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Evaluation of Anticancer Activity of *Acalypha wilkesiana* and *Codiaeum variegatum* (Euphorbiaceae) Against the A549 Lung Cancer Cell Line using MTT Assay

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Abstract

Cancer is perceived as one of the leading causes of death worldwide. Lung cancer is the leading cause of cancer-related deaths worldwide, accounting for the highest mortality rates among both men and women. Surgery, chemotherapy, and other cancer treatments have been known to reduce cancer-related deaths but induce adverse effects and are often incompletely effective and develop resistance to drugs. So it is necessary to seek alternative medicine. Many naturally derived products are shown to have anticancer activity. *Acalypha wilkesiana* and *Codiaeum variegatum* leaves were evaluated for anticancer effects on lung cell lines. An alcoholic extract of leaves of the plants Acalypha wilkesiana and *Codiaeum variegatum* was prepared by the Soxhlet extraction method by hot percolation and compared with penicillin-streptomycin for effect on the lung cancer cell line A549. Cytotoxicity at different extract concentrations was evaluated using the MTT assay. In the MTT assay, cells exposed to different concentrations of *A. wilkesiana* and *C. variegatum* extracts exhibited a significant decrease in cell viability, indicating moderate cytotoxicity.

KEYWORDS: *Acalypha wilkesiana*, *Codiaeum variegatum*, Anticancer activity, cell line, A549, MTT assay, etc.

Introduction

Cancer is perceived as one of the leading causes of death worldwide and has been the sole reason for accounting for nearly 10 million deaths in 2020**[1,2]**. Surgery, chemotherapy, and other cancer treatments have been known to reduce cancer-related deaths. Current drugs or other approaches to counteract chemotherapy-induced adverse effects are often ineffective, do not address potential long-term sequelae, or may even induce other side effects that only add to patient discomfort. Thus, extensive research is underway to identify more effective chemotherapy agents derived from naturally occurring compounds that can suppress or prevent carcinogenesis **[3]**.

There are different types of cancers in which abnormal cells divide uncontrollably and can infiltrate and destroy normal body tissues. The most common types of cancers are skin cancer, lung cancer, breast cancer, prostate cancer, pancreatic cancer, and colorectal cancer**[4]**. The leading cause of cancer death is lung cancer in both males and females, with approximately 2.2 million new cases and 1.8 million deaths worldwide in 2020 **[5]**. Not all cases of lung cancer are due to smoking. Still, the role of passive smoking is increasingly being recognized as a risk factor for lung cancer, leading to policy interventions to decrease undesired exposure of non-smokers to others' tobacco smoke. Emissions from automobiles, factories, and power plants also pose potential risks**[6]**. Lung cancer arises from the cells of the respiratory

epithelium and can be divided into two broad categories. Small cell lung cancer (SCLC) is a highly malignant tumor derived from cells exhibiting neuroendocrine characteristics and accounts for 15% of lung cancer cases. Non–small cell lung cancer (NSCLC), which accounts for the remaining 85% of cases, is further divided into 3 major pathologic subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Adenocarcinoma by itself accounts for 38.5% of all lung cancer cases, with squamous cell carcinoma accounting for 20% and large cell carcinoma accounting for 2.9%**[7]**. Cisplatin combination chemotherapy is currently considered a standard therapy for patients with advanced NSCLC. Although the chemotherapy response rate ranged from 20% to 35%, the overall survival ranged from approximately 6.9 to 11.3 months. Lung cancer patients often have resistance to cisplatin and metastasis. Drug resistance remains one of the predominant reasons for the failure of many cancer therapies, such as lung cancer**[8].**

Researchers have extensively explored drug development using natural products, and such approaches are frequent in cancer research **[9]**. The Plant Kingdom has been a source of

many clinically approved anticancer drugs, both natural and semisynthetic. Plants are a rich source of bioactive chemicals. Secondary metabolites, or phytochemicals, from plants have exhibited significant pharmacological activities over the years. Some of these anticancer agents are considered essential medicines by the World Health Organization. For example, vincristine and vinblastine, isolated from *Catharanthus roseus* (L.) G. Don, are useful agents against various types of lymphomas and solid tumors**[10, 11]**. The family Euphorbiaceae was founded by A.L. de Jussieu in 1789. *Euphorbiaceae*, the spurge family, is a large family of flowering plants with 300 genera and around 7,500 species. *Codiaeum variegatum* L. belongs to the family Euphorbiaceae and grows into shrubs and small trees in its native habitats of India, Malaysia, and some of the South Pacific islands**[12, 13]**. Aside from its main purpose in serving as an ornamental, the root decoction of *C. variegatum* is used to treat gastric ulcers; the leaves have antibacterial and antiamoebic properties and can be crushed and drunk to cure diarrhea, and in indigenous Malaysian medicine, the plant is used as an anti-infective and an anti-cancer agent**[14]**. *Acalypha wilkesiana* Muell Arg belongs to the *Euphorbiaceae* family, or the spurge family. Some other common names for *Acalypha wilkesiana* include copperleaf, Joseph's coat, and fire dragon**[15]**. The leaf of *Acalypha wilkesiana* has been reported to have medicinal properties for the treatment of malaria, dermatological, and gastrointestinal disorders. It is also used in the treatment of hypertension, especially in managing the abnormal sodium and potassium metabolism that accompany hypertension. The leaf poultice is considered effective for headaches, swellings, and colds in Trinidad **[16]**.

The study carried out by Halimah E. *et al. al showed that ethanolic extract of Acalypha wilkesiana possesses* cytotoxic activity against breast cancer cells. Similarly, Anim M.T. *et al. showed that the stem bark and leaf extracts of Codiaeum variegatum have anti-proliferative effects on leukemia (Jurkat), breast (MCF-7), and prostate (PC-3) cancer cell lines and may contain bioactive compounds with anticancer properties* **[13,17]**. This study aims to evaluate the anticancer activity of plant extracts from Acalypha wilkesiana and Codiaeum variegatum against the A549 lung cancer cell line using an in vitro MTT assay.

Materials and methods

Collection of plant material

The plant's leaves were collected from a local garden near Nagpur. Plant material authentication was conducted with assistance from a botanical specimen sheet provided by Dr. N. M. Dongarwar, affiliated with the Department of Botany at R. T. M. Nagpur University, Nagpur. Specimen numbers assigned to the authenticated sample numbers were *Acalypha wilkesiana* (231) and *Codiaeum variegatum* (233).

Preparation of plant extract

The leaves were detached from the plants and dried in the shade, away from sunlight. Then the coarse powder was extracted, with a measured amount (1kg) treated with petroleum ether at 50-60°C for 72 hours by hot percolation using a Soxhlet apparatus. Extraction solvents were heated in the bottom flask, vaporized into the sample thimble, condensed in the condenser, and dripped back. When the liquid reaches the siphon arm, it is again emptied into the bottom flask, and the process continues [18]. The residue left after petroleum extraction was dried and then extracted with 95% ethanol at 60-70°C for up to 72 hours using a Soxhlet apparatus. After concentrating the alcoholic extract, a brown residue was obtained and stored in a desiccator.

Screening for phytochemicals

The confirmatory qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds, including tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids [19-24].

Physical evaluation of plants

Determination of Ash value

Ash content in the investigated plant species *Acalypha wilkesiana* and *Codiaeum variegatum* was calculated by the methods given below:

Determination of total ash

Five grams of the ground plant material was taken in a silica crucible previously ignited and weighed. The ground plant material was then spread in a fine, even layer on the bottom of the crucible. It was then incinerated in a muffle furnace by gradually increasing the heat to dull red heat, until free from carbon, and then cooled and weighed. If a carbon-free ash could not be obtained in this way, the charred mass was exhausted with hot water. The residue was collected on an ash-less filter paper, which was then incinerated. The ash percentage was calculated with reference to the air-dried material.

$$\text{Total ash value} = [(W_2 - W)/W_1] \times 100\%$$

W = Weight of empty crucible, W_1 = Weight of sample taken, W_2 = Weight of crucible.

Determination of Water-Soluble Ash

100 mg of ash was boiled in 10 mL of distilled water for 5 minutes. The insoluble matter was collected in a silica crucible or on an ash-less filter paper. It was washed with hot water, then ignited to a constant weight at low temperatures. The weight of the insoluble matter was subtracted from the weight of the ash. The percentage of water-soluble ash was calculated with reference to the amount of ash taken.

$$\textbf{Water soluble ash} = [(W_3 - W)/W_1] \times 100\%$$

W_3 = Weight of crucible and acid insoluble ash; W_1 = Weight of sample taken; W = Weight of empty crucible

Determination of Acid Insoluble Ash

The total ash was boiled for 5 minutes in 25 mL of 10% HCl. The insoluble ash was collected in a silica crucible or on an ash-less filter paper. It was washed with hot water and then ignited and weighed. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight represents the acid-insoluble ash. The percentage of acid-insoluble ash was calculated with reference to the amount of ash taken.

$$\textbf{Acid insoluble ash} = [(W_2 - W)/W_1] \times 100\%$$

W_2 = Weight of crucible and acid-insoluble ash; W1 = Weight of sample taken; W = Weight of empty crucible[25, 26].

Determination of extractive value

Water-soluble extractives

Five grams of coarsely powdered, air-dried drug was macerated with 100 ml of water in a closed conical flask for 24 hours, shaken frequently for the first 6 hours, and then allowed to stand for 18 hours. This was filtered through Whatman filter paper, grade no. 100. Twenty-five milliliters of the filtrate was evaporated to dryness in a petri dish, dried at 105°C, and weighed. The percentage of water-soluble extractives relative to the air-dried material was calculated.

Alcohol soluble extractives

Five grams of air-dried and coarsely powdered drug was macerated with 100 ml of 70% ethanol in a closed conical flask for 24 hours, shaken frequently during the first 6 hours, and allowed to stand for 18 hours. This was filtered rapidly, with precautions against ethanol loss. Twenty-five milliliters of the filtrate was evaporated to dryness in a Petri dish, dried at 105 °C, and weighed. The percentage of alcohol-soluble extractives was calculated with reference to air-dried drugs.

Ether-soluble extractives

Five grams of air-dried, coarsely powdered drug were extracted with petroleum ether in a Soxhlet extractor for 20 hours. The ether extract was transferred to a petri dish and allowed to evaporate. It was dried at 105 °C to a constant weight. The percentage of ether-soluble extractives was calculated with reference to air-dried drugs**[27]**.

Anticancer activity

MTT Assay

Cytotoxicity of the provided samples on the A549 (Procured from NCCS Pune) cell line was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96-well plates for 24 h in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L-HIMEDIA) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781) at 37°C with 5% CO_2. The next day, cells were treated with different concentrations of the samples (as specified in the Excel sheet). The stock solutions of the samples were prepared in DMSO and further diluted to obtain different concentrations in incomplete Cell culture Medium (Without FBS). After 24 hours of incubation, MTT Solution (5 mg/ml) was added to the cell culture and incubated for 2 h. Cells without treatment were considered the Control, and cells without MTT were considered the Blank. At the end of the experiment, the culture supernatant was removed, and the cell layer matrix was dissolved in 100 µl of Dimethyl Sulfoxide (DMSO–SRL, Cat no. 67685) and read on an ELISA plate reader (iMark, Bio-Rad, USA) at 540 nm. IC_{50} was calculated using GraphPad Prism software. Images were captured under an inverted microscope (Olympus EK2) using an AmScope digital camera (10 MP Aptima CMOS). 50% inhibitory concentration (IC_{50}) was presented as Mean ± SEM (Standard Error of Mean)**[28-31]**.

Calculations

$$\% \text{ Viable cells} = (A_{test} / A_{Control}) * 100$$

(A_{test} = Absorbance of test sample)

($A_{Control}$ = Absorbance of Control)

Determination of IC50 values

For highly active extracts with> 75% cytotoxicity against various cancer cell lines and a human normal cell line, different concentrations were prepared for dose-response studies. The results were used to calculate the IC50 values for each extract using probit analysis and SPSS (SPSS for Windows, statistical analysis software package/version 9/ 1989 SPSS Inc., Chicago, USA).

Result and discussion

Preliminary phytochemical analysis

Plants can be viewed as natural biosynthetic laboratories that produce a diverse array of compounds, including alkaloids, glycosides, volatile oils, tannins, saponins, flavonoids, sugars, and more, that exert physiological effects. The results of preliminary phytochemical screening are shown in Table 1.

Table No.1: Preliminary phytochemical screening of the alcoholic extract of copper leaf and croton plant.

Plant Constituent	**Test/ Reagent**	**Inference *A. wilkesiana* plant extract**	**Inference *C. variegatum* plant extract**	**Inference Mixture of extract**
Alkaloids	Dragendorff's reagent	+	+	+
	Mayer's reagent	+	+	+
	Wager's test	+	+	+
Carbohydrate	Molisch' Test	+	+	+
	Benedict's Test	+	+	+
Flavonoids	Shinoda's Test	+	+	+
	Lead acetate test	+	+	+
Amino acid	Ninhydrin Test	+	+	+
Glycoside	Borntrager's Test	+	+	+
Tannis	Ferric chloride Test	+	+	+
	Lead acetate Test	+	+	+
Proteins	Millon's Test	+	+	+
	Xanthoproteic's Tese			
Pytosterols	Salkowaski Test	+	+	+
Saponin	Foam test	+	+	+
Phenols	Gelatin test	+	+	+

'+'indicates: present; '-' indicates: absent

Physical Evaluation

Ash value

The ash value of plants *Acalypha wilkesiana* and Codiaeum variegatum under study was determined by three different forms, viz. Total ash, water-soluble ash, and acid-insoluble ash, and the results are presented in Table 2.

Table No. 2: The ash value of plants *Acalypha wilkesiana* and Codiaeum variegatum.

Ash value	*A.wilkesiana* plant % w/w	*C. variegatum* plant %. w/w
Total ash value	7.56	6.78
Water soluble ash value	3.21	2.29
Acid insoluble ash value	1.67	1.23

Extractive value

The extractive values of the leaves of plants *Acalypha wilkesiana* and *Codiaeum variegatum* are shown in Table 3.

Table No. 3: The extractive values of the leaves of plants *Acalypha wilkesiana* and *Codiaeum variegatum*.

Extractive value	*A. wilkesiana*	*C. variegatum*
Water soluble value	0.17	0.14
Alcohol soluble value	0.56	0.49
Petroleum ether soluble ash value	0.1	0.11

The highest extractive value was found in alcohol followed by water and the minimum extractive value in petroleum ether. The above results indicate the presence of a higher number of polar compounds than nonpolar. The high water-soluble extractive value indicates the presence of acids, sugars, and inorganic compounds, and the high alcohol-soluble extractive value indicates the presence of polar constituents such as steroids, phenolics, glycosides, and flavonoids.

MTT Assay

In the current study, the cytotoxic effect of an alcoholic extract mixture of *Acalypha wilkesiana* and *Codiaeum variegatum* on the lung cancer cell line A549 was determined using an MTT assay at a concentration range of 10-1000 µg/ml after 24 h of treatment. Cell viability was assessed using the MTT assay. Each control and extract was assayed in quadruplicate, and the percent of cell survival in the negative control was assumed to be 100 (Table 4, Fig 1).

Table No. 4: The absorbance of different concentrations of *Acalypha wilkesiana* and *Codiaeum variegatum* and standard drug Doxorubicin on lung cancer cell line A549.

	Percentage Cell Viability of A549					
	***Acalypha wilkesiana* and *Codiaeum variegatum* Extract**			**Standard drug Doxorubicin**		
Sample Conc.	**Mean**	**SEM**	**N**	**Mean**	**SEM**	**N**
0	100	6.860721	4	100	6.260725	4
1	89.72603	6.388616	4	56.52150	6.488615	4
10	79.79452	4.982455	4	45.5140	4.122235	4
50	78.42466	6.903326	4	32.5644	4.903326	4
100	73.9726	5.201272	4	13.5747	4.201272	4
250	57.87671	5.167338	4	5.67575	4.167338	4
500	38.0137	6.161212	4	4.55325	4.161212	4
1000	17.46575	2.045259	4	2.3558	2.045259	4

N- Number of Test Replicates, SEM - Standard Error of Mean

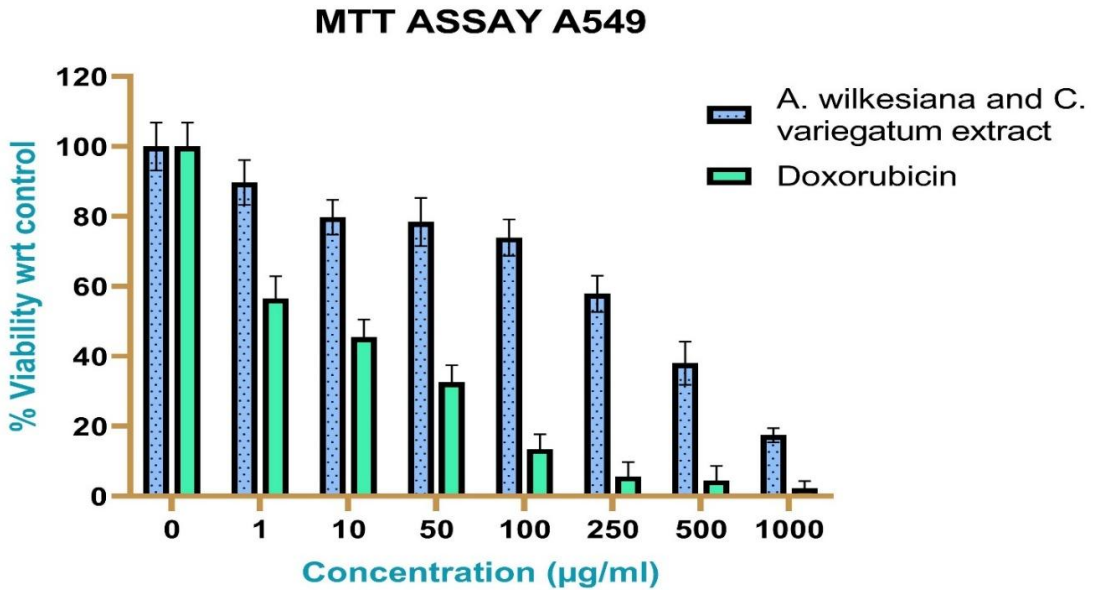

Figure No.1: Cytotoxicity of *A. wilkesiana* and *C. variegatum* combined extract and standard drug against Lung cancer cell line A549. Data are presented as mean ± SEM (n = 4).

Evaluating the anticancer activity of plant extracts is essential for safe treatment. It enables identification of the intrinsic toxicity of the plant and the effects of acute overdose. An in vitro cytotoxicity test using A549 lung cancer cell lines was performed to screen for potentially toxic compounds that affect basic cellular functions and morphology. The extract (*A. wilkesiana* and *C. variegatum*) showed in vitro growth inhibition effects on cancer cell lines.

quadruplicates by serial dilution. Among these seven concentrations, the 1000 µg/ml extract was the most effective at producing percentage growth inhibition. The IC50 values of ethanol extracts of *A. wilkesiana* and *C. variegatum* on lung (A549) cancer cell lines were found to be 274.6 ± 0.085, indicating a 50% inhibitory concentration.

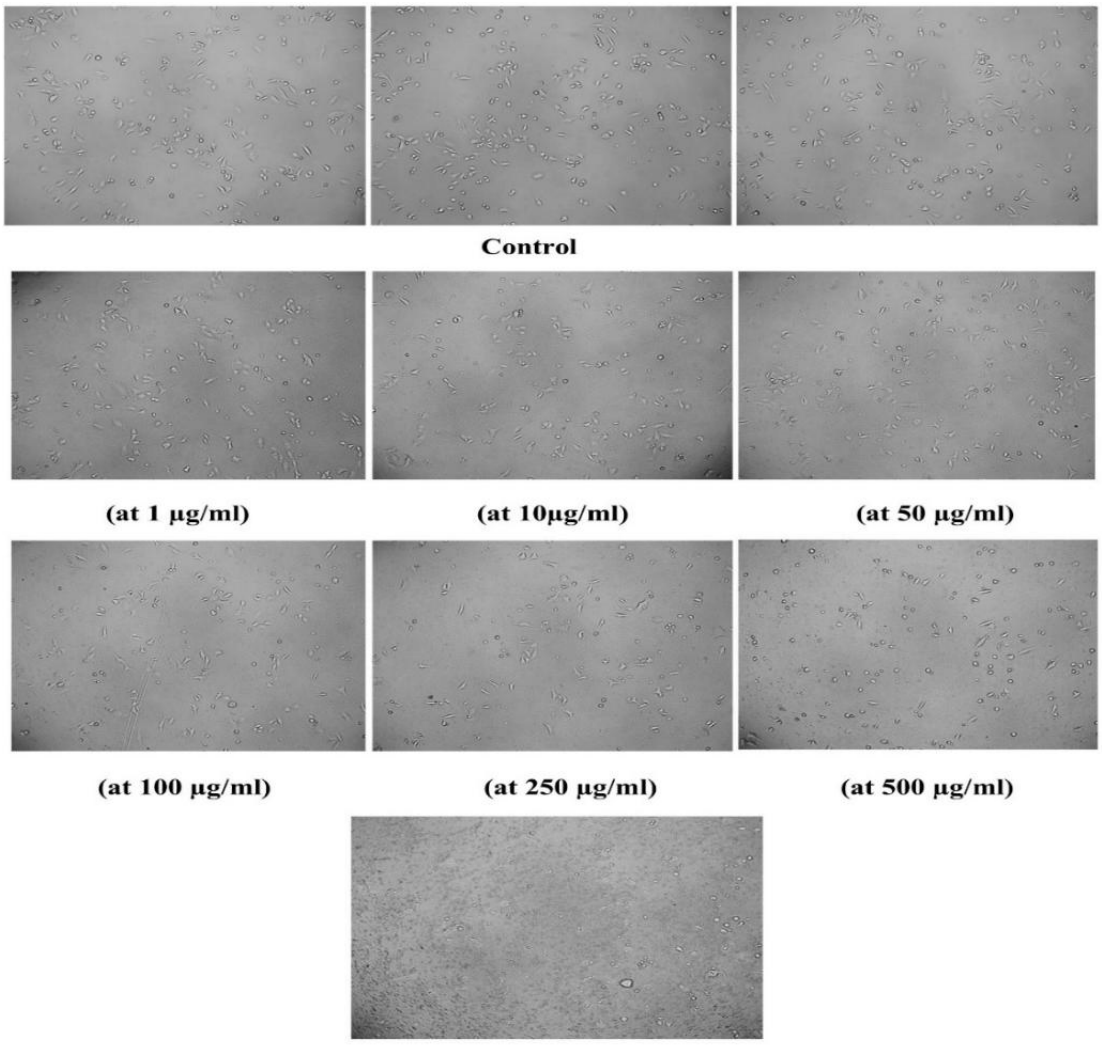

Figure No.2: Morphological changes showing inhibition of A549 lung cancer cell lines.

Conclusion

This study provides information on the phytochemical constituents, physicochemical properties, and cytotoxic effects of *C. variegatum* and *A. wilkesiana*. From the study, the leaves of *C. variegatum* and *A. wilkesiana* exhibited moderate anticancer activity.

In this study, the initial phytochemical analysis of ethanolic extracts from *Acalypha wilkesiana* and Codiaeum *variegatum* plants revealed the presence of alkaloids, glycosides, carbohydrates, flavonoids, amino acids, tannins, polyesters, proteins, and saponins. These components were identified through various phytochemical tests. The presence of alkaloids and flavonoids was responsible for anticancer activity. Flavonoids are involved in the induction of apoptosis through suppression/promotion of various factors, including Ras-ERK and PI3K-Akt signaling pathways and genes such as Bax, Bcl-2, and p53, which are involved in the proliferation of cancer cells, and play an important role in combating the extent of the disease by promoting apoptosis in cancer cells.

The assessment of anticancer activity was conducted using the MTT assay in A549 cells. Based on the MTT assay

results, the A549 cell line exposed to different concentrations of the extract exhibited moderate cytotoxicity.

References

[1]. Mandour YM, Refaat E, Hassanein HD. Anticancer activity, phytochemical investigation and molecular docking insights of *Citrullus colocynthis* (L.) fruits. Sci Rep. 2023;13:20038. doi:10.1038/s41598-023-46867-6.

[2]. Gadade JP, Patil SA. Cytotoxic effect of *Rotheca serrata* on cancer cell lines MCF-7 and neuroblastoma SH-SY5Y. J Cancer Res Ther. 2024;20(1):181-188. doi:10.4103/jcrt.jcrt_1325_22.

[3]. Motadi LR, Choene MS, Mthembu NN. Anticancer properties of *Tulbaghia violacea* regulate the expression of p53-dependent mechanisms in cancer cell lines. Sci Rep. 2020;10(1):12924. doi:10.1038/s41598-020-69722-4.

[4]. Alhawiti NM, Alramadhan WH, Boadi W, Myles EL. Anticancer activity of *Peganum harmala* and *Haloxylon salicornicum* leaf extracts on lung cancer A549 and prostate cancer PC-3 cell lines. Am J Plant Sci.2023;14:1360-1374. doi:10.4236/ajps.2023.1411092.

[5]. Pandi A, Mamo G, Getachew D, Lemessa F, Kalappan VM, Dhiravidamani S. A brief review on lung cancer. Int J Pharma Res Health Sci. 2016;4(1):907-914.

[6]. Dela Cruz CS, Tanoue LT, Matthay RA. Lung cancer: epidemiology, etiology, and prevention. Clin Chest Med. 2011;32(4):605-644. doi:10.1016/j.ccm.2011.09.001.

[7]. Mohammadi A, et al. Anacyclus pyrethrum extract significantly destroyed lung cancer cell line (A549) by inducing apoptosis. J Herb Med. 2023;39:100649. doi:10.1016/j.hermed.2023.100649.

[8]. Kumar G, Gupta R, Sharan S, Roy P, Pandey DM. Anticancer activity of plant leaves extract collected from a tribal region of India. 3 Biotech. 2019;9(11):399. doi:10.1007/s13205-019-1927-x.

[9]. Jiménez-González V, Benítez G, Pastor JE, López-Lázaro M, Calderón-Montaño JM. Evaluation of anticancer activity of 76 plant species collected in Andalusia (Spain) against lung cancer cells. Plants (Basel).2023;12(18):3275.doi:10.3390/plants12183275.

[10]. Bamola N, Verma P, Negi C. A review on some traditional medicinal plants. Int J Life Sci Sci Res. 2018;4(1):1550-1556. doi:10.21276/ijlssr.2018.4.1.7.

[11]. The Editors of Encyclopaedia Britannica. Euphorbiaceae. Encyclopaedia Britannica [Internet]. [cited 2026 Aug 25]. Available from: https://www.britannica.com/plant/Euphorbiaceae.

[12]. Stamps RH, Osborne LS. Croton production and use. EDIS. 2003;(7):ENH878/EP106. doi:10.32473/edis-ep106-2003.

[13]. Anim MT, Larbie C, Appiah-Opong R, Aning A, et al. Extracts of *Codiaeum variegatum* (L.) A. Juss is cytotoxic on human leukemic, breast and prostate cancer cell lines. J Appl Pharm Sci. 2016;6(11):87-93. doi:10.7324/JAPS.2016.601114.

[14]. Lim SW, Ting KN, Bradshaw TD, Zeenathul NA, Wiart C, Khoo TJ, et al. *Acalypha wilkesiana* extracts induce apoptosis by causing single-strand and double-strand DNA breaks. J Ethnopharmacol. 2011;138(2):616-623. doi:10.1016/j.jep.2011.10.005.

[15]. Ogbonna OA, Ogbonna SUA, Ugwu EI, Onyimba IA, Ogbonna CIC, Njoku AI, et al. Biochemical analysis and antimicrobial activity of ethanolic and aqueous leaf extracts of *Acalypha wilkesiana* var. *morea* Müll. Arg. (copperleaf). J Bot Res. 2021;4(1):131-137. doi:10.36959/771/570.

[16]. Halimah E, Hendriani R, Indradi B, Sofian FF. Cytotoxicity of ethanol extract and its fractions from *Acalypha wilkesiana* against breast cancer cell MCF-7. J Adv Pharm Educ Res. 2022;12(1):17-20. doi:10.51847/G2bMkvc6PO.

[17]. Mahire SP, Patel SN. Extraction of phytochemicals and study of its antimicrobial and antioxidant activity of *Helicteres isora* L. Clin Phytosci. 2020;6:40. doi:10.1186/s40816-020-00156-1.

[18]. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. J Exp Pharmacol. 2023;15:51-62. doi:10.2147/JEP.S379805.

[19]. Kokate CK. Practical pharmacognosy. 13th ed. Mumbai: Vallabh Prakashan; 2009. p.107-109.

[20]. Bijekar SR, Gayatri MC. Phytochemical profile of *Codiaeum variegatum* (L.) Bl. Int J Pharmacol Pharm Sci. 2014;2(3):22-31.

[21]. Khandelwal KR. Practical pharmacognosy: techniques and experiments. 19th ed. Pune: Nirali Prakashan; 2008. p.149-156.

[22]. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 53rd ed. Pune: Nirali Prakashan; 2017. p.7.15-7.19.

[23]. Rahimah SB, Djunaedi DD, Soeroto AY, Bisri T. The phytochemical screening, total phenolic contents, and antioxidant activities in vitro of white oyster mushroom (*Pleurotus ostreatus*) preparations. Open Access Maced J Med Sci. 2019;7(15):2404-2412.

[24]. Grover N, Meena R, Patni V. Physicochemical evaluation, phytochemical screening and chromatographic fingerprint profile of *Woodfordia fruticosa* (L.) Kurz extracts. Int J Pharm Sci Res. 2014;5(7):2772-2782.

[25]. Pant DR, Pant ND, Saru DB, Yadav UN, Khanal DP. Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of *Pterocarpus marsupium* Roxburgh. J Intercult Ethnopharmacol.2017;6(2):170-176. doi:10.5455/jice.20170403094055.

[26]. Chandel HS, Pathak AK, Tailang M. Standardization of some herbal antidiabetic drugs in polyherbal formulation. Pharmacogn Res. 2011;3(1):49-56. doi:10.4103/0974-8490.79116.

[27]. Morgan DML. Tetrazolium (MTT) assay for cellular viability and activity. Methods Mol Biol. 1998;79:179-184.

[28]. van Meerloo J, Kaspers GJL, Cloos J. Cell sensitivity assays: the MTT assay. Methods Mol Biol. 2011;731:237-245.

[29]. Fotakis G, Timbrell JA. In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicol Lett. 2006;160(2):171-177. doi:10.1016/j.toxlet.2005.07.001.

[30]. Tihăuan BM, Berca LM, Adascălului M, Martínez Sanmartin A, Nica S, Cimponeriu D, et al. Experimental in vitro cytotoxicity evaluation of plant bioactive compounds and phytoagents: a review. Rom Biotechnol Lett. 2020;25(4):1832-1842. doi:10.25083/rbl/25.4/1832.1842.

[31]. Sharma V, Pracheta. Microscopic studies and preliminary pharmacognostical evaluation of Euphorbia neriifolia L. leaves. Indian J Nat Prod Resour. 2013;4(4):348-357.

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