Heliyon 6 (2020) e04209

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Chemical, biological and *in silico* assessment of *Ocimum viride* essential oil

Madhulika Bhagat [a,*], Monica Sangral [a], Ajay Kumar [a], Rafiq A. Rather [a], Khushboo Arya [b]

[a] School of Biotechnology, University of Jammu, Jammu, 180006, India
[b] Department of Biochemistry, Lucknow University, Uttar Pradesh, 226003, India

ARTICLE INFO

Keywords:
Essential oil
Antimicrobial agent
Biomedical materials
Cancer research
Pharmaceutical science
Toxicology

ABSTRACT

Aims: *Ocimum viride* Willd. (family: *Lamiaceae*) is a member of the genus Ocimum, an aromatic annual and perennial herb with numerous culinary, horticultural and ethno-medicinal benefits. This study aims to explore the chemical properties of leaf essential oil (EO) from *Ocimum viride* and to evaluate its antimicrobial and anticancer potential.

Main methods: Characterization of essential oil was done by GCMS, antimicrobial by agar well diffusion methods, *in vitro* cytotoxicity evaluation by MTT assay, cell death analysis was done by DNA fragmentation, cell cycle analysis, nuclear morphology analysis and molecular docking studies were also conducted.

Key findings: Essential oil from aerial parts (leaf) of *Ocimum viride* revealed high content of oxygenated monoterpenes, notably thymol (~50%) and γ-terpinene (~18%). Further, antibacterial analysis showed that among all the evaluated bacterial species EO showed highest sensitivity against the *Bacillus subtilis* and was also found most effective against HT-29 colon cancer cell line with IC50 value of ~0.034 ± 0.001μL/mL. Mechanistic studies revealed that EO inhibits the growth of HT-29 colon cancer cells probably through induction of irreparable DNA damage leading to subsequent cell death in apoptotic manner. Molecular docking analysis also supports the *in vitro* studies conducted by indicating the interaction of thymol with Sec A protein of *Bacillus subtilis* cell wall as well as with Beclin protein responsible for apoptotic corpse clearance.

Significance: Taken together, our results indicate that EO possesses potent antimicrobial and anticancer properties, and may find applications as effective antibacterial and in cancer therapeutics.

1. Introduction

Essential oils are the aromatic, volatile and complex liquids obtained from different parts of plants. These are secondary metabolites that are primarily involved in defensive mechanisms in plants and usually consists terpenes (mono, sesquiterpenes and diterpens), alcohol, esters, citons, acids, apoxides, aldehyde, sulfide, amine etc. Apart from their various uses in cosmetics, pharmaceuticals, perfumes, food and alcoholic beverages, these are also reported for various biological properties such as antimicrobial, analgesic, antioxidant, antiviral, antispasmodic, carminative, analgesic, anti-inflammation etc (Blowman et al., 2018; Bhagat et al., 2018; Brnawi et al., 2018; Govindarajan et al., 2018; Hosseini et al., 2017; Ko et al., 2017; Moy et al., 2017). As antibiotic and chemotherapeutic drug resistance has become a global concern, essential oils and other phyto-products have gained interest, mainly due to their suitable chemical characteristics and biological activities. Various scientific investigators are now focused on elucidating the specific mode of action of essential oils components as well as to explore the synergistic effect of essential oil components and also with the existing antibiotic or chemotherapeutic drugs that may provide an alternative approach to rectify the emerging drug resistance.

Genus *Ocimum* (Family: Lamiaceae formerly Labiatae) has been placed among the most important traditional medicinal plants owing to its numerous reported medicinal properties. This genus is a great source of many naturally occurring essential oils and aromatic compounds, containing approximately 150 species of herbs and shrubs from the tropical regions of Asia, Africa, and Central and South America (Dubey et al., 1997; Martins et al., 1999). Among these reported species only nine of these species have been identified in India (Lemus-Mondaca et al., 2012), while *Ocimum sanctum* is widely known species reported profusely in Indian System of Medicine with numerous medicinal properties such as in cardiopathy, homeopathy, asthma, bronchitis, vomiting, skin diseases etc (Ekundayo, 1986; Khosla and morphology, 1986). In this study, we have explored a comparatively less explored species of genus *Ocimum* i.e., *Ocimum viride* Willd., native of Africa and then introduced into India. *Ocimum viride* specie, is cultivated in Jammu-Tawi and reported to give

* Corresponding author.
E-mail address: madhulikasbt@gmail.com (M. Bhagat).

https://doi.org/10.1016/j.heliyon.2020.e04209
Received 12 December 2019; Received in revised form 15 January 2020; Accepted 9 June 2020

maximum oil yield (0.4%) at full bloom stage with highest percentage of thymol (55.12%) in the oil that can be used as a substitute for thyme-ajowan oil (Khare, 2007). Traditionally, leaves are known for the treatment of febrifugal and used as a remedy for coughs, fevers, diarrhea, digestive problems, wounds healing, hypertension, convulsions, oxidative stress, diabetes, while leaves oil as antiseptic (Khare, 2007). Additionally, it is reported for the presence of terpenes predominantly (Singh et al., 2015) as well as for anti-malarial activity (Fokou et al., 2015), cytotoxic activity (Selvi et al., 2015) and for repellence property against *Aedes aegyptii* (Asiamah and Botchey, 2019) etc.

The aim of this study was to elucidate the chemical profile of essential oil (EO) from aerial parts of *Ocimum viride* Willd. and to evaluate its antibacterial and anticancer potential.

2. Materials and methods

2.1. Plant material

Collection of fresh aerial plant material (leaves) was done from fields of CSIR-Indian Institute of Integrative Medicine, Jammu, India.

2.1.1. Isolation of essential oil

The aerial parts (leaves) of the *Ocimum viride* Willd. were harvested at inflorescence initiation stage from fields of CSIR-Indian Institute of Integrative Medicine, Jammu, India. The plant was deposited at the herbarium of department of Botany, University of Jammu, Jammu with voucher number (HBJU-16097). EO was extracted immediately after collection of plant material by hydro-distillation using modified clevenger apparatus for 30 min to 2 h (Bhagat et al., 2016). The oil samples were dried over anhydrous sodium sulphate (Na2SO4) and stored at 4 °C for future analysis.

2.1.2. Gas chromatography-mass spectrometry (GC-MS) analysis

Analysis of the essential oil was carried out at CSIR-Indian Institute of Integrative Medicine, India, using GC-MS 4000 (Varian, USA) system equipped with CP-SIL 8CB column (30 m × 0.32mm i.d., 1μm film thickness). Injector temperature was maintained at 230 °C. Oven temperature programme used was holding at 60 °C for 5 min, heating to 250 °C at 3 °C/min and keeping the temperature constant at 250 °C for 10 min. Helium was used as a carrier gas at a constant flow of 1.0 ml/min and an injection volume of 0.20 μL was employed. The MS scan parameters included electron impact ionization voltage of 70eV, a mass range of 40–500 m/z. The identification of components of the essential oil was based on comparison of their mass spectra with those of NIST05 (version 2.0) library.

2.2. In vitro study

2.2.1. Antibacterial activity assay

The antibacterial activity of EO was determined by agar well diffusion method (Pesavento et al., 2015). The bacterial isolates were first grown by streaking on a nutrient agar plate and incubated for 24 h at 37 °C before use. Cell suspension was prepared by dissolving loop full of culture from the culture plate in autoclaved nutrient broth. Four wells were then bored into each agar plate using a sterile 5 mm diameter cork borer and filled with 20μl solution of 1 μL/mL essential oil. Plates were incubated at 37 °C for 24 h and later observed for zones of inhibition. The effect of EO was compared with that of positive control chloramphenicol to determine the sensitivity of bacterial growth. Each sample was used in duplicate for determination of antibacterial activity.

2.2.1.1. Minimum inhibition concentration (MIC) assay. The MIC of the *O. viride* leaves EO was determined according to the broth dilution technique. Standardized suspensions of the most susceptible bacterial strain was inoculated into a series of sterile tubes of nutrient broth containing two-fold dilutions of EO and incubated at 37 °C for 24 h. The MIC was read as the least concentration that inhibited the growth of the test organisms.

2.2.2. Cytotoxicity assay

Various cancer as well as normal cell lines were procured from American Type Cell Culture (ATCC, USA) and the cell line repository of National Center for Cell Sciences (NCCS, Pune-India). Initial screeining of *in vitro* cytotoxic effect of EO against DU-145 (prostate), HEP-2 (liver), IMR-32 (neuroblastoma), HT-29 (colon), 502713 (colon) and SW-620 (colon) was measured by tetrazolium-based colorimetric assay which measures the reduction of the tetrazolium salt MTT into a blue formazan product, mainly by the activity of the mitochondrial cytochrome oxidase and succinate dehydrogenase (Carmichael et al., 1987). Typically, 100 μL of cells suspension were plated at a density of approximate 2 ×104 cells per well in a 96-well plate, and were maintained at 37 °C in a 5% CO2 humid incubator for 24 h. Different concentrations of EO were added to each group (triplicate wells) and were incubated for 24 h, followed by addition of l0 μL (5 mg/mL) of MTT dye solution to each well for 4 h. After removal of the unused MTT dye, cells were treated with 100 μL DMSO and the absorbance at 570 nm was measured using ELISA reader. The cytotoxicity was calculated relative to the control (treated with 0.1% DMSO) and expressed as the concentration of drug inhibiting cell growth by 50% (IC50). All tests were run in triplicates. Among all the screened cancer lines for cytotoxicity, and most effective cancer cell line was further selected for the mechanistic studies.

2.2.2.1. Evaluation of anticancer mechanisms

2.2.2.1.1. Total genomic DNA fragmentation analysis. HT-29 colon cells after treatment with different concentrations of essential oil (0.1 μL/mL) for 24h were centrifuged at 1500 rpm for 10 min and washed with Dulbecco's PBS. The resultant cell pellet was suspended in 250 μL of lysis buffer (10mM EDTA, 50mM Tris–HCl, 0.5% SDS). Lysed cells were then supplemented with proteinase-K (500 μg/mL) at 55 °C for 1h and followed by incubation with 200 μg/mL DNAase-free RNase at 37 °C for 90 min. The DNA was extracted with 250 μL of phenol:-chloroform:isoamyl alcohol (25:24:1) for 1 min and centrifuged at 12,000 rpm for 5 min. The aqueous phase was further extracted with chloroform: isoamyl alcohol (24:1) and centrifuged. DNA was precipitated from aqueous phase with 0.1 volume of 2M NaCl and 2.5 volumes of chilled ethanol and kept at 20 °C overnight. The precipitated DNA was centrifuged at 12,000 rpm for 10 min and dissolved in Tris–EDTA buffer (pH 8.0) and electrophoresed in 1.5% agarose gel at 50V for 90 min. The gel was photographed using Bio-Rad Gel documentation system (Tong et al., 2004).

2.2.2.2. DNA cell cycle analysis. Cell cycle analysis was performed in accordance with the method reported earlier (Park et al., 2007). Colon HT-29 cells (106 cells/mL) in their exponential growing phase were dispended in a six well plate and maintained for 24 h in incubator. Cells were then treated with EO (0.1 μL/mL) and incubated for further 24 h. Cells were trypsnised, centrifuged and washed with PBS. Cells were fixed in 70% ethanol, washed with PBS and then incubated with propidium iodide (PI; 25 μg/mL). RNA was removed using RNAase at 37 °C for 30 min. The percentages of cells having the sub-G1 population were measured using BD-LSR flow cytometry equipped with blue (488 nm) excitation from argon laser.

2.2.2.3. Nuclear morphology analysis. Morphological changes of apoptotic cells were also examined using fluorescence microscopy. The HT-29 cells were treated with the 0.1 μg/ml of the essential oil and further cells were harvested, fixed with absolute ethanol, and stained with Hoechst 33258 for 15 min at 37 °C. The cells were then visualized using fluorescence microscopy (Olympus 1 × 70, Tokyo, Japan) with UV excitation at 300–500 nm. Cells containing condensed and/or

fragmented nuclei were considered to be apoptotic cells (Chazotte, 2011).

2.2.3. Molecular docking studies

Protein structures were retrieved from RCSB PDB in .pdb text format and various possible binding sites for thymol were predicted from COACH server. PyMOL, a molecular visualization tool, was used to identify desired protein sequences in the target protein for binding affinities with thymol. Different analogs of thymol were also searched in ZINC database and analysed for their potential to bind Sec A protein (bacterial protein secretory (Sec) pathway) and Beclin 2 protein (Beclin gene binds to the apoptosis-inhibiting protein Bcl-2). The structure of thymol was retrieved from the Zinc Database in .mol2 format. The drug-like property of thymol was measured using Lipinski filter. The protein and ligand molecule were imported in Mauler Virtual Docker and visualized for drug interaction sites by using Molegro visualize (Bhagat et al., 2018).

2.2.4. Statistical analysis

Numerical data have been presented as mean ± standard deviation (SD). Statistical analysis of numerical data between control versus treatments (*) was performed by applying Student's t-test. *P* values of <0.05 (*) and <0.01 (**) were considered as statistically significant.

3. Results

3.1. GC-MS analysis of the essential oil of O. viride

The chemical composition of the EO was analyzed by GC-MS and results obtained are indicated in the chromatogram. The chemical composition of EO revealed that the majority of the detected compounds were terpenes in nature. Out of these, thymol (~50%), gamma-terpinene (~18%), and para cymene (~11%) were detected as major compounds whereas alpha pinene, alpha thujene, sabinene, beta pinene, alpha terpinene, limonene, 4-thujenol, o-isopropenyl toluene, caryophyllene, bicyclo (5.3.0) decane, 2-methylene, alpha guaiene, alpha panasinsen and caryophyllene oxide were present in relatively small amounts.

3.2. Assesment of antibacterial activity

Antibacterial activity was assessed against five gram positive and five gram negative bacterial strains (Table 1). Essential oil strongly inhibited the growth of *Micrococcus luteus* (zone of inhibition; 11 ± 0.4mm), *Bacillus subtilis* (zone of inhibition; 14 ± 0.5mm), *Psudomonas alcaligenes* (zone of inhibition; 10 ± 0.6mm), *Bacillus cereus* (zone of inhibition; 12 ± 0.6mm) whereas moderate growth inhibitory activity was observed against *Staphylococcus aureus* (zone of inhibition; 04 ± 0.2mm), *Enterococcus faecalis* (zone of inhibition; 06 ± 0.2mm), *Pseudomonas aeruginosa* (zone of inhibition; 05 ± 0.3mm), *Alcaligenes denitrificans* (zone of inhibition; 06 ± 0.3mm) while no significant antibacterial effect was observed against *Escherichia coli* and *Campylobacter coli*. Chloramphenicol was used as reference antibiotic and results were comparable to the standard used. Overall, essential oil possesses potent antibacterial effect against the broad range of gram positive and gram negative bacteria. Further minimum inhibition concentration of most susceptible strain *Bacillus subtilis* was found to be 0.5 µl/ml of the essential oil.

Table 1. Antibacterial activity of leaf essential oil from *Ocimum viride*.

Antibacterial assay		
Zones of inhibitions (mm)		
Bacterial strains	EO (0.1 µl/ml)	Chloramphenicol
	Essential oil	(positive control)
1. *Micrococcus luteus*	11 ± 0.4	25 ± 1.05
2. *Staphylococcus aureus*	04 ± 0.2	18 ± 0.5
3. *Escherichia coli*	–	15 ± 0.5
4. *Enterococcus fecalis*	06 ± 0.2	18 ± 0.43
5. *Campylobactor coli*	–	28 ± 0.9
6. *Bacillus subtilis*	14 ± 0.5	15 ± 0.55
7. *Bacillus cereus*	12 ± 0.6	08 ± 0.25
8. *Alcaligenes denitrificans*	06 ± 0.3	21 ± 0.75
9. *Pseudomonas aeruginosa*	05 ± 0.3	16 ± 0.09
10. *Pseudomonas alcaligenes*	10 ± 0.5	15 ± 0.6

3.3. Assesment of in vitro cytotoxic effect

Colorimetric MTT assay was evaluated to elucidate the cytotoxic potential of essential oil at different concentrations (0.01–0.1 µl/ml). A dose dependent growth inhibition was observed by essential oil against human cancer cell lines with IC_{50} value of 0.042 ± 0.004 µl/ml against prostate (DU-145), IC_{50} value of 0.052 ± 0.006 µl/ml against liver (HEP-2), IC_{50} value of 0.045 ± 0.002 µl/ml against neuroblastoma (IMR-32), IC_{50} value of 0.034 ± 0.001 µl/ml against colon (HT-29), IC_{50} value of 0.040 ± 0.005 µl/ml against colon (502713) and IC50 value of 0.064 ± 0.007 µl/ml against colon (SW-620) respectively (Figures 1A and 1B). However, comparatively no significant inhibitory effect was observed on the proliferation of normal monkey kidney cell line (CV-1) (IC_{50}–1.92 µl/ml). Our findings indicated essential oil-induced cell death in a dose-dependent manner in all cancer cell lines.

3.4. Effect of essential oil on total genomic DNA fragmentation in HT 29 cells

Genomic DNA fragmentation was done to elucidate the intrinsic apoptotic cell death. DNA fragmentation is a key event of apoptosis which distinguish apoptotic cells from necrotic ones. Nucleases play an important role in DNA cleavage resulting into a distinguishing ladder pattern on the agarose gel. Obtained results showed that treatment with essential oil significantly increase internucleosomal DNA cleavage in HT-29 colon cancer cells which qualitatively was observed by DNA ladder formation on agarose gel (Figure 2). Lane 1 (L1) DNA ladder was not observed and intact genomic DNA appeared near the well at the top of the lanes in control (untreated) cells lane1 (L1), different concentration of the essential oil (L2) 0.1 µl/ml, (L3) 0.25 µl/ml and (L4) 0.5 µl/ml showed DNA ladder formation indicating cell death by apoptosis (Figure 2).

3.5. Effect of essential oil on cell cycle distribution in HT 29 cells

In order to understand the growth inhibitory effect of essential oil, flow cytometry was done to elucidate the DNA cell cycle analysis using PI staining method. The untreated colon HT-29 cells showed 7.69 % of cells in the sub G1 phase (Figure 3A). When treated with the essential oil of *O. viride* (0.1 µl/ml), the HT-29 cell population in the sub G1 phase increased significantly up to 78.16% (Figure 3C) thereby showing more cell death in the sub G1 phase. Drug camptothecin (1µM) was used as positive control to further validate our experiment (Figure 3B). Accordingly percentage of cell in sub G1 phase has increased on treatment of cells with essential oil (Figure 3D).

3.6. Effect of essential oil on determination of morphological changes in HT 29 cells

Nucleic acid staining with Hoechst 33258 revealed typical apoptotic nuclei, which exhibited highly fluorescent condensed chromatin in cells treated with the essential oil (Figure 4). The morphological changes and cell death of HT 29 cells were observed at concentrations of 0.1 µl/mL. Most cells were detached from the dishes, and cell rounding and shrinking occurred at the same concentration of the essential oil. Number

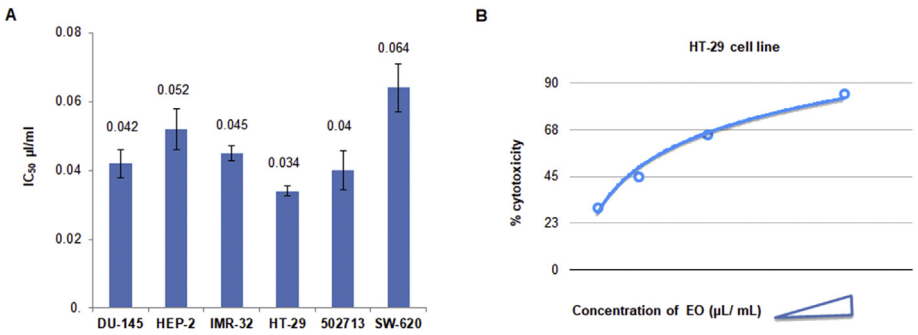


Figure 1. Cytotoxicity profile of *Ocimum viride* (leaf) essential oil against various cancer cell lines. (A) *In vitro* cytotoxic activity of *Ocimum viride* (leaf) essential oil against human cancer cell lines viz., DU-145 (prostate), HEP-2 (liver), IMR-32 (neuroblastoma) and HT-29, 502713, SW-620 (colon). (B) Cytotoxicity of EO against the most susceptible cell line HT29.

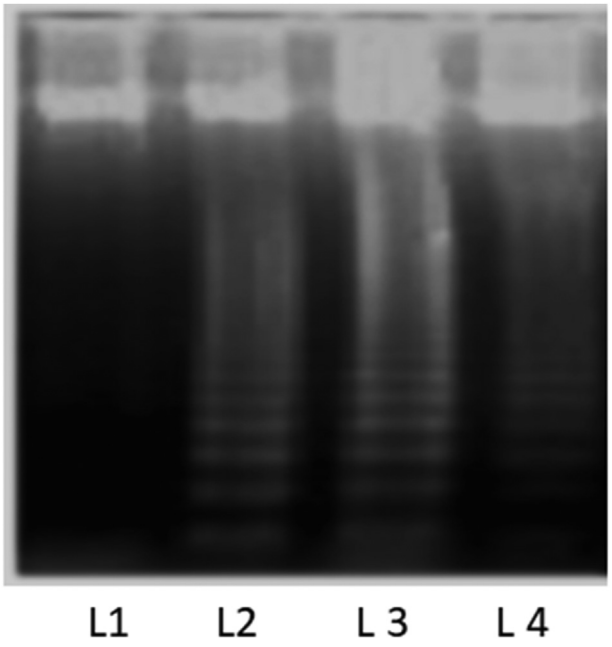


Figure 2. DNA fragmentation assay in human colon cancer cell line (HT-29) cells by *Ocimum viride* (leaf) essential oil. Lane 1 (Untreated cells), Lane 2 (Camptothecin 1 µM), Lane 3 (Camptothecin 3 µM), Lane 4 (*O.viride* essential oil 0.1 µl/ml).

of Hoeschst positive nuclei upon treatment with essential oil was found higher in comparison of untreated sample as indicated in Table 2.

3.7. *In silico prediction of mechanism by molecular docking analysis*

To understand the possible mechanism behind the antibacterial activity of EO against the bacteria *Bacillus subtilis*, molecular docking analysis was performed for the major constituent present in EO i.e., for thymol against bacterial proteins SecA, (general protein secretory (Sec) pathway, the peripheral membrane ATPase SecA, which provides the energy for the translocation of preproteins across the bacterial cytoplasmic membrane). It was found that thymol shows the best binding affinity with bacterial sec A protein. With the protein Sec A ATPase, an interaction was observed with minimal H-Bond energy of -2.5 kcal/mol by many components of different analogs of thymol followed by the scoring function in order to evaluate the interactions between sec A ATPase and thymol. Docking energy was used to identify the correct binding pose and then ranked the most befitting target ligand complex based on their binding affinity and RMSD value (root-mean-square deviation) that is the measure of the average distance between the atoms (usually the backbone atoms) of superimposed. The RMSD Matrix dialog can be used to quickly inspect deviations between molecules in the workspace. In addition to the standard measure Pairwise Atom-Atom RMSD (by ID), two variants Pairwise Atom-Atom RMSD (checking all automorphisms) and Pairwise Atom-Atom RMSD (by nearest unmatched neighbor) of the RMSD measure were tried to take intrinsic symmetries of the molecule into account when calculating RMSD. In this study we used Pairwise Atom-Atom RMSD (checking all automorphisms), to calculate the RMSD and with the help of software Molegro Virtual Docker (MVD) the binding energy of thymol was predicted. Different analogs of thymol were used but the best analog (zinc id - 967515) obtained on the basis predicted binding energy and other binding parameters like hydrogen bond interaction and electrostatic interaction was selected. After docking thymol was found to interact with protein Sec A with binding energy -15 kcal/mol with the active site Lys284, Trp275, Leu269, Arg19,Glu277, pro270 (Figures 5A and 5B). Three bonding site between ligand and cavity1 portion containing part of protein (Thr141, Asn93, Ile20) having respective solvation energy (-3.54,-8.31, 4.89) and respective bond types are Thr141 and C3 of drug non polar hydrophobic bond having bond length 1.53225 Asn93 and oxygen of drug having covalent bond of length 0.68 (covalent bonds are always tighter) Ile20 and c9 of drug having non polar hydrophobic bond of length 1.52998 was observed. Similarly, thymol was tried to docked with protein Beclin 2, that functions in autophagy via interaction of class III PI3K complex components and Bcl-2. It was found that thymol shows the best binding affinity with protein Beclin 2 CCD homodimer. Three main bonds are involved, first bond between Tyr21 (chain A) and ligand of energy -1.143154 & length 3.31369 a hydrogen bond having properties of donor rotable second bond between N7 of Gln198 and ligand is non polar with length 0.68 *A and sp2 hybridisation at 120* angle third bond is also non polar between Arg209 and ligand having 0.68 length sp2 hybridisation and 120° angle (Figure 5C).

4. Discussion

Demand and use for natural compounds with relevant medicinal properties have been increasing worldwide. Essential oils (EOs) and other phytoproducts are examples of natural products that have gained interest, mainly due to their suitable chemical characteristics and various biological activities. Various studies reported that essential oil act as natural bioactive substances and can induce sensitive growth inhibition against microbes as well as against cancer cells via inducing apoptosis (Cabral and Lemos, 2018). In this study we attempted to explore the potential of essential oil from *Ocimum viride* (leaves) as an antibacterial and anticancer agent. Earlier reports claim to contain a large number of aromatic components mainly thymol, eugenol, cymene and limonene in essential oil of *Ocimum viride* (Ali and Setzer, 2013), our results are also in accordance to these reports indicating thymol (a terpene) as major constituent in the essential oil obtained from the aerial part of *Ocimum viride*.

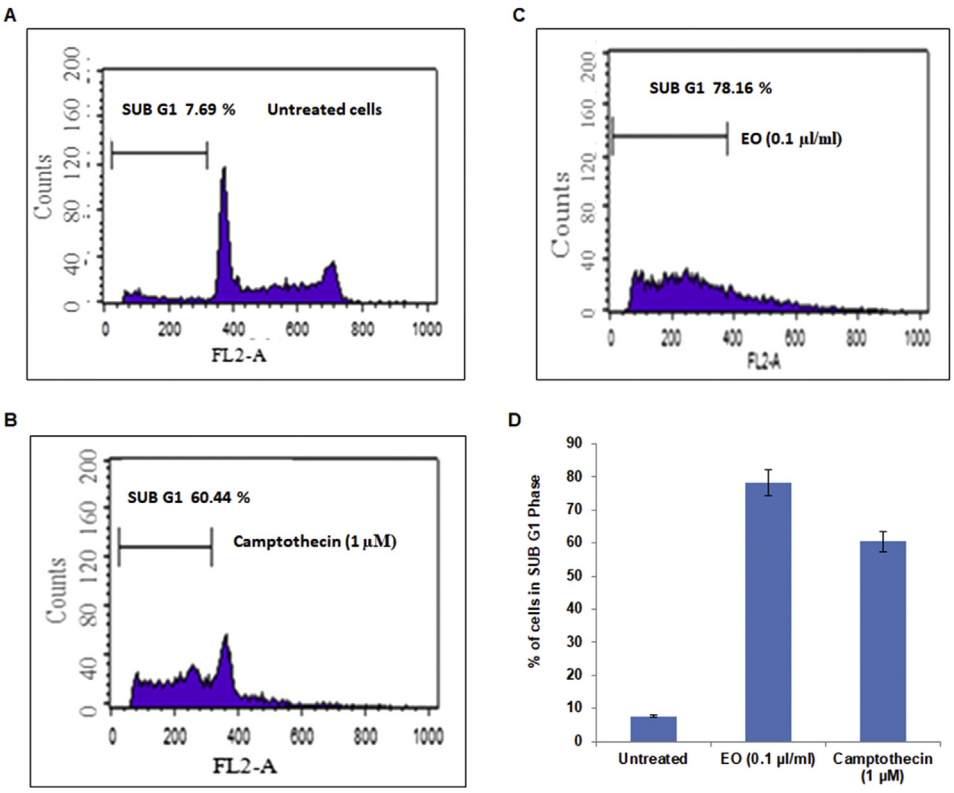


Figure 3. Cell cycle analysis of *Ocimum viride* (leaf) essential oil against human colon cancer cell line (HT29). (A) Untreated HT29 cells. (B) Positive control, cells treated with camptothecin (1µM). (C) HT-29 cells treated with 0.1 µl/ml essential oil. (D) Percentage of cells in sub G1 phase.

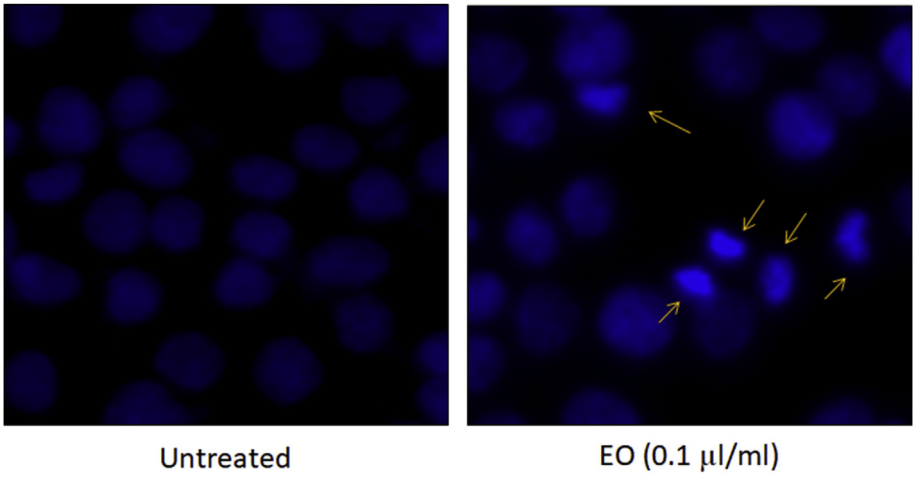
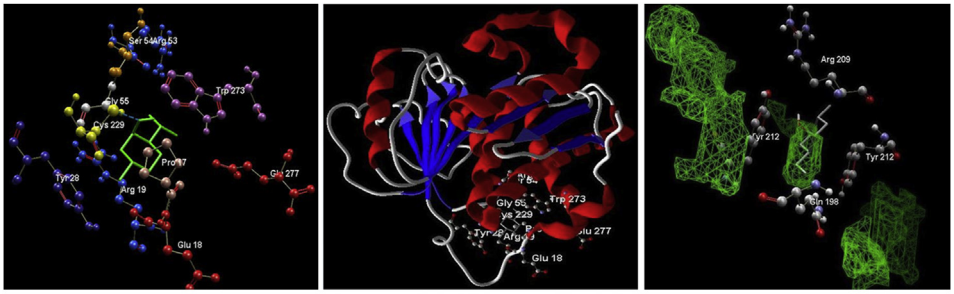


Figure 4. Morphological changes in human colon cancer cell line (HT29) cells by *Ocimum viride* (leaf) essential oil with Hoechst 33258.

Thymol has been extensively explored as a potential antibacterial and anticancer agent (Carocho, 2016; Islam et al., 2019; Moon et al., 2017). Additionally, isolataed essential oil of *Ocimum viride* comparatively showed highest sensitivity towards *Bacillus subtilis* pathogen (14 ± 0.5mm) with MIC value of 0.5 µl/ml. Further, the relationship between the major component of essential oil and antibacterial activity were drawn with the help of molecular docking studies, here a well known Sec pathway protein (Sec A) was docked with thymol to predict the best binding efficiency. The molecular docking of thymol with bacterial Sec A protein resulted in best binding energy of -15 kcal/mol with the active site Lys284, Trp275, Leu269, Arg19, Glu277 and Pro270. Since bacterial secA is an ATP-driven motor protein that is responsible for post translational translocation of proteins by and possibly serving as receptor for thymol thereby causing interference in protein transport in bacteria (Steinberg et al., 2018). In recent years Sec A has been widely recognized as a druggable target as more than 30% of bacterial proteins are destined to function at the cell envelope or outside the cell. Sec-pathway is

Table 2. Identification of Hoeschst positive nuclei upon treatment with *Ocimum viride* (leaf) essential oil.

S. No.	Condition	Number of Hoeschst positive nuclei per field ± s.d.
1.	Untreated	7 ± 1
2.	Essential Oil (0.1µ/ml)	33 ± 4



A B C

Figure 5. Molecular docking analysis of thymol of *Ocimum viride* (leaf) essential oil. (A) Against the bacterial Sec A ATPase. Green ligand is surrounded by amino acid residues with which ligand has hydrogen bonds. The amino acids are labeled as xxx123. In the binding moiety Lys, Trp, Leu, Arg, Glu and Pro are placed at 284, 275, 269, 19, 277 and 270 positions respectively. (B) Against the bacterial Sec A ATPase. Docking view of 2-Isopropyl-5-methylcyclohexanol against secondary structure of 3RPH with red colour showing helix. (C) Against human Beclin 2 CCD homodimer. Green ligand is surrounded by amino acid Tyr21 residue having hydrogen bond, Gln198 residue having non polar bond and Arg209 residue having non polar bond.

universally conserved in bacteria (as well as in the endoplasmic reticulum (ER) of eukaryotes and thylakoid membranes in plants) and is the primary route for protein export (De Waelheyns et al., 2014; Driessen and Nouwen, 2008). The vast majority of the proteins have functionally diverse roles such as nutrient uptake, excretion, metabolism, cell structure, communication, virulence and bacterial defense are known to transported via the ubiquitous secretion (Sec-) pathway (De Waelheyns et al., 2014) further making it interesting antimicrobial target. Moreover, we also sought to determine the mechanism by which essential oil of *Ocimum viride* exert its antiproliferative properties against HT-29 human colon cancer cells. Initially, MTT assay a commonly used endpoint method to assess the cytotoxicity was applied and here the viable cells with active metabolism converted MTT into a purple colored formazan product with an absorbance maximum at 570 nm. On cell death, they lose the ability to convert MTT into formazan, and color formation is observed that serves as a useful and convenient marker for viable cells (Mosmann, 1983). In this study, cytotxicity of the essential oil was evaluated against six cancer cell lines *viz*., prostate (DU-145), liver (HEP-2), neuroblastoma (IMR-32) and colon (HT-29, 502713, SW-620) and dose-dependent cytotxic response was observed against all the cancer cell lines. Here the highest ability to reduce MTT to the formazan derivative was observed against colon (HT-29) cancer cell line with IC50 value of 0.034 $\pm$ 0.001 µl/ml indicating the prominent cytotoxic/antiproliferative effect of the essential oil. As, induction of apoptosis in cancer cells is now considered as an indicator of the cancer treatment response, this study also elucidated the mode of cell death by EO was against colon (HT-29) cancer cells. Apoptosis is recognized for the physiological cell death that is accompanied by a specialized series of cellular events such as chromatin condensation, DNA fragmentation, cytoplasmic membrane blebbing and cell shrinkage (De Waelheyns et al., 2014; Galluzzi et al., 2018). The internucleosomal cleavage of DNA into oligonucleosomal size fragments is considered a biochemical hallmark of apoptosis. DNA isolated from treated HT-29 colon cancer cells, showed DNA fragments smearing away from the wells in agarose gel electrophoresis, forming a discreet ladder with the increase in concentrations of the essential oil. The genomic integrity is maintained by protecting cell division through the regulation of cell cycle checkpoints. However, the detection of DNA damage may force cells to undergo apoptosis rather than division (Visconti et al., 2016). The flow cytometric analysis of cell cycle distribution in our study indicated the increased population in the G2/M-phase and S-phase after 24 h treatment by inhibitory concentration of essential oil as compared to control. Sub-G1 peaks, which represent the cell population containing apoptotic nuclear fragments, were markedly increased after treatment that may be due to the possibility of DNA damage and failure of repair mechanism in the cells, thus blocking the cell cycle at the S-phase and preventing cell entry into the proliferative phase thus effecting the HT-29 cancer cell proliferation. In addition, fluorescent staining with Hoechst 33258 showed increased and consistent nuclear condensation and shrinkage, cell growth inhibition and apoptotic body formation in EO treated colon cancer cells in comparison to control cells that showed rounded intact nuclei. Similar findings on the *Ocimum viride* essential oil were also reported by Sharma and coworkers against COLO 205 cells (Sharma et al., 2016), other species from Ocimum genus such as *Ocimum basilium* showed *in vitro* cytotoxicity against cancer cells (Zarlaha et al., 2014), *Ocimum sanctum* showed to trigger cell death by apoptosis against human breast, liver and oral cancer cells etc (Manaharan et al., 2016; Sharma et al., 2016; Shivpuje et al., 2015). Molecular docking analysis was performed to confirm the binding efficacy of thymol from essential oil to Beclin protein that functions in autophagy, it interacts with class III PI3K complex components and Bcl-2 and lysosomal degradation pathway (Domínguez-Martín et al., 2018; Kim et al., 2017; Li et al., 2017). Hence, the current study supports that *Ocimum viride* essential oil induced antibacterial effect as well as anticancer effect in HT-29 colon cancer cells.

5. Conclusions

The present study displayed the therapeutic activity of essential oil from *Ocimum viride* (leaves) as antibacterial agent against pathogen *Bacillus subtilis* and anticancer agent against colon (HT 29) cancer cell line as confirmed by both *in vitro* and molecular docking studies providing the evidence for its therapeutic behavior.

Declarations

Author contribution statement

M. Bhagat: Conceived and designed the experiments.
M. Sangral: Performed the experiments; Wrote the paper.
A. Kumar: Performed the experiments; Contributed reagents, materials, analysis tools or data.
R. Rather: Analyzed and interpreted the data.
K. Arya: Performed the experiments.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

Authors would like to acknowledge the Department of Bioinformatics and the Center facility of School of Biotechnology that is created by the support of UGC-SAP, MHRD-RUSA, DST-FIST, PURSE grants, University of Jammu, for their support to carry out the research work and Instrumentation division, CSIR-IIIM-Jammu, J&K (India).

References

Ali, N., Setzer, W.N., 2013. Pharmacological activities of basil oil: a review. Recent progress in medicinal plants essential oils–II, 37, 286–306.
Asiamah, E.A., Botchey, M.A., 2019. Repellent and mosquitocidal properties of smoke from oil and non-polar extracts of Ocimum viride leaf against Aedes aegyptii. J. App. Sci. 19, 618–623.
Bhagat, M., Gupta, S., Jamwal, V.S., Sharma, S., Kattal, M., Dawa, S., et al., 2016. Comparative Study on Chemical Profiling and Antimicrobial Properties of Essential Oils from Different Parts of Eucalyptus Lanceolatus.
Bhagat, M., Sangral, M., Arya, K.A., Rather, R.A., 2018. Chemical Characterization, Biological Assessment and Molecular Docking Studies of Essential Oil of Ocimum Viride. JB, p. 390906.
Blowman, K., Magalhães, M., Lemos, M., Cabral, C., Pires, I., 2018. Anticancer properties of essential oils and other natural products. Evidence-Based Comp. Alter. Med. 2018.
Brnawi, W.I., Hettiarachchy, N.S., Horax, R., Kumar-Phillips, G., Seo, H.S., Marcy, J., 2018. Comparison of cinnamon essential oils from leaf and bark with respect to antimicrobial activity and sensory acceptability in strawberry shake. J. Food Sci. 83, 475–480.
Cabral, C., Lemos, M.F., 2018. Natural Products as a Source for New Leads in Cancer Research and Treatment.
Carmichael, J., DeGraff, W.G., Gazdar, A.F., Minna, J.D., Mitchell, J.B., 1987. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. J. Cancer Res. 47, 936–942.
Carocho, M.S., 2016. TESIS DOCTORAL Desarrollo de nuevos productos alimenticios: incorporación de extractos de plantas como ingredientes funcionales y conservantes naturales Development of new foods: incorporation of plant extracts as. UNIVERSIDAD COMPLUTENSE DE MADRID.
Chazotte, B., 2011. Labeling nuclear DNA using DAPI. J. Cold Spring Harbor Proto. 2011 pdb. prot5556.
De Waelheyns, E., Economou, A., Anné, J., 2014. Antibiotic targeting of the bacterial secretory pathway. Biochim. Biophy. Acta (BBA)-Mol. Cell Res. 1843, 1762–1783.
Domínguez-Martín, E.M., Díaz-Lanza, A.M., Faustino, C., 2018. Anticancer hybrid combinations: mechanisms of action, implications and future perspectives. J. Current Pharama. Design 24, 4312–4333.
Driessen, A.J., Nouwen, N., 2008. Protein translocation across the bacterial cytoplasmic membrane. J. Annu. Rev. Biochem. 77, 643–667.
Dubey, N., Kishore, N., Jaya, V., Lee, S., 1997. Cytotoxicity of the essential oils of Cymbopogon citratus and Ocimum gratissimum. Indian J. Pharma. Sci. 59, 263–264.
Fokou, P.V.T., Nyarko, A.K., Appiah-Opong, R., Yamthe, L.R.T., Addo, P., Asante, I.K., et al., 2015. Ethnopharmacological reports on anti-Buruli ulcer medicinal plants in three West African countries, 172, 297–311.
Galluzzi, L., Vitale, I., Aaronson, S.A., Abrams, J.M., Adam, D., Agostinis, P., et al., 2018. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on. Cell Death 25, 486, 2018.
Govindarajan, M., Vaseeharan, B., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., Al-Anbr, M.N., et al., 2018. High efficacy of (Z)-γ-bisabolene from the essential oil of Galinsoga parviflora (Asteraceae) as larvicide and oviposition deterrent against six mosquito vectors, 25, 10555–10566.
Hosseini, Z., Lorigooini, Z., Rafieian-Kopaei, M., Shirmardi, H.A., Solati, K., 2017. A review of botany and pharmacological effect and chemical composition of Echinophora species growing in Iran. J. Pharma. Res. 9, 305.
Islam, M.T., Khalipha, A.B., Bagchi, R., Mondal, M., Smrity, S.Z., Uddin, S.J., et al., 2019. Anticancer activity of thymol: a literature-based review and docking study with Emphasis on its anticancer mechanisms, 71, 9–19.
Khare, C., 2007. Ocimum viride Willd. In: Khare, C. (Ed.), Indian Medicinal Plants. Springer, New York, NY.
Kim, K.-Y., Park, K.-I., Kim, S.-H., Yu, S.-N., Park, S.-G., Kim, Y., et al., 2017. Inhibition of autophagy promotes salinomycin-induced apoptosis via reactive oxygen species-mediated PI3K/AKT/mTOR and ERK/p38 MAPK-dependent signaling in human prostate cancer cells, 18, 1088.
Ko, Y.-J., Ahn, G., Ham, Y.-M., Song, S.-M., Ko, E.-Y., Cho, S.-H., et al., 2017. Anti-inflammatory effect and mechanism of action of Lindera erythrocarpa essential oil in lipopolysaccharide-stimulated RAW264. 7 cells, 16, 1103.
Lemus-Mondaca, R., Vega-Gálvez, A., Zura-Bravo, L., Ah-Hen, K., 2012. Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: a comprehensive review on the biochemical, nutritional and functional aspects. J. Food Chem. 132, 1121–1132.
Li, H., Chen, C., 2017. Inhibition of autophagy enhances synergistic effects of Salidroside and anti-tumor agents against colorectal cancer. J. BMC Comp. Alter. Med. 17, 538.
Manaharan, T., Thirugnanasampandan, R., Jayakumar, R., Kanthimathi, M., Ramya, G., Ramnath, M.G., 2016. Purified essential oil from Ocimum sanctum Linn. triggers the apoptotic mechanism in human breast cancer cells. J. Pharmacognosy Magazine 12, S327.
Martins, A.P., Salgueiro, L.R., Vila, R., Tomi, F., Cañigueral, S., Casanova, J., et al., 1999. Composition of the essential oils of Ocimum canum, O. gratissimum and O. minimum, 65, 187–189.
Khosla and morphology, 1986. Inter-relationship Studies of Different Species of the Genus Ocimum. J. Plant Anat. Morp.
Moon, H., Kim, N.H., Kim, S.H., Kim, Y., Ryu, J.H., Rhee, M.S., 2017. Teriyaki sauce with carvacrol or thymol effectively controls Escherichia coli O157: H7, Listeria monocytogenes, Salmonella Typhimurium, and indigenous flora in marinated beef and marinade. J. Meat Sci. 129, 147–152.
Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.
Moy, R.L., Levenson, C., 2017. Sandalwood album oil as a botanical therapeutic in dermatology. J. Clin. Aesthetic Derm. 10, 34.
Ekundayo, O., 1986. Essential oils VIII. Volatile constituents of the leaves of Ocimum viride. J. Plant Medica 52, 200–202.
Park, W.-H., Han, Y.-W., Kim, S.-W., Kim, S.-H., Cho, K.-W., Kim, S.Z., 2007. Antimycin A induces apoptosis in As4. 1 juxtaglomerular cells. J. Cancer Lett. 251, 68–77.
Pesavento, G., Calonico, C., Bilia, A., Barnabei, M., Calesini, F., Addona, R., et al., 2015. Antibacterial activity of Oregano, Rosmarinus and Thymus essential oils against Staphylococcus aureus and Listeria monocytogenes in beef meatballs, 54, 188–199.
Selvi, M.T., Thirugnanasampandan, R., Sundarammal, S.J., 2015. Antioxidant and cytotoxic activities of essential oil of Ocimum canum Sims from India. J. Saudi Chem. Soc. 19, 97–100.
Sharma, P., Prakash, O., Shukla, A., Singh Rajpurohit, C., VasudevP, G., Luqman, S., et al., 2016. Structure-activity relationship studies on holy basil (Ocimum sanctum l.) based flavonoid orientin and its analogue for cytotoxic activity in liver cancer cell line Hepg2, 19, 656–666.
Shivpuje, P., Ammanangi, R., Bhat, K., Katti, S.J.T., 2015. Effect of Ocimum sanctum on oral cancer cell line: an in vitro study. J. Contemp. Dental Prac. 16, 709–714.
Singh, P., Kalunke, R.M., Giri, A.P., 2015. Towards comprehension of complex chemical evolution and diversification of terpene and phenylpropanoid pathways in Ocimum species. J. RSC Adv. 5, 106886–106904.
Steinberg, R., Knüpffer, L., Origi, A., Asti, R., Koch, H.-G., 2018. Co-translational protein targeting in bacteria. J. FEMS Microbiol. Lett. 365, fny095.
Tong, X., Lin, S., Fujii, M., Hou, D.-X., 2004. Echinocystic acid induces apoptosis in HL-60 cells through mitochondria-mediated death pathway. J. Cancer Lett. 212, 21–32.
Visconti, R., Della Monica, R., Grieco, D., 2016. Research CC. Cell cycle checkpoint in cancer: a therapeutically targetable double-edged sword. J.Experiment. Clin. Cancer Res. 35, 153.
Zarlaha, A., Kourkoumelis, N., Stanojkovic, T.P., Kovala-Demertzi, D., 2014. Cytotoxic activity of essential oil and extracts of Ocimum basilicum against human carcinoma cells .Molecular docking study of isoeugenol as a potent cox and lox inhibitor. Digest J. Nanomat. Biostruc. (DJNB) 9 (3).