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Vetiveria zizanioides attenuates proliferation, migration, and metastasis of human cervical cancer: In vitro and In Silico analysis

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Research Article

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Vetiveria zizanioides attenuates proliferation, migration, and metastasis of human cervical cancer: In vitro and In Silico analysis

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Running title: Vetiveria zizanioides and cervical cancer

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Abstract

Cancer remains a significant global health issue, even with substantial advancements in treatment and diagnostic techniques. Consequently, this research seeks to identify new, cost-effective drugs that exhibit minimal side effects on cancer cells. Medicinal plants serve as promising candidates for the discovery of novel compounds for cancer therapy. To effectively tackle this serious health challenge, it is essential that early detection methods are paired with affordable and clinically effective medications. South India harbors the perennial grass Vetiveria zizanioides, which is a member of the Gramineae family. The use of Vetiver root infusion has been medicinal in curing spermatorrhea, reducing fever, promoting sweating, improving digestion, relieving spasms, regulating menstruation, tightening tissues, purifying blood, and relieving urinary difficulties. The anti-cancer effects of Vetiveria zizanioides on human cervical cancer cells were examined. Cells were exposed to different concentrations of ethanol extract from Vetiveria zizanioides (100-500 µg/ml) for duration of 72 hours. Higher concentration of Vetiveria zizanioides significantly reduced cervical cancer cell viability. Vetiveria zizanioides significantly increased apoptotic cells. Higher concentration of Vetiveria zizanioides reduced the larger wounds of cancer cells. Vetiveria zizanioides enhanced the expression of caspase-3 while decreasing the expression of MMP-2 and MMP-9. Considering all the gathered data, it is proposed that Vetiveria zizanioides demonstrated effectiveness in inhibiting the proliferation, migration, and metastasis of human cervical cancer cells.

Keywords: Vetiveria zizanioides; cervical cancer; Apoptosis; cell viability; proliferation

Introduction

Cervical cancer is a common gynecological cancer that develops within the epithelial tissue of the uterine cervix (Sung et al., 2021). Higher deaths due to this disease were documented, with nearly 90% occurring in low- and middle-income nations. The elevated incidence in these areas is primarily influenced by limited access to advanced healthcare services and delayed diagnoses, which lead to higher mortality rates among disadvantaged women (Hull et al., 2020). Cervical cancer ranks as the fourth most prevalent cancer among women globally and represents a major cause of death (Hamid et al., 2025). The resistance of drugs presents a significant challenge in the management of cervical cancer (Burmeister et al., 2022). It promotes relapse and increases mortality by diminishing the sensitivity of tumours to antineoplastic medications. As tumour cells develop resistance, the effectiveness of the drugs decreases, resulting in inadequate therapeutic responses and ultimately, treatment failure (Nikolaou et al., 2018).

Asia is the native region of the perennial grass Vetiveria zizanioides, commonly referred to as Vettiveru (David et al., 2019). This plant is a member of the Gramineae family. It can grow up to 1.5 meters in height, particularly in fertile marshy soils and along riverbanks. The cultivation of vetiver began in South India for the purpose of extracting aromatic oil from its roots. Over the years, this practice has spread globally (Gunasekar et al., 2025). The soothing properties and positive effects of vetiver and its root oil on mental and emotional health have been recognized for a long time by traditional medical systems (Barcellos-Silva et al., 2025). The infusion of vetiver root has been utilized for medicinal purposes to treat spermatorrhea, lower fever, promote sweating, improve digestion, alleviate spasms, regulate menstrual cycles, tighten tissues, purify blood, and relieve urinary issues (Gunasekar et al., 2025).

Recently, the use of vetiver roots in electric room coolers has been on the rise. Furthermore, vetiver roots are recommended for addressing skin ailments and are often incorporated as a body exfoliant in Ayurvedic treatments (De Tollenaere et al., 2021). Currently, there is a lack of information regarding the anticancer effects of ethanol extracts from Vetiveria zizanioides against HeLa cells. Consequently, the anti-cancer activity of ethanol extracts from Vetiveria zizanioides was evaluated in HeLa cells.

Materials and Methods

Materials

We gathered fresh roots of Vetiveria zizanioides from Chidabaram, Tamil Nadu, India. The roots of Vetiveria zizanioides were collected and subsequently washed under running water to eliminate any soil contaminants. After being shade-dried, the subterranean roots were mechanically processed into a powder using an electric blender, and then sieved through a 40 mesh. The finely milled root was kept in an airtight container for extraction purposes.

Preparation of root extracts

Ethanol was employed as a solvent for the extraction of the dried root powder of Vetiveria zizanioides. The extraction process utilized Soxhlet equipment to extract the roots of Vetiveria zizanioides. Initially, 50 g of dried root powder was weighed and placed into the thimble, where it was dissolved in 250 ml of ethanol extraction solvent. This mixture was cycled continuously at 700°C for duration of eight hours over a period of three days. Subsequently, the concentrations of the semi-solid extract were collected, filtered, and evaporated using a water bath set at 650°C to yield the appropriate crude extracts. The resulting crude dried extracts were then weighed and accurately labeled in 30 ml vials. The extract was stored at 40°C in a refrigerator until needed (Amarasiri et al., 2021).

GC-MS Analysis

The crude root extracts of aqueous and ethanolic solvents of Vetiveria zizanioides were further utilized for GC-MS analysis to identify the bioactive ingredients (Gomathi et al., 2015). The Identification of components was accomplished searching plant compound library (Karousa et al., 2026).

Cell culture

HeLa cancer cells were obtained from ATCC. Following this, the cells were cultured in RPMI medium enriched with 10% FBS and 1% antibiotics, and maintained in a CO2 incubator at 37˚C.

Sulforhodamine B (SRB) assay

The SRB assay was employed to investigate the cytotoxic effects of Vetiveria zizanioides on human cervical HeLa cancer cells (Hanifa et al., 2022). In summary, HeLa cervical cancer cells were cultured in 96-well plates at a density of 1.6 × 10^5 cells per well for a duration of 24 hours, followed by treatment with varying concentrations of Vetiveria zizanioides (ranging from 100 to 500 µg/ml) for 72 hours. Absorbance was recorded at 515 nm to assess cell viability.

Apoptosis

Apoptosis was assessed using ethidium bromide/acridine orange staining (EB/AO). In summary, HeLa cervical cells were cultured and treated with varying dose of Vetiveria zizanioides (300-500 µg/ml) for duration of 72 hours. Then, cells were stained with AO and EB and observed under a microscope (Liu et al., 2015).

Wound healing assay

The assay for wound healing on cancer cells was performed in accordance with the method outlined earlier (Migliaccio, 2023). HeLa cervical cancer cells were cultured and wound was induced using sterile tips. The remaining cells were treated with varying dose of

Vetiveria zizanioides (300 µg/ml, 400 µg/ml, and 500 µg/ml) for a period of 72 hours. Finally, the extent of migration was assessed.

Migration assay

Migration assay on human cervical cell was measured according to previously reported method (Zhu et al., 2018; Migliaccio et al., 2023). Briefly, HeLa cervical cancer cells were culture in 6-well plates (1.6×10^6 cells/well) for 24 h, and then treated with various concentrations of Vetiveria zizanioides (300-500 µg/ml) for 72 h.

RT-PCR

RNA was extracted from homogenate of HeLa cervical cancer cells utilizing Trizol-Reagent. The process of reverse transcription was carried out using a cDNA synthesis kit. Table 1 illustrates the particular primers utilized for the amplification of caspase-3, MMP-2, MMP-9, and caspase-3. The relative expression ratio was calculated based on the change-in-threshold ($-\Delta\Delta CT$) method (Marema et al., 2026).

Immunofluorescence

The immunofluorescence assay was conducted on cervical cancer cells were performed following the methodology previously described (Ji et al., 2022). In summary, HeLa cervical cancer cells were cultured for duration of 24 hours, after which they were treated with varying concentrations of Vetiveria zizanioides (300-500 µg/ml) for 72 hours. Finally, the expression was observed under a microscope.

Molecular docking studies

A molecular docking study was performed to investigate the binding mode and interactions between the isolated compounds and the active site of the apoptotic activator Bcl-2 protein (Usha et al., 2014). The Bcl-2 protein's crystallographic structure was obtained from the PDB databank and altered utilizing a number of modifications to produce the matching PDBQT file. Using the HyperChem software to sketch the target compounds'

structures and the semi-empirical PM3 approach to optimize them, the most stable conformations were found. For the Bcl-2 receptor, a grid box measuring 42 × 42 × 42 points was chosen, with the centre coordinates at x (17.225), y (2.752), and z (15.481). The default values were used for the grid and docking parameters. Lastly, a Molecular Operating Environment was used to evaluate the collected findings files.

Statistical analysis

The data is displayed as means ± SEM. The differences between the control group and the subjects treated with Vetiveria zizanioides were assessed using Student's t-test and analysis of variance. A p-value of less than 0.05 was deemed statistically significant.

Results

The ethanolic root extract of Vetiveria zizanioides was subjected to GC-MS analysis revealed the chemical composition. The chromatogram showed peaks which confirmed the presence of 10 different compounds with different retention time. The compound such as Epizizanone, 4-methyl-1-(1-meth), 2, 3-Dihydro-benzofuran, Khusimol, Eugenol, Rosifoliol, Pluchidiol, n-Hexadecanoic acid, Pentadecanoic acid and 18-Nonadecenoic acid. We conducted an analysis of the anti-proliferative and apoptotic effects induced by Vetiveria zizanioides in HeLa cells. The SRB assay was employed to evaluate the impact of Vetiveria zizanioides on these cells. The cells were exposed to varying concentrations of Vetiveria zizanioides (100 to 500 µg/ml) over a period of 72 hours. The observed cell viability rates were 98.5%, 91.2%, 78.5%, 61.4%, and 42.6% at 100, 200, 300, 400 and 500 µg/ml of Vetiveria zizanioides, respectively (Figure 1A & B, $P< 0.05$). These results indicate a significant inhibitory effect of Vetiveria zizanioides on the proliferation of cervical cancer cells.

We employed EB/AO staining technique to investigate the impact of Vetiveria zizanioides on the apoptosis cancer cells. The influence of Vetiveria zizanioides on HeLa cell

apoptosis is presented in Table 2 and Figure 2. HeLa cells incubated with 300 µg/ml of Vetiveria zizanioides exhibited an increase in early apoptotic cells (1.5%) and total apoptotic cells (6.1%) compared to the control (Table 2, $P < 0.05$). Treatment with 400 µg/ml resulted in an increase in early apoptotic cells (8.8%) and apoptotic cells (14.1%) relative to the control (Table 2). Furthermore, treatment with 500 µg/ml of Vetiveria zizanioides led to an increase in early apoptotic cells (9.5%) and apoptotic cells (23%).

Vetiveria zizanioides exhibited a significant inhibitory effect on migration of human cervical cancer cells in a dose-dependent manner. The application of 300, 400, and 500 µg/ml of Vetiveria zizanioides markedly reduced the size of the wounds (Figure 3). Furthermore, the transwell assay validated the decrease in the migration of cancer cells. The treatment of HeLa cells with 200 µg/ml of Vetiveria zizanioides led to a modest reduction in cell motility compared to the untreated cells. In contrast, the treatment with 300, 400, and 500 µg/ml of Vetiveria zizanioides led to significant reductions in cancer cell motility by 23.2%, 35.6%, and 47.5%, respectively (Figure 4). The treatment with Vetiveria zizanioides increased the mRNA expression of caspase-3 by 20%, 40%, 60%, and 90% at respective concentrations in HeLa cells (Figure 5). Additionally, Vetiveria zizanioides decreased the mRNA expression of MMP-2 by 8%, 23%, 47%, and 62% at respective concentrations in HeLa cells (Figure 5). Furthermore, the treatment with Vetiveria zizanioides also significantly reduced the mRNA expression of MMP-9 by 7%, 22%, 38%, and 55% at respective concentrations in HeLa cells (Figure 5).

In the context of immunofluorescence analysis, treatment with Vetiveria zizanioides resulted in a notable increase in the protein expression of caspase-3 by 14%, 58%, and 89% at respective concentrations in HeLa cells. Additionally, Vetiveria zizanioides reduced protein expression of MMP-2 by 9%, 15%, 34%, and 54% at respective concentrations in HeLa cells. Furthermore, the treatment significantly diminished the protein expression of MMP-9 by

11%, 29%, and 48% at respective concentrations in HeLa cells (Figures 6-7). The Bcl-2 protein plays a crucial role in regulating apoptosis, and its overexpression in various types of cancer contributes to the survival of cancer cells. Bcl-2 is considered a promising target for the development of anticancer drugs, since its inhibition can facilitate apoptosis. The data indicated a stable complex between the ligand and the Bcl-2 protein, with Khusimol demonstrating the lowest binding energy value of −9.72 Kcal/mol. The most effective molecule, rosifoliol, exhibited favourable binding interaction energy of -9.58 with the Bcl-2 receptor (Table 3).

Discussion

Cervical cancer is a prevalent form of gynaecological cancer that arises in the epithelial tissue of the cervix of the uterus (Sung et al., 2021). The elevated incidence in these areas is primarily influenced by limited access to advanced healthcare services and delayed diagnoses, which lead to higher mortality rates among disadvantaged women (Hull et al., 2020). The resistance of drugs presents a significant challenge in the management of cervical cancer (Burmeister et al., 2022). Natural products have traditionally been essential in the discovery of anticancer drugs, with approximately 60% of the chemotherapeutics used in clinical settings originating from or being inspired by plant-derived compounds (2024). In relation to this, essential oils, which are intricate combinations primarily consisting of volatile compounds derived from medicinal or aromatic plants, contain a diverse array of bioactive natural compounds characterized by numerous structures and chemistries that may function as potential chemotherapeutics (de Sousa et al., 2023; Pavithra et al., 2024). Recent research indicates that numerous components of essential oils exhibit antiproliferative, pro-apoptotic, anti-inflammatory, antioxidant, and anti-angiogenic properties. These effects are mediated through mechanisms that include mitochondrial dysfunction, modulation of oxidative stress, and disruption of critical oncogenic signalling pathways (Sharma et al., 2023).

GC–MS profiling was utilized to determine the phytochemicals found in the ethanol extract of Vetiveria zizanioides. The predominant metabolites identified included Epizizanone, 4-methyl-1-(1-meth), 2, 3-Dihydro-benzofuran, Khusimol, Eugenol, Rosifoliol, Pluchidiol, n-Hexadecanoic acid, Pentadecanoic acid, and 18-Nonadecenoic acid. Previous studies have documented fingerprint markers of vetiver oil, such as rosifoliol and khusimol, all of which were consistently detected in our investigation (Chahal et al., 2015; Karousa et al., 2026). We investigated the anti-proliferative and apoptotic effects triggered by Vetiveria zizanioides in HeLa cells. Results demonstrated that Vetiveria zizanioides reduced the proliferation of HeLa cells and reduced MMP-2 and MMP-9 expression, indicating a decrease in cell migration. Our findings are consistent with those reported by Fadhillah et al. (2022), which indicated that treatment with Vetiveria zizanioides oil diminished cervical cancer cell proliferation and exhibited properties as an anticancer agent.

MMPs are released from tumour cells during the process of metastasis, resulting in the degradation of the extracellular matrix, which subsequently facilitates the invasion of cancer cells into lymphatic vessels and the bloodstream (Juurikka et al., 2019). Scarano et al. (2015) have indicated that elevated MMP-9 and MMP-2 expressions in the advanced stages of cancer may serve as potential biomarkers for the diagnosis of various cancers. Researchers have found that bioactive compounds can inhibit the metastasis by decreasing the activity of MMP-2 and MMP-9 (Godyla-Jabłoński et al., 2025). In this study, we analysed the anti-proliferative effect of ethanol extract of Vetiveria zizanioides against cervical cancer cells. The AO/EB staining technique is the most dependable method for detecting apoptosis. (Liu et al., 2015). Apoptosis is a crucial cellular process that eliminates unwanted, damaged, and cancerous cells (Wong 2011). Hanifa (2022) reported that Vetiveria zizanioides inhibits migration and promotes apoptosis in cancer cells. Our findings align with earlier research that

illustrates the cytotoxic capabilities of Vetiveria zizanioides against various cancer cells (Jha et al., 2024; Chitra et al., 2014; Powers et al., 2018).

Conclusion

Considering all the gathered data, it is proposed that the ethanol extract of Vetiveria zizanioides demonstrated effectiveness in inhibiting the proliferation, migration, and metastasis of human cervical cancer.

Author Contribution declaration

All authors contributed to the study conception and design. The manuscript draft was written by M. P, and V. A. Material preparation, data collection and analysis was performed by P. Goud and P.C. N. All authors read and approved the final.

Conflict of Interest

Authors declare that they have no conflict of interest.

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Figure legends

Figure 1: The therapeutic impact of the ethanol extract from Vetiveria zizanioides on proliferation of HeLa cells demonstrated by sulforhodamine B (SRB) assay.

Figure 2: The therapeutic impact of ethanol extract derived from Vetiveria zizanioides on the apoptosis of HeLa cells is demonstrated through EB/AO staining.

Figure 3: The therapeutic impact of ethanol extract obtained from Vetiveria zizanioides on the migration of HeLa cells is demonstrated through a wound healing assay.

Figure 4: The therapeutic impact of ethanol extract obtained from Vetiveria zizanioides on the migration of HeLa cells is demonstrated through a wound healing assay.

Figure 5: Therapeutic effect of ethanol extract obtained from Vetiveria zizanioides on the mRNA expression of caspase-3, MMP-9 and MMP-2 in HeLa cells.

Figure 6: Therapeutic effect of ethanol extract obtained from Vetiveria zizanioides on protein expression of caspase-3, MMP-9 and MMP-2 in HeLa cells.

Figure 7: Therapeutic effect of ethanol extract obtained from Vetiveria zizanioides on protein expression of caspase-3, MMP-9 and MMP-2 in HeLa cells.

Figure 8: Molecular docking interactions involving compounds obtained from the ethanol extract of Vetiveria zizanioides. Diagrams illustrating protein-ligand interactions, including hydrogen bonds, hydrophobic interactions, and van der Waals contacts.

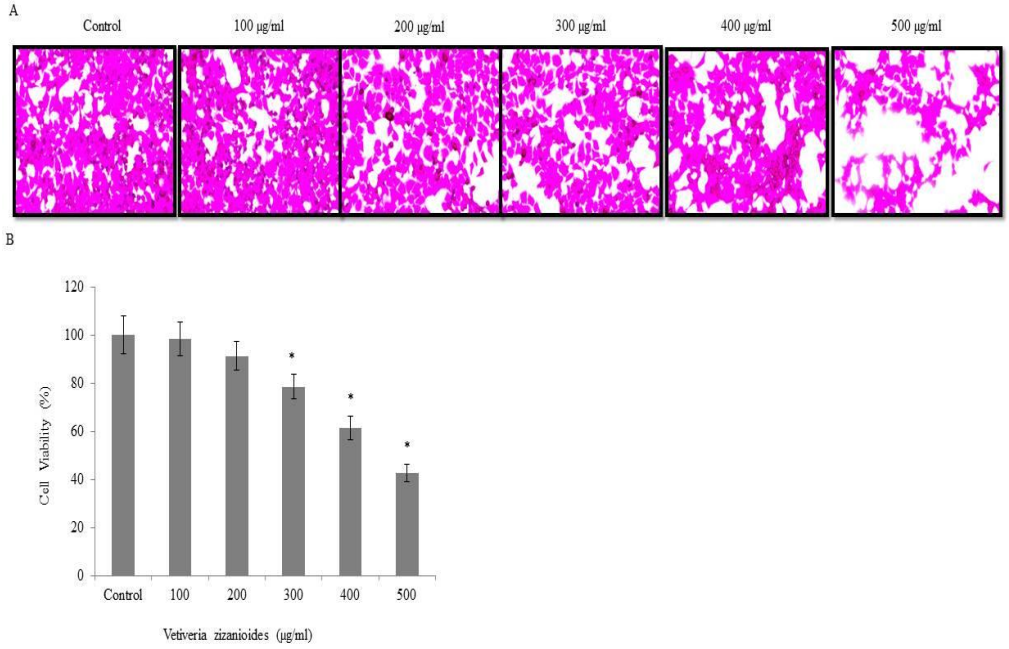

A
Control
100 µg/ml
200 µg/ml
300 µg/ml
400 µg/ml
500 µg/ml
B
Cell Viability (%)
120
100
80
60
40
20
0
Control
100
200
300
400
500
Vetiveria zizanioides (µg/ml)

Figure 1

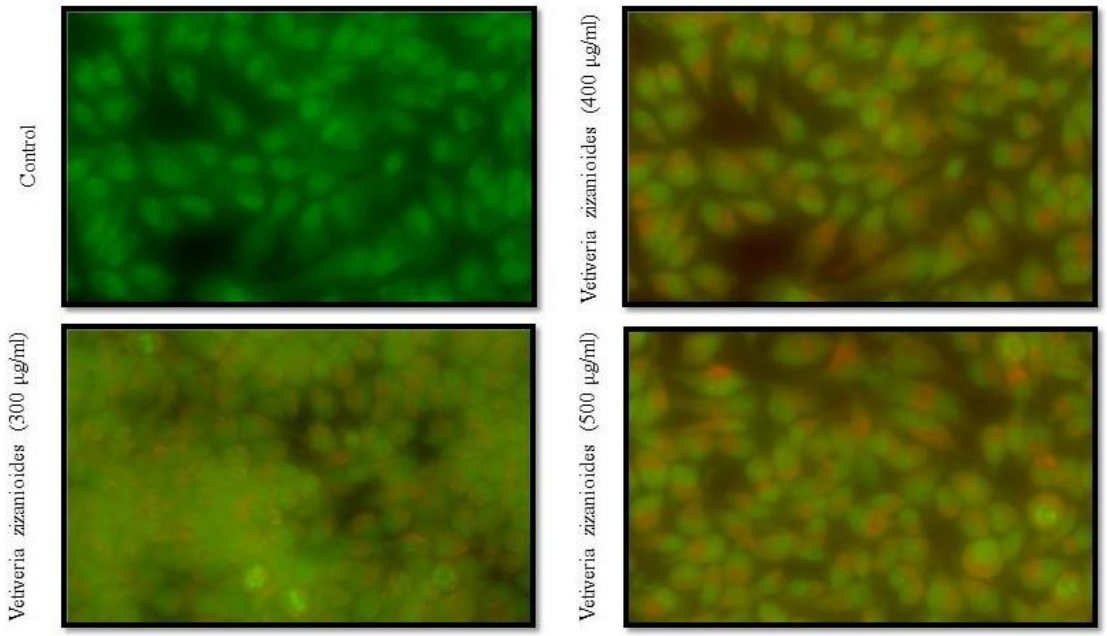

Control
Vetiveria zizanioides (400 µg/ml)
Vetiveria zizanioides (300 µg/ml)
Vetiveria zizanioides (500 µg/ml)

Figure 2

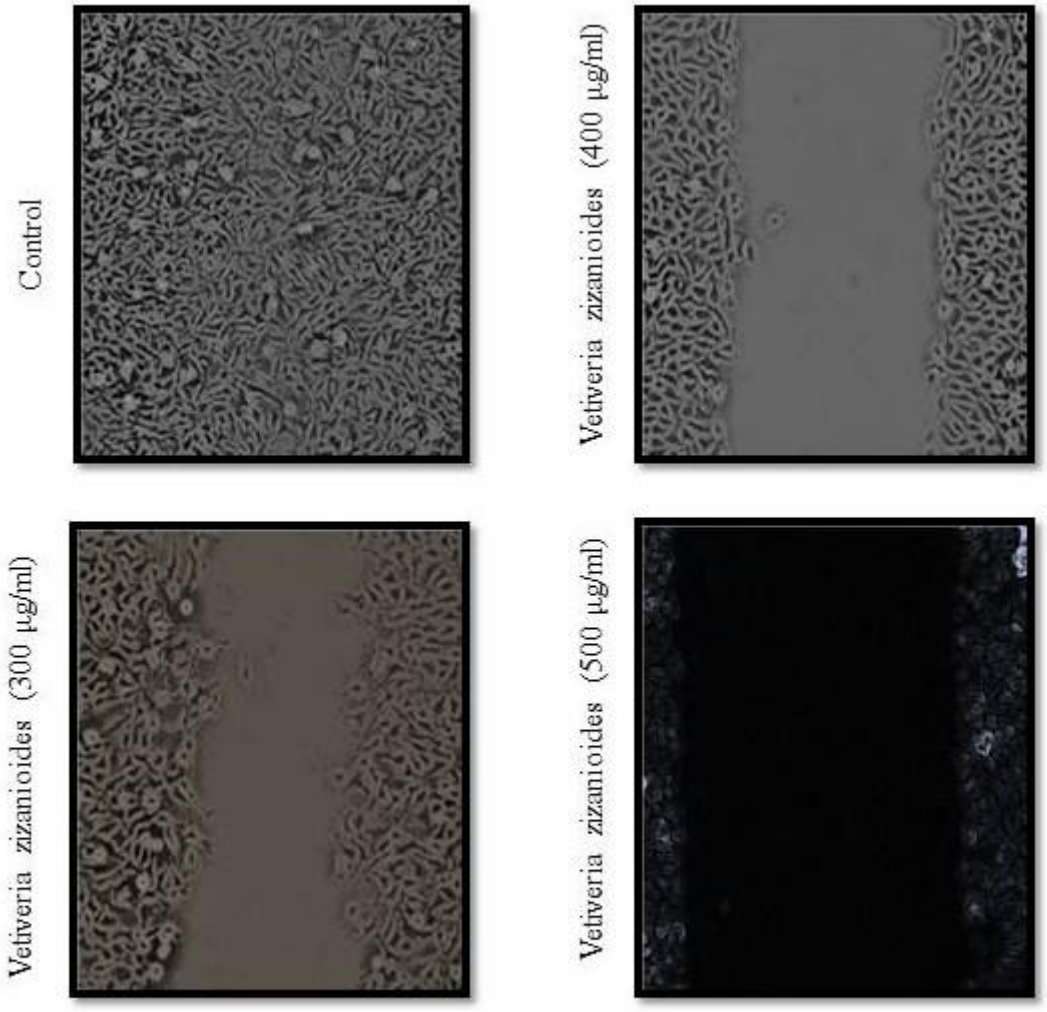

Control
Vetiveria zizanioides (400 µg/ml)
Vetiveria zizanioides (300 µg/ml)
Vetiveria zizanioides (500 µg/ml)

Figure 3

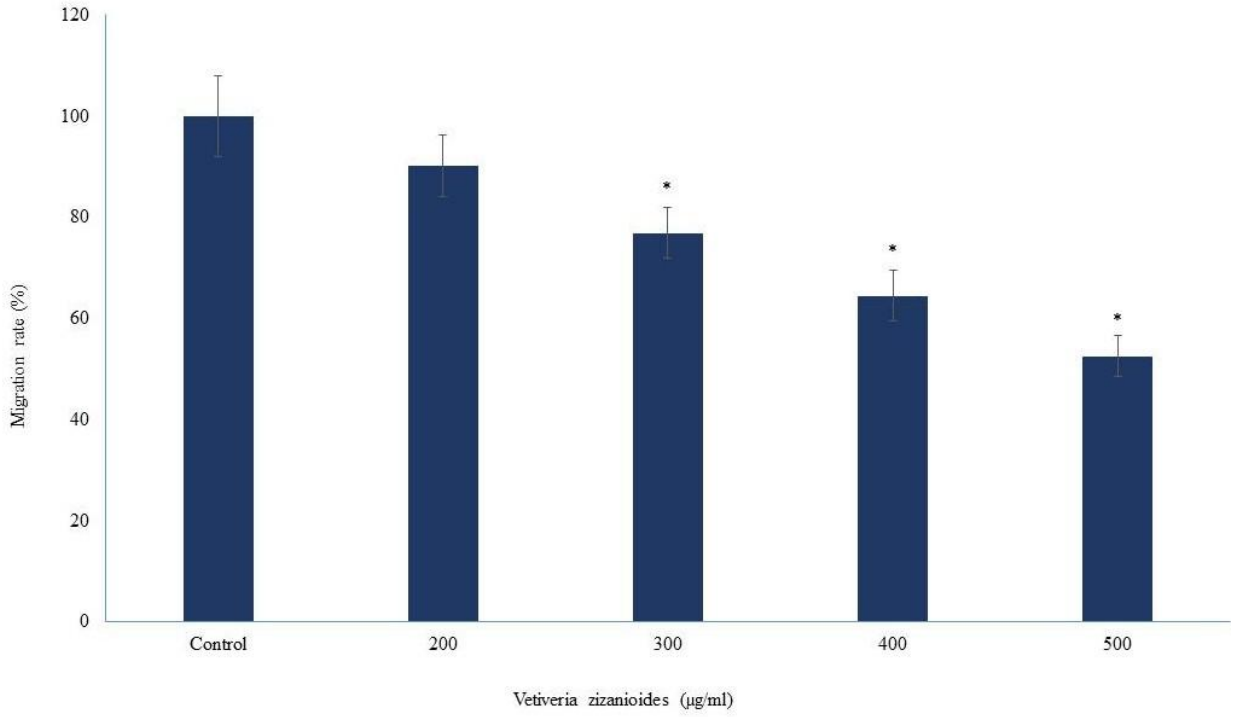

120
100
80
60
40
20
0
Migration rate (%)
*
*
*
Control
200
300
400
500
Vetiveria zizanioides (µg/ml)

Figure 4

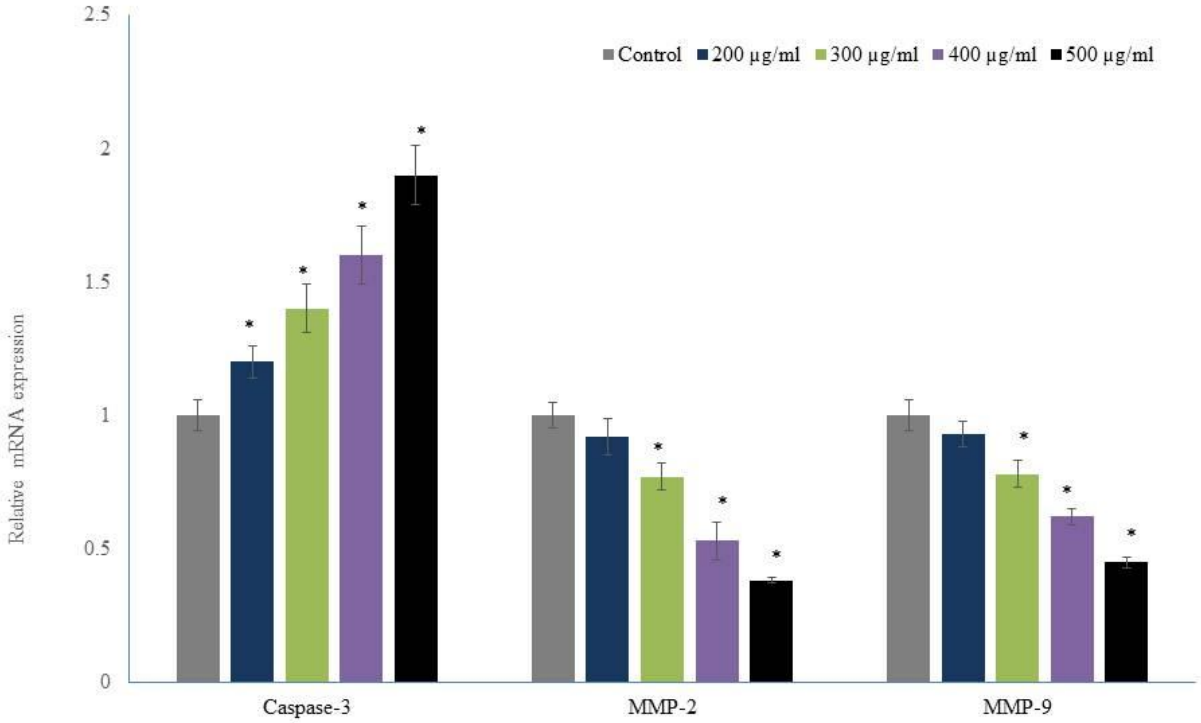

Control
200 µg/ml
300 µg/ml
400 µg/ml
500 µg/ml
Relative mRNA expression
2.5
2
1.5
1
0.5
0
Caspase-3
MMP-2
MMP-9

Figure 5

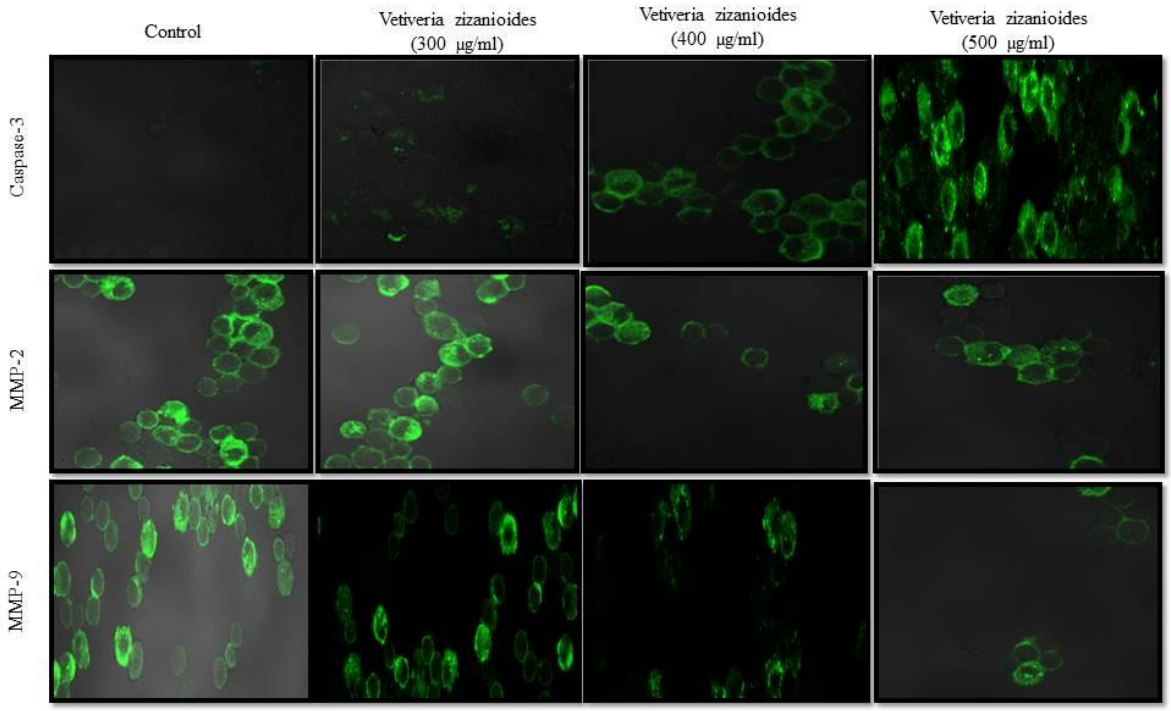

Control
Vetiveria zizanioides (300 µg/ml)
Vetiveria zizanioides (400 µg/ml)
Vetiveria zizanioides (500 µg/ml)
Caspase-3
MMP-2
MMP-9

Figure 6

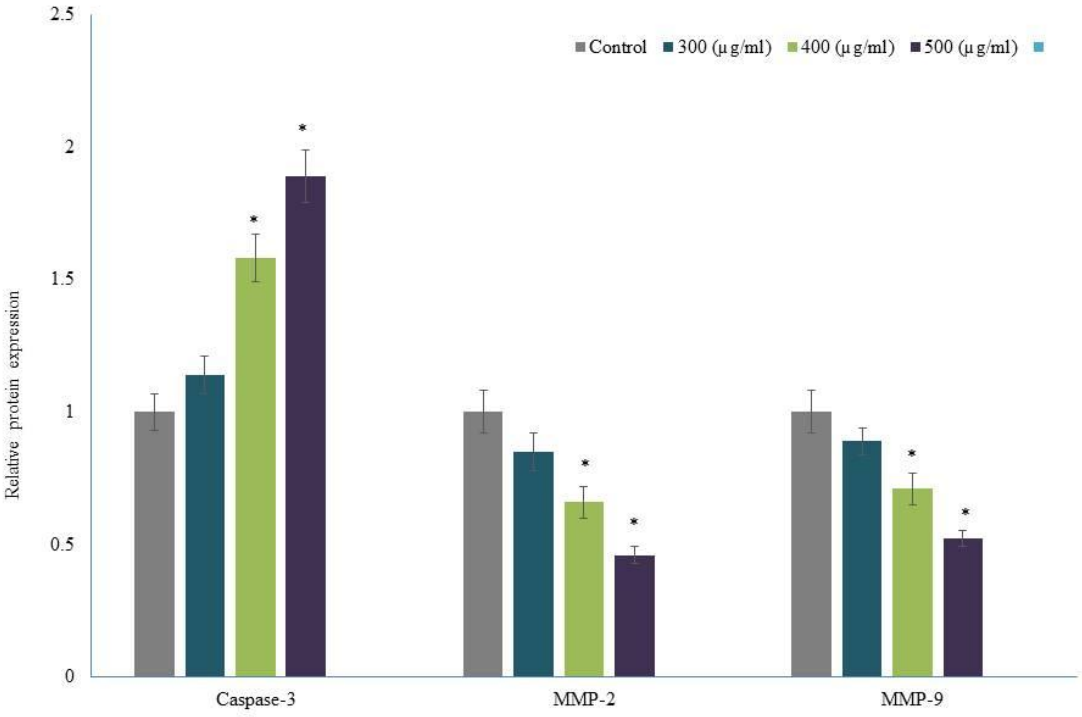

Control
300 (µg/ml)
400 (µg/ml)
500 (µg/ml)
Relative protein expression
2.5
2
1.5
1
0.5
0
Caspase-3
MMP-2
MMP-9

Figure 7

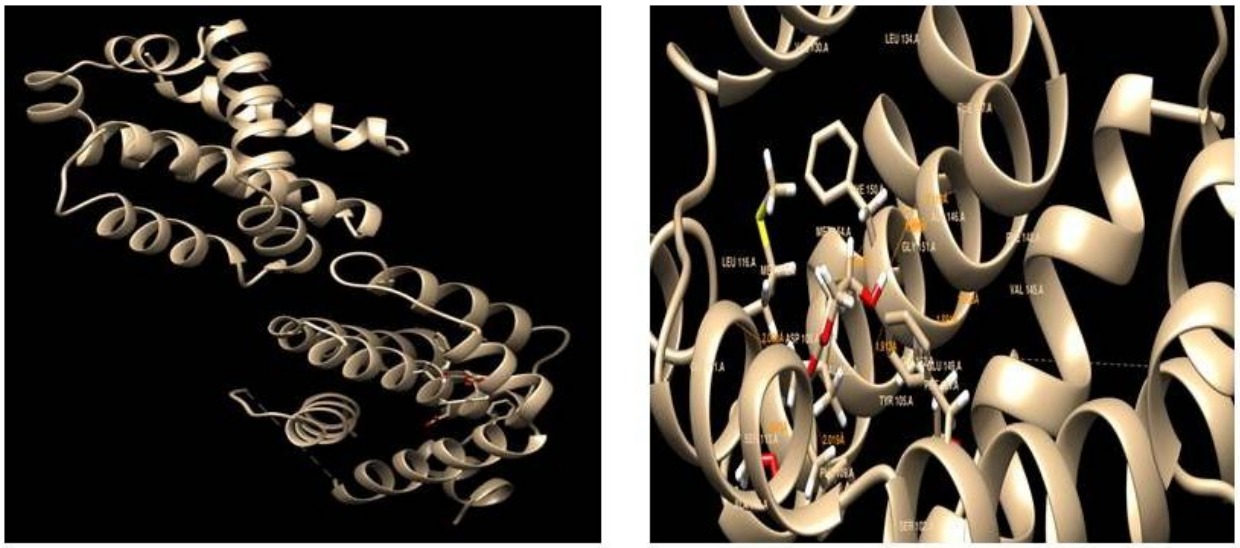


Figure 8

Table 1: A compilation of RT-PCR primers utilized in this research.

Marker	Forward primer	Reverse primer
GAPDH	5'-CAAGATCATCAGCAATGCCTCC-3'	5'-GCCATCACGCCACAGTTTCC-3'
Caspase-3	5'-ATGGTTTGAGCCTGAGCAGA-3'	5'-GGCAGCATCATCCACACATAC-3'
MMP-2	5′-CTCATCGCAGATGCCTGGAA-3′	5′-TTCAGGTAATAGGCACCCTTGAAGA-3′
MMP-9	5′-CAC TGT CCA CCC CTC AGA GC-3′	5′-CAC TTG TCG GCG ATA AGG-3′

Table 2: Percentage of live, early apoptotic, late apoptotic and necrotic human cervical cancer cells stained by EB/AO

Groups	Live cells	Early apoptotic	Late apoptotic	Necrotic
Control	96.1 ± 4.8	0.7 ± 0.05	1.6 ± 0.1	1.6 ± 0.11
Vetiveria zizanioides (300 µg/ml)	85.6 ± 4	1.5 ± 0.11*	6.1 ± 0.5*	6.8 ± 0.5*
Vetiveria zizanioides (400 µg/ml)	68.6 ± 3.8*	8.8 ± 0.8*	14.1 ± 2.2*	8.5 ± 0.8*
Vetiveria zizanioides (500 µg/ml)	55.5 ± 3*	9.5 ± 0.6*	23 ± 2.1*	12.2 ± 1.1*

*P< 0.05

Table 3: The binding energy values (Kcal/mol) of compounds in the active site of Bcl-2 protein

Ligand	Docking binding energy (Kcal/mol)
Epizizanone	-9.32
Khusimol	-9.72
4-methyl-1-(1-meth),	-9.53
2, 3-Dihydro-benzofuran	-9.27
Eugenol	-9.48
Rosifoliol	-9.58
Pluchidiol	-9.25
n-Hexadecanoic acid,	-9.51
Pentadecanoic acid	-9.42
18-Nonadecenoic acid	-9.29