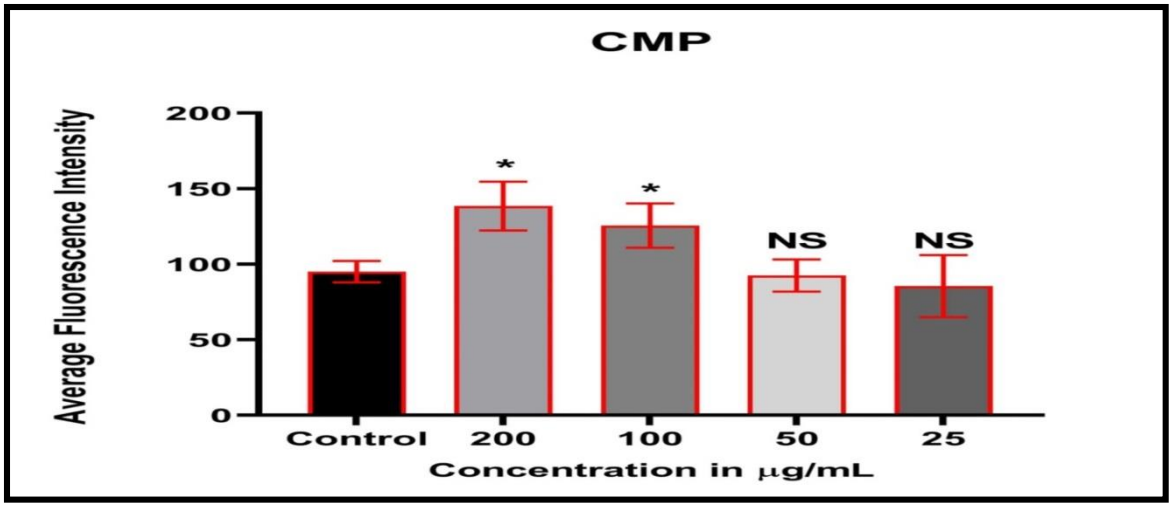


Fig.No. 5: Cell membrane permeability of MCF-7 after 24 hr of exposure to the alcoholic extract at 37ºC p ≤ 0.05, NS: Non-Significant n= 3.

Mitochondrial Membrane Potential (MPP)

Breast cancer cells MCF-7 appeared noteworthy contrasts when treated with a concentration of 200 g/mL of the extricate when compared to untreated cell (361.5±12.0, 249±33.9) whereas at concentrations of 100, 50, and 25 there were no significant differences. Comes about appeared that treatment with the alcoholic extract of *Vitex negundo* caused a noteworthy diminish in mitochondrial layer potential (MMP) in MCF-7 a concentration-dependent way, with the most greatest decrease occurring at a concentration of 200 µg/ml. This diminish shows impeded mitochondrial work, supporting the speculation that the extract invigorates the inborn pathway of apoptosis. Past considers have affirmed that the misfortune of MMP could be a basic step in actuating apoptosis in cancer cells .Since it is straightforwardly connected to the enactment of Bax/Bak proteins and expanded porousness of the mitochondrial layer, which leads to apoptosis [23].

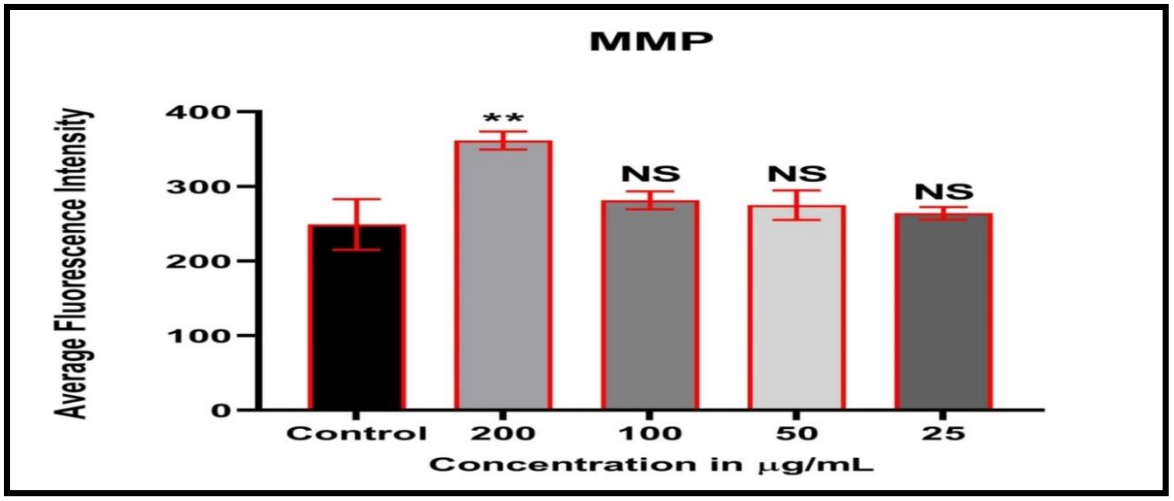


Fig.No. 6: Cell membrane permeability of MCF-7 after 24 hr of exposure to the alcoholic extract at 37ºC p≤0.05, NS: Non-Significant n= 3.

Cytochrome c Release

cytochrome- c was released when MCF-7 cells were treated with the extract was altogether expanded at the concentration of 200 ug/mL, which was (673.5±10.6) and at the concentration of 100 g/mL, it was (542.5±7.7) compared to untreated cells (494±19.7), while there was no statistically significant difference when treated with concentrations of 50 and 25 µg/mL (511.5±4.9 , 512.5±3.5) respectively, as shown in the Table (3) and the Fig. (7).Cytochrome- c may be a dissolvable protein that ties pitifully to the external surface of the inward film of mitochondria, where its release is linked to the conversion of electron transport to the formation of superoxide [24]. Discharge of cytochrome c into the cytosol invigorates the arrangement of the mitochondrial apoptosome, a high-molecular-weight complex that actuates caspases. Subsequently, the exchange of

cytochrome c to the cytosol is an occasion related with modified cell passing [25].These comes about give advance prove that apoptosis is mitochondria-dependent. The alcoholic extricate actuated apoptosis through actuation of caspases-8, -9 and -3/7, upregulation of Bax and downregulation of Bcl-2 protein [26].

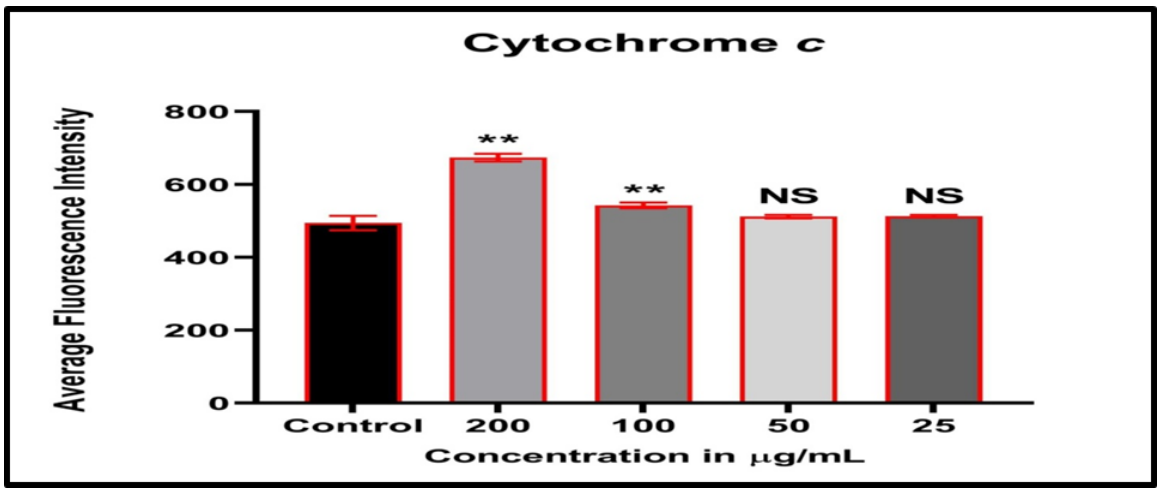


Fig.No. 7: Cytochrome c Release of MCF-7 after 24 hr of exposure to the alcoholic extract. at 37ºC $p \leq 0.05$, NS: Non-Significant n= 3.

5. CONCLUSION

The research results show that the presence of biologically active phytochemicals in the extract of *Vitex negundo* has a potent and toxic effect on MCF-7 cells. These chemical compounds inhibited the cell cycle at sub-G2 and prevented the cell from entering the G2 phase, thus inhibiting the cell cycle and promoting apoptosis. Finally the possibility of using *Vitex negundo* to treat breast cancer as a potential therapeutic drug.

CONFLICTS OF INTEREST: Authors have no conflicts of interest to declare.

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