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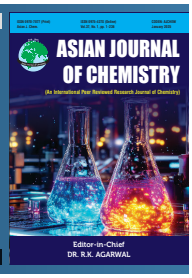

Isolation and Characterization of Anticancer Properties of Compounds from the Leaf Extracts of *Vitex negundo*

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Vitex negundo belongs to the family of Lamiaceae and is popularly known as sambhaloo or Chinese chaste tree. It is widely used as Indian medicinal shrub to prevent a variety of ailments including cancer. The aim of the study was to investigate the anticancer activity of solvent extracts and fractionates of *Vitex negundo* (VN) leaves against human lung carcinoma epithelial cells A549 by MTT assay. The leaves were extracted with various solvents by ultrasonic-assisted extraction method, in the sequel of non-polar to polar solvents from *n*-hexane, chloroform, ethyl acetate and ethanol:water (1:1). Among the four solvent extracts, the ethanol:water (1:1) extract has shown the significant anticancer activity (IC_{50} = 44.31 ± 0.61 at 5 to 300 µg/mL). Four components were isolated from ethanol:water (1:1) extract. The structure of these compounds were elucidated and identified as 4-OH benzoic acid (VN-EtOH+H_2O-P1), negundoside (VN-EtOH+H_2O-P2), isoorientin (VN-EtOH+H_2O-P3) and agnuside (VN-EtOH+H_2O-P4) by NMR, LCMS, FTIR and melting point. Among the four fractionated products, isoorientin showed the significant cytotoxic activity against A549 cells with IC_{50} value 18.50 ± 0.76 µM/mL. The standard drug, cisplatin IC_{50} values for anticancer activity of crude extracts (IC_{50} value 13.45 ± 0.084 µM/mL) were compared with that of pure compounds (IC_{50} value 14.27 ± 0.143 µM/mL). The bioactive compound, isoorientin (VN-EtOH+H_2O-P3) as well as the standard drug, doxorubicin treated with normal human dermal fibroblast (HDF) cells after 48 h, with an IC_{50} values of 416.57 ± 0.01µM/mL and 19.8 ± 0.01 µM/mL).

Keywords: Anticancer activity, *Vitex negundo*, MTT assay, Cytotoxicity, PREP-HPLC.

INTRODUCTION

Plants are of great interest in today's times as they are the important repositories of natural and miraculous molecules with significant pharmacological properties. These pharmacological properties such as antimicrobial, antioxidant, anticancer, anti-inflammatory, antidiabetic, *etc.* [1]. Nowadays, traditional medicines and pharmaceuticals hold limited impor-tance due to the differing response mechanisms of the body among individuals [2]. Thus, different researchers across the globe are concentrating on the exploration of novel molecules and drugs [3], which can be utilized effectively to combat the infections and different diseases being spread. The present study was performed to screen the solvent extracts for the anticancer activity of *Vitex negundo* [4], a non-native shrub [5] that blooms more effectively in sun. It has a loosely branched, in an open vase shape with interesting foliage. It can be found growing in the wastelands of the urban area of Telangana state, India and in mixed groves on hill slopes with elevations ranging from 600 to 6500 feet. The plant is known to have some medicinal and pharmacological properties, as revealed by local healers [6,7].

In search for the anticancer metabolites from the family of Lamiaceae [8], the *n*-hexane, chloroform, ethyl acetate and ethanol + water extracts (1:1) [9] of *Vitex negundo* were assayed on the A549 lung hypotriploid alveolar basal epithelial cell lines (human lung adenocarcinoma cell line) *in vitro*. One of the most prevalent cancer in the world is lung cancer. Lung cancer prevalence was higher in men (16.8%) than in women, according to the WHO survey reported in 2012 [10]. Currently, fewer than 18% of patients survive and 85% of patients had non-small cell lung cancer, comprising adenocarcinoma (40%), squamous cell carcinoma (25%), large cell carcinoma (10%) and patients with small cell lung cancer (10%).

Vitex negundo, also called a five-leaved chaste tree, is scientifically termed as *Vitex negundo* and commonly known as "Nallavalli" in Telugu and "Nallanochi" in Tamil language.

Nirgundi is widely used for pain management and treating headaches, inflammation, rheumatoid arthritis, bronchitis, spleen enlargement, cold, cough and fever. Nirgundi leaves, roots, flowers and bark are utilized in various herbal concoctions in the form of juices, pastes, powders and oils to cure disorders [11,12]. In the present study, four bioactive compounds were isolated from the leaves of *Vitex negundo* extract, and their anticancer activities against human lung carcinoma epithelial cells (A549) were investigated using the MTT assay. The isolated compounds were characterized by nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-mass spectrometry (LC-MS) analysis, and their structures were elucidated.

EXPERIMENTAL

The leaves of *Vitex negundo* (VN) were collected from The Keesara in the Medchal-Malkajgiri district of Telangana state, India. The plant was authenticated by Dr. Madhusudan Reddy, Assoc. Prof., Yogi Vemana University, Kadapa, and its authentication number is [Polathala (Kadapa) AMR4830]. The leaves were washed with tap water, shade dried and then the dried leaves were ground using a mixer grinder. The obtained powder was used for solvent extractions.

Solvents *viz.* hexane, chloroform, ethyl acetate and ethanol were purchased from SD Fine Chemicals, India. The chemicals including Dulbecco's Modified Eagle medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), trypsin, EDTA and phosphate buffered saline were procured from Sigma-Aldrich, USA. Gibco FBS was collected from clinically examined healthy animals under veterinary supervision, using the strictest aseptic collection techniques. Additionally, 25 mL and 75 mL flasks, along with 96-well plates, were purchased from Eppendorf India.

Preparation of solvent extracts: The powdered leaves of the plant material was kept under sonication (ultrasonic-assisted extraction) in different solvents namely *n*-hexane, chloroform, ethyl acetate and ethanol:water (1:1) [13] for 30 min (4 times/24 h) and followed by filtration. The filtrates obtained were concentrated using a rortary evaporator at 35 ºC under vaccum [14-17]. The liquid extracts were further dried to get extracts (5 to 300 µg/mL) and were screened for *in vitro* anticancer activity using the MTT assay.

Purification of solvent extracts: The extracts were further fractionated using gradient column purification (PREP-HPLC) with 0.1% formic acid in aqueous solution and acetonitrile applied sequentially for complete purification of the compounds. The purification was carried out over Kromosil C18 (25 × 150 mm, 7 µM) column with, 0.1% formic acid in water as the buffer and acetonitrile used as the eluents. The gradient elution was as follows: 10% acetonitrile for the first 1 min, increasing to 18% over 10 min, holding at 18% till 15 min and then increasing to 90% in 3 min, reaching 90% at 18 min. Four fractions were eluted between 10 min to 16 min, which were lyophilized [18] at 80 ºC. The obtained pure bioactive compounds were designated as (i) VN-ETOH+H_2O-P1, (ii) VN-ETOH+ H_2O-P2, (iii) VN-ETOH+H_2O-P3 and (iv) VN-ETOH+H_2O-P4 (Fig. 1). The purification was carried out on a Waters prep system with an fraction collector-III, modules 2489 UV-Vis wavelength detector, 2545 Binary pump and ChromoScope software.

Experimental procedures for identification

LC-MS analysis: The obtained bioactive compounds were analyzed by ultra performance liquid chromatography coupled with a single quadrupole (SQD) MS system [19]. An ACQUITY BEH C18, 2.1 mm × 50 mm, 1.7 µm column was used with 0.1% formic acid in water as mobile phase A (MP-A) and 0.1% formic acid in 100% acetonitrile as mobile phase B (MP-B). The gradient programme was as follows: time/% of B: 0/3, 0.4/3, 7.5/98, 9.5/98, 9.6/3, 10/3 was carried out using a diode array Max plot (Fig. 2).

NMR analysis: The ^1H, ^{13}C NMR, COSY, HSQC, HMBC and ROESY1D spectra of four bioactive molecules were recorded using a Bruker Avance-III HD 500 MHz NMR spectrometer equipped with a sensitive broad band observe probe (BBO) [20]. The chemical shifts for both ^1H and ^{13}C NMR are stated on the δ scale in ppm, with tetramethylsilane (TMS) serving as

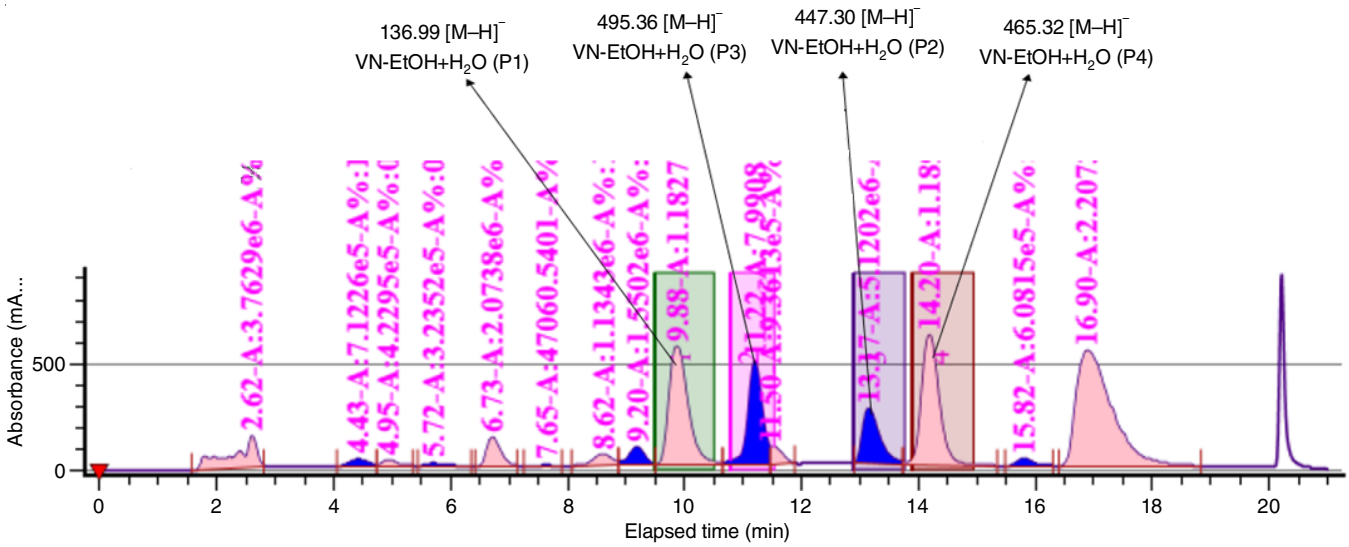

Fig. 1. Purification of VN-ethanol + water (1:1) extract

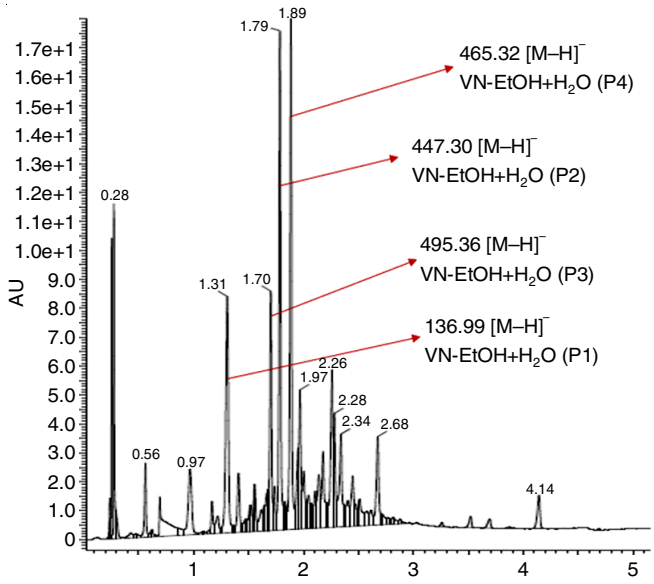

Fig. 2. LC-MS data for VN-ethanol + water (1:1) extract

the internal standard. The ^{1}H NMR spectra were calibrated using the TMS signal at δ 0.00 ppm and the ^{13}C NMR spectra were calibrated to δ 39.50 ppm in DMSO-d_6 [21].

Cell line maintanance: The A549 epithelial cancer cell line was procured from NCCS, Pune, India and maintained them in DMEM + 10% FBS with antibiotics (penicillin/streptomycin; 0.5 mg/mL concentration) in atmosphere of 5% CO_2/95% air at 37 ºC.

The extracted and purified compounds were weighed separately and dissolved in DMSO. The media was adjusted to a final concentration of 1 mg /mL for the compound and from 5 to 300 µg/mL series of concentrations were used to treat the cells.

Anticancer activity: Two independent experiments were conducted to evaluate the viable cells in triplicate using plant extractswith the concentrations ranging from 5 to 300 µg/mL. Trypan blue assay was used to to assess the viable cell number in the suspensions after trypsinization. The cell density of 5 × 10^3 per well with 100 µL media was taken using hemocytometer in a 96 well plate and permited to grow at 37 ºC for overnight. The fresh media of 100 µL containing the plant extract was added after the removal of the old media to all treated wells. After 2 days of incubation, The old media was discorded and fresh media containing 0.5 mg/mL MTT solution was added to each well and incubated at 37 ºC for 3 h [22]. Viable cells having active mitochondria shown the formation of coloured formazan crystals from MTT reduction at the end of incubation [23-27]. Using an ELISA reader, the optical density (OD) of DMSO solubilized formazan crystals was recorded at 570 nm. Finally, the growth inhibition by the plant extract was measured using the following formula:

$$\text{Inhibition } (\%) = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

The equation y = mx + c (where y = 50 and m, c values are derived from the viability graph) was used to determine the IC_{50} value of the plant extract in a linear regression graph [28].

RESULTS AND DISCUSSION

Isolation of obtained bioactive components: VN-EtOH +H_2O-P1, a pale brown crystalline powder, was identified as 4-hydroxy benzoic acid. Yield: 0.1 g; m.p.: 188-193 ºC, exact mass: 138.12 g/mol, HRMS: 137.0445 $[M-H]^-$, chemical formula: $C_7H_6O_3$, mass: 137.05 $[M-H]^-$; IR (KBr, ν_{max}, cm^{-1}): 3394.72 (-OH *str.*), 1681.93 (-C=O), 1238.30 (-C-O).

VN-EtOH+H_2O-P2, a pale green crystalline powder, was identified as negundoside, an iridoid glycoside. Yield: 0.05 g; m.p.: 148-152 ºC. exact mass: 496.5 g/mol, HRMS: 495.1518 $[M-H]^-$, chemical formula: $C_{23}H_{28}O_{12}$, Mass-495.15 $[M-H]^-$; IR (KBr, ν_{max}, cm^{-1}): 3257.77 (-OH *str.*), 2872.01 (carboxylic-OH), 1720.50 (-C=O) and 1269.16 (-C-O).

VN-EtOH+H_2O-P3, a pale yellow crystalline powder, was identified as isoorientin, a flavonoid glycoside. Yield: 0.08 g; m.p.: 233-237 ºC, exact mass: 448.38 g/mol, HRMS: 447.2279 $[M-H]^-$, chemical formula: $C_{21}H_{20}O_{11}$, Mass 447.23 $[M-H]^-$; IR (KBr, ν_{max}, cm^{-1}): 3379.29 (-OH *str.*), 1614.42, 1490.97 (Ar -C-C-) and 1080.10 (-C-O-C-).

VN-EtOH+H_2O-P4, an off-white crystalline powder, was identified as agnuside, an iridoid glycoside. Yield: 0.1 g; m.p.: 145-149 ºC, exact mass: 466.44 g/mol, HRMS: 465.2046 $[M-H]^-$, chemical formula: $C_{22}H_{26}O_{11}$, Mass 465.20 $[M-H]^-$; IR (KBr, ν_{max}, cm^{-1}): 3441.01 -OH *str.*), 1701.22 (C=O) and 1602.85, 1450.47 (Ar-C-C-), 1130.29 (-C-O-C-).

The ^{1}H and ^{13}C NMR data of all the four isolated bioactive compounds *viz.*, (i) VN-ETOH+H_2O-P1, (ii) VN-ETOH+H_2O-P2, (iii) VN-ETOH+H_2O-P3 and (iv) VN-ETOH+H_2O-P4 are shown in Tables 1 and 2.

Anticancer activity of bioactive components: The studies revealed that the ethanol:water (1:1) extract possessed effective significant anticancer activity in a dose dependent manner amongst all extracts studied. The results (IC_{50} values) of the anticancer activity of extracts are shown in Table-3. The ethanol:water (1:1) extract (Fig. 3) was shown significant anticancer activity (IC_{50} value 44.31 µg/mL). The active extract was isolated into four fractions *via* gradient column chromatography (PREP-HPLC) resulted into four compounds *viz.* VN-EtOH+H_2O-P1, P2, P3 and P4 which were tested for anticancer activity on epithelial A549 cell lines. The standard drug cisplatin was used and its IC_{50} value was found to be 13.45 ± 0.084 µM. The results correlate with previous studies in a similar context [29,30].

The effective fractionated compounds from ethanol:water (1:1) were further tested for anticancer activity on epithelial A549 cell lines and among the four fractionated compounds tested, VN-EtOH+H_2O-P3 (IC_{50} value 18.50 µM/mL) showed the most significant anticancer activity (Fig. 4). This compound demonstrated significant cytotoxic activity against A549 cells. The IC_{50} values (Table-4) were reported for the evaluated compounds, indicating the concentration necessary for 50% inhibition of cell growth, and the study attempted to ascertain the maximum inhibition of A549 lung cancer cells by the most active fraction.

Toxicity study: The toxicity study was performed for VN-EtOH+H_2O-P3 (isoorientin) on human dermal fibroblast (HDF)

TABLE-1
^{1}H AND ^{13}C NMR SPECTRAL DATA FOR THE COMPOUNDS VN-EtOH+H_2O-P1 AND VN-EtOH+H_2O-P2

4-Hydroxy benzoic acid (VN-EtOH+H_2O-P1)				Negundoside (VN-EtOH+H_2O-P2)			
Atom No.	Type of atom	^{1}H Chemical shift (ppm), coupling constant (*J*)	^{13}C chemical shift (ppm)	Atom No.	Type of atom	^{1}H Chemical shift (ppm), coupling constant (*J*)	^{13}C chemical shift (ppm)
1, 5	CH	7.79 (d, 8.5 Hz, 2H)	131.4	1	CH	3.2 (t, 8.5 Hz, 1H)	70.12
2, 4	CH	6.82 (d, 8.5 Hz, 2H)	114.99	2	CH	3.29 (m, 1H)	77.43
3	C	–	161.42	3	O	–	–
6	C	–	122.17	4	CH	4.84 (d, 8.0 Hz, 1H)	95.94
7	C	–	167.54	5	CH	4.70 (t, 8.5 Hz, 1H)	73.39
8	O	–	–	6	CH	3.49 (t, 8.5 Hz, 1H)	74.14
9	OH	12.0 (hump, 1H)	–	7	OH	5.28 (hump, 1H)	–
10	OH	12.0 (hump, 1H)	–	8	O	–	–
				9	OH	5.2 (hump, 1H)	–
				10	CH_2	3.74 (d, 11.3 Hz, 1H) 3.50 (dd, 11.3, 6.5 Hz, 1H)	60.83
				11	OH	4.63 (hump, 1H)	–
				12	O	–	–
				13	CH	5.29 (d, 2.0 Hz, 1H)	93.46
				14	O	–	–
				15	CH	7.03 (s, 1H)	148.72
				16	C	–	112.29
				17	CH	2.73 (m, 1H)	29.84
				18	CH	1.97 (m, 1H)	50.54
				19	C	–	167.27
				20	O	–	–
				21	OH	11.0 (hump, 1H)	–
				22	CH_2	2.03 (m, 1H) 1.25 (m, 1H)	28.98
				23	CH_2	1.50 (m, 2H)	39.78
				24	C	–	77.75
				25	CH_3	1.13 (s, 3H)	24.23
				26	OH	4.62 (s, 1H)	–
				27	C	–	164.68
				28	C	–	120.65
				29	O	–	–
				30, 34	CH	7.72 (d, 8.0 Hz, 2H)	131.26
				31, 33	CH	6.80 (d, 8.0 Hz, 2H)	115.08
				32	C	–	161.54
				35	OH	10.22 (hump, 1H)	–

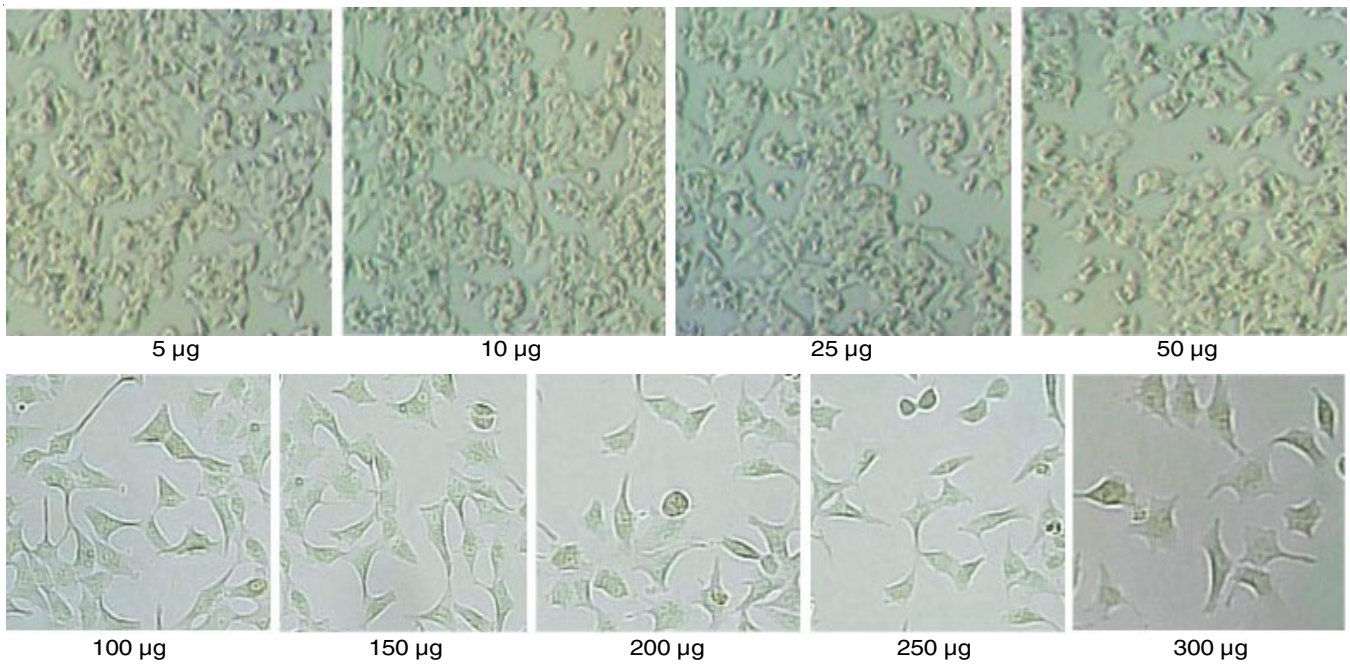

Fig. 3. Microscopic images of VN-ethanol:water (1:1) extract against A549 cell lines (5 to 300 µg/mL)

TABLE-2
^{1}H AND ^{13}C NMR SPECTRAL DATA FOR THE COMPOUNDS VN-EtOH+H_2O-P3 AND VN-EtOH+H_2O-P4

Isoorientin (VN-EtOH+H_2O-P3)				Agnuside (VN-EtOH+H_2O-P4)			
Atom No.	Type of atom	^{1}H Chemical shift (ppm), coupling constant (*J*)	^{13}C chemical shift (ppm)	Atom No.	Type of atom	^{1}H Chemical shift (ppm), coupling constant (*J*)	^{13}C chemical shift (ppm)
1	CH	3.12 (t, 9.0 Hz, 1H)	70.59	1	CH	3.04 (td, 9.0, 5.0 Hz, 1H)	70.03
2	CH	3.20 (t, 8.5 Hz, 1H)	78.92	2	CH	3.10 (dd, 9.5, 5.6 Hz, 1H)	77.17
3	CH	4.04 (d, 9.0 Hz, 1H)	70.16	3	O	–	–
4	CH	4.59 (d, 9.8 Hz, 1H)	73.01	4	CH	4.53 (d, 7.8 Hz, 1H)	98.29
5	O	–	–	5	CH	2.99 (td, 8.6, 5.0 Hz, 1H)	73.33
6	CH	3.17 (m, 1H)	81.55	6	CH	3.16 (td, 9.0, 4.6 Hz, 1H)	76.61
7	C	–	108.85	7	CH_2	3.39 (dt, 11.0, 5.6 Hz, 1H) 3.65 (dd, 11.0, 5.6 Hz, 1H)	61.11
8	OH	4.67 (hump, 1H)	–	8	OH	4.38 (t, 5.6 Hz, 1H)	–
9	CH_2	3.70 (d, 11.0 Hz, 1H) 3.41 (dd, 11.0, 5.7 Hz, 1H)	61.46	9	OH	4.94 (d, 5.0 Hz, 1H)	–
10	OH	4.67 (hump, 1H)	–	10	OH	4.96 (d, 5.0 Hz, 1H)	–
11	OH	4.67 (hump, 1H)	–	11	OH	4.98 (d, 5.0 Hz, 1H)	–
12	OH	4.67 (hump, 1H)	–	12	O	–	–
13	C	–	160.65	13	CH	4.86 (d, 7.0 Hz, 1H)	95.76
14	C	–	103.36	14	O	–	–
15	C	–	156.16	15	CH	6.37 (d, 6.0 Hz, 1H)	140.34
16	CH	6.47 (s, 1H)	93.46	16	CH	5.07 (dd, 5.5, 4.2 Hz, 1H)	104.65
17	C	–	163.6	17	CH	2.56 (m, 1H)	44.81
18	OH	13.56 (s, 1H)	–	18	CH	2.83 (t, 7.5 Hz, 1H)	46.69
19	OH	9.81 (hump, 1H)	–	19	CH	4.35 (t, 5.0 Hz, 1H)	80.58
20	C	–	181.83	20	CH	5.80 (s, 1H)	132.28
21	CH	6.67 (s, 1H)	102.76	21	C	–	139.8
22	C	–	163.6	22	OH	5.16 (d, 5.6 Hz, 1H)	–
23	O	–	–	23	CH_2	4.88 (d, 14.5 Hz, 1H) 4.90 (d, 14.5 Hz, 1H)	62.04
24	O	–	–	24	O	–	–
25	C	–	121.38	25	C	–	165.13
26	CH	7.40 (d, 2.0 Hz, 1H)	113.27	26	C	–	120.18
27	C	–	145.72	27	O	–	–
28	C	–	149.69	28, 32	CH	7.87 (d, 8.5 Hz, 2H)	131.54
29	CH	6.90 (d, 8.2, 1H)	116.02	29, 31	CH	6.87 (d, 8.5 Hz, 2H)	115.37
30	CH	7.42 (dd, 8.2, 2.0 Hz, 1H)	118.94	30	C	–	162.05
31	OH	9.81 (hump, 1H)	–	33	OH	10.29 (broad hump, 1H)	–
32	OH	9.81 (hump, 1H)	–				

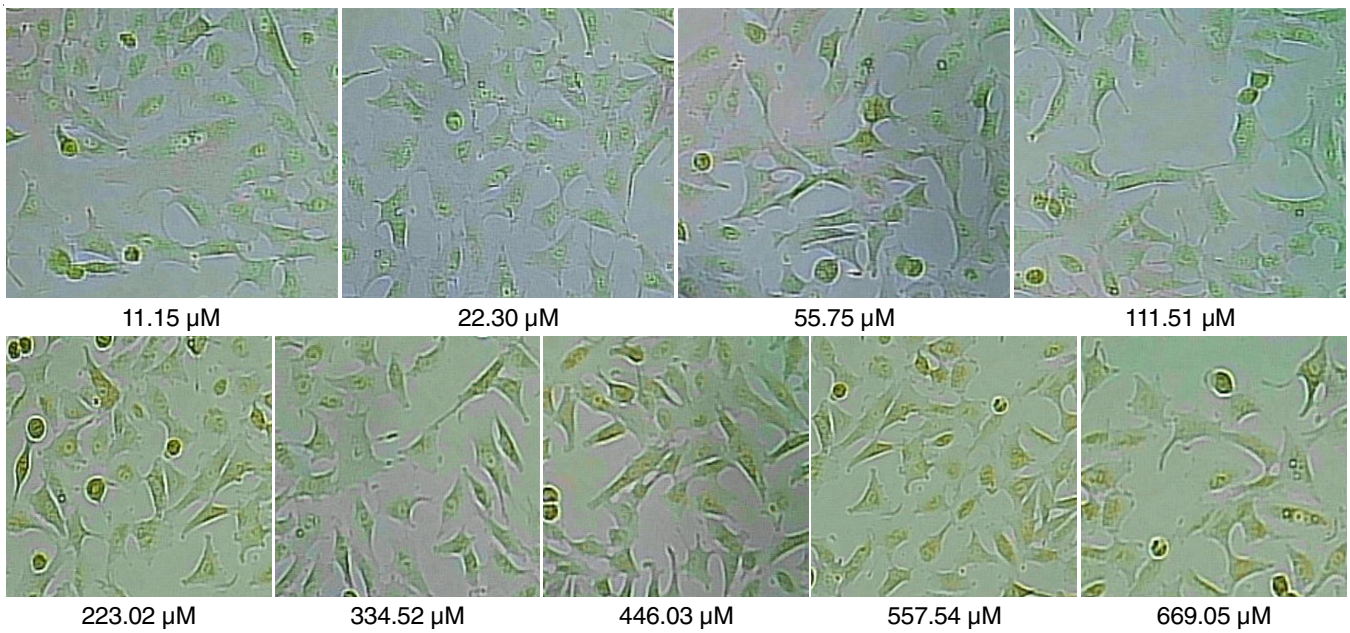

Fig. 4. Microscopic images of isoorientin (VN-EtOH+H_2O-P3) against A549 cell lines [5 (11.15) to 300 (669.05 µM/mL) µg/mL]

TABLE-3
CELL LINE A549 CELLS TREATED WITH SOLVENT EXTRACTS SHOWING THE IC_{50} VALUES (5 to 300 µg/mL)

Compounds (extracts)	IC_{50} (µg/mL)	Max inhibition
VN-Hexane	63.03	82.83
VL-Chloroform	79.12	77.40
VN-Ethyl acetate	65.70	82.83
VN-Ethanol + water (1:1)	44.31	80.52

TABLE-4
CELL LINE A549 CELLS TREATED VN-ETHANOL: WATER (1:1) FRACTIONATES SHOWING THE IC_{50} VALUES IN THE (5 (11.1 µM) TO 300 (600 µM) µg/mL)

Compounds	IC_{50} (µM/mL)	Max inhibition
VN-EtOH+H_2O-P1 (4-OH Benzoic acid)	51.40	77.33
VN-EtOH+H_2O-P2 (Negundoside)	21.32	68.91
VN-EtOH+H_2O-P3 (Isoorientin)	18.50	71.84
VN-EtOH+H_2O-P4 (Agnuside)	83.06	76.43

cell lines at different concentrations [5 µg/mL (11.1 µM) to 300 µg/mL (669 µM)]. The IC_{50} value of 416.57 ± 0.01 µM for the VN-EtOH-H_2O-P3 compound against HDF cells after 48 h of treatment and doxorubicin was used as the standard control in the study, exhibited an IC_{50} value of 19.8 ± 0.01 µM (Table-5), indicated its cytotoxicity threshold. This value suggests that at higher concentrations (closer to the IC_{50}). The compound begin to exhibit a significant cytotoxic effect on HDF cells. However, below this threshold, it appears to be non-toxic. Moreover, the presence of isoorientin (VN-EtOH-H_2O-P3), proven to be non-toxic, strengthens the safety profile, at least *in vitro* with reference to the microscopic images of VN-EtOH +H_2O-P3 (isoorientin) (Fig. 5a) and the standard control (Fig. 5b). Both compounds treated on HDF (human dermal fibroblast) cell lines at different concentrations.

The bioactive compounds VN-EtOH+H_2O-P1, VN-EtOH +H_2O-P2, VN-EtOH+H_2O-P3 and VN-EtOH+H_2O-P4 isolated from *V. negundo* leaves have great potent to act against the lung cancer cell lines. Among the four isolated compounds, VN-EtOH +H_2O-P3 (isoorientin) exhibited the most potent apoptotic activity. The results indicated that isolated VN-EtOH+H_2O-P3 of EtOH+H_2O-leaf extract of *V. negundo* showed significant anticancer activity against the lung cell lines, A549 based on marked inhibition of cancer cell line. Further,

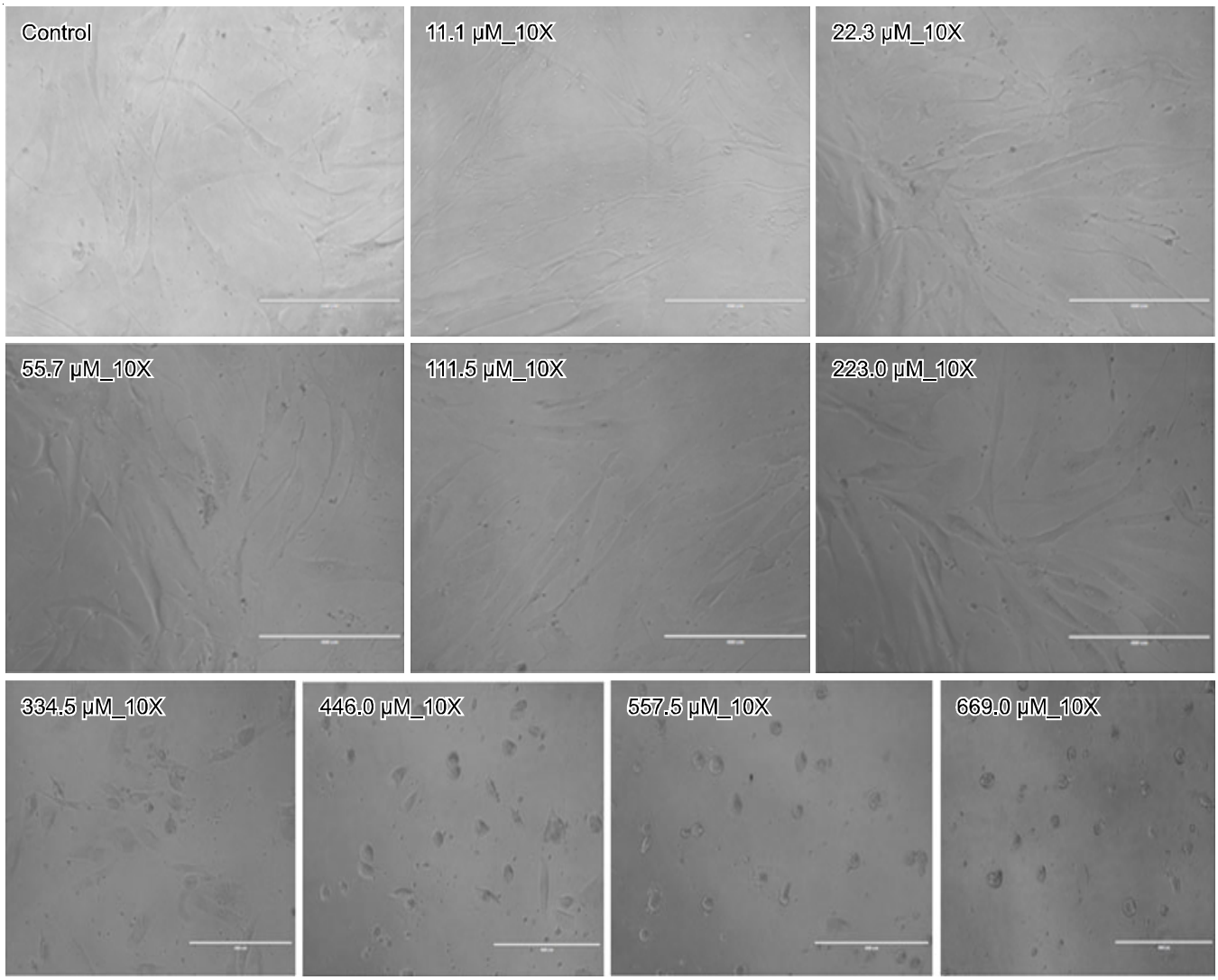

Fig. 5a. The microscopic images for VN-EtOH+H_2O-P3-isoorientin treated HDF cells post 48 h treatment

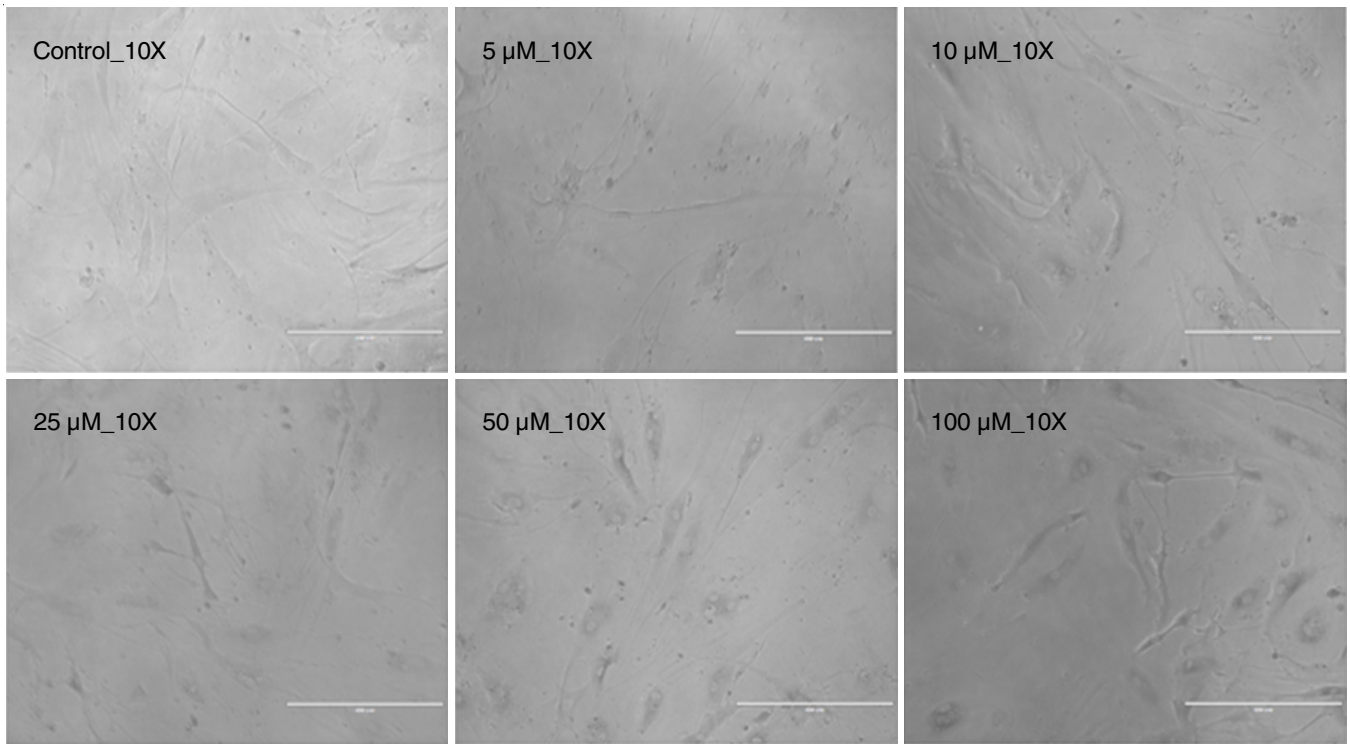

Fig. 5b. The microscopic images for standard control doxorubicin treated HDF cells post 48 h treatment

the compound showed a low level of cytotoxicity even at highest concentration against the HDF cells (Table-5).

TABLE-5
CELL LINE HDF CELLS TREATED POST 48 h TREATMENT, THE IC_{50} VALUES

(a) VN-EtOH+H_2O-P3-Isoorientin			(b) Doxorubicin used as standard control		
Conc. (µM)	Cell viability (%)	IC_{50} (µM)	Conc. (µM)	Cell viability (%)	IC_{50} (µM)
Control	100		Control	100	
11.1	88.48		5	53.36	
22.3	88.73		10	50.87	19.8 ± 0.01
55.7	83.02		25	47.38	
111.5	83.02	416.57 ± 0.01	50	46.63	
223.0	55.51		100	44.13	
334.5	53.60				
446.0	44.41				
557.5	41.84				
669.0	30.24				

Conclusion

The results in this study indicated that ethanol:water (1:1) extracts from the leaves of *Vitex negundo* plant contain the flavonoid isoorientin, which has shown potential anticancer activity. The cumullative data from the anticancer studies provide substantial evidence for the role of VN-EtOH+H_2O-P3 (isoorientin) in the treatment of lung cancer. Isoorientin has been identified as a significant compound in the leaves of *V. negundo* plants, positioning it as a potential therapeutic agent. However, further research, including medicinal and discovery chemistry strategies then preclinical trials will be essential to explore its biological and pharmacological activities as well as the mechanisms underlying its potential for treating and preventing lung cancer. The present study aims to isolate and screen the novel molecules that are present in such extracts. The study will thus provide the way to identify and characterize the molecule(s) responsible for anticancer activity. Further, the evaluated toxicity of the compound VN-EtOH+H_2O-P3 (isoorientin) showed a low level of cytotoxicity even at higher concentrations *i.e.* 11.1 µM/mL to 669 µM/mL against the HDF cell line. The determined IC_{50} value supports its efficacy against HDF cell lines when compared to the standard doxorubicin.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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