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# Biological Activities of Essential Oil of *Vitex altissima* Leaves and Inhibition Potential towards Phosphoinositide-3 Kinase (PI3K) Enzyme by Molecular Docking

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This study aims to employ molecular docking to verify the efficacy of a few bioactive compounds of pharmacological significance extracted from *Vitex altissima*. GC/MS and GC/FID were used for chemical profiling. An *in vitro* study for anticancer potential done using MTT assay with DLD-1 cancer cell line and L929 normal cell line. All the bioactive compounds were docked against phosphoinositide-3 kinase (PI3K) using AutoDock 4.2.6 software. The identified 22 compounds (89.91%) with *allo*-aromadendrene (29.82%), *E*-phytol (16.08%) α-humulene (14.04%), β-caryophyllene (4.18%), α-santalol (3.64%) and spanthulenol (2.23%) as the dominant sesquiterpenoid compounds. The study indicates effective anticancer and antioxidant potential with the oil. Apoptosis evaluation revealed that the cells experience necrosis after treatment with *Vitex altissima* leaf oil. The deregulation of enzyme PI3K is liable for various types of cancers. The ADMET properties of respective compounds reveal their pharmacokinetic properties. All compounds subjected to docking satisfied Lipinski's rule of five, indicating the scope for their use as oral drugs.

**Keywords: *Vitex altissima*, Essential oil, Chemical composition, Cytotoxicity, Molecular docking, Phosphoinositide-3 kinase.**

## INTRODUCTION

Medicinal plants were thought to be the source of many medications and many of today's prescription for medications are still of botanical, which is found increasing day by day all over the world [1-3]. Around 80% of the population around the world rely primarily on traditional medicine to treat life-threatening diseases and is still used to find novel drug candidates because it is the golden standard of medical practice for humans [4-6]. Moreover, the natural products of botanical origin have continued to deliver exclusive structural diversity in contrast to standard combinatorial chemistry, which illustrates a great opportunity for discovering new low molecular weight lead compounds. *In silico* approaches such as molecular docking and virtual screening supports the efforts to analyze the therapeutical perspectives of bioactive compounds of botanical origin, with advanced computer technologies [7-11]. Since all the pharmaceutical effects in *Vitex altissima* have not been a prominent focus of previous literature, it remains the most significant aim of this investigation.

The genus *Vitex*, which belongs to the Verbenaceae family, contains over 250 species of trees and shrubs found all over the world [12]. Majority of aromatic *Vitex* species are used in traditional medicine [13-15]. *Vitex altissima* is one among the six species of *Vitex,* commonly seen in Kerala state, India [16]. It is locally known as 'Mayila' that grows in alluvial substrates near riversides in the deciduous forest [17,18]. Flowering of the plant usually occurs in March-July and fruit-bearing season is during September-January [16,19]. Traditionally, the leaves of *V. altissima* have been used to treat medical conditions such as ulcers, allergies, swelling, wounds, urinary infections and so on [20]. The leaves of *V. altissima* are reported to be used in the treatment of rheumatism [21].

A broad range of biological activities of various solvent extracts of dried *V. altissima* leaves such as antimicrobial, anti-larvicidal, antiviral, anti-inflammatory has already been reported. A new iridoid glycoside, isolated from ethyl acetate extract, which was obtained after the sequential extraction of *V. altissima* leaves in Soxhlet apparatus, showed potent antioxidant activity,

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whereas its parent ethyl acetate extract exhibited significant anti-inflammatory activities [22].

Essential oil studies were reported from a number of *Vitex* species. The essential oil studies on *V. negundo* leaves reports viridifloral, β-caryophyllene, sabinene, 4-terpineol, γ-terpinene, caryophyllene oxide, 1-ocatene-3-ol and globulol as the major compounds [23-27]. The study of essential oil of *V. agnus-castus* L. seeds reports the presence of 1,8-cineole, sabinene, α-pinene, β-farnesene, β-caryophyllene oxide, β-caryophyllene as the major components [28]. Essential oil collected from the upper part of *V. rivularis*, an African species, identifies sesquiterpene hydrocarbons as the main class of compounds and germacrene D as the major component [29].

The essential oil of *V. agnus-castus* seeds was found to exhibit potency against different pathogenic *Candida* species and so could effectively replace synthetic antifungal agents [28]. The petroleum ether extract and methanol extract of *V. trifolia* leaves were the ones having better anticancer activity against MCF-7 cancer cell lines [30]. Casticin [31,32] an important anticancer compound has been isolated from various plant parts of *Vitex* genus such as fruits and leaves of *V. trifolia*, seeds of *V. agnus-castus* and leaves of *V. negundo* [33].

The intention of this work was to carry out chemical evaluation of the South Indian *V. altissima* leaf oil by GC-MS and to identify its biological activities. This is an area that has not been done previously on the plant from a particular geographical area. However, the biological studies using computational studies as well as the pharmacological analysis with reference to phosphoinositide-3 kinase enzyme inhibition, anticancerous and antioxidant activity are limited. Therefore, this investigation is intended to unveil the aforesaid aspects with strong molecular evidence on the same.

## EXPERIMENTAL

Plant materials were collected from the hilly area of Thiruvananthapuram city near the Western Ghats of India. The collection point is situated between north latitudes 8.7533 ºN and east longitude 77.0263ºE, Palode, Thiruvananthapuram in April 2021. The samples were authenticated by Dr. T. Sabu, Technical Officer of the Jawaharlal Nehru Tropical Botanic Garden & Research Institute and the voucher specimen (No. 37346) was deposited at the herbarium of JNTBGRI.

**Extraction of volatile oil:** Freshly collected *V. altissima* leaves were cut into small pieces and then subjected to hydrodistillation for a period of 5 h using Clevenger apparatus (5 L). After hydrodistillation, the volatile essential oil collected was dried over anhydrous $Na_2SO_4$ and stored at 4 ºC in dark.

**Gas chromatography/flame-ionization detection (GC/FID) analysis:** Essential oil of *V. altissima* leaves (100 µL) was diluted to 3 mL using acetone. About 1 µL of this solution was injected onto a Shimadzu GC-2010 Plus Gas Chromatograph (Shimadzu, Japan) with AOC-20i auto-injector and Flame Ionization Detector, fitted with an Rxi-5 Sil MS capillary column (5% phenyl and 95% dimethyl polysiloxane, non-polar, 30 m × 0.25 mm i.d., 0.25 mm film thickness, Restek USA). GC operation conditions: injection mode, split; split ratio, 50:1; injector temperature, 270 ºC; oven temperature programme, 60-250 ºC (3 ºC/min); hold time 2 min at 250 ºC; carrier gas, $N_2$ at 3 mL/min; detector temperature 270 ºC [34,35]. *V. altissima* leaf essential oil injections (1 µL each) were repeated thrice under similar experimental conditions. Relative percentages of individual components present in *V. altissima* leaf essential oil were obtained from the peak area-percent report of volatiles from GC-FID data.

**Gas chromatography/mass spectroscopy analysis:** Essential oil of *V. altissima* leaves (1 mL) was introduced by splitless injection on a Hewlett Packard 6890 gas chromatograph (Hewlett-Packard, USA), fitted with an HP-5 (5% phenyl 95% dimethylpolysiloxane, non-polar, 30 m × 0.32 mm, i.d., 0.25 mm film thickness) capillary column, coupled with a Model 5973 mass detector. Injector temperature, 220 ºC; transfer line, 240 ºC; oven temperature programme, 60-246 ºC (3 ºC/min); carrier gas, He at 1.4 mL/min were the GC-MS operation temperature conditions. Mass spectra: Electron Impact ($EI^+$) mode, 70 eV with a mass range of 40 to 450 *m/z*; ion source temperature, 240 ºC. Linear retention indices (LRIs) of constituents of leaf oil in the *V. altissima* HP-5 column were determined using standard $C_5$-$C_{30}$ hydrocarbons (Aldrich Chemical Co., USA). Individual components were identified by Wiley 275.L database matching, comparison of LRIs and by comparison of mass spectra of constituents with reported data [34-36].

**Antioxidant activity:** Sodium nitroprusside (5 mmol $L^{-1}$) in phosphate buffered saline (pH 7.4) was mixed with different concentrations (125-2000 µg/mL) of *V. altissima* leaf oil. From the stock, a concentration of 10 mg/mL was incubated at 25 ºC for 30 min. A control without the test compound, but an equivalent amount of distilled water was also taken. After 30 min, 1.5 mL of incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% $H_3PO_4$ and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of nitrate with sulphanilamide and subsequent coupling with N-1 naphthyl ethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard [37-39]. The $IC_{50}$ calculations were done with ED50 PLUS V1.0 Software.

**Antioxidant activity:** If the test sample possesses antioxidant properties, then the colour of DPPH changes from pink to yellow. The reduction capacity can be evaluated by spectrophotometry [40], by measuring the decrease in absorbance of DPPH at 517 nm. Ascorbic acid was used as standard and the antioxidant activity of leaf essential oil was expressed in terms of inhibition concentration. Hence as the inhibition concentration value or $IC_{50}$ value decreases, the higher is the antioxidant activity.

For assay, the reagent was prepared by dissolving 4 mg of DPPH in 100 mL of methanol to form a 0.1 mM DPPH solution. Different concentrations of samples such as 12.5-200 µg/mL from stock solution were made up to a final volume of 20 µL with DMSO and 1.48 mL DPPH (0.1 mM) solution was added to each of the mixtures. The reaction mixture was incubated in dark conditions at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. A DPPH solution (3 mL) was taken as the control and the $IC_{50}$ calculations were done with ED50 PLUS V1.0 Software.

**Cell culture and MTT assay:** Human colorectal adenocarcinoma (DLD-1) cells and fibroblast (L-929) cells were obtained from the National Centre for Cell Science, India and cultured separately in 25 mL tissue culture flasks in Dulbecco's modified Eagles medium (DMEM) (Sigma-Aldrich, USA), supplemented with 10% foetal bovine serum, sodium bicarbonate (Merck, Germany), L-glutamine and antibiotic solution. The antibiotic solution consists of pencillin (100 U/mL), amphoteracin B (2.5 μg/mL) and streptomycin (100 μg/mL). Cell lines were cultured at 37 ºC in a humidified $CO_2$ incubator with 5% $CO_2$ (NBS Eppendorf, Germany).

DLD-1 and L-929 lines were harvested from 25 mL tissue culture flasks in completely DMEM medium supplemented with 10% FBS, L-glutamine, sodium bicarbonate and an antibiotic solution containing pencillin (100 U/mL), streptomycin (100 μg/mL) and amphoteracin B (2.5 μg/mL). The cell concentration was adjusted to $5 \times 10^3$ cells/100 μL. The cells (DLD-1 and L-929) were shown in 96 well plates with $5 \times 10^3$ cells/100 μL per well and incubated for 24 h. Then, the spend medium was removed and 100 μL of complete growth medium with various doses (6.25, 12.5, 25, 50 and 100 μg/mL) of *V. altissima* leaf essential oil in 0.1% DMSO or standard anticancer drug 5-flurouracil (5-FU) were added in triplicate to respective wells. A set of control wells added with 100 μL medium containing 0.1% DMSO with respective cell lines was also maintained. After 48 h of incubation in a humidified $CO_2$ incubator with 5% $CO_2$ at 37 ºC, the spent medium was removed and added with 100 μL fresh medium with MTT (5 mg/mL) and incubated in a $CO_2$ incubator for 4 h. Optical densities were measured by using microplate reader (ELISA plate reader, ERBA, Germany) at 540 nm, on a DMSO solution of MTT formazan products [41].

**Morphological observations of DLD-1 and L-929 cell lines:** The cell lines were observed under a phase-contrast microscope after 48 h of incubation to assess nuclear condensation.

**Acridine orange (AO)/ethidium bromide (EB) staining:** The cultured cell lines (DLD-1) were incubated at 37 ºC in a humidified 5% $CO_2$ incubator (NBS Eppendorf, Germany) for 24 h to allow cell adherence. After the incubation, DLD-1 cells were treated with sample at a final concentration of sample *V. altissima* leaf essential oil, 19.45 μg/mL ($LC_{50}$ concentration) for 24 h, the cells were washed by cold PBS and then stained with a mixture of acridine orange (100 μg/mL) and ethidium bromide (100 μg/mL) at room temperature for 10 min. The stained cells were washed twice with 1X PBS and observed by a fluorescence microscope in a blue filter of fluorescent microscope (Olympus CKX41 with Optika Pro5 camera) to discriminate between live, apoptotic and necrotic cells. DNA-binding dyes acridine orange (AO) and ethidium bromide (EtBr) were used for the morphological detection of apoptotic and necrotic cells. Acridine orange (AO) and ethidium bromide (EtBr) (Sigma, USA) DNA-binding dyes were used to detect apoptotic and necrotic cells [41].

### Molecular modeling study

**Enzyme target preparation:** Protein data bank (PDB) (http://www.rcsb.org) was used to obtain the target protein. For this study, one of the protein's chains (chain A) was used to improve ligand binding accuracy [42]. Also, for better ligand binding, interfering hetero molecules were also removed from the protein [43]. The enzyme phosphoinositide-3 kinase (PI3K) (PDB Id: 3S2A) was applied as the target drug with 2.30 Å resolution using XRD.

**Phosphoinositide-3 kinase:** Enzyme PI3K deregulation responsible for various types of cancers also forms the reason for chemotherapy resistance. The biocomponents of *V. altissima* leaves along with reference compounds selected as ligands, were docked with enzyme, which paves way for a promising strategy of a combatant against cancer by targeting the PI3K signaling pathway [44].

**ADMET properties of ligands:** The properties of bioactive compounds in the extract oil of *V. altissima* leaves, such as absorption, distribution, metabolism and excretion (ADME) were determined using Swiss ADME, a free software, that supports studies on drug development processes [44].

**Molecular docking:** The major bioactive compounds of *V. altissima* leaf oil and standard 5-fluorouracil were docked against the phosphoinositide-3 kinase enzyme using the bioinformatics software AutoDock 4.2.6, which is a reliable automated software that works on the Lamarckian genetic algorithm to understand both protein-protein and protein-ligand interactions [45,46].

Docking was done with the search space of grid box size of 40 Å × 40 Å × 40 Å and 0.375 Å of grid spacing. The total number of grid points per map was 64000 and the grid box size of the PIK3 enzyme was × center: 20.525, y center: 1.548 and z center: 34.032, respectively [47].

Accelrys' Discovery Studio 3.1 is a free visualizer that contains a wealth of information about ligand-receptor interactions. This software was used to create two-dimensional and three-dimensional interaction images of docked proteins in this study. The protein-protein interactions, macromolecular engineering, pharmacophore modelling, antibody modelling and optimization simulations were all covered [46-49].

## RESULTS AND DISCUSSION

The current study used computational molecular docking studies in order to explore the presence of the rich chemical constitutents of *V. altissima* leaf essential oil for pharmacological purposes. Previous studies points out that the essential oil of *V. altissima* leaves is a rich source of sesquiterpene hydrocarbons. From reported works on different species of *Vitex* genus, it is understood that there are certain compounds common in all those species [14,15]. β-Caryophyllene, caryophyllene oxide, globulol, sabinene, *allo*-aromadendrene, cadinol, β-eudesmol and germacrene D were present in *V. trifolia, V. negundo*, *V. rivularis* and *V. agnus-castus* [23,28-30]. All the compounds were also detected in the present investigation. In addition to these compounds, linalool, palustrol, spathulenol, humulene epoxide, α-santalol, viridiflorol, phytol and bicyclogermacrene were also seen in the oil in large proportions. Among all these compounds, *allo*-aromadendrene, α-humulene, E-phytol and β-caryophyllene are found present in higher concentrations, which forms the basis of their high medicinal values.

A total of 37 compounds were detected by gas chromatographic analysis of *V. altissima* leaf essential oil. Following data base matches and interpretation of GC-MS data, 22 compounds were identified, accounting for 89.91% of the oil. *allo*-Aromadendrene (29.82%) α-humulene (14.04%), α-santalol (3.64%), β-caryophyllene (4.18%) and spathulenol (2.23%) are the major sesquiterpenes present in the essential oil obtained from leaves. The major diterpene identified in *V. altissima* essential oil was E-phytol (16.08%). GC/FID and GC/MS data are shown in Table-1. Linalool is the only monoterpene identified, with less than 1% yield. The total ion chromatogram of *V. altissima* leaf essential oil is presented in Fig. 1, the percentage of different classes of compounds present in leaf essential oil in Figs. 2 and 3 gives chemical structures of the major compounds.

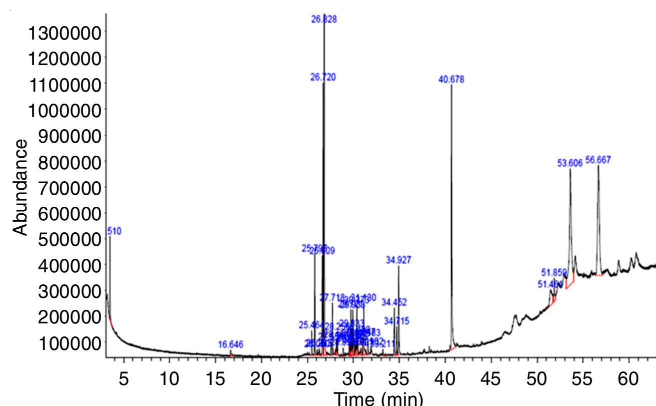



Fig. 1. TIC of *V. altissima* leaves essential oil

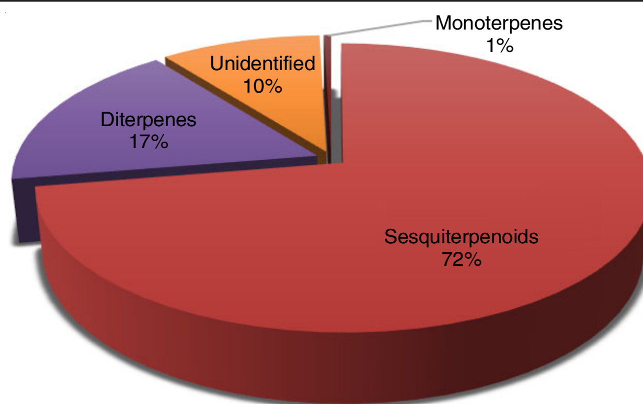



Fig. 2. Percentage of different classes of compounds present in *V. altissima* leaves essential oil

In comparison with the results of essential oil of other *Vitex* species, it is evident that qualitative and quantitative differences exist regarding the major and minor components. High content of sesquiterpene increases the biological importance of *V. altissima* leaves and demands further studies on the essential oil. The high proportion of *allo*-aromadendrene and E-phytol in the leaf oil makes it valuable for aromatherapy, medicines, food, antiseptic and cosmetic industries. A comparative study of *V. altissima* with these species is shown in Table-2. α-Humulene, β-caryophyllene and caryophyllene oxide are the common compounds found to be present in all the species of *Vitex*. Linalool was found to be present in all species, except in *V. agnus-castus*. Spathulenol is another general compound, which is not present in *V. rivularis Gurke* and *V. diversifolia*.

TABLE-1
CHEMICAL COMPOSITION OF THE *V. altissima* LEAF OIL

| Peak No. | Components | Retention time | Linear retention index | Retention index literature | Area (%) | Identification |
|---|---|---|---|---|---|---|
| 1 | Linalool | 16.65 | 1101 | 1095 | 0.46 | RI, MS |
| 2 | α-Gurjunene | 25.46 | 1407 | 1409 | 1.21 | RI, MS |
| 3 | *trans*-Caryophyllene | 25.79 | 1420 | 1417 | 4.18 | RI, MS |
| 4 | Aromadendrene | 26.28 | 1439 | 1439 | 0.305 | RI, MS |
| 5 | α-Humulene | 26.72 | 1456 | 1452 | 14.04 | RI, MS |
| 6 | *allo*-Aromadendrene | 26.83 | 1460 | 1458 | 29.82 | RI, MS |
| 7 | Bicyclogermacrene | 27.72 | 1495 | 1500 | 1.1 | RI, MS |
| 8 | Palustrol | 29.56 | 1573 | 1567 | 0.92 | RI, MS |
| 9 | Spathulenol | 29.72 | 1579 | 1577 | 2.23 | RI, MS |
| 10 | Caryophyllene Oxide | 29.83 | 1584 | 1582 | 1.09 | RI, MS |
| 11 | Globulol | 29.93 | 1588 | 1590 | 1.96 | RI, MS |
| 12 | Viridiflorol | 30.14 | 1597 | 1592 | 0.85 | RI, MS |
| 13 | 5-epi-7-epi-α-Eduesmol | 30.37 | 1607 | 1607 | 2.21 | RI, MS |
| 14 | Humulene epoxide II | 30.49 | 1612 | 1608 | 0.95 | RI, MS |
| 15 | *trans*-Isolongifolanone | 30.82 | 1627 | 1625 | 1.61 | RI, MS |
| 16 | Caryophylla-4(12),8(13)diene-5α-ol | 31.05 | 1636 | 1639 | 2.8 | RI, MS |
| 17 | Caryophylla-4(12),8(13)diene-5β-ol | 31.13 | 1640 | 1639 | 2.25 | RI, MS |
| 18 | Allohimachalol | 31.58 | 1660 | 1661 | 1.19 | RI, MS |
| 19 | (Z)-α-Santalol | 31.9 | 1674 | 1674 | 3.64 | RI, MS |
| 20 | Drimenone | 34.45 | 1791 | 1792 | 0.79 | RI, MS |
| 21 | E-Phytol | 40.67 | 2007 | 1942 | 16.08 | RI, MS |
| 22 | *trans*-Isoeugenyl phenyl acetate | 51.46 | 2693 | 2297 | 0.23 | RI, MS |
| | Total | | | | 89.91 | |

Individual components were identified by Wiley 275.L database matching, comparison of LRIs and by comparison of mass spectra of constituents with published data.

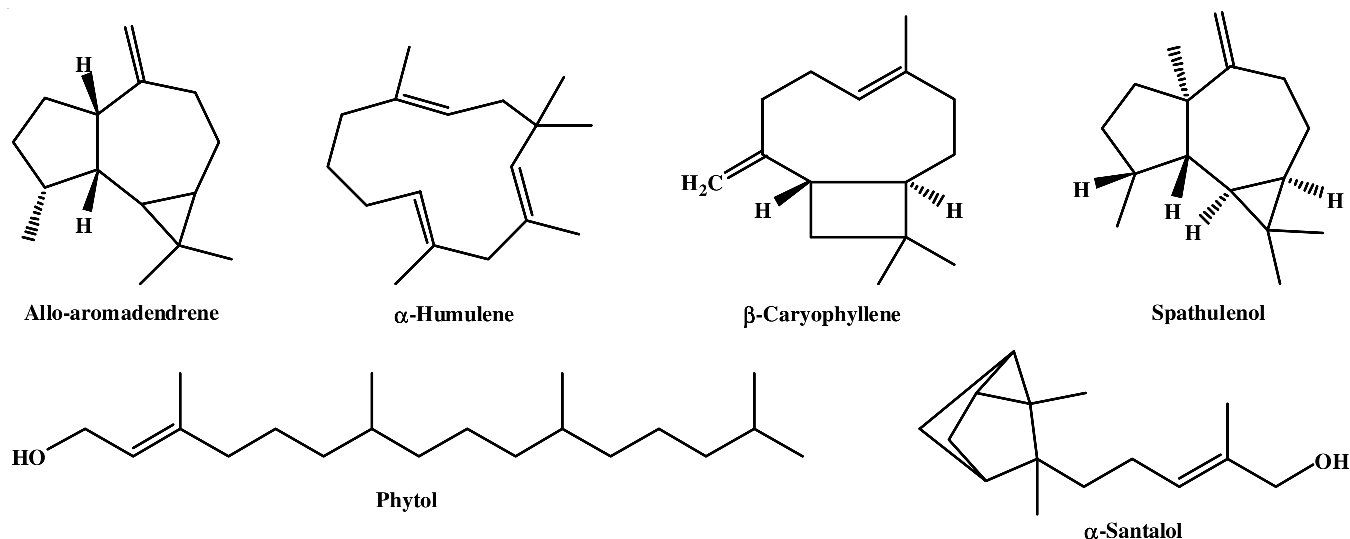



Fig. 3. Major compounds in the essential oil of *V. altissima* leaves

TABLE-2
COMPARATIVE STUDY OF COMPOUNDS WHICH PRESENT IN THE *Vitex* SPECIES

| No. | Compounds in *V. altissima* | Percentage of compounds | | | | | |
|---|---|---|---|---|---|---|---|
| | | *V. altissima* | *V. negundo* | *V. trifolia* | *V. diversifolia* | *V. agnus-castus* | *V. rivularis Gurke* |
| 1 | Linalool | 0.46 | 0.97 | 0.39 | 0.3 | – | 2.6 |
| 2 | α-Gurjunene | 1.21 | 0.22 | – | – | – | – |
| 3 | β-Caryophyllene | 4.18 | 13.65 | 17.54 | 1.91 | 5.02 | 7.3 |
| 4 | Aromadendrene | 0.31 | – | Trace | – | 1.37 | – |
| 5 | α-Humulene | 14.04 | 0.53 | 0.65 | 2.8 | 1.03 | 2.6 |
| 6 | *allo*-Aromadendrene | 29.82 | – | 0.83 | 0.2 | – | – |
| 7 | Bicyclogermacrene | 1.1 | – | 4.72 | – | – | – |
| 8 | Palustrol | 0.92 | – | – | – | – | – |
| 9 | Spathulenol | 2.23 | 1.26 | 4.57 | - | 1.01 | – |
| 10 | Caryophyllene Oxide | 1.09 | 1.25 | 1.81 | 3.2 | 5.82 | 0.9 |
| 11 | Globulol | 1.96 | 17.27 | – | – | – | – |
| 12 | Viridiflorol | 0.85 | 1.10 | – | – | – | – |
| 13 | 5-epi-7-epi-α-Eduesmol | 2.21 | – | – | 0.3 | – | – |
| 14 | Humulene epoxide II | 0.95 | – | – | – | – | – |
| 15 | *trans*-Isolongifolanone | 1.61 | – | – | – | – | – |
| 16 | Caryophylla-4(12),8(13) diene-5α-ol | 2.8 | – | – | – | – | – |
| 17 | Caryophylla-4(12),8(13)diene-5β-ol | 2.25 | – | – | – | – | – |
| 18 | Allohimachalol | 1.19 | – | – | – | – | – |
| 19 | (Z)-α-Santalol | 3.64 | – | – | – | – | – |
| 20 | Drimenone | 0.79 | – | – | – | – | – |
| 21 | E-Phytol | 16.08 | – | – | 0.2 | - | 4.1 |
| 22 | *trans*-Isoeugenyl phenyl acetate | 0.23 | – | – | – | – | – |

Table-2 also provides a brief outlook of essential oils of major *Vitex* species, which were already reported in comparison with *V. altissima* leaf essential oil. Among the species mentioned here, only *V. altissima* is rich in sesquiterpene. *V. negundo* contain both mono and sesquiterpenes, in which γ-terpine, β-caryophyllene, β-caryophyllene oxide, globulol, are the major compounds [50]. *V. rivularis*, a subgenus of *Vitex*, commonly seen in western tropical and subtropical African countries, is a sesquiterpene rich species with E-phytol, β-caryophyllene, α-humulene, caryophyllene oxide and linalool, that are also observed in *V. altissima* leaves [29].

**Antioxidant activity:** The antioxidant activity of *V. altissima* essential oil from leaves was determined using the nitric oxide and DPPH radical Scavenging assay, with standards gallic acid and ascorbic acid, respectively. *V. altissima* leaf essential oil exhibited an $IC_{50}$ value of 834.07 µg/mL compared to gallic acid which was 833.11 µg/mL. The nitric oxide method results show that *V. altissima* leaf essential oil is as effective as or more effective than gallic acid as an antioxidant. The oil competes with oxygen for NO in reactions, preventing the formation of nitrite and peroxy nitrite anions [51]. Graphical representation of antioxidant activity of *V. altissima* leaves' essential oil at various concentration is provided in Fig. 4. Similar results were obtained from the DPPH radical scavenging assay of essential oil of *V. altissima* leaves, with higher antioxidant activity (92.12 ± 2.19 µg/mL) than the standard ascorbic acid

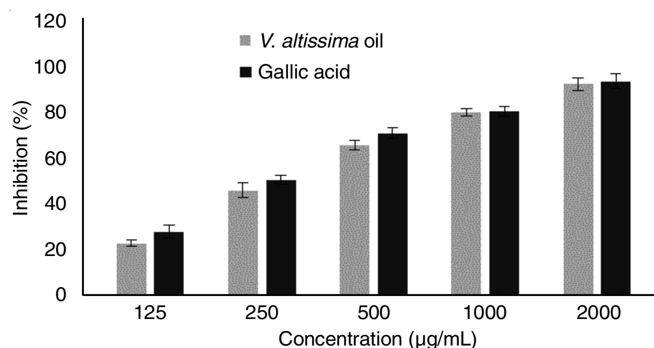



Fig. 4. Nitric oxide scavenging assay of essential oil *V. altissima* leaves at various concentrations

(49.72 ± 0.360 µg/mL). The concentration-dependent DPPH assay on *V. altissima* essential oil and standard ascorbic acid are presented in Fig. 5.

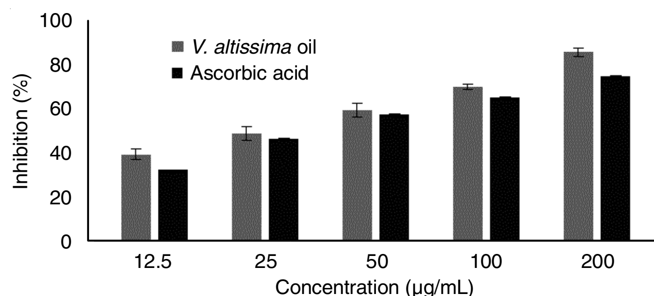



Fig. 5. DPPH scavenging assay of essential oil *V. altissima* leaves at various concentrations

**Anticancer activity:** The MTT assay of *V. altissima* leaf essential oil against human colorectal adenocarcinoma (DLD-1) and murine fibroblast (L929) cell lines at various concentrations reveals the significant activity. The $LC_{50}$ value of *V. altissima* oil against DLD-1 cell line was 19.45 µg/mL and the same against normal cell line L929 shows 100.93 µg/mL. 5-Fluorouracil was used as the corresponding positive control. The analytic data showed that essential oil of *V. altissima* leaves induce dose dependent *in vitro* cytotoxicity against DLD-1 and L929 cell lines. Thus, *V. altissima* leaf essential oil might be a good competitor for advanced therapeutic strategy against cancer. The cultured DLD1 cell lines with DMSO were treated with *V. altissima* leaf oil for 24 h, stained with acridine orange-ethidium bromide dye, resulting in green membranes for the living control cells and orange-red membranes for the cells. The percentage of viability of leaf essential oil observed at MTT assay is graphically represented in Fig. 6. The photomicrographic images of DLD-1 and L929 cells at different concentrations are presented in Fig. 7, while the photographic images of different stages of DLD1 cell line during apoptosis are provided in Fig. 8. The essential oil of *V. altissima* leaves exhibits significant cytotoxicity against cancer cells and thus could serve as a good candidate for advanced cancer remedial tactics, subject to further investigations.

**Molecular docking studies:** Regarding the effective research on plant-based products for pharmacology, the applications of advanced computational strategies like molecular docking to

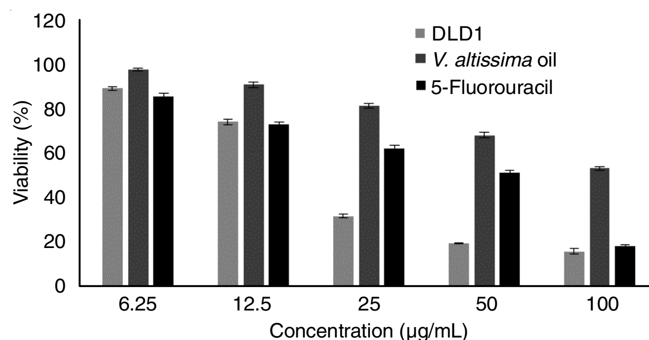
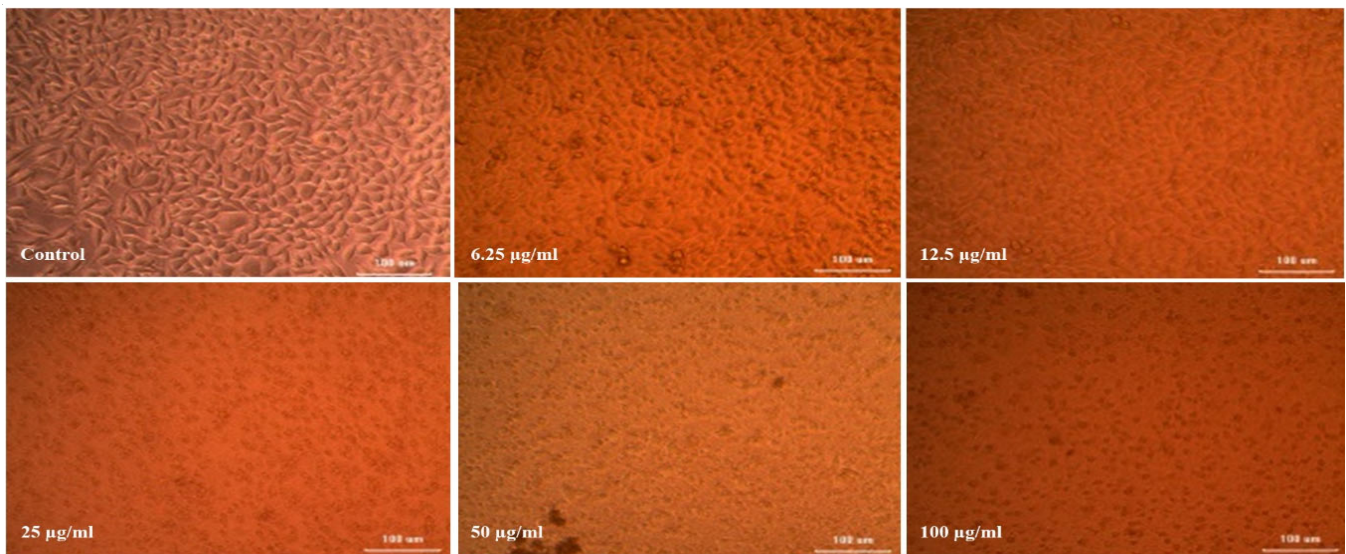



Fig. 6. Percentage of cell viabililty of *V. altissima* leaf essential oil, against normal and cancer cell lines

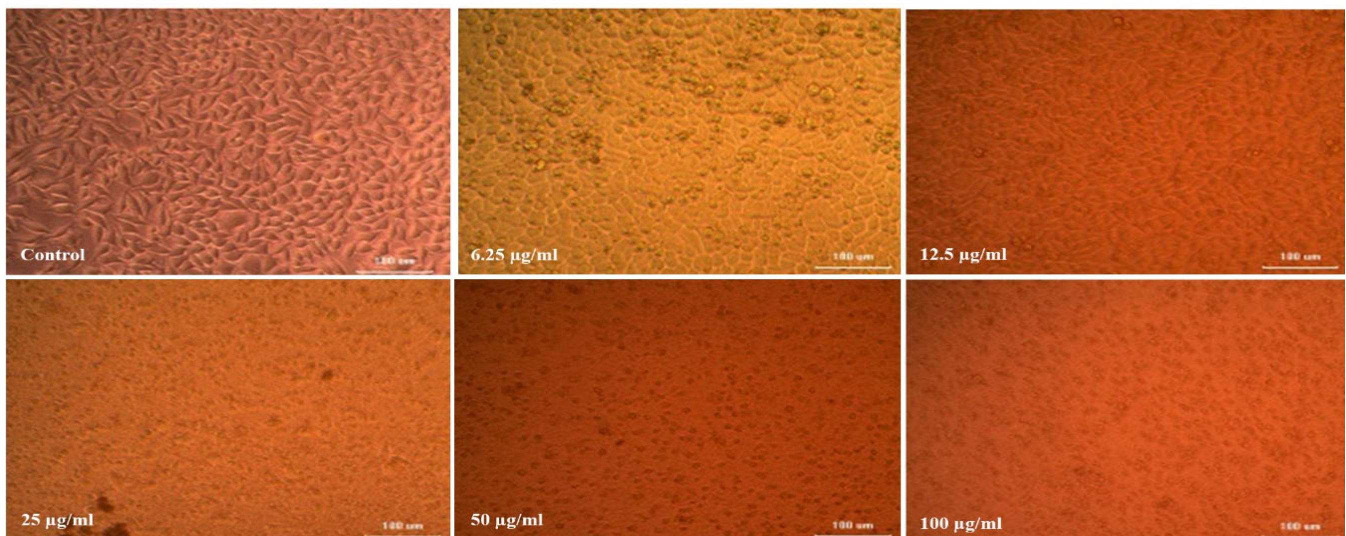
analyze various biological activities must address the structural and *in vitro* studies of such phytochemical constituents. In this work, the studies were carried out with the help of AutoDock 4.2. The inhibition potential of major bioactive components of *V. altissima* fresh leaves was found to be considerably high. Fig. 9 illustrates the 2D and 3D interactions of bioactive compounds spanthulenol, α-Santalol, α-humulene, allohimachalol and standard drug 5-fluorouracil, respectively with their lowest binding energies against phosphoinositide-3 kinase. The molecular docking studies reveals that the binding of bioactive components of essential oil and PI3K could be explained by the detected inhibitor activity. The structure PI3K complexed with the essential oil components such as β-caryophyllene, *trans*-isolongifolanone, linalool, α-santalol and drimenone indicates that hydrogen bonding and hydrophobic interactions, *i.e.* weak protein-ligand interactions, play an influential role in stabilizing energetically favoured protein-ligand interactions. The complex structure outlines the importance of amino acids like ALA 885, ILE 879, MET 953 and PHE 961 in hydrophobic interactions and hydrogen bonding. Best fit was found out by using the visualizing tool Discovery studio 3.1. The enzyme was re-docked with the synthetic inhibitor 5-fluorouracil (5-FU) to validate the docking procedure. The binding affinity of 5FU against PI3K was computed as -4.10 Kcal/mol, almost all the studied natural compounds exhibit better docking results than the synthetic drug 5-FU.

From molecular docking, it was found that all major bioactive components possess hydrogen bonding and van der Waals interactions with aromatic residues in the PI3K active site (Table-3). The inhibition results show little differences with the standard drug 5-FU due to the less catalytic anionic site interaction of bioactive components with the active site of an enzyme.

The pharmacokinetic analysis of compounds of *V. altissima* leaves essential oil and extract using ADMET properties were studied. Table-4 shows the Lipinski properties of bioactive compounds. Table-5 shows the ADME properties and Table-6 show the toxicity properties of the major bioactive compounds in *V. altissima* leaves. The results thus, demand further studies on the possible ethnomedicinal use of *V. altissima* leaves.The findings suggest the application of *V. altissima* leaves essential oil as a promising candidate for neurological complications. The results, however, demand further studies on the possible ethnomedicinal use of *V. altissima* leaves.




(a) DLD-1




(b) L929

Fig. 7. Photographic images of cancer cell (DLD1) and normal cell line (L929) against *V. altissima* leaves essential oil

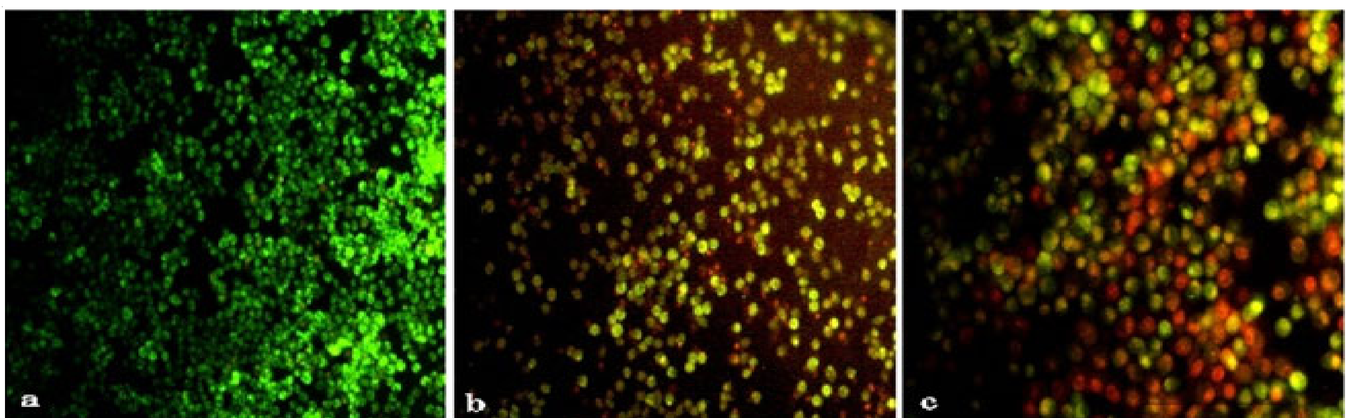
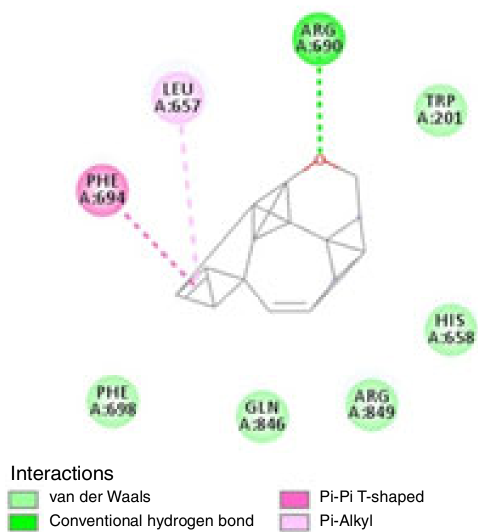
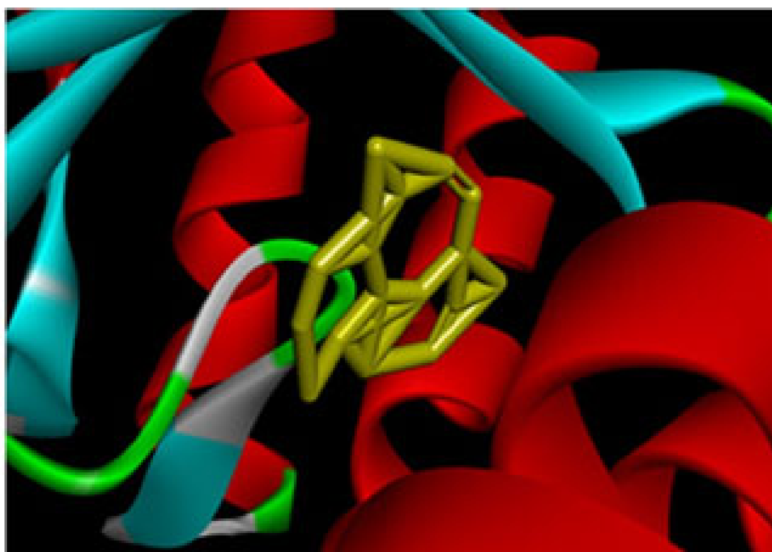
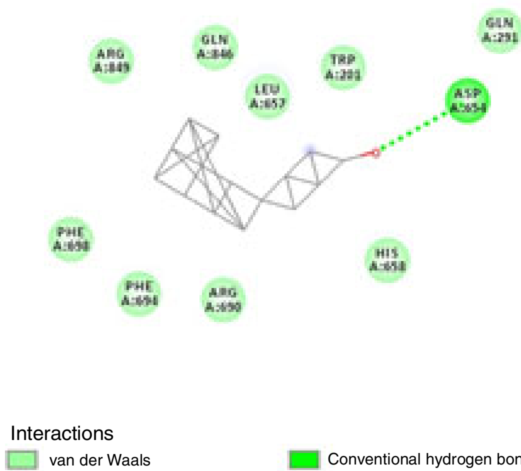


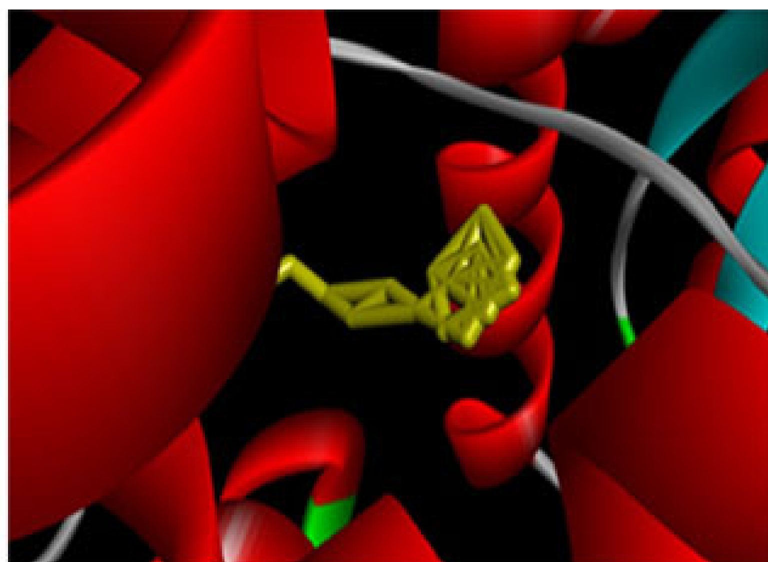

Fig. 8. Photographic images of different stages of DLD 1 cells, (a) DLD 1 cells treated with 0.1% DMSO (vehicle control) for 24 h and photographed under a fluorescent microscope using acridine orange-ethidium bromide staining. The green colour with intact cell membrane is live cells, (b) DLD 1 cells treated with *V. altissima* leaf essential oil for 24 h and photographed under a fluorescent microscope using acridine orange-ethidium bromide staining. The cells which present as orange-red colour with membrane blebbing is represent for dead cells, (c) DLD 1 cells treated with standard 5FU appeared in yellowish-red means dead cells



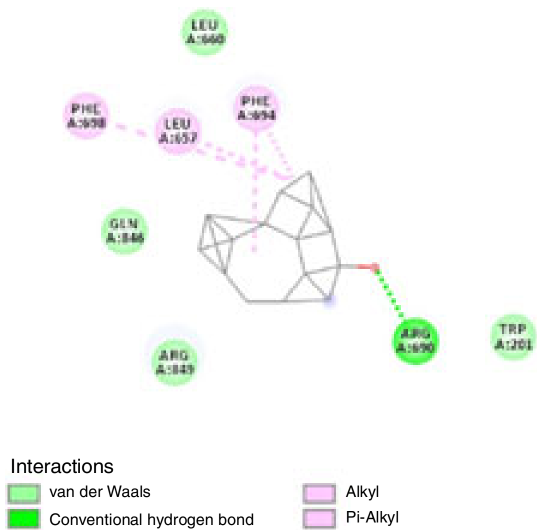



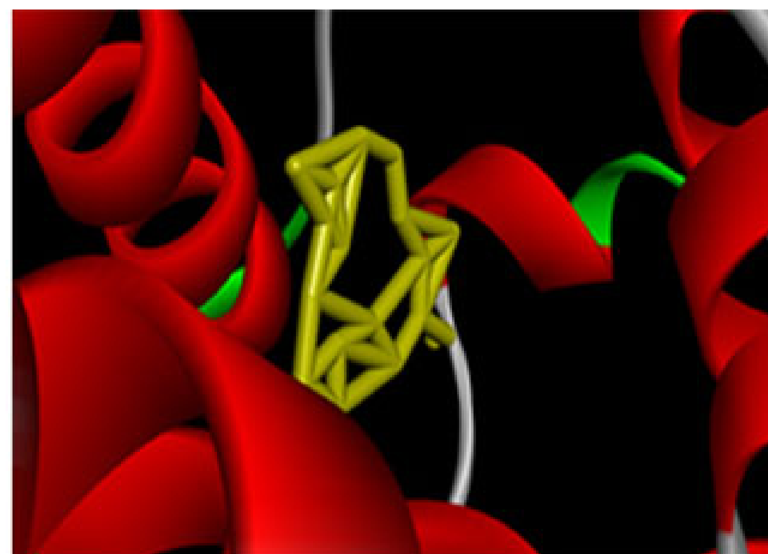
(a)



(b)



(c)

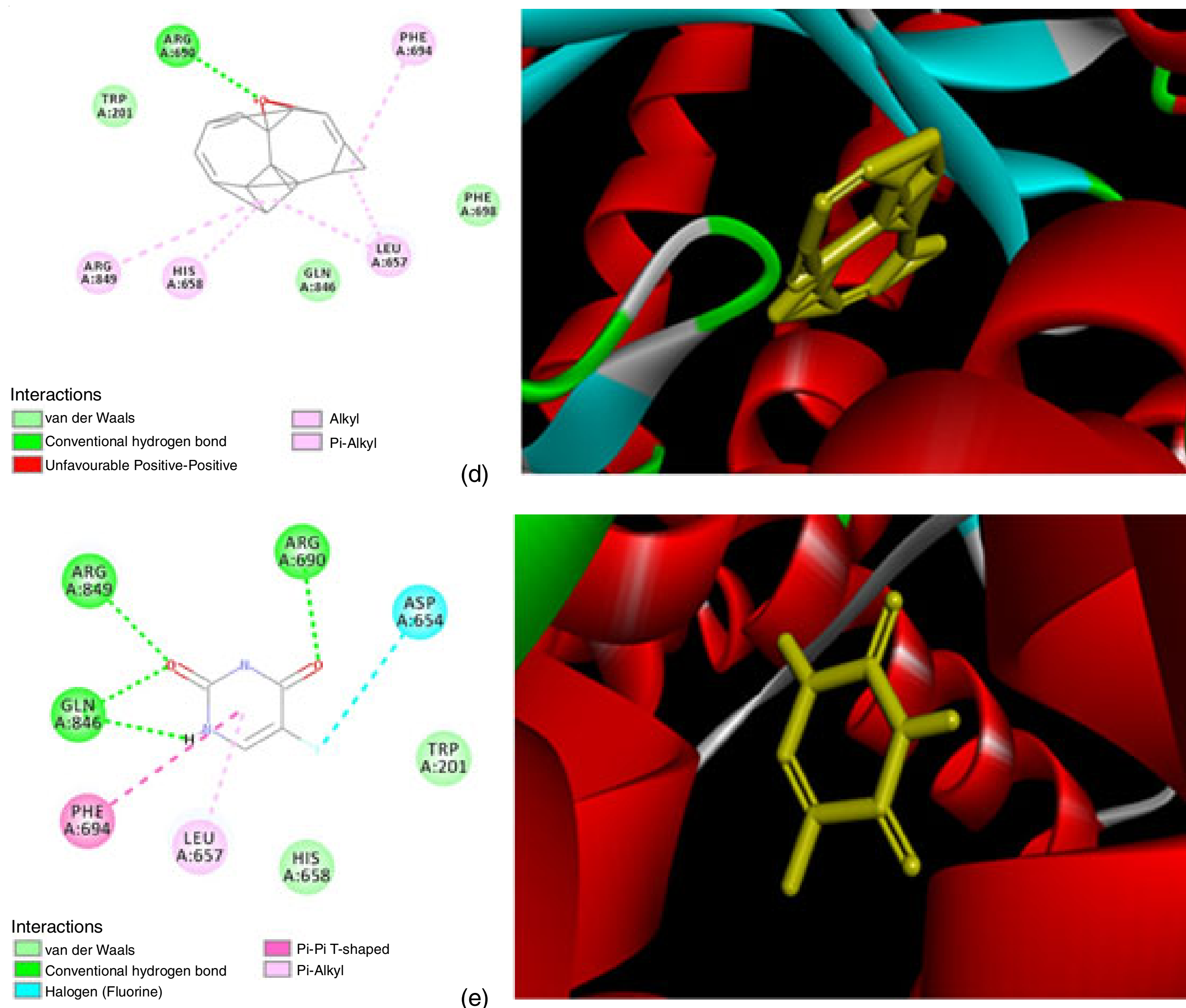



Fig. 9. Two dimensional and three-dimensional interactions of (a) spanthulenol, (b) α-santalol, (c) α-humulene, (d) allohimachalol and (e) 5-FU against the enzyme PI3K

## Conclusion

The essential oil extracted from *V. altissima* leaves was found to be enriched with prominent anticancer and antioxidant properties. About 22 compounds were identified using GC-MS, which includes globulol, sabinene, *allo*-aromadendrene, cadinol, β-eudesmol, linalool, palustrol, spathulenol, humulene epoxide, α-santalol, viridiflorol, E-phytol and bicyclogermacrene as the predominant compounds. All the docked compounds were found to be satisfied the Lipinski's rule of five indicating the scope for its use as an oral drug. The high percentage of sesquiterpenes increases the biological importance of *V. altissima* leaves and demands further studies on the essential oil.

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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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TABLE-3
MOLECULAR DOCKING RESULT ANALYSIS OF BIOACTIVE COMPOUNDS FROM ESSENTIAL OIL OF *V. altissima* LEAVES AGAINST PHOSPHOINOSITIDE-3 KINASE (PI3K) (PDB Id: 3S2A)

| No. | Compound | Binding energy (Kcal/mol) | Number of H bonds | H-bond interactions | Total polar and non-polar bonding |
|---|---|---|---|---|---|
| 1 | Linalool | -5.15 | 1 | TRP 201 | LEU 657, ARG 690, ASP 654, HIS 658, GLN 291 |
| 2 | α-Gurjunene | -6.84 | 0 | – | ARG 849, GLN 846, ARG 690, PHE 694, PHE 698, LEU 657, LEU 660 |
| 3 | β-Caryophyllene | -7.04 | 0 | – | LEU 657, PHE 694, LEU 660, ARG 690, PHE 698, GLN 846, ARG 849 |
| 4 | Aromadendrene | -6.91 | 0 | – | PRO 1040, ASP 837, ARG 839, GLN 840, GLY 1038, LEU 843, LYS 668, GLN 665, THR 1037, LEU 661 |
| 5 | α-Humulene | -7.02 | 1 | ARG 690 | ARG 849, GLN 846, PHE 698, LEU 657, PHE 694, TRP 201, LEU 660 |
| 6 | *allo*-Aromadendrene | -6.91 | 0 | – | ARG 849, LEU 660, ARG 690, GLN 846, PHE 694, LEU 657, PHE 698 |
| 7 | Bicyclogermacrene | -7.04 | 0 | – | GLN 846, PHE 698, ARG 690, LEU 657, PHE 694, ARG 849, HIS 658, TRP 201 |
| 8 | Palustrol | -7.55 | 0 | – | ARG 690, LEU 660, PHE 694, LEU 657, ARG 849, GLN 846, PHE 698 |
| 9 | Spathulenol | -7.68 | 1 | ARG 690 | PHE 698, GLN 846, ARG 849, HIS 658, TRP 201, LEU 657, PHE 694 |
| 10 | Caryophyllene oxide | -7.21 | 1 | ARG 690 | TRP 201, ARG 849, PHE 698, LEU 657, PHE 694, GLN 846, LEU 660 |
| 11 | Globulol | -7.80 | 0 | – | ARG 849, GLN 846, HIS 658, PHE 694, LEU 657, ARG 690, TRP 201, HIS 658 |
| 12 | Viridfloral | -7.14 | 1 | ARG 690 | GLN 846, ARG 849, LEU 657, PHE 698, TRP 201, PHE 694 |
| 13 | Humulene epoxide II | -7.37 | 0 | – | LEU 660, PHE 694, LEU 657, PHE 698, GLN 846, ARG 690, TRP 201, ARG 849 |
| 14 | *trans*-Isolongifolanone | -7.95 | 1 | ARG 690 | LEU 657, ARG 849, GLN 846, TRP 201, HIS 658, PHE 698, PHE 694 |
| 15 | Allohimachalol | -8.03 | 1 | ARG 690 | PHE 694, TRP 201, ARG 849, HIS 658, GLN 846, LEU 657, PHE 698 |
| 16 | α-Santalol | -7.42 | 1 | ASP 654 | ARG 849, GLN 846, LEU 657, TRP 201, GLN 291, HIS 658, ARG 690, PHE 694, PHE 698 |
| 17 | Drimenone | -7.76 | 1 | ARG 690 | PHE 694, LEU 657, PHE 698, ARG 849, GLN 846 |
| 18 | E-Phytol | -6.37 | 1 | ARG 277 | TRP 201, ARG 294, TRP 292, LYS 298, HIS 295, GLN 291, GLU 279, GLY 790 |
| 19 | 5-Fluorouracil | -4.10 | 3 | GLU 846, ARG 690, ARG 849 | ASP 654, TRP 201, HIS 658, LEU 657 |

TABLE-4
LIPINSKI PROPERTIES OF BIOACTIVE COMPOUNDS FROM ESSENTIAL OIL OF *V. altissima* LEAVES

| No. | Compound | Molecular weight (< 500 Da) | Log P (< 5.6) | H-bond donor (< 5) | H-bond acceptor (< 10) | Molar refractivity (40-130) |
|---|---|---|---|---|---|---|
| 1 | Linalool | 154.25 | 2.66 | 1 | 1 | 50.44 |
| 2 | α-Gurjunene | 204.35 | 4.27 | 0 | 0 | 67.14 |
| 3 | β-Caryophyllene | 204.35 | 4.24 | 0 | 0 | 68.78 |
| 4 | Aromadendrene | 204.35 | 4.34 | 0 | 0 | 67.14 |
| 5 | α-Humulene | 204.35 | 4.26 | 0 | 0 | 70.42 |
| 6 | *allo*-Aromadendrene | 204.35 | 4.34 | 0 | 0 | 67.14 |
| 7 | Bicyclogermacrene | 204.35 | 4.15 | 0 | 0 | 68.78 |
| 8 | Palusterol | 222.37 | 3.46 | 1 | 1 | 68.82 |
| 9 | Spathulenol | 220.35 | 3.26 | 1 | 1 | 68.34 |
| 10 | Caryophyllene oxide | 220.35 | 3.68 | 0 | 1 | 68.34 |
| 11 | Globulol | 222.37 | 3.42 | 1 | 1 | 68.27 |
| 12 | Viridfloral | 222.37 | 3.42 | 1 | 1 | 68.82 |
| 13 | Humulene epoxide II | 220.35 | 3.71 | 0 | 1 | 69.91 |
| 14 | Trans isolongifolanone | 220.35 | 3.64 | 0 | 1 | 67.3 |
| 15 | Allohimachalol | 222.37 | 3.58 | 1 | 1 | 70.16 |
| 16 | α-Santalol | 220.35 | 3.57 | 1 | 1 | 68.04 |
| 17 | Drimenone | 224.34 | 2.82 | 1 | 2 | 66.06 |
| 18 | E-Phytol | 296.53 | 6.22 | 1 | 1 | 98.94 |

TABLE-5
ADME PROPERTIES OF VARIOUS BIOACTIVE COMPOUNDS

| No. | Compound | Water solubility | BBB permeability | Caco-2 permeability | GI absorption | Human intestinial absorption HIA (%) | Skin permeability |
|---|---|---|---|---|---|---|---|
| 1 | Linalool | Soluble | Yes | 29.35 | High | 100 | -0.895 |
| 2 | α-Gurjunene | Soluble | No | 23.63 | Low | 100 | -0.978 |
| 3 | β-Caryophyllene | Soluble | No | 23.64 | Low | 100 | -1.120 |
| 4 | Aromadendrene | Soluble | Yes | 23.49 | Low | 100 | -0.991 |
| 5 | α-Humulene | Soluble | Yes | 11.26 | Low | 100 | -0.655 |
| 6 | *allo*-Aromadendrene | Soluble | Yes | 23.49 | Low | 100 | -0.991 |
| 7 | Bicyclogermacrene | Soluble | No | 23.40 | Low | 100 | -0.984 |
| 8 | Palustrol | Soluble | Yes | 54.57 | High | 100 | -1.504 |
| 9 | Spathulenol | Soluble | Yes | 54.42 | High | 100 | -1.331 |
| 10 | Caryophyllene oxide | Soluble | Yes | 52.51 | High | 100 | -1.750 |
| 11 | Globulol | Soluble | Yes | 54.57 | High | 100 | -1.369 |
| 12 | Viridfloral | Soluble | Yes | 54.57 | High | 100 | -1.936 |
| 13 | Humulene epoxide II | Soluble | Yes | 52.26 | High | 100 | -0.499 |
| 14 | *trans*-Isolongifolanone | Soluble | Yes | 32.80 | High | 100 | -1.781 |
| 15 | Allohimachalol | Soluble | Yes | 40.12 | High | 100 | -1.305 |
| 16 | α-Santalol | Soluble | Yes | 18.57 | High | 94.81 | -1.796 |
| 17 | Drimenone | Soluble | Yes | 28.20 | Low | 100 | -1.169 |
| 18 | E-Phytol | Soluble | No | 37.62 | Low | 100 | -0.523 |

TABLE-6
TOXICITY PROPERTIES OF VARIOUS BIOACTIVE COMPOUNDS

| No. | Compound | Algae toxicity | AMES toxicity | hERG inhibition | Carcinogenicity (mouse) | Carcinogenicity (rat) |
|---|---|---|---|---|---|---|
| 1 | Linalool | 0.034 | Mutagen | Low risk | Negative | Negative |
| 2 | α-Gurjunene | 0.013 | Non-mutagen | Medium risk | Negative | Positive |
| 3 | β-Caryophyllene | 0.024 | Mutagen | Low risk | Negative | Negative |
| 4 | Aromadendrene | 0.017 | Mutagen | Medium risk | Negative | Positive |
| 5 | α-Humulene | 0.020 | Non-mutagen | Low risk | Positive | Negative |
| 6 | *allo*-Aromadendrene | 0.017 | Mutagen | Medium risk | Negative | Positive |
| 7 | Bicyclogermacrene | 0.018 | Mutagen | Medium risk | Positive | Positive |
| 8 | Palustrol | 0.019 | Non-mutagen | Low risk | Positive | Negative |
| 9 | Spathulenol | 0.026 | Mutagen | Low risk | Positive | Negative |
| 10 | Caryophyllene oxide | 0.025 | Mutagen | Medium risk | Positive | Positive |
| 11 | Globulol | 0.021 | Non-mutagen | Low risk | Negative | Positive |
| 12 | Viridfloral | 0.021 | Non-mutagen | Low risk | Negative | Negative |
| 13 | Humulene epoxide II | 0.025 | Mutagen | Medium risk | Positive | Negative |
| 14 | *trans*-Isolongifolanone | 0.032 | Non-mutagen | Low risk | Negative | Positive |
| 15 | Allohimachalol | 0.016 | Mutagen | Low risk | Positive | Positive |
| 16 | α-Santalol | 0.025 | Mutagen | Low risk | Positive | Positive |
| 17 | Drimenone | 0.025 | Non-mutagen | Low risk | Negative | Positive |
| 18 | E-Phytol | 0.001 | Non-mutagen | Low risk | Positive | Negative |


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