

Integrative Phytochemical and Cytotoxic Dissection of *Desmanthus Virgatus* Against Human Lung Adenocarcinoma

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ABSTRACT

The anticancer activity of the ethanolic extract of *Desmanthus virgatus* was explored against the human lung cancer cell line A549 by phytochemical, insilico, and in vitro methods. The presence of phenolics, flavonoids, tannins, saponins, and alkaloids was confirmed by preliminary phytochemical screening. The GC-MS analysis revealed the presence of major bioactive compounds such as dl- α -tocopherol and ergosta-4,6,22-trien-3-ol. Molecular docking analysis showed strong binding affinity with a cancer-related protein target, which supported the anti proliferative activity. The MTT assay showed dose-dependent cytotoxicity with an IC₅₀ value of 42.79 μ g/mL. Morphological alterations in treated cells confirmed apoptosis. The results suggest that *D.virgatus* is a potential natural source for anticancer compounds.

Keywords: A549 cell line; Anticancer activity; GC-MS analysis; Molecular docking.

INTRODUCTION

Cancer is a life-altering category of more than 100 diseases in which genetic mutations result in the uncontrolled growth of abnormal cells, as opposed to normal cell division and apoptosis, which form tumors that invade surrounding tissues and metastasize through blood or lymph, originating from anywhere in the trillions of cells in the body. Cells normally grow in an orderly fashion and die when they are damaged, but in cancer, there is an abnormality in which malignant (cancerous) tumors develop and spread, as opposed to benign tumors that remain localized, do not regrow after being removed, and still cause severe problems such as brain tumors, while blood cancer, such as leukemia's, develop no solid tumors.⁽¹⁾⁽²⁾

Lung cancer is a significant global health issue as it is both very common and very fatal, with around 2.2 million new cases and 1.8 million deaths recorded worldwide in 2020, accounting for about 11-12% of

all cancers and about 18% of all cancer deaths, and remains the leading cause of cancer deaths for both men and women in many regions of the world. ⁽³⁾ Lung cancer is described as a malignant process originating from cells in the lung tissue, most often from the epithelial cells lining the airways or alveoli, and is limited to primary bronchopulmonary malignancies and not to malignancies metastasizing to the lungs from other sites. Biologically, it is described as the uncontrolled growth and division of abnormal cells that develop genetic and epigenetic mutations, proliferate to form tumors, impair normal lung functions, invade adjacent tissues, spread through lymphatic and vascular routes, and metastasize to other distant sites such as lymph nodes, brain, bone, liver, and adrenal glands. The paramount significance in public health terms is not only due to its high disease burden but also because a large proportion of cases are preventable, as active smoking of tobacco is the main cause and accounts for 80-90% of deaths due to lung cancer in most populations, with the risk rising

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

with the duration, intensity, and pattern of smoking, even years after quitting. ⁽⁴⁾ Besides active smoking, second-hand smoking is another important cause of lung cancer in never smokers, while other important modifiable risk factors include exposure to radon gas, a naturally occurring radioactive gas that can accumulate in buildings, occupational carcinogens such as asbestos, arsenic, chromium, nickel, silica, diesel exhaust, and certain industrial chemicals (such as Asbestos, Hexavalent chromium (Cr VI)), air pollution, and previous chest radiation. Non-modifiable risk factors like genetic predisposition, inherited or acquired driver mutations, family history of lung cancer, and existing lung diseases like chronic obstructive pulmonary disease, pulmonary fibrosis, and recurrent pneumonia also make the individual more susceptible to the disease by providing a constantly inflamed and genetically unstable microenvironment in the lungs. ⁽⁵⁾ All these factors contribute to the development of chronic inflammation, cumulative DNA damage, genomic instability, activation of oncogenes, inactivation of tumor suppressor genes, and disruption of crucial signaling pathways that regulate cell proliferation, apoptosis, and repair, ultimately leading to malignant transformation. Clinically, lung cancer can be insidious in its early presentation and thus presents late in its diagnosis because, in its early stages, there may be no symptoms or only vague, non-specific ones such as a cough or fatigue; but when it does present, common symptoms include a progressive cough, hemoptysis or rust-colored sputum, pleuritic chest pain precipitated by coughing or deep breathing, shortness of breath, wheezing, hoarseness, unexplained weight loss, anorexia, fatigue, recurrent or persistent respiratory infections such as bronchitis or pneumonia, and, in advanced disease, symptoms of metastatic disease such as bone pain or neurological dysfunction, as well as those due to paraneoplastic syndromes (PNS). In terms of pathological and clinical classification, lung cancer can be generally categorized into two major histological types according to microscopic characteristics: small cell lung cancer, which consists of undifferentiated neuroendocrine-type cells that grow aggressively and metastasize early, and non-small cell lung cancer, which constitutes 80-90% of all cases and encompasses adenocarcinoma, squamous cell carcinoma, and large cell carcinoma that vary in terms

of cell of origin, location, and growth characteristics. Taken together, the prevalence, strong association with modifiable risk factors, late stage at presentation, aggressive biological behavior, and high mortality rate clearly indicate that lung cancer is one of the most pressing problems in contemporary clinical practice. ⁽⁶⁾

Owing to the numerous challenges faced in the treatment of cancer, scientists have shifted their focus towards natural treatments, as they hope to reduce the side effects. Although the plant-based products have shown promising results in the treatment of cancer, their use in human patients is still awaited (Desai et al., 2008). Since ancient times, alternative treatments for diseases have been provided by indigenous plants. Recent research has revealed that approximately 65% of anticancer medications have been developed from natural compounds that were derived from plants (Mbele et al., 2017).

Desmanthus virgatus (L.) Wild. (Figure:01) is a perennial legume of the family Fabaceae (Leguminosae) and order Fabales, also known as wild tanton or prostrate bundle flower, and is widely distributed in tropical and subtropical areas. ⁽⁷⁾ Traditionally, *Desmanthus* species and other species of the Fabaceae family have been used as protein-rich forage and in folk medicine as general tonics and for the treatment of various ailments such as fever, constipation, muscular pain, and wound healing. ⁽⁸⁾ Recently, *D. virgatus* has gained considerable attention from the scientific community because of its extensive pharmacological properties, especially from leaf extracts, which have shown strong α -glucosidase inhibitory activity with IC_{50} values as low as 7.14 μ g/mL, thus justifying its use in the treatment of diabetes. ⁽⁹⁾ Phytochemical analysis using LC-MS has revealed an extensive secondary metabolite composition, including more than 25 major flavonoids such as luteolin-7-glucoside, quercetin, and other phenolic compounds, which are responsible for its antifungal ⁽¹⁰⁾, antiviral ⁽¹¹⁾ and it has flavonoids that may presence of antibacterial properties. ⁽¹²⁾ Although the direct evidence of anticancer properties of *D. virgatus* is not yet abundant, the significance of *D. virgatus* in oncology can be inferred from the presence of bioactive flavonoids, particularly luteolin derivatives, which



have been extensively reported in the literature for their ability to induce apoptosis,⁽¹³⁾ cell cycle arrest, proliferation inhibition, angiogenesis, and metastasis in various cancer cell lines, including lung, liver, breast, cervical, and colorectal cancers, through the

modulation of key signaling pathways like PI3K/Akt, NF- κ B, and p53. Thus, the high flavonoid content and established bioactivity of *D. virgatus* make it a promising candidate for future anti-proliferative and chemopreventive research.



Figure:01 (Flowers of *Desmanthus virgatus*(L.) Wild.)

MATERIALS AND METHODS:

COLLECTION OF PLANT MATERIAL:

The flowers of *D. virgatus* were collected from a rural area of Salem, Tamil Nadu, India. The same sample of powdered flowers was used for the identification of phytochemical constituents and the in vitro study of the anti-proliferative activity of *D. virgatus*.

PHYTOCHEMICAL EXTRACTION FROM DESMANTHUS VIRGATUS:

The inflorescences of *D. virgatus* were carefully collected and processed using shade desiccation for 3

days, followed by pulverization to a fine granulometry and exhaustive phytochemical extraction using sequential maceration with a total of 650 mL of absolute ethanol. The maceration process took place over a 5-day incubation period with periodic agitation to ensure optimal solute distribution; the combined ethanolic percolates were then concentrated under reduced pressure using rotary evaporation, resulting in a viscous mass that was hermetically sealed and stored at 4-8°C to prevent oxidative breakdown and microbial growth.⁽¹⁴⁾(Figure:02)



Figure:02 (Summary of the procedure developed to obtain the ethanolic extract of the plant)

PHYTOCHEMICAL ANALYSIS OF DESMANTHUS VIRGATUS CRUDE EXTRACTS:

Phytoconstituents analysis of the crude plant extracts was carried out according to standard procedures for phenolic compounds, flavonoids, saponins, tannins, and alkaloids. ⁽¹⁵⁾

GC-MS ANALYSIS OF DESMANTHUS VIRGATUS:

The crude extracts of *D. virgates* were subjected to analysis using GCMS QP2010SE to determine the functional groups present in the crude extracts. The column temperature was maintained at 50°C, the injection temperature at 250°C, the ion source temperature at 200°C, and the interface temperature at 250°C. The pressure was held constant at 68.1 kPa, the total flow volume at 16.2 ml/min, and the solvent cut time was set to 3.50 min. The sample, prepared in ethanol with a 1:10 split ratio, was injected at a volume of 1 µl. Based on the mass spectrum obtained, a NIST library search was performed to determine the phyto compounds present in the crude extracts.

IN SILICO STUDY WITH PROTEIN

Data generated from the GC-MS analysis were identified using the PubChem web database to determine the compound's name and chemical structure. Preparation was done using Biovia Discovery Studio to obtain a sterile protein. Furthermore, Autodock Vina was used to carry out a molecular docking test and determine the binding affinity value. After molecular docking, the compound was interacted with and visualized using Biovia Discovery Studio to determine the position and type of interaction formed. Then, each of these compounds was tested for Lipinski Drug likeness and pharmacokinetics to determine its potential as a bioactive compound with Lipinski's five rules through the website <https://www.swissadme.ch/>. Bio computational molecular docking analysis was used to assess the anti-cancer activity of compounds that fulfilled the Drug likeness and pharmacokinetic tests. The receptor used in silico study was anti-proliferative with PDB (Protein Data Bank) code 4foc. ⁽¹⁶⁾

CELL LINE AND CELL CULTURE

A549 cells were procured from NCCS. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO₂ at 37 °C until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells is checked and centrifuged. Further, 50,000 cells /well were seeded in a 96-well plate and incubated for 24 hrs at 37 °C, 5 % CO₂ incubator. ⁽¹⁷⁾

EVALUATION OF CELL CYTOTOXICITY OF DESMANTHUS VIRGATUSEXTRACTS

The cells were treated with trypsin, and the cell concentration was adjusted to 5×10^5 cells/mL using the appropriate growth medium containing 10% Fetal Bovine Serum. Then, 100 µL of this suspension (about 50,000 cells per well) was added to each well of a 96-well plate. After 24 hours, the liquid above the cells was removed, the cell layer was washed once with fresh medium, and 100 µL of different concentrations of the test drugs was added to the wells. The plates were incubated at 37°C in a 5% CO₂ atmosphere for another 24 hours. After this incubation, the drug solutions were discarded, and 0.05 mg of MTT was added to each well, followed by incubation for 4 hours under the same conditions. The liquid was then removed, and 100 µL of DMSO was added to dissolve the purple formazan crystals formed by living cells, with gentle shaking to help the process. The absorbance was measured at 570 nm using a microplate reader. The percentage of growth inhibition was calculated using the standard formula, and the IC₅₀ value (the concentration of drug required to inhibit 50% of cell growth) was obtained from the dose-response curve for each cell line. ⁽¹⁸⁾

RESULTS AND DISCUSSION:

Phytochemical screening of crude extracts of *D. virgates* syndicated the presence of various compounds such as phenolic compounds, flavonoids, saponins, tannins, and alkaloids. (Table:01)



Phytochemical constituents	Observation
Phenolic compound	+
Flavonoids	+
Saponins	+
Tannins	+
Alkaloids	+

Table:01 (+) – presence, (-) – absence

Determination of the extract's secondary metabolites using GC-MS

The GC-MS chromatogram of the ethanolic extract of *D. virgatus* revealed 78 peaks(Figure:03) for different phytoconstituents, including Terpenoid, terpene, Phenolic, aromatic compound, Alkaloids, nitrogen-

containing compound, Fatty acid, fatty ester, Alcohols, Esters, carbohydrates, Nitrile, Steroids, and sterol. The identification of these phytoconstituents was done by matching their retention time (RT), peak area, peak height, and mass fragmentation patterns with those of known compounds in the National Institute of Standards and Technology (NIST) library (Table 02). Of these 78 compounds, dl- α . -Tocopherol was found to be the most abundant, accounting for 33.352% of the peak area. Other prominent compounds were Ergosta-4,6,22-trien-3. α . -ol (32.876% peak area), 9,12,15-Octadecatrienoic acid (25.953% peak area), Phytol (22.078 %peak area), and Neophytadiene (18.754 %peak area) are listed in Table 02. The structures of the identified compounds are shown in Figure-04

S.No	Retention time [min]	Name of compound	Molecular formula	Molecular weight(g/mol)
1	33.352	dl- α .-Tocopherol ⁽¹⁹⁾	$C_{29}H_{50}O_2$	430.7
2	32.876	Ergosta-4,6,22-trien-3. α .-ol ⁽²⁰⁾	$C_{28}H_{44}O$	396.6
3	25.953	9,12,15-Octadecatrienoic acid ⁽²¹⁾	$C_{18}H_{30}O_2$	278.4
4	22.078	Phytol ⁽²²⁾	$C_{20}H_{40}O$	296.5
5	18.754	Neophytadiene ⁽²³⁾	$C_{20}H_{38}$	278.5

Table.02

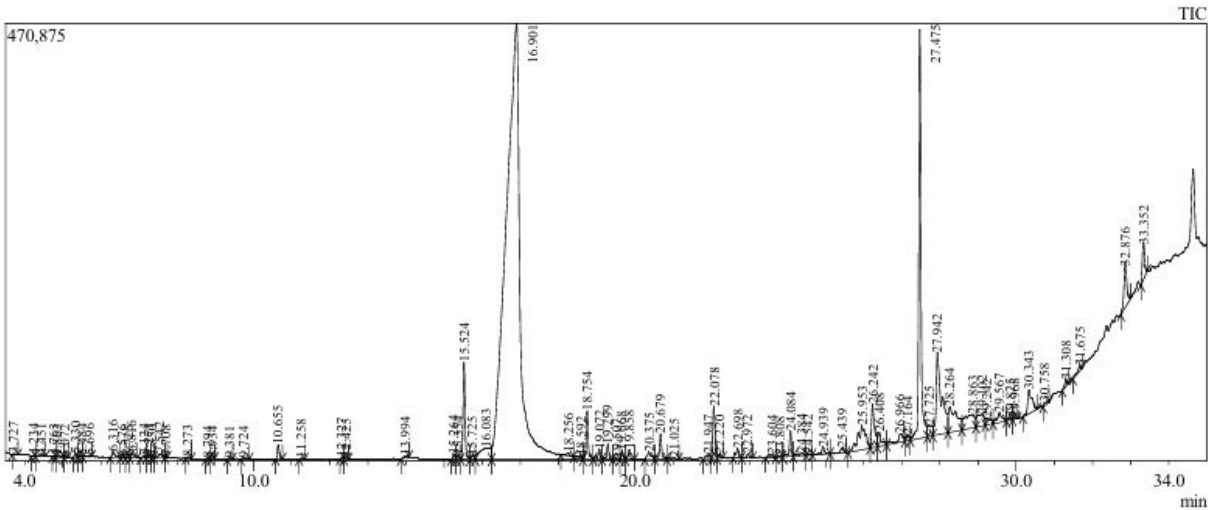


Figure: 03 (78 peaks that shown GCMS analysis of the ethanolic extract of *D. virgatus*)



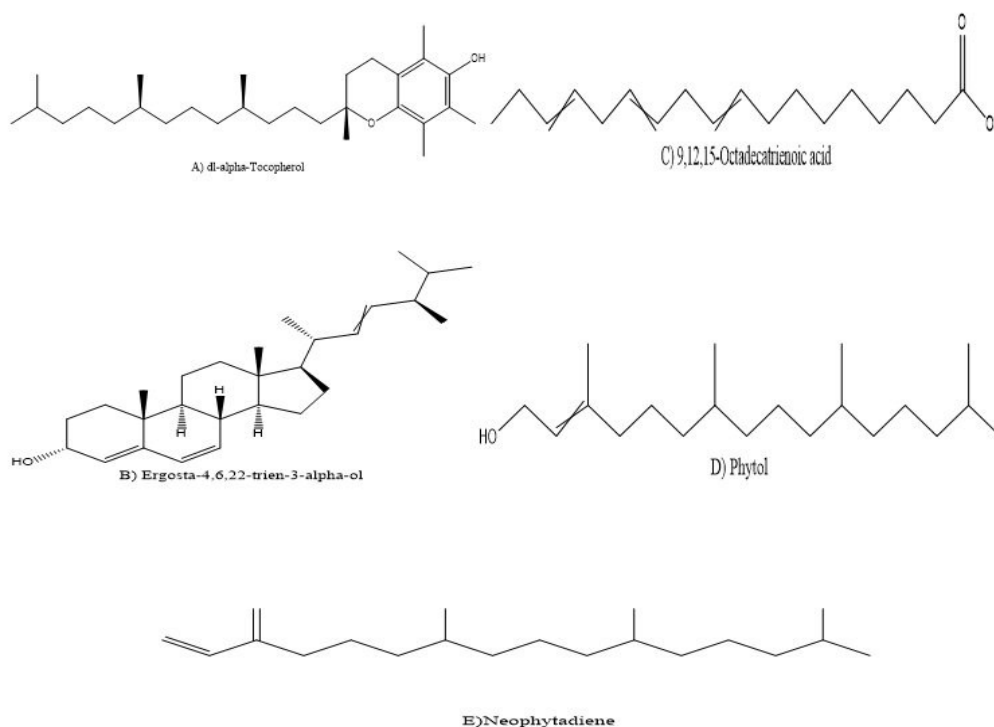


Figure:04 (structures of identified compounds)

In Silico Study with Protein 4foc

In general, protein structures in the Protein Data Bank (PDB) contain solvent molecules in the form of water and other residues (Figure:05).

Therefore, it is necessary to remove water molecules so as not to interfere when docking simulations are carried out and to ensure that what really interacts is the ligand and receptor. It is also necessary to add hydrogen. In order to get the docking condition closer to pH 7, hydrogen atoms are also added. (Figure:06)

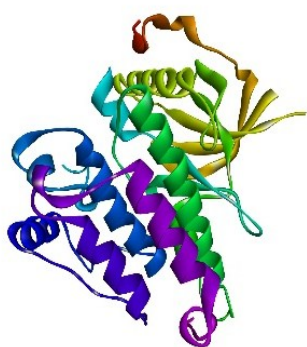
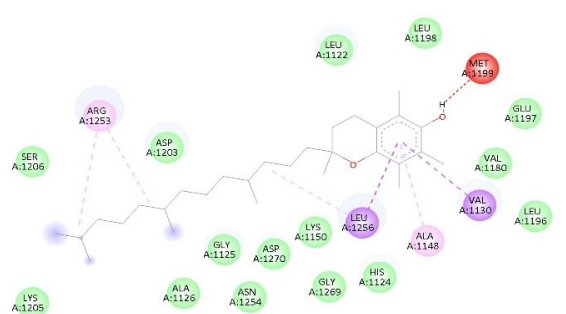
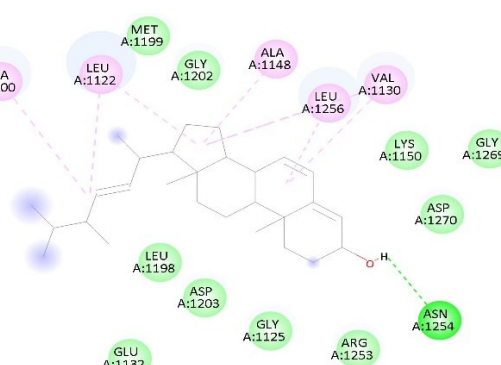


Figure:05

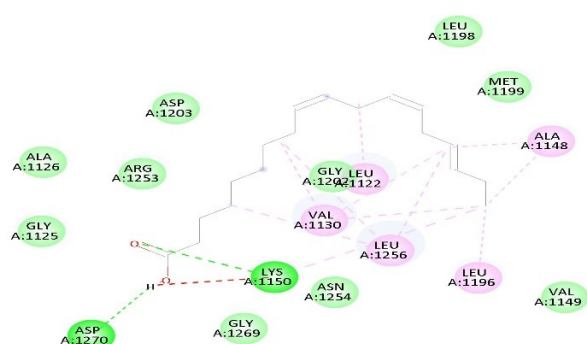
S.No	Name of compound	Binding affinity (kcal/mol)	H-bond donor	H-bond acceptor	Molar refractivity	LogP	H-bond donor	PubChem CID
1	dl-.alpha.-Tocopherol	-7.9	1	2	139.27	8.27	1	2116
2	Ergosta-4,6,22-trien-3.alpha.-ol	-8.9	1	1	127.47	6.42	1	20831069
3	9,12,15-Octadecatrienoic acid	-6.1	1	2	88.99	5.09	1	860
4	Phytol	-6.0	1	1	8.94	6.25	1	5280435
5	Neophytadiene	-6.1	0	0	97.31	7.07	0	10446

Table:03

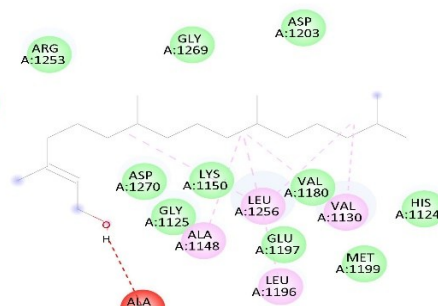
(A)dl-.alpha.-Tocopherol



(B)Ergosta-4,6,22-trien-3. alpha. -ol



(C)9,12,15-Octadecatrienoic acid



(D)Phytol

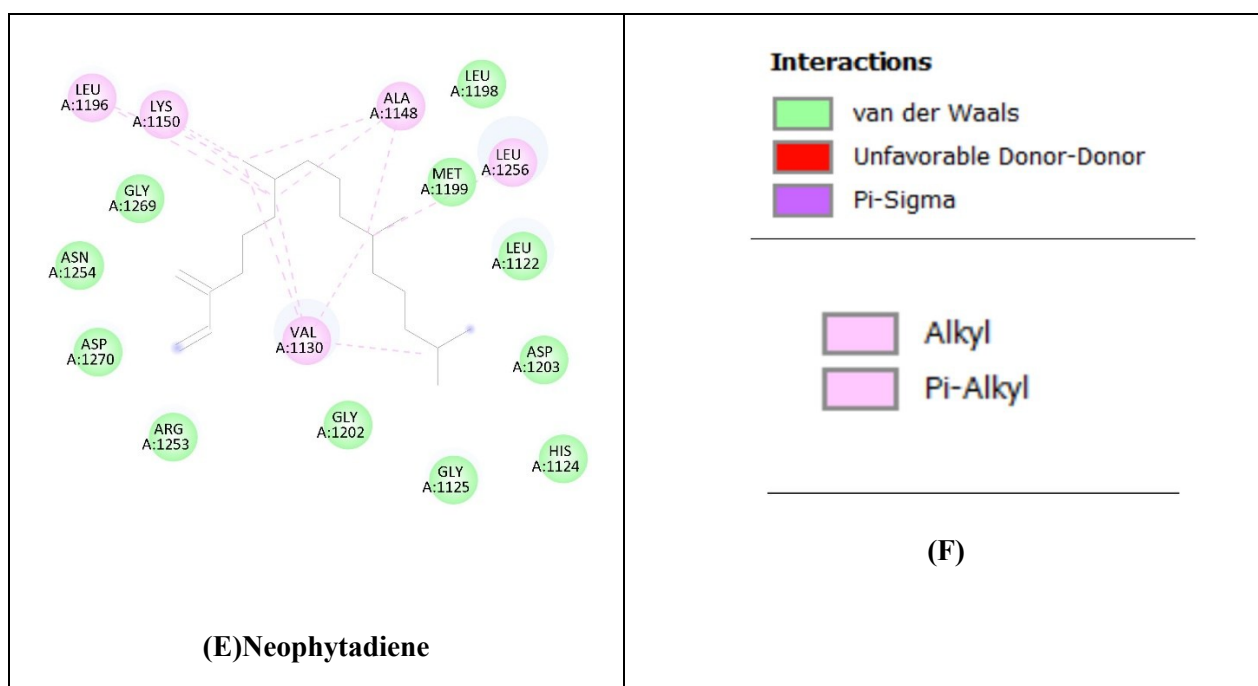


Figure:06 (Visualization of ligand-receptor interaction of potential compound)

Based on the binding affinity values in the table, the test compound with the lowest binding affinity value is Phytol with a value of -6.0. While the highest binding affinity value is Ergosta-4,6,22-trien-3. α -ol at -8.9. While other compounds are included in the range. Judging from the data above, these 5 test compounds are considered to have potential in anti-proliferative activity. Also, the pharmacokinetic properties of the above-mentioned compounds were studied using Swiss ADME software and found to be in the acceptable range of the Lipinski rule of five. (Table 03)

In Vitro anti-proliferative Assay

The cytotoxic effects of the *D. virgatus* extract on A549 lung cancer cells were evaluated using the MTT

assay. The untreated control cells showed an optical density (OD) of **0.634**, representing 100% viable cells. Cells were treated with varying concentrations (50–500 $\mu\text{g/ml}$) of the extract for 24 hours (Figure:08). The extract significantly reduced the viability of A549 cells in both a dose- and time-dependent manner. The results exhibited that the crude extract showed potent cytotoxic activity against the A549 lung cancer cell line, with a promising IC_{50} of **42.79 $\mu\text{g/mL}$** compared to doxorubicin (14.80 $\mu\text{g/mL}$) as the standard drug shown in graph (Figure:07). For crude plant extracts or newly isolated natural compounds, IC_{50} values in the range of **20–50 $\mu\text{g/mL}$** are generally considered biologically active and worthy of further investigation.

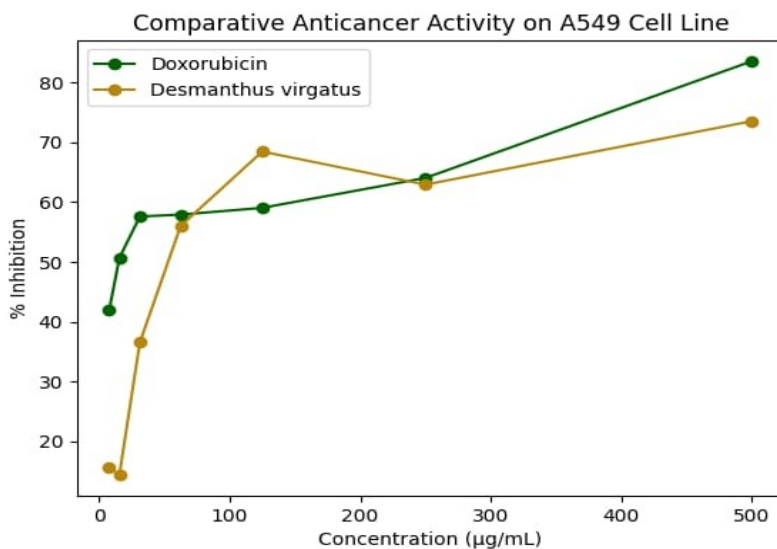


Figure:07

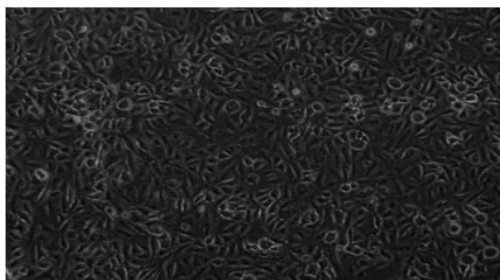


Figure:08(A)

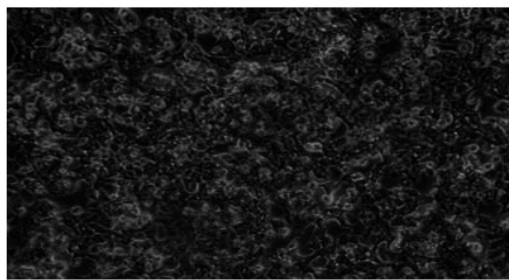


Figure:08 (B)

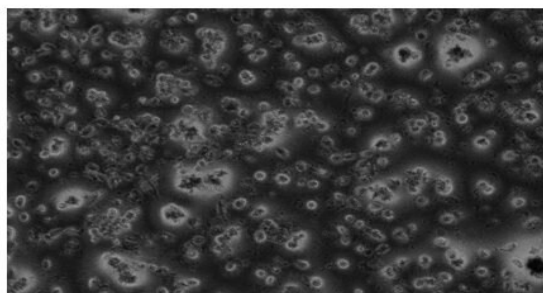


Figure:08(C)

Figure:08 (A) Control healthy A549 cells show normal adherent morphology, while drug-treated cells (B) A549 cells treated with Doxorubicin display comparable morphological alterations, indicating significant antiproliferative activity. (C) A549 cells treated with crude extracts of *D. virgatus* exhibit pronounced cytotoxic changes with cell shrinkage and detachment.

DISCUSSION

The present investigation proved the anticancer effect of the ethanolic extract of the title plant on the human

lung adenocarcinoma cell line A549 by in silico and in vitro studies, and also by the phytochemical investigation of the title plant extract, in which the title extract contained phenolic compounds,



flavonoids, tannins, saponins, and alkaloids, which have shown strong anticancer and antioxidant properties. Phenolic compounds in the title extract have shown strong anticancer potential by having a good ability to cause apoptosis in cancer cells. Compounds like dl- α -tocopherol, ergosta-4,6,22-trien-3-ol, 9,12,15-octadecatrienoic acid, phytol, and neophytadiene were found to be bioactive using GC-MS. Most of the compounds found have previously been related to anti-inflammatory, antioxidant, and cytotoxic effects. Since the mentioned compounds are found to be present in the extract, it can be suggested that the anti-cancer effects may be due to the synergistic effects of different compounds. The molecular docking study performed on cancer-related protein targets (PDB ID: 4FOC) revealed promising binding affinity for the compounds ergosta-4,6,22-trien-3-ol (-8.9 kcal/mol) and dl- α -tocopherol (-7.9 kcal/mol). The binding affinity values are a clear indication of the interaction between the ligand and the protein, which might occur as a potentially inhibiting factor for the survival/proliferation of cancer cells. Moreover, the result from the Swiss ADME indicated that the chosen compounds were in accordance with the rule of five, as described by Lipinski. The in vitro MTT assay indicated a specific amount of decrease in the percentage of survival of A549 cells in a dose-dependent manner, as the IC₅₀ was 42.79 μ g/mL. According to the criteria considered, crude extracts that have an IC₅₀ below 50 μ g/mL are considered biologically active. Moreover, the morphological observation indicated a clear result, as the cells exhibited a shrunken, rounded, and detached appearance, similar to a cytotoxic/apoptotic effect, as opposed to normal, adhering cells. Although the standard drug showed more pronounced effects, the plant extract exhibited notable anti-proliferative effects.

REFERENCES

1. Leong, S. P., & Witte, M. H. (2024). Cancer metastasis through the lymphatic versus blood vessels. *Clinical & experimental metastasis*, 41(4), 387–402. <https://doi.org/10.1007/s10585-024-10288-0>
2. Sathishkumar, K., Chaturvedi, M., Das, P., Stephen, S., & Mathur, P. (2022). Cancer incidence estimates for 2022 & projection for 2025: Results from National Cancer Registry Programme, India. *The Indian journal of medical research*, 156(4&5), 598–607. https://doi.org/10.4103/ijmr.ijmr_1821_22
3. Li, C., Lei, S., Ding, L., Xu, Y., Wu, X., Wang, H., Zhang, Z., Gao, T., Zhang, Y., & Li, L. (2023). Global burden and trends of lung cancer incidence and mortality. *Chinese medical journal*, 136(13), 1583–1590. <https://doi.org/10.1097/CM9.0000000000002529>
4. Siddiqui F, Vaqar S, Siddiqui AH. Lung Cancer. [Updated 2023 May 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK482357/>
5. Tang, F. H., Wong, H. Y. T., Tsang, P. S. W., Yau, M., Tam, S. Y., Law, L., Yau, K., Wong, J., Farah, F. H. M., & Wong, J. (2025). Recent advancements in lung cancer research: a narrative review. *Translational lung cancer research*, 14(3), 975–990. <https://doi.org/10.21037/tlcr-24-979>
6. Tesfaw, L. M., Dessie, Z. G., & Mekonnen Fenta, H. (2023). Lung cancer mortality and associated predictors: systematic review using 32 scientific research findings. *Frontiers in oncology*, 13, 1308897. <https://doi.org/10.3389/fonc.2023.1308897>
7. Patel AB, Kumar R, Singh P. Growth, yield, and quality of hedge lucerne (*Desmanthus virgatus*) under different management practices. *Forage Res.* <https://forageresearch.in/wp-content/uploads/2023/06/487-493.pdf>
8. MacRoberts MH, MacRoberts BR. *Desmanthus virgatus* (Fabaceae): new to Louisiana. *Phytoneuron*. 2011;2011-54:1–3. <https://www.phytoneuron.net/PhytoN-Desmanthus.pdf>
9. Tukiran, Tukiran & Hendrawan, Syahrul & Wardana, Andika. (2026). Inhibitory activity of the ethyl acetate extract of *Desmanthus virgatus* (L.) Willd. leaves against α -glucosidase enzyme: In silico and in vitro studies. *Journal of Pharmacy & Pharmacognosy Research*. https://doi.org/10.56499/jppres_14.1.2419
10. Tukiran, T., Farkhiyah, L., & Dewistamara, M. G. (2025). Antifungal Activity of Ethanol Extract *Desmanthus virgatus* (L.) Leaves In Silico and In



- Vitro. *Fabad Eczacılık Bilimler Dergisi*, 50(2), 329-342.
<https://doi.org/10.55262/fabadeczacilik.1586787>
11. Hendrawan SL, Ambarwati C, Tukiran T. Effectiveness of *Desmanthus virgatus* leaf ethyl acetate extract and antiviral drugs as antirabies based on in silico study. *J Pijar Mipa*. 2025;20(4):616–625.
<https://doi.org/10.29303/jpm.v20i4.9024>
 12. do Nascimento, J. B., & da Costa, J. G. M. (2025). Flavonoids: A Review of Antibacterial Activity Against Gram-Negative Bacteria. *International journal of microbiology*, 2025, 9961121.
<https://doi.org/10.1155/ijm/9961121>
 13. Rauf, A., Wilairatana, P., Joshi, P. B., Ahmad, Z., Olatunde, A., Hafeez, N., Hemeg, H. A., & Mubarak, M. S. (2024). Revisiting luteolin: An updated review on its anticancer potential. *Heliyon*, 10(5), e26701.
<https://doi.org/10.1016/j.heliyon.2024.e26701>
 14. Abubakar, A. R., & Haque, M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of pharmacy & bioallied sciences*, 12(1), 1–10.
https://doi.org/10.4103/jpbs.JPBS_175_19
 15. Shaikh, Junaid & Patil, Matsyagandha. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*. 8. 603-608.
<https://www.chemijournal.com/archives/2020/vol8issue2/PartI/8-2-34-147.pdf>
 16. Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry*, 31(2), 455–461.
<https://doi.org/10.1002/jcc.21334>
 17. Präbst, K., Engelhardt, H., Ringgeler, S., & Hübner, H. (2017). Basic Colorimetric Proliferation Assays: MTT, WST, and Resazurin. *Methods in molecular biology* (Clifton, N.J.), 1601, 1–17. https://doi.org/10.1007/978-1-4939-6960-9_1
 18. Maurya, D. K., Nandakumar, N., & Devasagayam, T. P. (2011). Anticancer property of gallic acid in A549, a human lung adenocarcinoma cell line, and possible mechanisms. *Journal of clinical biochemistry and nutrition*, 48(1), 85–90.
<https://doi.org/10.3164/jcbrn.11-004FR>
 19. Heinonen, O. P., Albanes, D., Virtamo, J., Taylor, P. R., Huttunen, J. K., Hartman, A. M., Haapakoski, J., Malila, N., Rautalahti, M., Ripatti, S., Mäenpää, H., Teerenhovi, L., Koss, L., Virolainen, M., & Edwards, B. K. (1998). Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. *Journal of the National Cancer Institute*, 90(6), 440–446.
<https://doi.org/10.1093/jnci/90.6.440>
 20. Taank, Y., & Agnihotri, N. (2025). Exploring the anticancer potential of ergosterol and ergosta-5,22,25-triene-3-ol against colorectal cancer: Insights from experimental models and molecular mechanisms. *Biochemical pharmacology*, 240, 117061.
<https://doi.org/10.1016/j.bcp.2025.117061>
 21. Yan, H., Zhang, S., Yang, L., Jiang, M., Xin, Y., Liao, X., Li, Y., & Lu, J. (2024). The Antitumor Effects of α -Linolenic Acid. *Journal of personalized medicine*, 14(3), 260.
<https://doi.org/10.3390/jpm14030260>
 22. Yu, J., Jin, F., Tang, Y., & Huang, Y. (2025). In Vitro Anticancer Activity of Phytol on Human Non-Small Cell Lung Cancer A549 Cells. *Integrative cancer therapies*, 24, 15347354251344592.
<https://doi.org/10.1177/15347354251344592>
 23. Rajeswaran, S., & Rajan, D. K. (2025). Neophytadiene: Biological activities and drug development prospects. *Phytomedicine: international journal of phytotherapy and phytopharmacology*, 143, 156872.
<https://doi.org/10.1016/j.phymed.2025.156872>

HOW TO CITE: Balagopalan T. G., Vinisha A. P., Nandha Kishore V. S., Tharun S., Priya K., Integrative Phytochemical and Cytotoxic Dissection of *Desmanthus Virgatus* Against Human Lung Adenocarcinoma, *J. Pharm. Sci.*, 2026, 2 (3), 492-502.
<https://doi.org/10.5281/zenodo.19217709>

