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Euphorbia tirucalli leaf extract induces apoptosis and inhibits cell migration in HSC-3 tongue cancer cells

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Abstract

Objective: Therefore, this study was conducted to evaluate the effect of ETLE on cell viability, apoptosis, and cell migration in human oral squamous carcinoma (HSC)-3 tongue cancer cells.

Material and Method: ETLE was prepared using the maceration technique. HSC-3 cells were treated with ETLE at concentrations of 2%, 10%, and 50%. Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, while apoptosis was analyzed through the sub-G1 assay. A cell migration assay was also conducted to assess the effect of ETLE on cell migration.

Results: ETLE significantly reduced HSC-3 cell viability in a concentration-dependent manner. ETLE increased the percentage of apoptotic cells in a concentration-dependent manner as well. Additionally, ETLE significantly inhibited cell migration, with the 50% ETLE-treated group showed minimal migration, comparable to the doxorubicin-treated group.

Conclusion: ETLE could exhibited significant cytotoxic effects on HSC-3 tongue cancer cells by inducing apoptosis and inhibiting migration, suggesting its potential as an anti-cancer agent for tongue cancer cell.

Keywords: Apoptosis, Cell migration, Euphorbia tirucalli, HSC-3, Tongue cancer
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Introduction

Oral squamous cell carcinoma (OSCC), particularly in the tongue, is one of the most aggressive and prevalent forms of head and neck cancer,[1] often characterized by high recurrence and metastasis rates.[2] Although there have been advancements in treatment options such as surgery, radiation, and chemotherapy, the prognosis for advanced OSCC remains poor due to the cancer's resistance to conventional therapies and its potential for local invasion and distant spread.[3] This situation emphasizes the urgent need for alternative therapeutic strategies to effectively target this cancer.

In response to this need, the search for new therapeutic agents has led to the exploration of natural products known for their potential anticancer properties[4] and fewer side effects.[5] Among these, Euphorbia tirucalli has garnered attention due to its traditional medicinal uses and its richness in various bioactive compounds.[6] Notably, the most abundant and significant compounds in E. tirucalli are diterpenes, particularly phorbol esters, which have demonstrated cytotoxic effects against various cancer cells.[7] These compounds have been shown to induce apoptosis by activating intrinsic pathways that promote programmed cell death.[7]

Understanding the mechanisms of apoptosis is essential, as this process plays a crucial role in eliminating damaged or cancerous cells.[8] It is characterized by a series of biochemical events that lead to cell shrinkage, DNA fragmentation, and ultimately, cell death.[9,10] Furthermore, in addition to inducing apoptosis, the ability of certain compounds to influence cell migration is of great importance in cancer treatment, as metastasis remains the leading cause of cancer-related mortality.[11] Thus, effective therapeutic agents must not only induce apoptosis but also inhibit the migratory behavior of cancer cells to prevent tumor spread.[12] In this context, E. tirucalli presents an intriguing opportunity for investigation, particularly regarding its effects on the mechanisms of apoptosis and cell migration.

Prior research has examined the effects of E. tirucalli leaf extract (ETLE) on various cancer cell lines, including those associated with breast[13], colorectal[13] and melanoma cancers.[14] However, no studies have examined the effects of ETLE on oral squamous cell carcinoma (OSCC), particularly in tongue cancer cells. Furthermore, the apoptotic mechanisms induced by ETLE remain largely undefined and require further investigation. Therefore, this study was conducted to evaluate the effect of ETLE on cell viability, apoptosis, and cell migration in human oral squamous carcinoma (HSC)-3 tongue cancer cells.

Material and Methods

Preparation of E. tirucalli Leaf Extract

The leaves of E. tirucalli were acquired from Indonesian Institute for Testing Instrument Standard for Spices, Medicinal, and Aromatic Plant, Ministry of

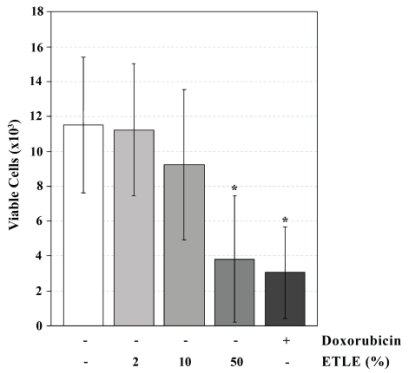

Figure 1. ETLE decreased the numbers of HSC-3 viable cells in a concentration-dependent manner. HSC-3 cells were starved for 12 hours, followed by treatment with/without 3 µM Doxorubicin or ETLE in various concentrations for 24 hours. Viable cells were assessed using MTT assay, as detailed in the Methods. Data are presented as mean±standard deviation (n=6). *Significant (Tukey's post hoc test, p<0.05) compared with Sham group.

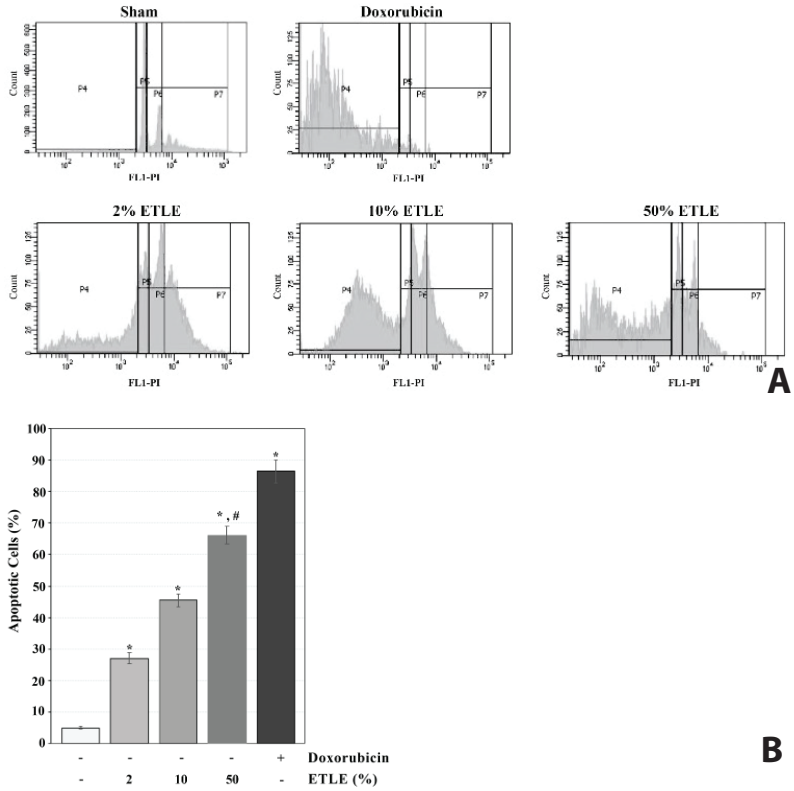

Figure 2. ETLE increased the percentage of HSC-3 apoptotic cells in a concentration-dependent manner. HSC-3 cells were starved for 12 hours, followed by treatment with/without 3 µM Doxorubicin or ETLE in various concentrations for 24 hours. Apoptotic cells were assessed using Sub-G1 assay, as detailed in the Methods, A. Histograms of treated HSC-3 cells in Sub-G1, G1, S, G2/M phases, B. The percentage of HSC-3 apoptotic cells. Data are presented as mean±standard deviation (n=6). *Significant (Tukey's post hoc test, p<0.05) compared with Sham group. #Significant (Tukey's post hoc test, p<0.05) compared with Doxorubicin group. P4: Sub-G phase; P5: G1 phase, P6: S phase; P7: G2/M phase.

Agriculture. The ETLE was prepared through a maceration process, in which the plant material was finely chopped, dried, and extracted with 96% ethanol. The resulting extract was filtered and concentrated using a rotary evaporator, and the final ETLE was stored at 4°C.

HSC-3 Cell Culture

HSC-3 cell culture was conducted as previously reported method.15 The HSC-3 cell line was grown Dulbecco's modified eagle medium (DMEM) (Sigma-Aldrich, St. Louis, Missouri, USA) enriched with 50 U/mL penicillin (Sigma-Aldrich), 50 µg/mL streptomycin (Sigma-Aldrich), and 10% fetal bovine serum (FBS) (Sigma-Aldrich). The cells were maintained in a humidified environment at 37°C with 5% CO2. Once the HSC-3 cells reached approximately 80% confluence, the cells were detached with trypsin-ethylenediamine tetraacetic acid (EDTA) solution (Sigma-Aldrich).

Cell Viability Assay

Cell viability assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, following previously reported method.15 HSC-3 cells were seeded into 96-well plates (5x103 cells/well) and treated with/without 2, 10, or 50 % ETLE or 3 µM Doxorubicin (Global Dankos Farma, Jakarta, Indonesia) for 24 hours. Following the treatment, MTT solution was added to reach a final concentration of 0.5 mg/mL, and the cells were incubated for 4 hours. The solution was then discarded, and the formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO). The absorbance of the dissolved formazan was measured at an optical density of 570 nm using a microplate reader (Bio-Rad, Hercules, USA). Each experimental group was analyzed in sextuplicate.

Sub-G1 Assay

Apoptotic HSC-3 cells were quantified using the sub-G1 assay to examine the cytotoxic effects of ETLE, following previously reported method.15 The treated HSC-3 cells were collected and suspended in a hypotonic fluorochrome solution (50 µg/mL of propidium iodide (Fujifilm Wako, Osaka, Japan), 0.1% Triton X-100 (Sigma-Aldrich), and 0.1% sodium citrate (Wako, Osaka, Japan)). The cell suspensions were then incubated in the dark for 30 minutes. The fluorescence of individual nuclei was analyzed using a FACSCanto II flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA), recording a total of 100,000 events.

Migration Cell Assay

Thin tube (Thermo Fisher, Waltham, MA, US) was placed on the surface of a 12-well plate, and the HSC-3 cell line was seeded into the well. After removing the tube, an area free of cell adhesion was created and documented using a phase-contrast microscope at 4x and 10x magnifications. The HSC-3 cells were then treated and incubated for 24 hours. Following incubation, the area free of cell adhesion was documented again with the phase-contrast inverted microscope

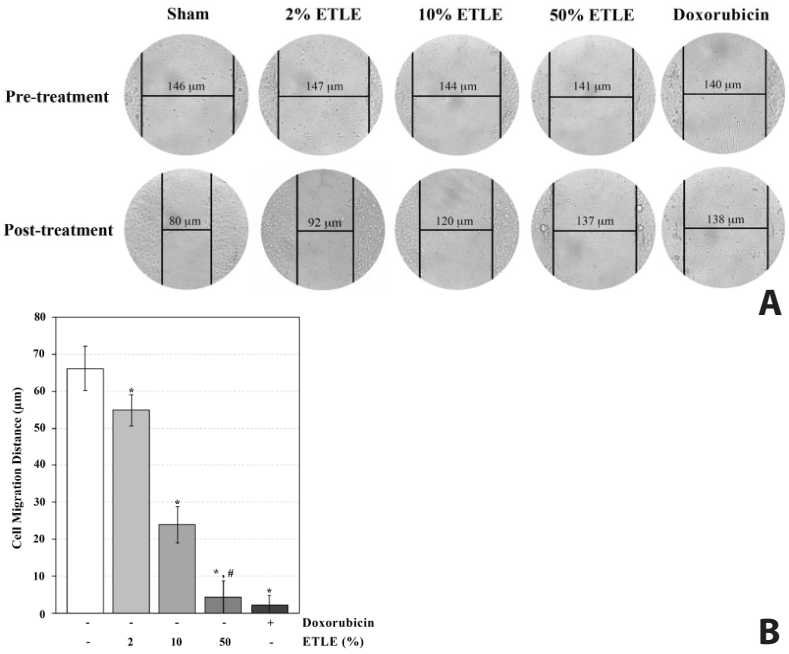

Figure 3. ETLE inhibited the migration distance of HSC-3 cells in a concentration-dependent manner. HSC-3 cells were seeded into a 12-well plate and a thin tube was used to create a defined area free of cell adhesion. After removing the tube, the cells were treated with/without 3 µM Doxorubicin or ETLE at various concentrations for 24 hours, A. Area free of cell adhesion in pre- and post-treatment that was assessed using phase-contrast microscopy, B. Change in migration distance of HSC-3 cells. Data are presented as mean±standard deviation (n=6). *Significant (Tukey's post hoc test, p<0.05) compared with Sham group. #Significant (Tukey's post hoc test, p<0.05) compared with Doxorubicin group.

(Zeiss, Jena, Germany) at the same magnifications.

Statistical Analysis

Statistical analysis was performed using the Shapiro-Wilk test for normality. For data that exhibited a normal distribution, one-way ANOVA was conducted, followed by Tukey's post hoc test, meanwhile, for data that did not follow a normal distribution, the Kruskal-Wallis test was conducted, followed by Mann-Whitney's post hoc test.

Results

ETLE Decreased HSC-3 Viable Cells

Numbers of HSC-3 viable cells in Doxorubicin group (3.049±2.633) were significantly lower (Tukey's post hoc test, p=0.004) than the ones in the Sham group (11.500±3.884) figure 1. ETLE treatment could decrease the numbers of HSC-3 viable cells significantly (ANOVA, p=0.001) in a concentration-dependent manner. The numbers of HSC-3 viable cells in 2% (11.239±3.780; Tukey's post hoc test, p=1.000) and 10% (9.235±4.319; Tukey's post hoc test, p=0.823) ETLE-treated groups were not significantly different than the ones in the sham group, meanwhile the numbers of HSC-3 viable cells in 50% ETLE-treated group (3.833±3.617) were significantly lower (Tukey's post hoc test, p=0.011) than the ones in the Sham group. Additionally, the numbers of HSC-3 viable cells in the 50% ETLE-treated group (Tukey's post hoc test, p=0.996) were not significantly different than the ones in the Doxorubicin group.

ETLE Increased HSC-3 Apoptotic Cells

The histogram figure 2A showed an increase in the percentage of cells in the Sub-G1 phase was observed in the Doxorubicin group. In contrast, the Sham group had most cells in the G1 phase (P5) with minimal apoptosis. In the 2% ETLE-treated group, there was a noticeable reduction in apoptotic cells, then in the 10% and 50% ETLE-treated groups, the percentage of cells in the Sub-G1 phase further decreased. The calculated percentage data were presented in figure 2B.

The percentage of apoptotic HSC-3 cells in Doxorubicin group (86.383±3.616%) was significantly higher (Tukey's post hoc test, p=0.000) than the ones in the Sham group (5.017±0.449%) figure 2B. ETLE treatment could increase the percentage of HSC-3 apoptotic cells significantly (ANOVA, p=0.000) in a concentration-dependent manner. The percentage of apoptotic HSC-3 cells in 2% (27.133±1.657%), 10% (45.517±2.051), and 50% (66.117±2.910%) ETLE-treated groups were significantly higher (Tukey's post hoc test, p=0.000) than the ones in the Sham group. Additionally, the percentage of apoptotic cells in the 50% ETLE-treated group was significantly lower (Tukey's post hoc test, p=0.000) than the ones in the Doxorubicin group.

ETLE Inhibited the Migration Distance of HSC-3 Cells

The Sham group showed the migration distance decreasing from 146 µm to 80 µm figure 3A. The 2% ETLE-treated group showed a slight migration distance, meanwhile the 10% and 50% ETLE-treated groups exhibited greater inhibition of migration distance. Notably, 50% ETLE-treated group resulted in minimal change in the migration distance, similar to the doxorubicin-treated group, which showed almost no change in migration distance.

The migration distance of HSC-3 cells in Doxorubicin group (2.757 mm) was significantly lower (Tukey's post hoc test, p=0.000) than the ones in the Sham group (66.167 mm) figure 3B. ETLE treatment could inhibited the migration distance of HSC-3 cells significantly (ANOVA, p=0.000) in a concentration-dependent manner. The migration distance of HSC-3 cells in 2% (54.833 mm), 10% (4.833 mm), and 50% (4.446 mm) ETLE-treated groups were significantly lower (Tukey's post hoc test, p=0.000) than the ones in the Sham group. Additionally, the migration distance of HSC-3 cells in the 50% ETLE-treated group

was significantly higher (Tukey's post hoc test, p=0.987) than the ones in the Doxorubicin group.

Doxorubicin or ETLE at various concentrations for 24 hours. A: Area free of cell adhesion in pre- and post-treatment that was assessed using phase-contrast microscopy. B: Change in migration distance of HSC-3 cells. Data are presented as mean±standard deviation (n=6). *Significant (Tukey's post hoc test, p<0.05) compared with Sham group. #Significant (Tukey's post hoc test, p<0.05) compared with Doxorubicin group.

Discussion

ETLE showed a cytotoxic effect in HSC-3 cells in concentration-dependent manner. The results from the MTT and sub-G1 assays indicated a decrease in viable HSC-3 cells attributable to the induction of apoptosis. These findings align with previous studies that reported ETLE's ability to induce apoptosis in MCF-7 (breast cancer),[13] HT-29 (colorectal cancer),[13] and SK-MEL-28 (melanoma cancer)[14] cell lines. IC50 for ETLE-induced apoptotic HSC-3 cells was 31.87 µg/mL, which was classified as moderate cytotoxicity (21-200 µg/mL).[15] The IC50 of ETLE-induced apoptotic HSC-3 cells was lower than those reported for CCLE-induced apoptotic MCF-7 cells (33.88 µg/mL) and CCLE-induced apoptotic HT-29 cells (34.86 µg/mL).

In this current study, ETLE not only induced apoptosis in HSC-3 cells but also inhibited their migration. This is important, as cell migration is a key factor in cancer metastasis,[16,17] suggesting that ETLE might be a promising therapeutic agent for preventing the spread of OSCC, especially in tongue cancer cells. The substantial reduction in migration distance in ETLE-treated groups, particularly at higher concentrations, indicated that ETLE may interfere with the cellular mechanisms that drive motility.[18] Moreover, the decreased migration distance compared with the Doxorubicin group suggested that ETLE could enhance the effectiveness of existing chemotherapy.[13] This dual action (inducing apoptosis and inhibiting cell migration) of ETLE may lead to improved treatment strategies, especially in cases resistant to standard therapies.[19]

The results of this study align with previous research demonstrating the potential of plant-derived compounds to induce apoptosis and inhibit cell migration in cancer cells.[20] The phytochemical coumestrol effectively targets human skin carcinoma cells by inducing mitochondrial-mediated apoptosis, leading to cell cycle arrest and inhibiting migration and invasion through modulation of the m-TOR/-PI3K/AKT signaling pathway.[21] Similarly, the ethanolic extract of Piper nigrum has been shown to inhibit MCF-7 cell migration by reducing the expression of matrix metalloproteinases (MMP) 9 and modulating genes related to MMP 2, VEGFA, and ICAMP1.[22]

Further studies are needed to clarify the specific molecular pathways through which ETLE affects cancer cells, as this may reveal new targets for intervention. Future studies should also evaluate the effects of ETLE in vivo to assess its potential as a systemic treatment for tongue cancer.

Conclusion

ETLE exhibited significant cytotoxic effects on HSC-3 cells by inducing apoptosis and inhibiting cell migration in a concentration-dependent manner. The IC50 value for ETLE in HSC3-cells was 31.87 µg/mL, indicating moderate cytotoxicity.

Acknowledgments

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Conflict of Interest

The authors report no conflict of interest.

References

1. Hayati N, Panjaitan CC, Sandra F. Microbiome in oral squamous cell carcinoma: Mechanisms and signaling pathways. Mol Cell Biomed Sci 2020;4: 52-60.
2. Eain HS, Kawai H, Nakayama M, et al. Double-faced CX3CL1 enhances lymphangiogenesis-dependent metastasis in an aggressive subclone of oral squamous cell carcinoma. JCI Insight 2024;9: e174618.
3. Galvis MM, Santos-Silva AR, Jardim JF, et al. Different patterns of expression of cell cycle control and local invasion-related proteins in oral squamous cell carcinoma affecting young patients. J Oral Pathol Med 2018;47: 32-39.
4. Sandra F, Hayuningtyas RA, Ranggaini D, et al. Elephantopus scaber Linn. leaf extract sensitizes doxorubicin in inducing apoptosis in HSC-3 tongue cancer cells through inhibiting survivin activity at Thr34. Indones Biomed J 2024;16: 372-378.
5. Tafrihani AS, Hasanah NU, Salsabila DU, et al. Cardamom essential oil extract suppress the progression of triple-negative breast cancer 4T1 cell line. Indones Biomed J 2023;15: 150-156.
6. de Souza LS, Tosta CL, Lemos BC, et al. Pharmacological potential of Euphorbia tirucalliL. latex in gastric adenocarcinoma: From an ethnopharmacological perspective into new products. Nat Prod Res 2024: 1-8.
7. de-Oliveira LF, Fuentefria AM, Fda SK, et al. Antifungal activity against Cryptococcus neoformans strains and genotoxicity assessment in human leukocyte cells of Euphorbia tirucalli L. Braz J Microbiol 2015;45: 1349-1355.
8. Sandra F, Hudono KF, Putri AA, et al. Caspase inhibitor diminishes caffeic acid-induced apoptosis in osteosarcoma cells. Indones Biomed J 2017;9: 160-164.
9. Hermawati E, Handini M, Ilmiawan MI, et al. Chlorogenic acid protects cell death in the cerebellum through anti-apoptotic protein Bcl2 in transient global ischemia cases. Mol Cell Biomed Sci 2024;8: 44-50.
10. Hajibabaie F, Abedpoor N, Mohamadynejad P. Types of cell death from a molecular perspective. Biol 2023;12: 1426.
11. Muhammad S, Antonius PA, Oktavian R, et al. Rapidly

growing ovarian granulosa cell tumor following complete debulking for suspected ovarian cancer with histopathology result of benign ovarian cyst. Mol Cell Biomed Sci 2023;7: 162-167.
12. Setiawan KW, Sandra F. Naïve T cells in immunosuppression diseases: Human immunodeficiency virus and cytomegalovirus. Mol Cell Biomed Sci 2018;2: 1-10.
13. Arsianti A. Cytotoxic effect of euphorbia tirucalli extract towards breast MCF-7 and colorectal HT-29 cancer cell lines. Int J Curr Res. 2023;15: 24921-24925.
14. Alves-da-Paz DP, Nagamine MK, Grande MPD, et al. Inhibitory effects of Euphorbia tirucalli lineu (Euphorbiaceae) diluted latex on human and canine melanoma cells. Evid Based Complement Alternat Med 2020;2020: 4093206.
15. Sandra F, Rizal MI, Dhaniar AY, et al. Cosmos caudatus leaf extract triggers apoptosis of HSC-3 cancer cells by decreasing bcl-2 and increasing bax. Indones Biomed J 2024;16: 285-291.
16. Ikawati M, Utomo RY, Hapsari NP, et al. Diosmin enhances the anti-migration activity of curcumin analog PGV-1 on colorectal cancer cells. Indones Biomed J 2024;16: 56-65.
17. Rahmawati DY, Dwifulqi H, Sandra F. Origin, stemness, marker and signaling pathway of oral cancer stem cell. Mol Cell Biomed Sci 2020;4: 100-104.
18. Stuelten CH, Parent CA, Montell DJ. Cell motility in cancer invasion and metastasis: Insights from simple model organisms. Nat Rev Cancer 2018;18: 296-312.
19. Tanumihardja M, Pakki E, Mandey F, et al. New formulation of devitalize paste containing Pulp Out for tooth application. J Dentomaxillofac Sci 2024;9: 156-160.
20. Bukit GAK, Riza A. Comparison of effectiveness of normal saline, aquades and mineral water as an irrigation solution in odontectomy of impacted mandibular third molar in University of Sumatera Utara Hospital. J Dentomaxillofac Sci 2022;7: 121-124.
21. Kuang W, Hu W, Ren H, et al. Plant derived coumestrol phytochemical targets human skin carcinoma cells by inducing mitochondrial-mediated apoptosis, cell cycle arrest, inhibition of cell migration and invasion and modulation of m-TOR/PI3K/AKT signalling pathway. Saudi J Biol Sci 2021;28: 2739-2746.
22. Buranrat B, Boontha S. Effect of Piper nigrum ethanolic extract on human breast cancer cell growth and cell migration. Pharmacogn Mag 2019;15: 538-546.