In silico and *in vitro* anti-cancer Study of *Coleus zeylanicus* Leaves Extract on PBMC and MCF7 cell lines - A potential reservoir of Bioactive molecules

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Abstract
Coleus zeylanicus is one of the plant species belonging to the family Lamiaceae with many bioactive characteristics and well-known bio-therapeutic applications. In this work, bioactive components of chloroform and ethanol leaf extracts of Coleus zeylanicus were analysed by GC-MS. The GC-MS chromatogram represents the presence of 12 phytochemical compounds. Further, the leaf extracts were investigated for in-vitro anti-cancer activity. MTT assay was experimented to understand the cytotoxicity ability of C.zeylanicus extract MCF7 and PBMC cells. The IC50 values of the extracts were found to be 64.81 ± 0.57 µg/ml and 87.92 ± 0.86µg/ml respectively.

Subsequently, the identified 12 compounds were docked against VEGF Receptor (1Y6A), BCL-2 (5JSN) and VEGF factor D (2XV7) in order to explore its inhibitory action by in silico anti-cancer property. In this study the phytochemical compounds were analysed by GC-MS and for in-vitro anti-cancer activity of this plant species. The result of this study may lead to create a path for of novel anti-cancer drugs development.

Keywords: *Coleus zeylanicus*, GC-MS, PBMC and MCF7 cells, *in silico* anti-cancer.

Introduction

Coleus zeylanicus is a member from Lamiaceae referred in India as "Iruveli" or "Karuver"[9]. The plant species has been reported in past decades for its therapeutic efficiency against digestive problems like abdominal discomfort and inflammation[1]. This plant is reported to cause astringent, common cold, fever, asthma, small pox, nausea, diarrhea, dysentery, stomach ulcer, dental infections, eye infections and infections caused by worms[3]. Diarrhea is treated and cured with the herbal juice extracted from the leaves and stems of *C.zeylanicus* along with honey[1,5]. It also has chronic, diaphoretic, diuretic, cholagogue and acute congestion of liver and biodegradable micro-biocides[7].

Cancer is considered to be one of the most life threatening and fatal diseases in the world. It has been reported that there are more than 100 types of cancer. Cancer is considered to be third leading cause for death in the world[13]. It is reported that 12.5% of the population died due to cancer. The mortality rate of cancer is very high, but many advancements came in treating the cancer and understanding its biological pathway at the cellular level[5]. The most common cancer diagnosed in women is breast cancer. South central Asian countries were reported to have highest incidence of breast cancer. Breast cancer is considered to be the common and malignant form of cancer in women.

Mostly 43.7% of breast cancers can be detected at earlier stage[3]. Research has been conducted to search for an alternative and efficient drug produced naturally by using the phytocompounds obtained from plants to slow down, prevent and also to avoid cost of chemotherapeutic agents, side effects and anticancer drugs. Medicinal plants can be used as an alternate source in providing an efficient therapeutic drug for treating cancer as there are more than 50% of cancer agents identified in plants.[2,6]

Various research investigations in accordance with cancer have been reported from various plants with medicinal value to find new bioactive molecules that have lesser side effects. With the above mentioned point, a study has been done to evaluate the anti-cancer activity by both *in vitro* and *in silico* approach to help Indian traditional way of treatment to combat cancer fatality.

Material and Methods

Extraction procedure: The extracts were prepared by collecting the leaves from the plant *Coleus zeylanicus*; the leaves were washed thoroughly with distilled water and kept for shade drying. After drying, the samples were powdered and refrigerated at 4℃ until further use. 10% (w/v) of crude fresh extract was dissolved in chloroform and ethanol and kept for 24 hrs with intermittent shaking. After 24 hr the extracts were filtered with Whatmann filter paper no. 1 and the obtained clear filtrate was evaporated till it becomes dry to form chloroform extracts and then refrigerated at 4℃ for further study.

GC-MS spectrum: In recent times, GC-MS has been employed as ideal method for the detection of compounds in various medicinal plants extracts in order to explore its bioactive compounds present in various plants having medicinal values. Chloroform and ethanol leaf extracts of *C. zeylanicus* were analysed. The GC-MS chromatogram of chloroform and ethanol leaf extracts of *C. zeylanicus* represented a total of 14 peaks corresponding to the compounds presents in the leaf extracts. Retention time,

peak area (%), height (%) and mass spectral fragmentation fingerprints pattern to that of available known compounds were cited in the National Institute of Standards and Technology (NIST) library. Molecular docking study was further taken to screen the lead compounds with anti-cancer activity before proceeding to *in vitro* anti cancer study.

***In silico* anti-cancer study by molecular docking**

Protein and Ligands preparation: Targets were identified as supported property of neoplastic cell escaping apoptosis, initiation of cancer signals by growth factors. Three protein targets were selected for studying interaction of small compounds that have potential to bind and reverse the function of those targets. *In silico* analysis of the identified compounds from the plant *Coleus zeylanicus* was studied for binding interaction against BCL2 which is an apoptic regulator. The three-dimension structural information about the protein targets was retrieved from protein data bank. All the heteroatoms and bounded ligands were far away from the native structure for molecular docking study.

The identified phytochemical compounds were chosen as ligands and their 3-D structure was retrieved from Pubchem NCBI database in SDF format from which they were converted to PDB format using Open Babel software. The binding efficiency of ligands was performed using AutoDock 4.2 software. Algorithm used for searching best conformers was Genetic Algorithm (GA). Maximum conformers for every compound interaction were set to 10. Results evaluated supported best conformer formed out of 10.

Compounds purification and Structure elucidation: Column chromatography was used for purification of ethanol and chloroform leaf extract on a packed silica gel (100-200 mesh - Merck) and compounds were eluted with mixture of solvents by focusing solvent polarity increasing parameters with chloroform, ethyl acetate, ethanol, methanol and distilled water in the used ration of 90:10, 80:20, 70:30, 50:50, 30:70, 20:80, 10:90. 5 fractions were collected. The isolated compound yield obtained was 25 mg in chloroform. Thin layer chromatography was used to predict the number of compounds in the collected fractions. The collected fractions were loaded on silica gel (0.2 mm thickness) 60 F_{254} coated aluminium plate with a eluting solvents ethyl acetate:methanol (2:1).

The TLC plates were derivatized by immersing the plate in vanillin sulfuric acid (1%) and then the plate heated on 105 ℃ for color formation. All the five collected fractions were analysed for compound structure elucidation using NMR method (Bruker 400 MHz, CD_3OD). In fraction 4, the three compounds were predicted by following ^1H NMR method.

***In vitro* anti-cancer study:** The anti-cancer activity was designed on PBMC and breast cancer cell line MCF-7 received from National Centre for Cell Sciences, Pune, India. The cell lines were cultured in Dulbeco's modified eagle medium (DMEM) and supplemented with 10% heat inactivated FBS, 2mM L-glutathione, penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5µg/ml) in humidified atmosphere with supply of 5% CO_2 at 37˚C until confluent. The cells were typs/850*963-[.3nized with trypsin EDTA solution. The stock cultures were grown in 25cm^2 flat bottles and the cell viability studies were carried out in 96 well microtiter plates.

Isolation and culturing of human PBMC: Human Peripheral Blood Mononuclear Cells (PBMC) mixtures of monocytes and lymphocytes were isolated by centrifugation method. Blood was diluted in PBS buffer in the ratio of 1:1 and this suspension was layered into histopaque-1077 and centrifuged (Harris 18/80, Sanyo, London UK,) for 30 minutes at 800 ×g and 4°C. PBMC were collected from the interface, lysed with 150 mM NH_4Cl and 10mM $NaHCO_3$. 0.1mM EDTA was maintained in RPMI-1640 medium. The cells were grown in RPMI-1640 medium containing 10% fetal calf serum (FCS). The medium was supplemented with glutamine (2mM), HEPS (20 mM), streptomycin (10,000µg/ml), penicillin (10,000µg/ml) and sodium bicarbonate (24 mM). Cells were grown in 25 ml flasks containing 12×10^6 cells/ml. The cells were kept in a humidified atmosphere at 37 °C containing 5% CO_2. Cells were used at 2×10^6 cells/ml.

Both PBMC and MCF-7 cells were treated with various concentrations (10-50 µg dissolved in DMSO) with and without extract for 24 hours of incubation. The final concentration of DMSO in the culture medium was monitored not to exceed to 0.05% in order to avoid toxicity of the cells.

***In vitro* Cytotoxicity by MTT assay:** The assay was designed as per the procedure described earlier. In this assay, by the action of mitochondrial reductase, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a water-soluble dye is converted into insoluble formazan having purple colour. The cells are then treated with an organic solvent such as DMSO used for solubilizing the cells.

Different concentrations of the oil ranging as 20µl, 40µl 80µl and 160µl were subjected for the *in vitro* analysis against MCF7 cells and Peripheral Blood Mononuclear Cells (PBMC). Constant cell number was maintained in PBMC. During this process, the tetrozolium compound MTT was reduced by mitochondrial dehydrogenase as a result of which the colour gets changed to purple.

Lysing solution like organic solvent was added to the wells to solubilize the formazan crystals. ELISA reader (E1x 800 ELISA reader, Biotech Instruments Inc., USA) was used to measure the absorbance and the absorbane was measured at 570 nm. The data was analyzed by plotting absorbance versus cell number in a graph. The rate of reduction of tetrozolium salt is directly proportional to the rate of cell

proliferation. The percentage of cytotoxicity was calculated by the formula [(A-B)/A×100] where A and B are the absorbance of control and treated cells respectively. The IC_{50} value was found. The results were determined by performing in triplicates for each extract.

Statistic analysis: Results are summarized as means ± S.E. of experiments. Comparison with essential oil and control of *Coleus zeylanicus* was observed by Analysis of Variance (ANOVA). Using the Tukey Kramer's test (INStat Graph Pad Software, Inc.,) significant differences were analyzed.

Results

MTT assay for cytotoxicity of the extracts with PBMC and MCF-7cells: MTT results against cancer cell lines MCF 7 and PBMC showed that as the concentration of oil increased the viability of cells decreased and it inhibited the proliferation of cells.

Optical microscope was used to analyze the cytopathic effect of the plant extract on MCF 7 cells. Cells which were not treated were observed to be elongated and attached smoothly on the surface of the culture. Some of the cells were found to form groups called colonies. After treating the cells with extracts for 24h, the cells were found to be round and lost cell contacts. Surface morphological changes causing cell detachment were observed. From this study it is clear that crude extracts obtained from the leaves of *Coleus zeylanicus* have efficient anticancer activity against the cell lines of breast cancer.

GC-MS spectrum summary: Bioactive compounds present in the extract of *Coleus Zeylanicus* were identified through GC-MS. The parameters such as area (%), molecular weight (MW), molecular formula (MF), retention time (RT), synonym, peak and structure were identified. 12 compounds were observed and their structures are tabulated in table 2. The physiochemical properties and structure compounds were obtained from PubChem database.

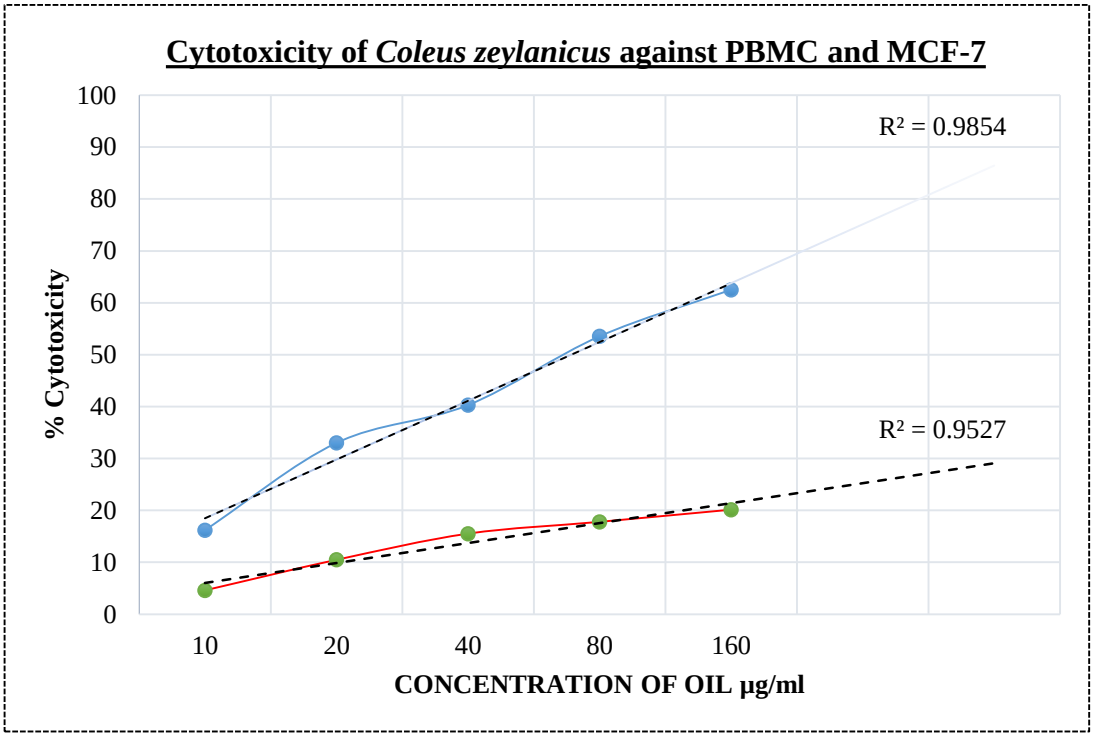

Figure 1: MTT assay was used to evaluate the cytotoxic activity of *C.zeylanicus* leaf extract on MCF7 and PBMC cells. The IC50 values of the extracts were found to be 64.81 ± 0.57 µg/ml and 87.92 ± 0.86µg/ml respectively. The chloroform extract has showed effective cytotoxicity, ($p<0.001$) (Table 1). The IC50 of (positive control) doxorubicin was found to be 1.09±0.05 µg/ml. the positive correlation between the concentration of the oil and cytotoxicity when the regression analysis showed above 0.9. Extract of transferred plant showed higher toxic profile than non-transferred plant

Table 1
Effect of crude extract/positive control against PBMC and MCF-7 cell line by MTT assay

Name of the extract	IC50 value (µg/ml)
Doxorubicin (Positive control)	1.09 ± 0.05
Coleus zeylanicus (PBMC)	87.92 ± 0.86
Coleus zeylanicus (MTT)	64.81 ± 0.57

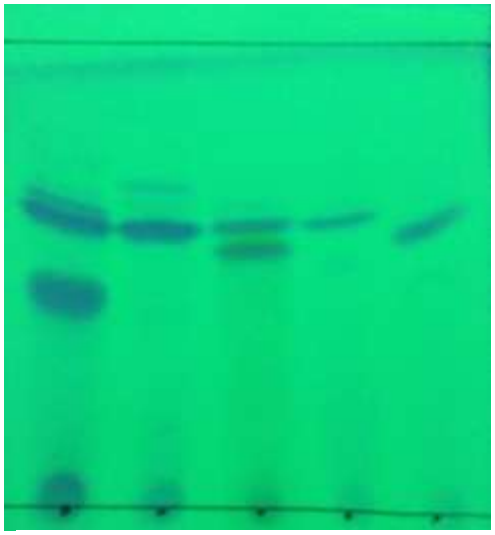

Figure 2: The collection five fractions from Column Chromatography were characterized on pre-coated plate in order to explore and elucidate the isolated compound structure

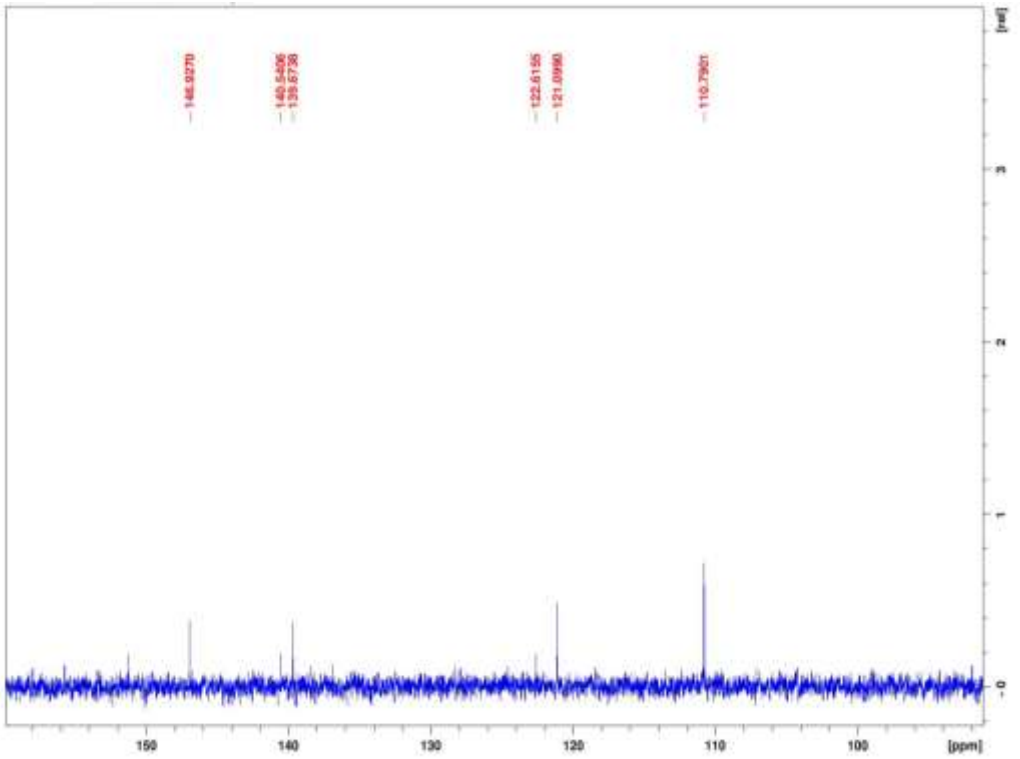

Figure 3: NMR Spectrum of fraction 4. Cubenol -^{1}H NMR (400 MHz, CD_3OD): δ H 0.76 (d, *J* = 6.7, CH_3-12 0.81 (d, *J* = 6.8, CH_3-13), 0.94 (d, *J* = 6.7, CH_3-15), 1.04 (m, CH_2-4), 1.25 (m, CH_2-7), 1.32 (m, CH_2-8), 1.23 (m, H-9), 1.46 (m, H-6), 1.64 (s, CH_3-14), 1.76 (m, H-11), 1.91 (m, CH_2-3), 2.05 (m, H-10), 5.35 (m, H-1). Widdrene - ^{1}H NMR (400 MHz, CD_3OD): δ 0.45 (2H, 0.39 (dd, *J* = 9.6, 8.1 Hz), 0.41 (dd, *J* = 9.6, 7.2 Hz)), 0.97-0.98 (6H, 0.97 (s), 0.97 (s)), 1.01 (3H, s), 1.18 (1H, dd, *J* = 8.1, 7.2 Hz), 1.38 (1H, ddd, *J* = 13.2, 10.3, 2.6 Hz), 1.31-1.54 (2H, 1.38 (ddd, *J* = 13.2, 10.3, 2.5 Hz), 1.49 (ddd, *J* = 13.2, 3.0, 2.6 Hz)), 1.46-1.72 (6H, 1.67 (s), 1.62 (ddd, *J* = 13.2, 3.0, 2.6 Hz), 1.52 (dtt, *J* = 13.1, 3.0, 2.5 Hz), 1.62 (dtt, *J* = 13.1, 10.3, 2.6 Hz)), 2.07 (1H, dd, *J* = 14.9, 9.8 Hz), 2.40 (1H, dd, *J* = 14.9, 9.5 Hz), 5.41 (1H, dd, *J* = 9.8, 9.5 Hz). Phytol - ^{1}H NMR (400 MHz, CD_3OD): δ 0.45 (2H, 0.39 (dd, *J* = 9.6, 8.1 Hz), 0.41 (dd, *J* = 9.6, 7.2 Hz)), 0.98 (6H, 0.97 (s), 0.96 (s)), 1.0 (3H, s), 1.18 (1H, dd, *J* = 8.1, 7.2 Hz), 1.39 (1H, ddd, *J* = 13.1, 10.2, 2.6 Hz), 1.54 (2H, 1.38 (ddd, *J* = 13.2, 10.3, 2.5 Hz), 1.49 (ddd, *J* = 13.2, 3.0, 2.6 Hz)), 1.72 (6H, 1.67 (s), 1.62 (ddd, *J* = 13.2, 3.0, 2.6 Hz), 1.52 (dtt, *J* = 13.1, 3.0, 2.5 Hz), 1.62 (dtt, *J* = 13.1, 10.3, 2.6 Hz)), 2.07 (1H, dd, *J* = 14.9, 9.8 Hz), 2.40 (1H, dd, *J* = 14.9, 9.5 Hz), 5.41 (1H, dd, *J* = 9.8, 9.5 Hz)

Protein Targets for Molecular docking: Molecular Docking was done with the vascular endothelial growth factor D, crystal structure of VEGFR2 and target protein BCL-2 apoptosis regulatoras which play important role in cell initiation and cell signaling in cancer.

Docking results: Tables 4 to 7 provide the results from docking of the drug like compounds with the receptor GL. The majority of the reactions were observed to hydrophobic in nature except hydrogen bonds present in levomenol and phytol.

Drug likeness: LogP is responsible for analog optimization and drug selection. Hence drugs are screened according to logP than other criterias. Lipophility plays a major role in determining absorption, distribution, penetration across vital membranes, metabolism and elimination (ADME). LogP of the drug compound for oral administration should follow some of the Lipinski's rule:

1. The size must be less than 5 (<5)
2. Molecular weight of the compound must be between 150 and 500 g/mol
3. polarity: TPSA between 20 and 130 Å
4. Solubility: The value of log *S* must not be higher than 6
5. Flexibility: It should not have more than 9 rotatable bonds.

SWISSADME was performed to identify the drug likeness and the results are tabulated in table 8. From the result it is clear that all compounds show LogS value less than 6 and LogP values not higher than 5.

Table 2
GC-MS chromatogram represented bioactive compounds present in the leaf extracts of *Coleus Zeylanicus.*

S.N.	Phyto-Compounds	Molecular weight (g/mol)	Area %	RT	Formula
1	GAMMA-GURJUNENE	204.35	100	6.6	$C_{15}H_{24}$
2	GAMMA-PATCHOULENE	204.35	66.83	5.2	$C_{15}H_{24}$
3	LEVOMENOL	222.37	31.99	6.7	$C_{15}H_{26}O$
4	CUBENOL	222.37	23.47	6.6	$C_{15}H_{26}O$
5	WIDDRENE	204.35	18.42	6.5	$C_{15}H_{24}$
6	GAMMA-HIMACHALENE	204.35	17.31	5	$C_{15}H_{24}$
7	BETA-YLANGENE	204.35	16.43	5.3	$C_{15}H_{24}$
8	ALPHA-BERGAMOTENE	204.35	16.2	4.7	$C_{15}H_{24}$
11	JUNIPENE	204.35	9.31	4.6	$C_{15}H_{24}$
12	PHYTOL	296.5	6.15	14.1	$C_{20}H_{40}O$

Table 3
Protein targets for molecular docking study

S.N.	Target	PDB ID	Protein Target
1.	VEGF Receptor	1Y6A	Crystal structure of VEGFR2
2.	BCL-2	5JSN	Apoptosis regulator
3.	VEGF factor D	2xv7	Vascular Endothelial Growth Factor D

Table 4
Molecular docking of compounds with receptor 6GL8

Compounds	Binding Energy	Inhibition Constant	Inter molecular energy	Internal Energy	Torsional Energy	Cluster RMS	Ref RMS
Cubenol	-6.51	16.78 µM	-7.11	-0.26	0.6	-0.26	19.62
Gamma Gurjunene	-6.4	20.22 µM	-6.7	-0.24	0.3	-0.24	19.17
Gamma Patchouline	-6.46	18.41 µM	-6.46	0	0	0	18.3
Widderine	-5.13	172.67 µM	-5.13	0	0	0	15.06
Phytol	-4.19	851.17 µM	-8.36	-1.19	4.18	-1.19	7.18
Ylangene	-6.65	13.34 µM	-6.95	-0.37	0.3	-0.37	18.42
Alphabergamotene	-6.22	27.71 µM	-7.11	-0.78	0.89	-0.78	18.51
Junipene	-7.13	5.97 µM	-7.13	0	0	0	17.83
Levomenol	-6.17	29.96 µM	-7.66	-0.5	1.49	-0.5	19.21

Table 5
Molecular docking of compounds with receptor 1Y6A

Compounds	Binding Energy	Inhibition Constant	Inter molecular energy	Internal Energy	Torsional Energy	Cluster RMS	Ref RMS
Gamma Gurjunene	-6.5	17.3 µM	-6.79	-0.24	0.3	0.1	34.43
Gamma Patchoulene	-6.51	17.02 µM	-6.51	0	0	0	32.73
Levomenol	-6.09	34.46 µM	-7.58	-0.52	1.49	0	34.76
Cubenol	-6.37	21.37 µM	-6.97	-0.22	0.6	0	32.13
Widdrene	-6.12	32.7 µM	-6.12	0	0	0	46.04
Gamma Himachalene	-6.25	26.43 µM	-6.25	0	0	1.21	32.89
Beta Ylangene	-6.49	17.45 µM	-6.79	-0.26	0.3	0	34.94
Alpha Bergamotene	-6.18	29.5 µM	-7.08	-0.46	0.89	0	60.48
Alpha Guaiene	-6.57	15.39 µM	-6.86	-0.22	0.3	0.1	33.96
Alpha Panasinsene	-6.03	37.87 µM	-6.03	0	0	0	32.8
Junipene	-6.53	16.27 µM	-6.53	0	0	0	32.93
Phytol	-4.1	989.97 µM	-8.27	4.18	4.18	0	42.5
Beta Elemene	-6.12	32.54 µM	-7.02	0.89	0.89	0	34.29

Table 6
Molecular docking of compounds with receptor 5JSN

Compounds	Binding Energy	Inhibition Constant	Inter molecular energy	Internal Energy	Torsional Energy	Cluster RMS	Ref RMS
Gamma Gurjunene	-6.6	14.64 µM	-6.89	-0.25	0.3	0.03	55.65
Gamma Patchoulene	-5.99	40.49 µM	-5.99	0	0	0	61.37
Levomenol	-5.47	97.1 µM	-6.93	-0.86	1.49	0	54.88
Cubenol	-5.91	46.76 µM	-6.3	-0.22	0.6	0	47.48
Widdrene	-6.16	30.65 µM	-6.16	0	0	0	53.91
Gamma Himachalene	-6.14	31.81 µM	-6.13	0	0	0.02	55.08
Beta Ylangene	-6.29	24.59 µM	-6.59	-0.29	0.3	0.02	53.22
Alpha Bergamotene	-5.8	56.1 µM	-6.69	-0.57	0.89	0	54.6
Alpha Guaiene	-6	40.18 µM	-6.29	-0.22	0.3	0	53.76
Alpha Panasinsene	-6.12	32.55 µM	-6.12	0	0	0	62.6
Junipene	-6.17	30.12 µM	-6.17	0	0	0.08	53.56
Phytol	-4.66	380.68 µM	-8.82	-1.42	4.18	0	55.56
Beta Elemene	-5.65	72.77 µM	-6.54	-0.54	0.89	0	54.6

The molecular weight of the compounds was found to be less than 500g/mol with rotatable bonds less than 9 except for phytol. From this result it is observed that except phytol all the other compounds contain drug like properties. These compounds except phytol can be analyzed at *in vitro* level.

Minimum binding energy showing compounds: Docking was performed for each molecule with 3 targets and the results were evaluated based on number of hydrogen bonds formed, binding efficiency which provides fundamental information about the interaction and its efficiency.

ADME Properties analysis: 3 molecules namely cubenol, widdrene, phytol were subjected to SWISS ADME bioinformatic tools to check biological and physical properties and results were evaluated [Table 8].

Only one compound CUBENOL was found to show all physical and biological properties when three compounds were tested on considering different parameters and this can be further used for treating cancer.

Discussion

Cancer is group of diseases which is widely being investigated and classified as many types based on different parameters such as part of body, type of tissue affected etc. Cancer is a disease which is characterized by uncontrolled or abnormal cell growth which has potential to spread and invade into different parts of body.

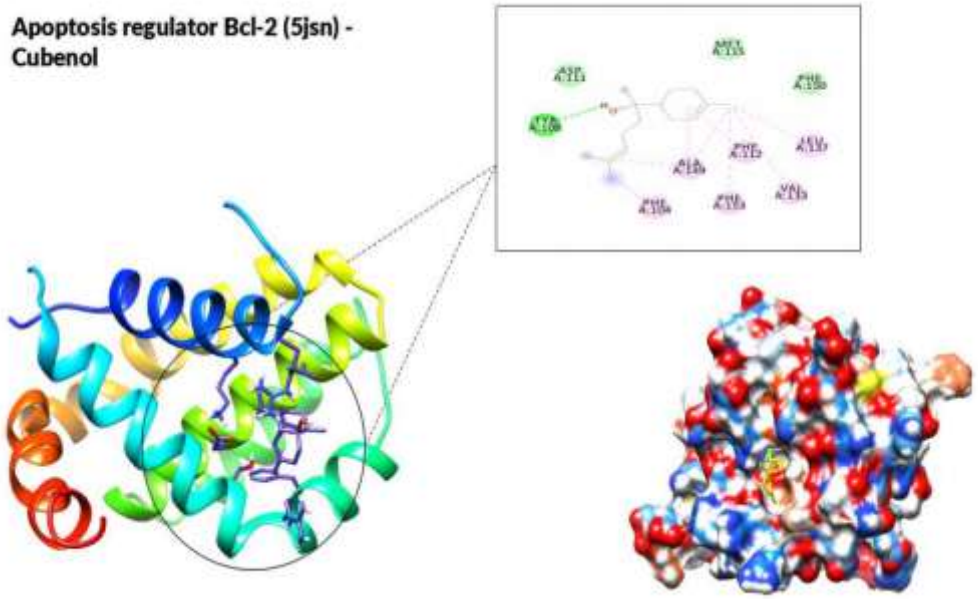

Figure 4: Apoptosis regulator Bcl-2 in complex with Cubenol

Table 7
Molecular docking of compounds with receptor 2XV7

Compounds	Binding Energy	Inhibition Constant	Inter molecular energy	Internal Energy	Torsional Energy	Cluster RMS	Ref RMS
Gamma Gurjunene	-5.97	42.13 µM	-6.27	-0.24	0.3	0	38.99
Gamma Patchoulene	-5.98	41.33 µM	-5.98	0	0	0.06	40.71
Levomenol	-5.32	126.72 µM	-6.81	-0.59	1.49	0	39.7
Cubenol	-5.83	53.1 µM	-6.43	-0.26	0.6	0	41.21
Widdrene	-5.37	116.21 µM	-5.37	0	0	0	41.59
Gamma Himachalene	-5.99	40.88 µM	-5.99	0	0	0	40.21
Beta Ylangene	-6.42	19.78 µM	-6.72	-0.29	0.3	0	39.99
Alpha Bergamotene	-5.69	67.8 µM	-6.58	-0.5	0.89	0	40.05
Alpha Guaiene	-5.37	115.76 µM	-5.67	-0.23	0.3	0.05	40.22
Alpha Panasinsene	-5.98	41.64µM	-5.98	0	0	0.01	41.01
Junipene	-5.43	104.56 µM	-5.43	0	0	0.04	40.98
Phytol	-3.23	4.29µM	-7.41	-1.04	4.18	0	40.56
Beta Elemene	-4.87	271 µM	-5.76	-0.6	0.89	0	40.62

Table 8
SWISSADME for identifying drug likeliness of compounds

Compounds	Smiles Notation	LogP	LogS	BBB Permeant	Lipinski Score	Mol.Wt (g/mol)	TPSA	Rotatable bonds
Cubenol	CC1CCC(C2C1(CCC(=C2)C)O)C(C)C	3.52	-3.48	Yes	Yes	222.22	20.23	1
Gamma Gujurene	CC1CCC(C2C1(CCC(=C2)O)C(C)C	4.33	-4.36	No	Yes:1	204.19	0	1
Levomenol	OC(CCC=C(C)C)(C)C1CCC(C)=CC1	3.76	-3.34	Yes	Yes	222.2	20.23	4
Widderine	C123C(C(C)=CCC1(C)CCCC3(C)C2	4.48	-4.11	No	Yes:1	204.19	0	0
Gamma Patcholene	C123C(C)(C)C(CCC3=C)CC1C(C)C2	4.48	-4.26	No	Yes:1	204.19	0	0
Phytol	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C	6.22	-5.98	No	Yes:1	296.53	20.23	13
Ylangene	CC1=CCC2C3C1C2(CCC3C(C)C)C	4.3	-3.86	Yes	Yes:1	204.35	0	1
Alphabergamotene	CC1=CCC2CC1C2(C)CCC=C(C)C	4.7	-4.97	No	Yes:1	204.35	0	3
Junipene	CC1(CCCC2(C3C1(C2=C)CC3)C)C	4.5	-4.31	No	Yes:1	204.35	0	0

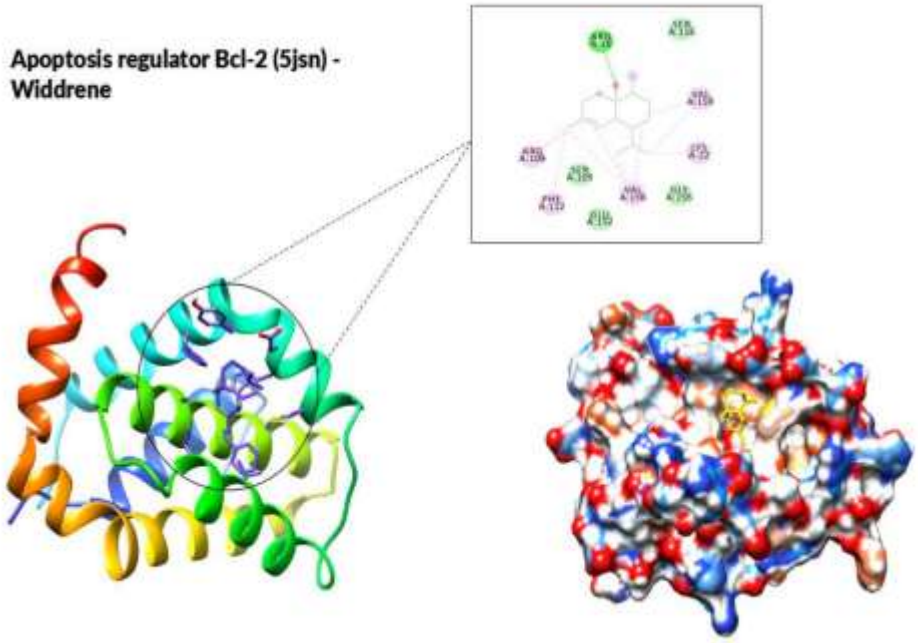

Figure 5: Apoptosis regulator Bcl-2 in complex with Widdrene

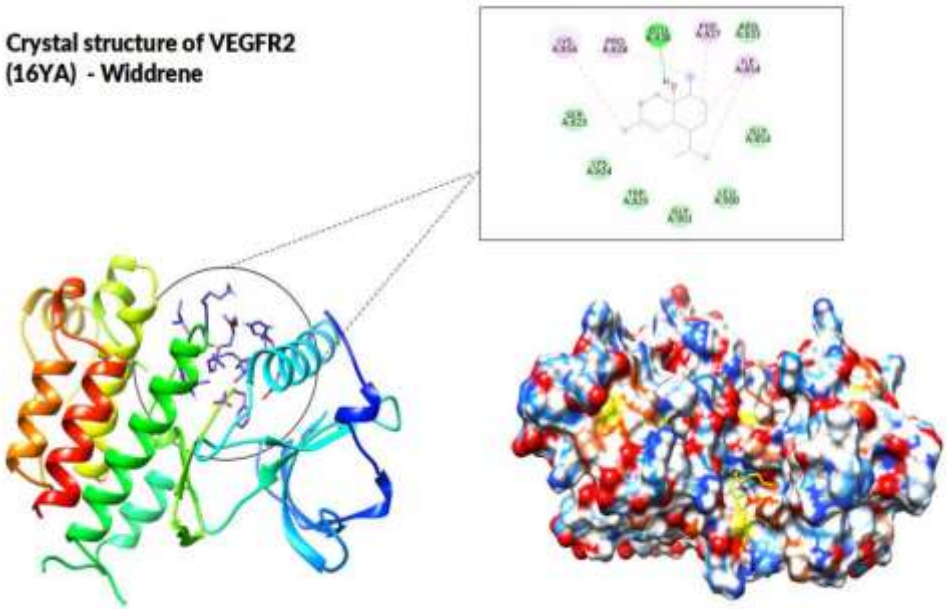

Figure 6: VEGFR2 Crystal structure in complex with Widdrene

Table 9
Binding efficiency of interacted molecules

S.N.	Target	Phytochemicals	Minimum binding energy	No. of Hydrogen Bonds
1	5JSN	CUBENOL	**-5.91**	1
		WIDDRENE	**-6.16**	1
2	2XV7	CUBENOL	**-5.83**	1
		WIDDRENE	**-5.37**	1
		PHYTOL	**-3.23**	1
3	1Y6A	WIDDRENE	**-6.12**	1
		PHYTOL	**-4.1**	2

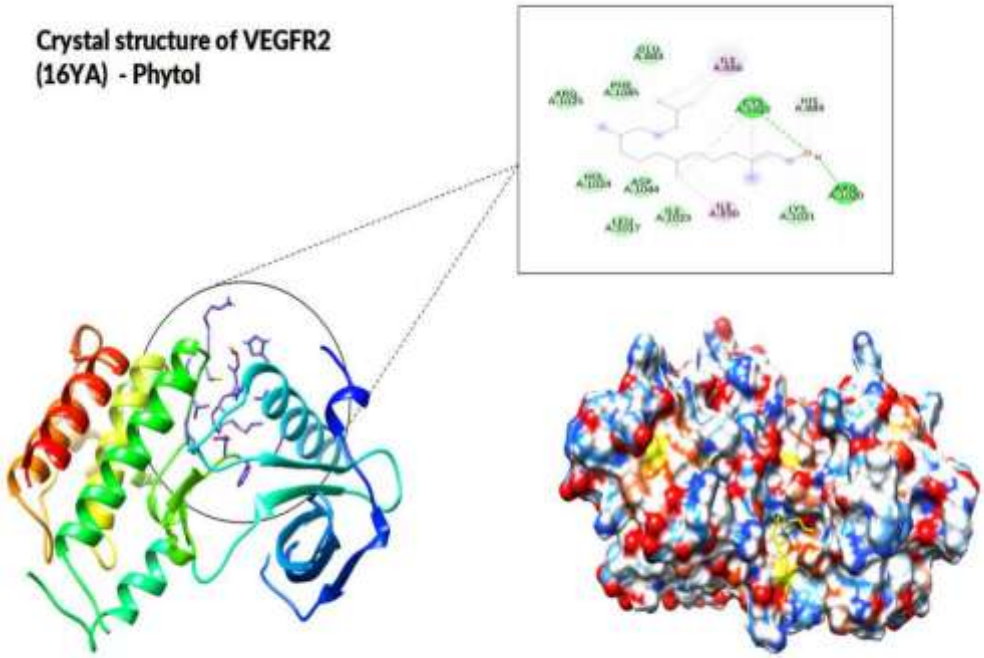

Figure 7: VEGFR2 Crystal structure in complex with Phytol

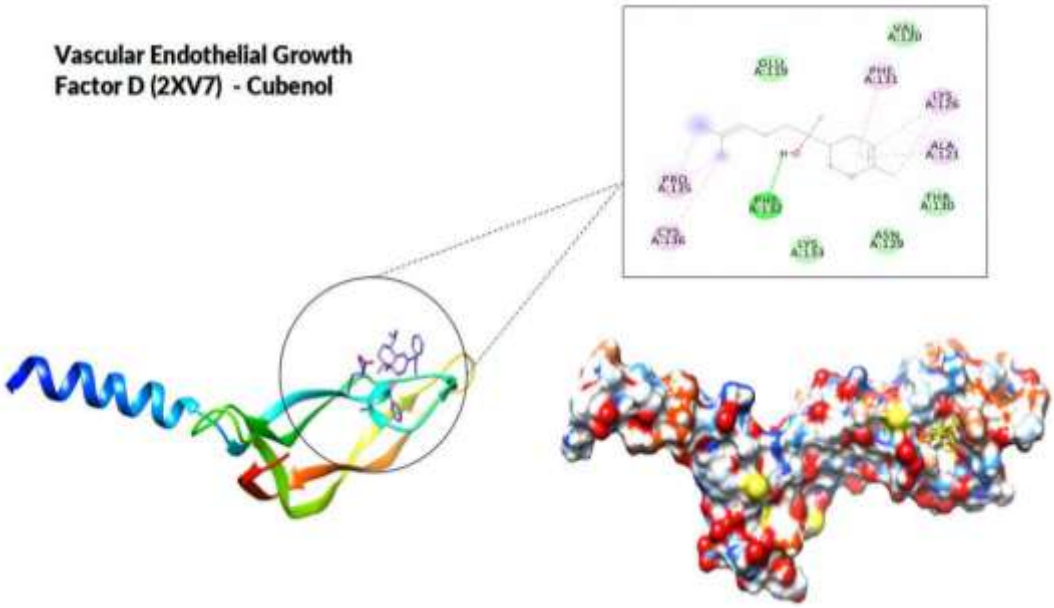

Figure 8: Vascular Endothelial Growth Factor D in complex with Cubenol

Table 10
Biological and Physical properties of widdrene, cubenol and phytol

Molecule	Mol. Wt	TPSA	Consensus Log P	Solubility	GI absorption	BBB Permeant	Log Kp (cm/s)	Lipinski	Lead Likeness
Cubenol	222.37	20.23	3.52	Soluble	High	Yes	-5.03	0	2
Widdrene	204.35	0	4.48	Moderate soluble	Low	No	-4.17	1	2
Phytol	296.53	20.23	6.22	Poorly soluble	Low	No	-2.29	1	2

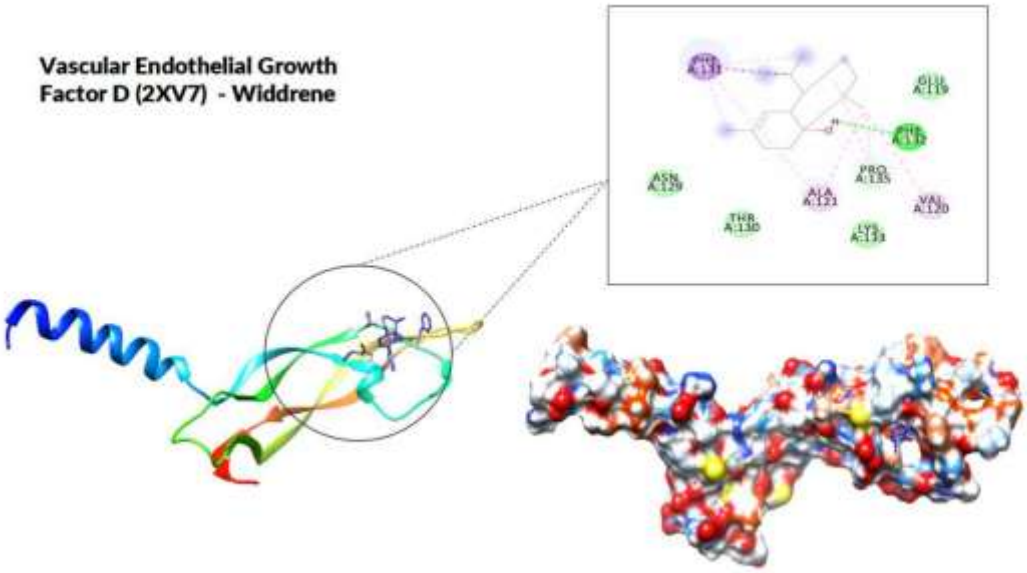

Figure 9: VEGFR2 Crystal structure in complex with Widdrene

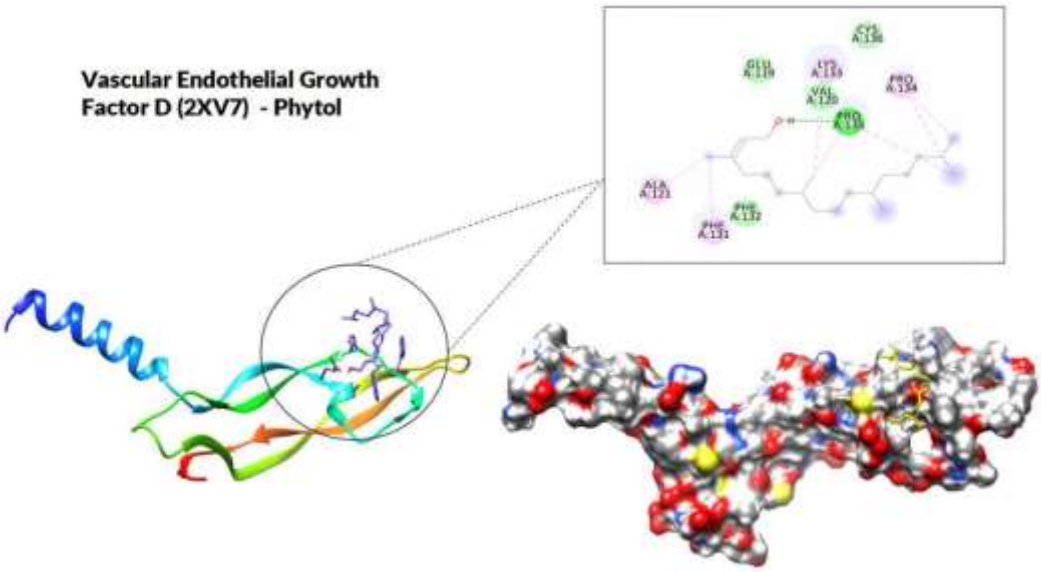

Figure 10: VEGFR2 Crystal structure in complex with Phytol

It has been most leading diseases over past few years. More than 100 types of cancers have been discovered and yet more types are to be discovered. Some cancers are genetic and some are hereditary (ovarian, breast cancers).

Treatment of this disease is possible with one form of anti-carcinogen or combination. Chemotherapy, radiation therapy, surgical process, immunotherapy, targeted therapy, or hormone therapy are available treatment for cancer. Various anti-cancer drugs have been found from the past decades from medicinal plants and reported high response towards cancer inhibitory action studied by *in vitro* and *in vivo* approach. Research is still going on for the finding of potential anti-cancer candidates. Phytochemicals with anti-cancer ability have been extracted from various medicinal plant species and being tested as targets for cancer. Thousands of phytochemicals have reported high potential for some cancer targets and used as candidate drug molecules for anticancer therapeutic approach with lesser side effects.

In this study we have focused on phytochemicals extracted from plant species *Coleus Zeylanicus* and these plant compounds have been cited known for their various biological activities.[8,10,13] GC-MS spectrum of leaves extract of *Coleus Zeylanicus* revealed the presence of 12 phytocompounds that belong to flavonoids, carbohydrates, anthocyanin, tannins, phenols, alkaloids, steroids classes. These bioactive compounds could be a reason for the anti-cancer activity of leaf extracts of *Coleus Zeylanicus.*

In referencing supports of this study, earlier some studies have been reported the potential antimicrobial activities of essential oil isolated from *Coleus Zeylanicus*. In addition, we have also investigated the *in silico* anti-cancer activity of leaves extract of *Coleus Zeylanicus.*

The above study evidences that *Coleus Zeylanicus*, plant enriched with various potential bioactive compounds could be potent pharmacological drug candidates having inhibitory actions like anti-microbial, anti-cancer, anti-oxidant, anti-inflammatory etc. Hence, the identification of phyto constituents from *Coleus Zeylanicus* is considered to be a significant medicinal activity and further studies including single compound purification and investigation of biological properties will be future bioprospecting to be studied in future.

Conclusion

Based on the obtained results, in which *in vitro* anti-cancer activity, PBMC and MCF-7 cell line models were used, it can be concluded that the leaves extract of *Coleus zeylanicus* possesses significant anti-cancer bioactivity for further extensive future work.

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(Received 28th January 2021, accepted 05th March 2021)