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Evaluation of cytotoxicity and apoptotic effects of *Catharanthus roseus* on the human lung cancer cell lines CaLu-6

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ABSTRACT

Background: Lung cancer is ranked as the fourth global important cause of cancer related deaths. Despite remarkable advances in drug design, the pharmaceutical industry grapples with significant scientific and financial challenges. While recent strides have been made in the development of chemically synthesized pharmaceutical drugs, nature continues to serve as a primary source of bioactive compounds. The synthesis and assessment of natural herbal remedies have been a focal point of cancer therapy research for many years. The synthesis and evaluation of natural herbal remedies have been a primary research for many years in cancer therapy and the obtained results support the continuous development of novel plant-derived products, mainly because natural herbal remedies have several benefits compared to traditional chemical pharmaceuticals.

Methods: This study aims to evaluate the potential cytotoxicity of ***Catharanthus roseus*** methanolic extract on Human Lung Cancer Cell Lines (CaLu-6). The evaluation was performed using apoptotic and membrane depolarization assays. In addition, the real time gene expression was used for assessing the levels of mRNA.

Results: The results demonstrated the possible and positive anticancerous activity of ***Catharanthus roseus*** methanolic extract compared to the positive control ($p<0.05$).

Conclusions: Our results support the ongoing exploration of novel plant-derived products for cancer therapy, emphasizing the benefits offered by natural herbal remedies over traditional chemical pharmaceuticals. Further research in this direction could lead to the development of more effective and less toxic treatments for lung cancer.

Keywords: apoptosis, lung cancer, mitochondrial membrane potential, real-time PCR

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INTRODUCTION

With an unfavourable prognosis and no recognised treatment at the moment, advanced non-small cell lung carcinoma (NSCLC) is the most common type of lung cancers. Lung cancer alone is responsible for more fatality cases than colon, prostate, and breast cancer put together. Due to the few available therapy options, survival times are frequently short [1]. Furthermore, the lung cancer prognosis is due also to the fact that more than half of patients present metastatic lung cancer at the point of diagnosis [2]. Finally, roughly 85% of newly discovered lung cancer cases are non-small cell lung cancer (NSCLC). The biggest risk factor for the development of lung cancer is considered to be the use of tobacco products. Furthermore, it was shown that exposures to passive smokes raises the risks of developing lung cancer for about 26% [3]. Family history, exposure to asbestos as well as exposure to dangerous substances like heavy metals, radon gas, and polycyclic aromatic hydrocarbons are other risk factors for lung cancer occurence. In the few past decades, non-small cell lung cancer (NSCLC) treatment involved radiation therapy, chemotherapy, surgery and their combinations. High toxicity and lack of selectivity of conventional anticancer medications make the development of alternative treatment a priority. In addition, because of their limited efficacy and patients' overall poor survival, searching for further treatment strategies is of great importance for overcoming such problems [4].

For numerous people, the traditional nutritional and medical treatment with herbs is still a major issue. The majority of anticancer drugs which exhibited high clinical efficiency were taken from plants and microorganisms. Their anticancer impacts depends on various mechanisms, like apoptosis induction, immune system modulation and angiogenesis inhibition [5].

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Catharanthus roseus (*C. roseus*) is among the most often used herbs in Asian countries, boasting more than 120 terpenoid indole alkaloids (TIAs) [6] with strong pharmacological impacts. With a rich history in traditional medicine, *C. roseus* was used for treating different diseases including cancer, diabetes, hypertension, fevers as well as hemostasis [7]. Key active chemicals found in *C. roseus* include vinblastine and vincristine (anticancer), ajmalicine (antihypertensive), and serpentine (sedative), with vinblastine and vincristine frequently combined with other medications to treat malignancies such as lymphomas and leukaemia [8].

In Asia, *Catharanthus roseus* (*C. roseus*) is one of the most frequently used herb. Because it includes more than 120 terpenoid indole alkaloids (TIAs), of which many have potent pharmacological effects, *C. roseus* is of great medicinal interest [6]. In many cultures, the medicinal herb *Catharanthus roseus*, formerly known as Vinca rosea L. (Apocynaceae), has been used to treat diabetes, cancer, hypertension, fever, and hemostasis [7]. Vinblastine and vincristine (anticancer), ajmalicine (antihypertensive), and serpentine (sedative) are some of the key active chemicals that were discovered in *C. roseus* in previous investigations [8]. The plant produces modest amounts of vinblastine and vincristine, currently known to be frequently combined with other medications to treat malignancies such as lymphomas and leukaemia [9,10].

In light of this, the major goal of this study is to determine the primary processes of cytotoxic activity of *C. roseus* and evaluate its cytotoxic and pro-apoptotic impacts.

METHODS

Extraction preparation

The *C. roseus* stems were purchased from the neighbouring market and underwent cleaning before being air-dried for (7-10) days. Subsequently, the dried stems were finely ground into powder and introduced into the Soxhlet device for extraction using methanol, ethanol, chloroform, ethyl acetate as well as hot distilled water. To ensure effective extraction of phytoconstituents, approximately 20g of material was utilised. The extraction process took place with 300 ml of respective solvents at 45°C for 24–30 hrs. Following lyophilization, extracts were reconstituted in (20) mg/ml dimethyl sulfoxide (DMSO).

Cell culture

The L-Glutamine-supplemented Dulbecco's Modified Eagle Medium (DMEM) was employed for the cultivation of Human lung cancer cell lines (CaLu-6). To prevent bacterial growth, 10% heat inactivated FBS (HiMedia), 1% Penicillins (10,000) U/ml and Streptomycins (10,000) mg/ml (HiMedia) were added to the media. All cells were kept at 37°C with 5% CO2. The forementioned prepared media and 96 well plates were used for cellular methods like cell culturing and seeding in addition to plant extractions and fraction dilutions.

Cell culture and sulforhodamine B assay

A DMEM-containing culture flask was kept at 37°C and 5% CO2. The sulforhodamine B (SRB) method was used to determine the cytotoxicity of the extract [10]. Cells were seeded at 7.5x103 cells/mL in 96 well plates (Falcon), and incubated for 24 hr at 37°C and 5% CO2. After that, a quantity of DMEM medium (100µL) was replaced with the same amount of the extract and Camptothecin (Cat No: 7689-03-4) at various concentrations (100, 75, 50, 30, 20, 10, 5, 2.5, 1.0, and 0.5µg/ml). Further, the plates were incubated for 48h at 37°C. After incubation, following the addition of 50µL of 50% cold trichloroacetic acid, both treated & non treated cells were fixed at 40°C for 1 hr. The plates were air-dried following cleaning with DW and coloring with 0.2% SRB for 20-30 mins at RT. Then, 1% acetic acid was used to wash the plates for the removal of the background color. Each plate well contained 200 µl of 10 mM Tris-base after air drying. The plates were shaken for 20 minutes in the photometer of ELISA. By applying the wave length of λ=492nm and a reference wavelength of λ=620nm, the absorbances were measured. The following equation was used to determine the effect on cell growth:

(GI)% = 1 - [OD (492 - 620) samples / OD (492 - 620) blanks]) x 100

where GI% = % Inhibition in growth; OD = optical density. IC50 values were determined by linear regression analyses.

Apoptosis by flow cytometry

CaLu-6 cells were grown in DMEM with1% streptomycin & 10% FCS. After 24 hrs following cell seeding (2x106 cells per well), various concentrations of methanolic extract (0.29, 29 and 290µg/ml) were applied. The positive control was rifampicin, and the negative control was DMSO. After 48 hours of incubation, cells were extracted and used to determine apoptotic activity quantity by the use of the kit of Annexin apoptosis from (Genetic Bioscience Co.). The fraction of apoptotic cells was determined using the Annexin V-FITC/PI assay [11].

Mitochondrial membrane potential (MMP) assay

The suitable fluorescent probe with flow cytometry technique was used to detect the 1%m shifts in mitochondrial trans-membrane potential generated by the active phytochemical components on CaLu-6 [12, 13].

The cells of CaLu-6 are seeded at 1x106/ml density and cultured in a 6-well plate for 48 hours. After incubation, methanolic extract and carbonyl cyanide chlorophenylhydrazone CCCP (positive control) was added to the mixture, followed by the incubation of the mixture for further 6 hrs at 37°C with 5% CO2. Then, 2mM JC-1 dye was applied for 15 min. at 37°C for the labeling of the treated cells, then the cells were rinsed with the warmed phosphate buffered saline (PBS). The flow cytometer outfitted with 488nm, 530nm and 585nm emission filters was used to evaluate the cells. The 10mM CCCP was used to treat the positive control included cells.

DNA fragmentation assay

For the purpose of DNA fragmentation studying, the cells of CaLu-6 were treated by active methanolic extract with positive control at IC50 to trigger apoptosis. According to the guidelines of the manufacturer, the HiMedia DNA purification kit was used to extract the DNA. After quantification, 2µgs of DNA was loaded on the 0.8% agarose gel to perform electrophoresis. The ethidium bromide (10g/mL) was used to stain the gel and the ultraviolet light was used to photograph it [14].

RNA extraction

CaLu-6 cells (5x105 cells/well) were incubated for 96 hours with the plant extract and 1% DMSO (negative control). These cells were then processed to extract the RNA. For extraction purposes, chloroform extracts were chosen because they displayed the greatest reduction rates. The RNeasy Mini (Qiagen 74104) kit was used for RNA isolation from confluent cells (treated & untreated) [15]. The examination was conducted under the instructions of the manual. The cells were trypsinized, centrifuged at 6000 rpm for 5 min and dissolved in 560µL of AVL buffer and 560µL of cold ethanols. Then, this mixture was centrifuged at 8000 rpm for about one min. After cleaning the columns with 700µl of washing buffer, it was incubated with AVE buffer. After elution, the RNA was kept at 20 degrees Celsius. The RNA was used for cDNA (260/280) synthesis, following qualitative UV spectrophotometer measurement.

cDNA synthesis

cDNA was synthesized following the guidelines of the manufacturer, while the SuperScript II Reverse Transcriptase enzyme was used. Random primers were used for reverse transcription and the resulting cDNA was used for subsequent real-time PCR.

Real-time PCR

Sigma-Aldrich supplied primers built with Primer3 software for use in the real-time test (Table 1). Malla Sudhakar et al. (2020) [16]. HiMedia's iQTM SYBR Green was used for real-time amplification. The reaction took 1µl of RT products and 600nM primers, totalling 12.5µl. Each reaction, as well as the negative controls, was repeated twice.

The samples were subjected to qPCR on the Corbett Research cycler (both control and treatment). For the amplification experiment, the following factors were used: 600nM vascular endothelial growth factors A (VEGFA) (forward: 5' TGCTCTACTTCCCCAAATCACT 3' with reverse: 5' CTCTCTGACCCCGTCTCTCT 3') as well as Arachidonate 5-Lipoxygenase (ALOX5) (forward: 5' AAGCGATGGAGAACCTGTTCA 3' with reverse: 5' GTCTTCCTGCCAGTGATTCATG 3'). The program was performed for about 35 cycles at 94°C for 60s, 65°C for 45s, and 72°C for 50s, utilizing 1.31µl of RNA products. To compare the expression of mRNA, reference genes glyceraldehyde-3-phosphate dehydrogenases (GAPDH) (forward: 5' GCTGAGTACGTCGTGGAGTC 3' with reverse: 5' CCCATTCCCCAGCTCTCATA 3'; 455bp) were amplified along with gene members. In the comparative assay, A Ct method was employed. The comparison analysis employed the ΔΔCt method, with Ct values normalized to the housekeeping gene (GAPDH).

Statistical analysis

The mean standard deviation method was used to calculate the study values, with triplicate performance of each test. The SPSS Software is used to do the statistical analysis. $p<0.05$ was used to analyse significant differences between the treatment and control groups.

Table 1. Primers used for gene expression studies by qPCR

Gene		Template strand	Length	Tm	GC %	Product length
VEGFA	FW	TGCTCTACTTCCCCAAATCACT	21	56.4	55	138
	RV	CTCTCTGACCCCGTCTCTCT	20	58.6	50	
ALOX5	FW	AAGCGATGGAGAACCTGTTCA	21	56.8	55	350
	RV	GTCTTCCTGCCAGTGATTCATG	20	56.8	50	
GAPDH	FW	GCTGAGTACGTCGTGGAGTC	20	60.18	60	455
	RV	CCCATTCCCCAGCTCTCATA	20	58.56	55	

Abbreviations: FW- forward primer, RV- reverse primer, Tm- melting temperature.

RESULTS

Cell culture and sulforhodamine B assay

The *C. roseus* extracts were assessed for their cytotoxic effects on CaLu-6 cancer cell lines using the SRB test [17, 18]. Figure 1 presents the cell-inhibitory activity of ethanol, methanol, chloroform, ethyl acetate, and aqueous *C. roseus* extracts. At 100µg/ml concentration, the aqueous and ethyl acetate extracts demonstrated low activity (30%) compared to the methanolic extract, which exhibited high activity against CaLu-6 cell lines. A comparable efficacy was shown in both ethanol extracts 68(±0.61) and chloroform extracts 65(±0.85), but at 100µg/ml were not significantly remarkable in comparison with the methanolic extracts and positive controls (92 ±0.11). A dose-dependent response was observed ($p<0.05$). The cancer cells were reduced by the methanolic extracts by 50% at (35.24±0.23) µg/mL concentration, with a significantly higher difference than the positive control 15.31(±1.83)µg/mL.

Apoptosis by flowcytometry

Figure 2 shows a substantial increase ($p<0.05$) in CaLu-6 cell apoptosis following treatment with methanolic extract (20%) of *C. roseus* against the control cell (7%), a

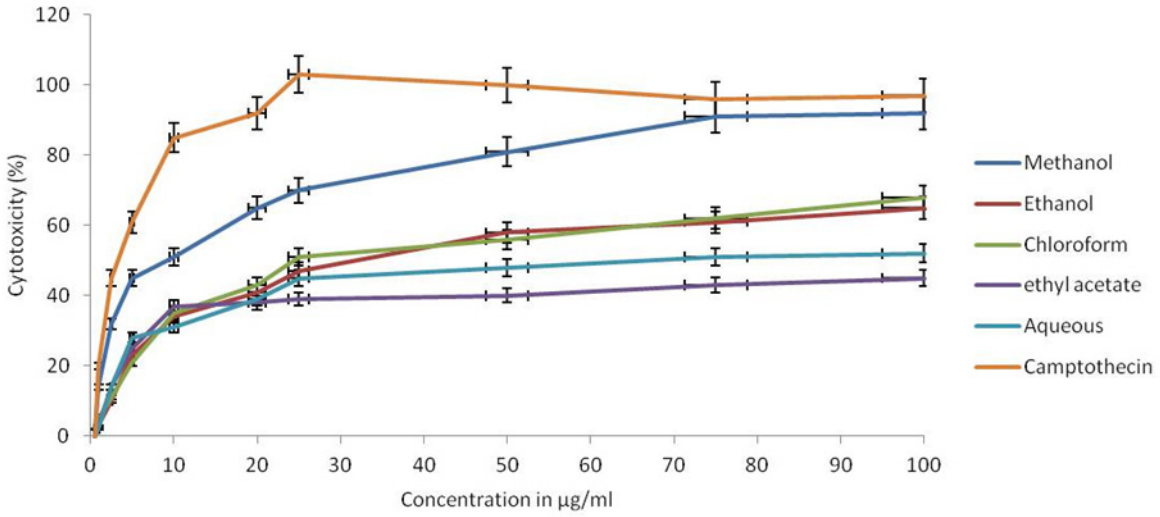


Figure 1. Cytotoxicity of the *C. roseus* extracts on CaLu-6 cells at various concentrations. All the values are average of triplicates and expressed as value ±SD. ($p<0.05$). Camptothecin was used as positive control.

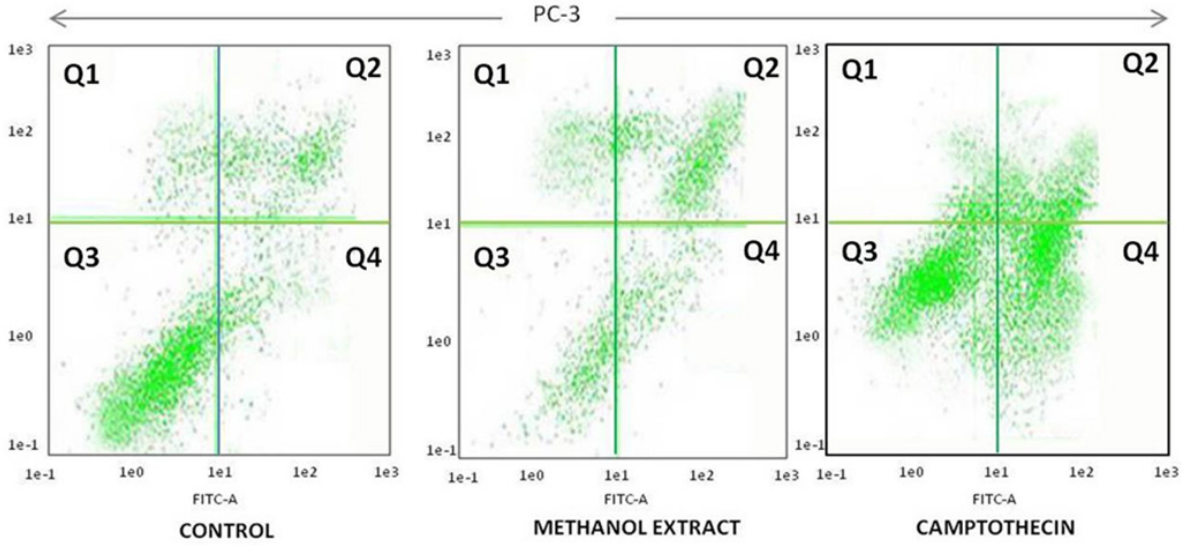


Figure 2. Dot plots of CaLu-6 after 6h of treatment with the *C. roseus* methanolic extract. The cells were treated with various concentrations of methanolic extracts for 6h ($p < 0.05$) and compared with the control cells. Only results of 35µg/ml were presented. All the values are average of three independent experiments. Q1: Quadrant represents unviable cells; Q2: cells in either late apoptosis or necrosis; Q3: viable cells: Q4: cells in early apoptosis/cell apoptosis.

result which was significantly different from the positive controls (25%). The viable cells were reduced in about 55% and 48% for the Camptothecin and methanolic extract respectively ($p<0.05$). Figure 3 illustrates the distribution of CaLu-6 cell following 6 hours treatment with the methanolic extract of ***C. roseus***.

Membrane depolarization on CaLu-6 lines

The drug-induced apoptosis includes mitochondrial alterations, such as oscillation in the mitochondrial membrane potentials (MMP) (1%m) [19]. Treatment with the ***C. roseus*** methanolic extract significantly increased the percentage of MMP depolarization in CaLu-6 cell lines. This suggests that mitochondrial membrane depolarization may be involved in CaLu-6 cellular death induced by the active extracts of the plant. The quantification percentage of methanol and positive controls were 11.59 and 17.89 respectively, in comparison with the control group 4.31 at ($p<0.05$), as shown in figure 4.

DNA fragmentation assay

The DNA fragmentation assay showed increased DNA shearing in CaLu-6 treated cells after 72 hrs of treatment with the ***C. roseus*** methanolic extract, which is match-

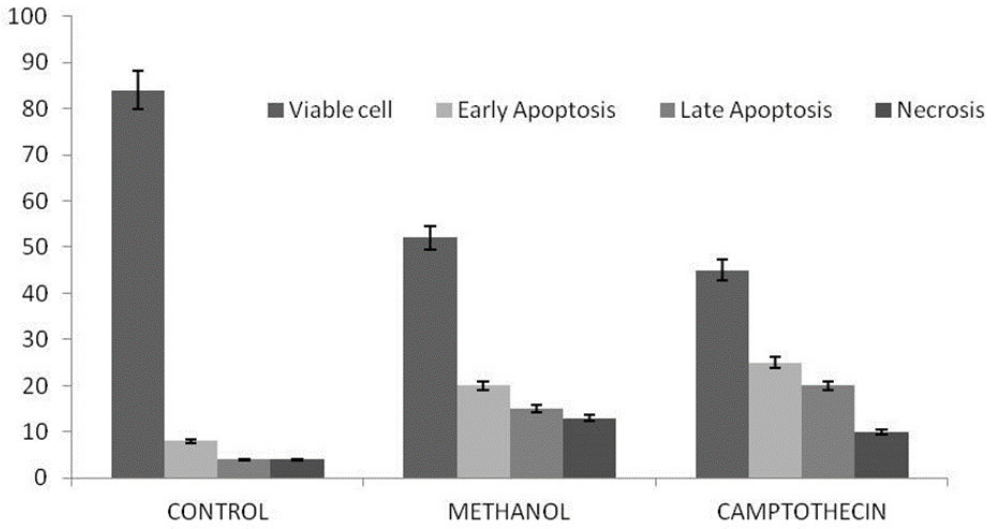


Figure 3. Percentage of cell distribution of CaLu-6 after 6h of treatment with *C. roseus* methanolic extracts. The cells were incubated with various concentrations of methanolic extracts for 6h ($p < 0.05$) and compared with the control cells. Only results of 35µg/ml were presented. All the values are average of three independent experiments.

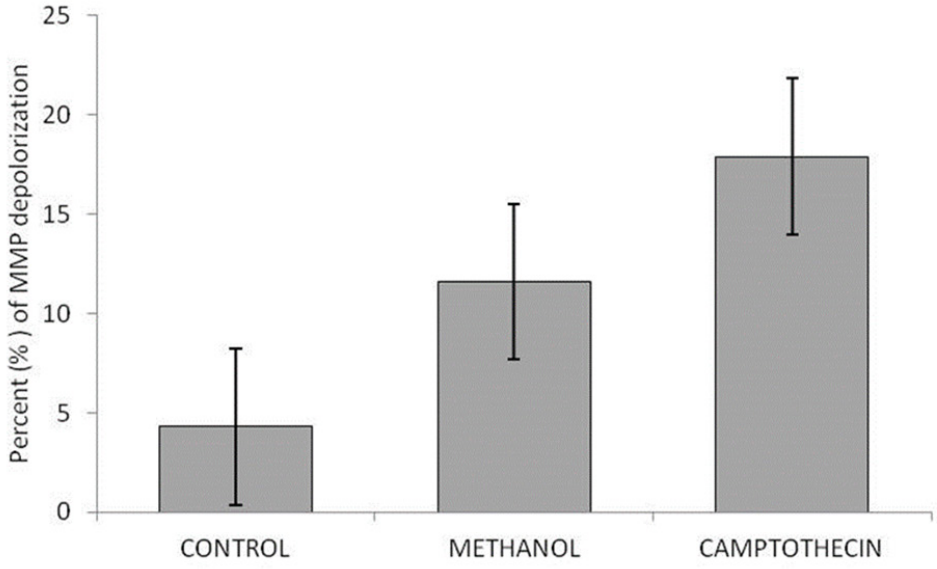


Figure 4. Percentage of depolarization induced by the *C. roseus* methanolic extracts treatment on CaLu-6 prostate cancer cell lines. CaLu-6 cell lines were incubated for 6h with the IC50 of methanolic extracts. Data represents means ± SE (n = 3) $p < 0.05$.

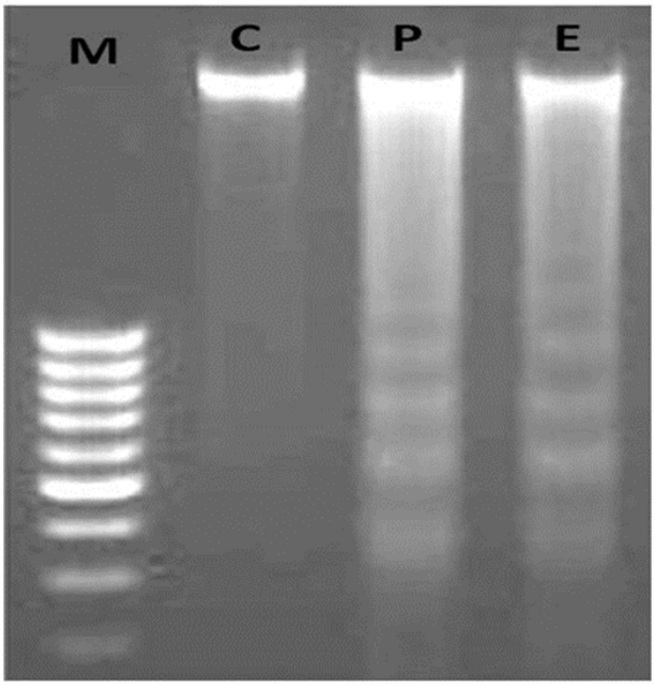


Figure 5. The defragmentation assay of the methanolic active extract on 0.8% agarose gel. C: control, P: positive control; E: C. roseus methanolic extract, M: Molecular marker (1000bp).

ing with the positive controls ($p<0.05$), as illustrated in figure 5. These results suggest the potent anticancer properties of *C. roseus* methanolic extracts, leading to the death of Human lung cancer cell line CaLu-6.

Gene expression

The methanolic extract significantly inhibited gene member mRNA expression by fivefold. Therefore, providing primary data may improve the credibility of these results. Figure 6 demonstrates that the methanolic extract downregulated the mRNA of both VEGF & ALOX5. The expression of VEGFA was decreased fivefold when compared with the positive control. Likewise, the expression of ALOX5 gene was four-fold less than the positive control. Figure 6 illustrates the considerable alteration of the expression level of treatment with methanolic extract treatment following 6 hr. incubation in comparison with the positive controls. Following 12 hr. treatment, the methanolic extract *C. roseus* revealed a lower activity in comparison with the positive controls ($p < 0.05$). Additional explanation on the molecular and cellular mechanisms should be involved, and the study limitations must focus on providng more comprehensive understanding of the study findings.

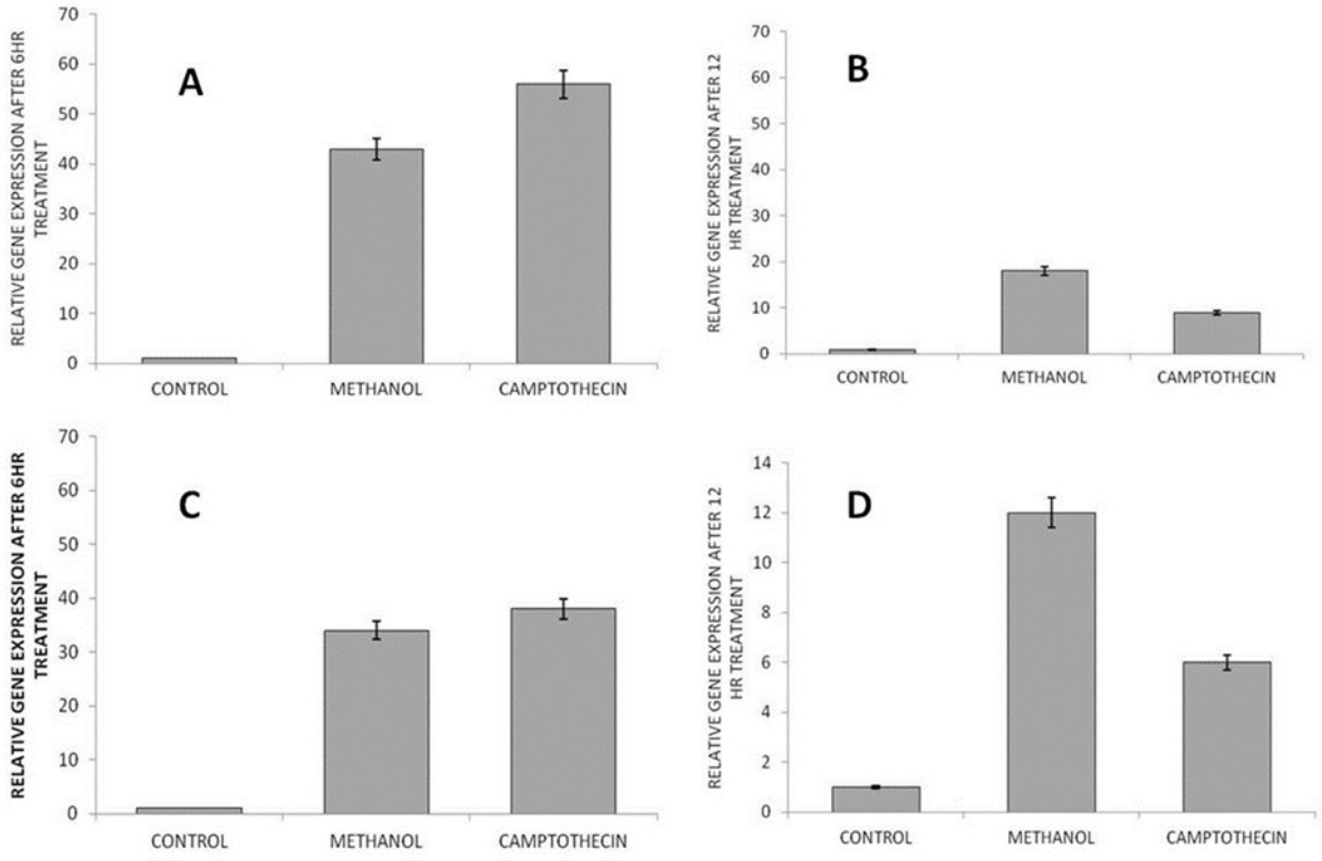


Figure 6. The in vitro biological activity of plant extracts through relative fold change expression. All data are means of three independent replicates ± SD (n = 3). ($p < 0.05$). A: VEGFA expression at 6h; B: VEGFA expression after 12h; C: ALOX5 expression at 6h; D: ALOX5 expression after 12h.

DISCUSSION

The current study investigated the antitumor impacts of *C. roseus* methanolic extract via cytotoxicity and apoptotic tests. The results showed a highly significant cell growth inhibition ($p<0.05$) when the methanolic extract was used as a treatment. The sulforhodamine B (SRB) method was used for assessing the in vitro chemosensitivity, that showed higher reproducibility and accuracy in comparison with the MTT method, even with a higher linearity. Such pre-clinical examinations are essential to assess the harmony of substances, particularly in the context of medical devices and products utilized in dentistry [20].

The methanolic extracts demonstrated significantly higher cytotoxic activities than positive controls in this research. These results were similar to a former study by Patel & Patel (2011) who used MTT with SRB techniques for assessing the cytotoxicity of Artocarpus heterophyllus methanolic extract. Similarly, (Patel et al., 2009) applied both methods for investigating Solanum nigrum methanolic extract cytotoxicity [21]. Such comparisons highlighted the robustness of the results in the context of the present literature.

Elaeagnus angustifolia (EA) is usually used in the Middle East as a medical plant for curing different human diseases [22]. In some studies, EA flower extract was shown to suppress the invasion and proliferation of HER2-positive oral and breast cancer cells. Moreover, early and late apoptosis are stimulated by EA extracts in a dose-dependent way through two triple-negative breast cancer cell lines [23].

Using Dorema Glabrum seed extracts as a treatment was also shown to be a generator of internucleosomal chromosomal DNA fragments, suggesting apoptosis [24]. A powerful role of *C.roseus* extract used to treat cancer cell lines was indicated by former studies due to its antiproliferation and anti-migration activities [25]. Consistent with these results, our study detected an increased apoptotic rate in malignant cell lines, which confirmed the consistency of our results with earlier researches.

For better acievements in the future, it is of importance to determine and discuss the limitations of the study. To assure the robustness and generalization of the current results, it is necessary to address every methodological constraint or confounding factor.

CONCLUSION

The present study gave an initial insight into the possible anticancer activitiy of *C. roseus* methanolic extract, thus, it is important to emphasize the necessity of carrying out further research for decisively supporting the current results. The validity of the present findings is significantly limited by the lack of comprehensive understanding of the involved individual chemical elements, in addition to a clarification of their exact molecular mechanisms of action.

For filling such a crucial information gap, future studies must focu on the identification and characterization of the phytoconstituents that exist in *C. roseus* which can have an effect on cancerous cells. In addition, entire examinations of the intricate molecular mechanism, especially targeted by such phytoconstituents within cancer cells are needed to confirm the effectiveness of the treatment with *C. roseus* as a feasible candidate for cancer therapeutic methods.

It can be concluded from the current study that it yielded promising primary results, and concentrated on the significance of rigorous scientific examinations and complete investigations to prove the possible therapeutic function of *C. roseus* in tumor management. A well-defined study postulate, along with thorough analysis and discussion of the present results, can serve as the basis to further understanding and clinical utilization of *C. roseus* in the field of cancer treatment.

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AUTHORS' CONTRIBUTION

OSS- analysis, data visualization and data management, writing the original draft

ASS- study conception and design, manuscript preparation

All authors have read the complete manuscript and take responsibility for the content of the manuscript.

CONFLICT OF INTEREST

None to declare.

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REFERENCES

1. Abizov EA, Tolkachev ON, Mal'tsev SD, Abizova EV. Composition of biologically active substances isolated from the fruits of Russian olive (Elaeagnus angustifolia) introduced in the European part of Russia. Pharm Chem J. (2008) 42:696-8. DOI: 10.1007/s11094-009-0203-5
2. Aggarwal BB, Ichikawa H, Garodia P, Weerasinghe P, Sethi G, Bhatt ID, et al. From traditional Ayurvedic medicine to modern medicine: identification of therapeutic targets for suppression of inflammation and cancer. Expert Opin Ther Targets. 2006 Feb;10(1):87-118. DOI: 10.1517/14728222.10.1.87
3. Bannazadeh Amirkhiz M, Rashtchizadeh N, Nazemieh H, Abdolalizadeh J, Mohammadnejad L, Baradaran B. Cytotoxic effects of alcoholic extract of dorema glabrum seed on cancerous cells viability. Adv Pharm Bull. 2013;3(2):403-8. DOI: 10.1155/2013/949871
4. Chaudhry GE, Md Akim A, Sung YY, Sifzizul TMT. Cancer and apoptosis: The apoptotic activity of plant and marine natural products and their potential as targeted cancer therapeutics. Front Pharmacol. 2022 Aug 10;13:842376. DOI: 10.3389/fphar.2022.842376
5. Chen R, Manochakian R, James L, Azzouqa AG, Shi H, Zhang Y, et al. Emerging therapeutic agents for advanced non-small cell lung cancer. J Hematol Oncol. 2020 May 24;13(1):58. DOI: 10.1186/s13045-020-00881-7
6. Couraud S, Zalcman G, Milleron B, Morin F, Souquet PJ. Lung cancer in never smokers--a review. Eur J Cancer. 2012 Jun;48(9):1299-311. DOI: 10.1016/j.ejca.2012.03.007
7. Don G. Catharanthus roseus. In: Ross IA, editor. Medicinal plants of the world: Chemical constituents, traditional and modern uses, vol 1. Totowa, New Jersey, USA: Humana Press; 2003. pp. 175-196.
8. Fouzat A, Hussein OJ, Gupta I, Al-Farsi HF, Khalil A, Al Moustafa AE. Elaeagnus angustifolia Plant Extract Induces Apoptosis via P53 and Signal Transducer and Activator of Transcription 3 Signaling Pathways in Triple-Negative Breast Cancer Cells. Front Nutr. 2022 Mar 18;9:871667. DOI: 10.3389/fnut.2022.871667
9. Hao J, Zheng L, Han Y, Zhang H, Hou K, Liang X, et al. Genome-wide identification and expression analysis of TCP family genes in Catharanthus roseus. Front Plant Sci. 2023 Apr 12;14:1161534. DOI: 10.3389/fpls.2023.1161534
10. Isenberg JS, Klaunig JE. Role of the mitochondrial membrane permeability transition (MPT) in rotenone-induced apoptosis in liver cells. Toxicol Sci. 2000 Feb;53(2):340-51. DOI: 10.1093/toxsci/53.2.340
11. Kheraldine H, Gupta I, Alhussain H, Jabeen A, Cyprian FS, Akhtar S, et al. Substantial cell apoptosis provoked by naked PAMAM dendrimers in HER2-positive human breast cancer via JNK and ERK1/ERK2 signalling pathways. Comput Struct Biotechnol J. 2021 May 7;19:2881-90. DOI: 10.1016/j.csbj.2021.05.011
12. Sudhakar M, Venkata Raman B. Bactericidal and Anti-biofilm Activity of Tannin Fractions Derived from Azadirachta against Streptococcus mutans. Asian J Appl Sci. 2020;13:132-43. DOI: 10.3923/ajaps.2020.132.143
13. Orellana EA, Kasinski AL. Sulforhodamine B (SRB) Assay in Cell Culture to Investigate Cell Proliferation. Bio Protoc. 2016 Nov 5;6(21):e1984. DOI: 10.21769/BioProtoc.1984
14. Patel RM, Patel SK. Cytotoxic activity of methanolic extract of Artocarpus heterophyllus against A549, Hela and MCF-7 cell lines. J App Pharm Sci. 2011;1:167-71.
15. Patel S, Gheewala N, Suthar A, Shah A. In-vitro cytotoxicity activity of Solanum nigrum extract against Hela cell line and Vero cell line. Int J Pharm Pharm Sci. 2009;1:38-46.
16. Pham HNT, Vuong QV, Bowyer MC, Scarlett CJ. Phytochemicals Derived from Catharanthus roseus and Their Health Benefits. Technologies. 2020;8(4):80. DOI: 10.3390/technologies8040080
17. Rajashekara S, Reena D, Mainavi MV, Sandhya LS, Baro U. Biological isolation and characterization of Catharanthus roseus (L.) G. Don methanolic leaves extracts and their assessment for antimicrobial, cytotoxic, and apoptotic activities. BMC Complement Med Ther. 2022 Dec 9;22(1):328. DOI: 10.1186/s12906-022-03810-y
18. Salam A, Sudhakar M. Cytotoxicity and expression studies of angiogenesis-promoting genes in cancer cell lines under the treatment of cancer candidate drugs, Asian J Pharm Clin Res2019;12(5):130-34. DOI: 10.22159/ajpcr.2019.v12i5.31929
19. Saleh AI, Mohamed I, Mohamed AA, Abdelkader M, Yalcin HC, Aboulkassim T, et al. Elaeagnus angustifolia Plant Extract Inhibits Angiogenesis and Downgrades Cell Invasion of Human Oral Cancer Cells via Erk1/Erk2 Inactivation. Nutr Cancer. 2018 Feb-Mar;70(2):297-305. DOI: 10.1080/01635581.2018.1412472
20. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020 Jan;70(1):7-30. DOI: 10.3322/caac.21590
21. Škubník J, Pavlíčková VS, Ruml T, Rimpelová S. Vincristine in Combination Therapy of Cancer: Emerging Trends in Clinics. Biology (Basel). 2021 Aug 31;10(9):849. DOI: 10.3390/biology10090849
22. Sottomayor M, Ros Barceló A. The Vinca alkaloids: From biosynthesis and accumulation in plant cells, to uptake, activity and metabolism in animal cells. In: Rahman A, editor. Studies in natural products chemistry (bioactive natural products) The Netherlands: Elsevier Science Publishers; 2005. pp. 813-857. DOI: 10.1016/S1572-5995(06)80041-4
23. Vajrabhaya Lo, Korsuwannawong S. Cytotoxicity evaluation of a Thai herb using tetrazolium (MTT) and sulforhodamine B (SRB) assays. J Anal Sci Technol. 2018;9:15. DOI: 10.1186/s40543-018-0146-0
24. Van der Heijden R, Jacobs DI, Snoeijer W, Hallard D, Verpoorte R. The Catharanthus alkaloids: Pharmacognosy and biotechnology. Current Medicinal Chemistry. 2004;11(5):607-28. DOI: 10.2174/0929867043455846
25. Zamzami N, Marchetti P, Castedo M, Zanin C, Vayssière JL, Petit PX, et al. Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death in vivo. J Exp Med. 1995 May 1;181(5):1661-72. DOI: 10.1084/jem.181.5.1661