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

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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 diterpenes), 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

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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 aegypti (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 antitumor 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 cleverenger apparatus for 30 min to 2 h (Bhagat et al., 2016). The oil samples were dried over anhydrous sulphuric acid (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 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 Culture Collection (ATCC, USA) and the cell line repository of National Center for Cell Sciences (NCCS, Pune-India). Initial screening 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 10 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 of 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 1 h and followed by incubation with 200 μg/mL DNase-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).
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 Bel-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 Maular 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, sabinen, beta pinene, alpha terpinene, limonene, 4-thujenol, o-isopropenyl toluene, caryophyllene, bicyclo (5.3.0) decane, 2-methylen, alpha guaiene, alpha panasines and caryophyllene oxide were present in relatively small amounts.

3.2. Assessment 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), Pseudomonas 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.

3.3. Assessment 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 IC50 value of 0.042 ± 0.004 µl/ml against prostate (DU-145), IC50 value of 0.052 ± 0.006 µl/ml against liver (HEP-2), IC50 value of 0.045 ± 0.002 µl/ml against neuroblastoma (IMR-32), IC50 value of 0.034 ± 0.001 µl/ml against colon (HT-29), IC50 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) (IC50=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 against colon (502713) and IC50 value of 0.064 µl/ml against colon (HT-29), 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

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

| Bacterial strains       | EO (0.1 µl/ml) | Chloramphenicol (positive control) |
|-------------------------|----------------|-----------------------------------|
|                         | Essential oil  |                                   |
| 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 faecalis| 06 ± 0.2       | 18 ± 0.43                         |
| 5. Campylobacter 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                          |
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.

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., thymol against bacterial proteins SecA, (general protein secretory (Sec) pathway, the peripheral membrane ATPase SecA, which provides 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 SecA 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 SecA 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 Åm and oxygen of drug having covalent bond of length 1.52998 Åm 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 Becl-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 Åm a hydrogen bond having properties of donor rotatable 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*.
Thymol has been extensively explored as a potential antibacterial and anticancer agent (Carocho, 2016; Islam et al., 2019; Moon et al., 2017). Additionally, isolated essential oil of *Ocimum viride* comparatively showed highest sensitivity towards *Bacillus subtilis* pathogen (14/60.5mm) 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 (0.1μl/ml) | 33 ± 4 |

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 Hoeschst 33258.
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 be 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, cytotoxicity 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 cytotoxic 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 ± 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; Galluzzo 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 (Viscotti 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; Shivpje 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.

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Competing interest statement

The authors declare no conflict of interest.


Additional information

No additional information is available for this paper.

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