Research Article

In vitro Anti-Cancer Activity of *Plectranthus amboinicus* (Lour.) Spreng. in DLA Cell lines

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ABSTRACT

Background: Today, the number of cancer patients is increasing because of lifestyle, food habits, radiation, etc. Chemotherapy and other medicines cause many problems in the normal body. Discovering new cancer drugs from nature is crucial. Many available medicines are chemically manufactured, leading to numerous side effects. Therefore, the current emphasis has shifted towards exploring natural products, including plants and plant-derived substances, to pave the way for a potentially safer future in cancer treatment. Many of the naturally occurring products have many medicinal properties as well as anticancer activities. Many investigations were carried out to study the anticancer activities of different plant products, including leaf, bark, root, rhizomes, tubers, etc., in search of new drugs. Phytochemicals are the compounds that are present in plants; they have antimicrobial, antifungal, anti-inflammatory, and anticancer activities. A study on the "toxicity of phytochemicals against intestinal and nasopharyngeal bacteria" was conducted in our lab during 2016. Methanol and acetone extracts of six medicinal plants (*Allium schoenoprasum, Cinnamomum zeylanicum, Plectranthus amboinicus, Ayapana triplinervis, Ocimum sanctum, and Piper nigrum*) in different concentrations were tested for their antibacterial properties. It was revealed that the methanol extract of *Plectranthus amboinicus* shows high antibacterial properties. **Aim:** The present study mainly aimed at investigating the phytochemicals present in the methanol extract of *P. amboinicus* and its anti-cancer activities on DLA cell lines. **Materials and Methods:** The plant powder is employed for both organoleptic study and qualitative phytochemical analysis. Following standard procedures, it was dispatched to the Amala Cancer Research Centre for additional anticancer studies. **Results:** The phytochemical analysis revealed that the methanol extract of *P. amboinicus* is rich in bioactive compounds with flavonoids, glycosides, terpenoids, steroids, tannins, quinones, reducing sugar, anthocyanin, phenol, and flavanol. The plant showed 4.2, 12.6, and 30.4% cytotoxicity in DLA cell lines at concentrations of 50, 100, and 200 µg/mL, respectively. There was no cell death in lower concentrations (10 µg, 20 µg). **Conclusion:** The results indicate that the plant exhibits a noteworthy variety of phytochemicals, and these compounds exert a direct impact on cancer cells at specific concentrations.

Keywords: *Plectranthus amboinicus*, Phytochemical, Anticancer activity, DLA cell lines.

INTRODUCTION

Medicinal plants have been employed in the treatment of numerous human and animal diseases since ancient times. They hold a significant role as an alternative to synthetic medicine in Indian Ayurveda. Even today, 60% of people around the globe rely on traditional herbal medicine.[1] A substantial number of leading active drug compounds are primarily derived from plant molecules.[2]

The importance of phytochemicals is increasing day by day. A large number of research discoveries are going on regarding the scavenging activities of phytoconstituents. The importance of future pharmacological valuations and the isolation of therapeutic antibiotics from plants

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was stressed.[3,4] The antimicrobial potential of 77 extracts from 24 plants was screened against 8 bacteria and 4 pathogenic fungi.[5] The analysis of phytochemicals against various human diseases was carried out.[6]

Cancer is a dangerous condition of the body, and the cases the world over are increasing day by day. A large number of deaths are caused by different types of cancer. The prevention of cancer relies significantly on the crucial role played by herbal medicines, which are readily available and relatively cost-effective. Approximately 62% of the anticancer drugs approved for commercial use between 1983 and 1994 out of the total 92 have a direct connection to their natural origin. Encouragement for research on plants exhibiting anticancer effects is substantial, aiming to unveil new drugs characterized by reduced toxic side effects and enhanced cost-effectiveness. Plant-derived natural compounds, encompassing flavonoids, terpenoids, alkaloids, saponins, tannins, glycosides, among others, have garnered significant attention in recent years. This attention is owed to their diverse pharmacological properties, inclusive of antioxidant and cancer-preventive effects. Given that free-radical damage is implicated in cancer development, certain natural products abundant in antioxidants have the potential to interact with these radicals, thereby preventing the associated damage.[7]

Effectively extracting drug candidates from natural product sources necessitates a meticulous selection of plants, extraction methods, and screening techniques geared towards the discovery of bioactive molecules.[8] A large majority of the wonder compounds in plants are undiscovered for their pharmacological activity. The extensive utilization of medicinal plants by tribal communities has not been adequately documented, hindering a comprehensive understanding of their phytochemical constituents and pharmacological potential.[9]

The members of the family Lamiaceae have been commercially important since the ancient period due to their aroma and the specific compounds they contain. Rich ethno-medicinal properties are possessed by various species such as Plectranthus, Salvia, Ocimum, Mentha, and others. In the Lamiaceae family, *Plectranthus amboinicus* (Loureiro) Spreng. stands out as one of the most extensively documented species.[4] Commonly known as Chempalaka, Njavara, Panikoorka, Kanjikoorka, Kannikoorka in Malayalam, Karpuravalli, Omavalli in Tamil, Patta Ajavayin, Patharcur in Hindi, and Country Borage in English, this succulent, aromatic perennial herb is highly distinctive. With its unique fragrance and pubescent nature, the plant features aromatic leaves. Widely distributed across India, it is commonly cultivated in gardens.The leaves of this plant have found application in treating various conditions, including malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, bronchitis, colic, and convulsions.[10] The plant was investigated for antifungal and antimicrobial activities by many researchers.[11-13] A novel abietanediterpene, 16-acetoxy-7-α,12-dihydroxy-8,12-abietadiene-11,14-dione, has been successfully isolated from the acetone extract of the root of *Plectranthus hereroensis*.[14] A study was conducted to investigate the phytochemical composition and larvicidal activity of various solvent leaf extracts of Plectranthus glandulosus against three significant vector mosquitoes-namely, *Anopheles gambiae*, *Aedes aegypti*, and *Culex*.[15] The anti-biofilm efficacy of *Plectranthus amboinicus* extract was carried out against the biofilm-forming *Streptococcus pyogenes* isolated from pharyngitis patients.[16]

The toxicity of phytochemicals in six medicinal plants (*Allium schoenoprasum, Cinnamomum zeylanicum, Plectranthus amboinicus, Ayapana triplinervis, Ocimum sanctum, and Piper nigrum*) in different concentrations was tested for their antibacterial properties against intestinal and nasopharyngeal bacteria in our laboratory.[12] The methanolic and acetonic plant extracts used in the study revealed that the methanolic extract of *Plectranthus amboinicus* has high antibacterial properties. There is only limited literature available on the anticancer studies of this plant in the DLA cell lines. Therefore, the present work was designed to comprehend the pharmacognostic features, chemical composition, and anti-cancer activity, the study aims to ascertain and validate the ethno-medical and pharmaceutical potential of *Plectranthus amboinicus*.

MATERIALS AND METHODS

Plant Material

Plectranthus amboinicus, chosen for the investigation, was gathered from Pandalam in the Pathanamthitta District of Kerala. The study focused specifically on the leaves of the plant.

Morphological study

The taxonomic identification and morphological analysis of the species *Plectranthus amboinicus* were studied using standard keys.[17] Observations were duly recorded.

Powder analysis

Fresh leaves of *Plectranthus amboinicus* were gathered in a polythene bag, and then meticulously washed with running tap water to eliminate any dirt. After drying at room temperature, the plant material was powdered

using an electronic blender and sifted through a fine mesh sieve. Subsequently, the resulting powder was employed for solvent extraction.

Organoleptic study

The assessment encompassed the examination of sensory organs and the macroscopic features of the plant material. Characteristics such as color, odor, taste, and overall nature were thoroughly evaluated.

Phytochemical screening

Extract Preparation and Percentage yield[18]

The extraction process involved subjecting approximately 15 g of powdered plant material to a Soxhlet apparatus with 100 mL of methanol. Subsequently, the extract was concentrated under reduced pressure and stored in the refrigerator for future use. The percentage of the crude extract was calculated using the following equation:

$$\text{Percentage yield}\,(\%) = \frac{\text{Weight of crude extract}}{\text{weight of sample}} \times 100$$

Qualitative analysis of the Extract

Various phytochemical constituents were assessed through the application of standard biochemical procedures.[19] These tests, conducted through standard biochemical procedures, aim to identify and characterize the diverse range of chemical compounds present in the plant extract, providing insights into its potential pharmacological activities. The plant extract was subjected to tests for the presence of various phytochemical constituents, including: Tannins, Saponins, Flavonoids, Alkaloids, Terpenoids, Phlobatannins, Glycosides, Simple phenolics, Quinones, Flavanols, Steroids, Xanthoproteins, Reducing Sugar, Anthocyanins, Oil, and Fat.

Anti cancer study by Trypan blue exclusion method

The anticancer effect of a crude methanol extract of *P. amboinicus* was studied using *DLA* cells. The crude methanol extracts from *P. amboinicus* at high concentrations damage the cells and make pores on the membrane through which Trypan blue enters. The damaged cells are stained with Trypan blue stain and can be distinguished from viable cells. Since live cells are excluded from staining, this method is also known as the dye exclusion method.[20]

Dalton's Lymphoma Ascites cells (DLA)

Preparations were made with crude methanol extract of *Plectranthus amboinicus* at varying concentrations (100, 500, and 1000 µg/mL).Cell suspensions (1×106 DLA cells in 0.1 mL) were introduced into tubes with different concentrations of test extracts (20, 50, 100, and 200 µg/mL). The volume was then adjusted to 1 mL using phosphate-buffered saline (PBS). In the control tube, only the cell suspension was present. The mixtures were then incubated for 3 hours at 37°C and supplemented with two drops of Trypan blue dye. Dead cells absorbed the blue color of Trypan blue, distinguishing them from live cells, which remained unaffected. The Trypan blue exclusion method was employed to assess the percentages of dead cells, with stained and unstained cell numbers counted separately.

$$\text{Percentage of dead cells} = \left[\frac{\text{Number of dead cells}}{\text{Number of viable cells} + \text{Number of dead cells}}\right] \times 100$$

RESULTS

Plant Morphology Analysis

An account of the morphological characters of *Plectranthus amboinicus is* given in Table 1.

Powder analysis

Organoleptic study

The plant powder exhibited the following characteristics:

Colour: Light green.

Smell: Astringent.

Taste: Lightly bitter.

Texture: Smooth.

Table 1: Morphological Characteristics of *Plectranthus amboinicus* (Lour.) Spreng.

Charachter	Description
Habit	Perennial herb.
Leaves	The opposite, aromatic, fleshy leaves measure 5.6x3.1 cm and are ovate or sub-orbicular. They have a cordate base, finely serrate margins, and an obtuse or acute apex. The petiole can reach up to 2 cm in length.
Flower	The flowers are shortly pedicelled and arranged in a dense thyrsoid panicle. The calyx, which is small and hispid, consists of an upper lip that is ovate and acute, while the lower lip has 4 acuminate lobes. The throat is glabrous within.
Petal	The corolla is purple and exhibits a 2-lipped structure, with a decurved tube.
Androecium	There are 4 stamens, arranged didynamously, with confluent anther cells.
Gynoecium	The ovary is 4-partite, and the stigma is 2-fid.
Fruit	The nutlets are smooth, orbicular, and ovoid, occurring in a set of four.

These observations provide valuable information about the visual, olfactory, and gustatory properties, as well as the texture of the plant powder.

Phytochemical screening

Extract-yield

In the given context, the yield of the methanol extract, prepared through Soxhlet extraction, is reported to be 4%. This indicates that 4% of the original plant material was obtained as the methanol extract.

Qualitative analysis of the Extract

In the methanol extract of the plant, a qualitative analysis of a total of 15 phytochemicals was conducted. Most of the compounds were detected in the extract, while phlobatannins, glycosides, steroids, gums, and mucilage were found to be absent. The specific phytochemical screening tests provided insights into the composition of the extract (Table 2).

The phytochemical analysis revealed that the methanolic extract of *P. amboinicus* is rich in bioactive compounds with flavonoids, glycosides, terpenoids, steroids, tannins, quinones, reducing sugar, anthocyanin, phenol, and flavanol. So it can be used as a natural drug for curing diseases like skin allergies, coughs, etc.

Table 2: The phytochemicals that have been tested in *Plectranthus amboinicus*.

Sl. No.	Phyto-constituents	Presence/Absence
1.	Flavonoids	+
2.	Glycosides	+
3.	Alkaloids	-
4.	Terpenoids	+
5.	Steroids	+
6.	Quinones	+
7.	Tannins	+
8.	Saponins	-
9.	Oils and fats	-
10.	Xanthoprotein	-
11.	Phlobatannins	-
12.	Reducing Sugar	+
13.	Anthocyanin	+
14.	Phenol	+
15.	Flavanol	+

Anticancer effects on DLA cells

The plant extract exhibits *in vitro* anticancer activity, with a proportional increase in the percentage of cell death as the concentration rises. The cytotoxicity rises in correlation with the increasing concentration of the drug. The methanol extract from *Plectranthus amboinicus* leaves displayed notable dose-dependent cytotoxicity against the tested cell lines, specifically DLA (Dalton's Lymphoma Ascites).The plant extracts showed 4.2, 12.6, and 30.4% cytotoxicity in DLA cell lines at concentrations of 50, 100, and 200 µg/mL, respectively. It showed high activity with increasing concentrations of the extract; there was no cell death at lower concentrations (10 µg, 20 µg) (Figure 1) (Plate 1).

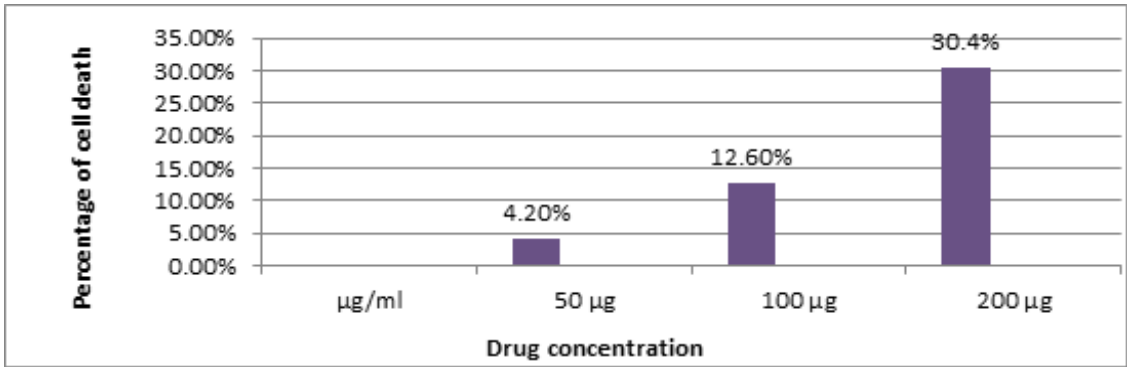
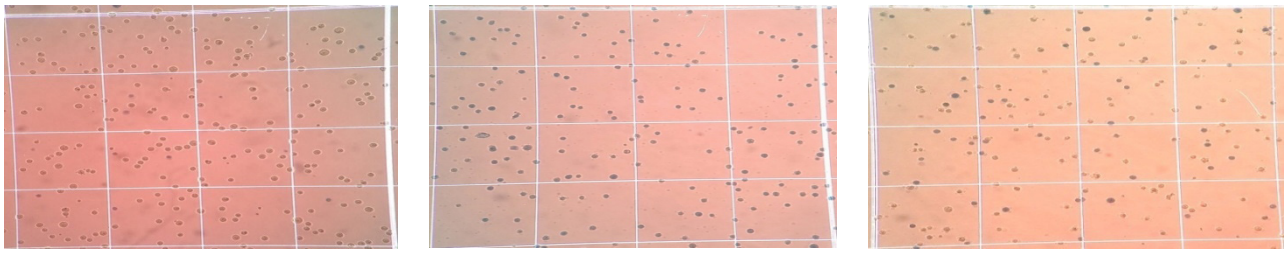


Figure 1: Percentage of cell death.



Live 100% (controlled) **100% Dead (controlled)** **30.4% Dead (200 µg)**

Plate 1: Effect of the extract on mouse DLA cell lines.

DISCUSSION

Many of the currently employed anticancer drugs are both highly toxic and costly, and the emergence of resistance mechanisms represents a significant challenge in cancer treatment. There persists a demand for the discovery of novel drug candidates that are not only more effective but also more readily available and less toxic. Plant extracts emerge as a crucial source of potentially valuable compounds, offering promise for the development of new and improved anticancer drugs.[21] DLA cells represent a rapidly growing carcinoma characterized by highly aggressive behavior, demonstrating the ability to proliferate in nearly all strains of mice. Ascitic tumor implantation induces local inflammatory reactions, fostering heightened vascular permeability. This process leads to the development of intense edema, cellular migration, and the progressive formation of ascitic fluid. Ascitic fluid is crucial for tumor growth as it serves as the direct nutritional source for tumor cells.[22] The extract obtained from *Plectranthus amboinicus* (Lour.) Spreng. demonstrated cytotoxic activity against the HeLa cell line.[23] A study employing HPLC-based metabolomics was conducted to identify cytotoxic compounds from *Plectranthus amboinicus* (Lour.) Spreng. against human breast cancer MCF-7 cells.[24] The decrease in viable cell count and the simultaneous rise in non-viable cancer cell count, shifting towards normal levels in the tumor host, indicate an apparent antitumor effect against EAC and DLA cells in mice.[25] The anticancer study's results indicated that the methanol extract derived from *Plectranthus amboinicus* leaves exhibited notable dose-dependent cytotoxicity, demonstrating remarkable anticancer activity against the tested cell lines, particularly DLA (Dalton's Lymphoma Ascites). The methanol extracts of *Plectranthus amboinicus* demonstrated cytotoxicity percentages of 4.2%, 12.6%, and 30.4% in DLA cell lines at concentrations of 50, 100, and 200 μg/mL, respectively. The *in vitro* anticancer study on DLA cell lines revealed high activity with increasing concentrations of the extract. Notably, no cell death was observed at lower concentrations (10 μg, 20 μg). The *in vitro* anticancer study on DLA cell lines revealed high activity with increasing concentrations of the extract. Notably, no cell death was observed at lower concentrations (10 μg, 20 μg).

The studied cells exhibited potential anticancer activity when treated with the selected plant. Previous studies on this plant, particularly in relation to oral carcinomas, have consistently demonstrated its effectiveness. Notably, in the *in vitro* anticancer study, the plant extract in methanol at a concentration of 200 micrograms proved highly effective, resulting in a 30.4% cell death rate. As the concentration of the extract increased, a significant decrease in viable cells was observed. The IC_{50} value of *P. amboinicus* against oral cancer cells was determined to be 53 micrograms per mL.[26] The ethanolic extract of *Plectranthus amboinicus* leaves contains flavonoid compounds that demonstrate exceptionally potent anti-cancer and antioxidant activities. Consequently, the ethanolic extract from these leaves exhibits robust antioxidant properties.[27] When administered to C57BL/6 mice via injection, the essential oil of *P. amboinicus* demonstrated a robust chemotherapeutic effect on the B16F-10 melanoma cell line.[28] Calculated IC_{50} values for cytotoxic activity against breast (MCF-7) and colorectal (HT-29) cell lines were 53±0.01 mg mL^{-1} and 87±0.01 mg mL^{-1}, respectively.[29] The current study primarily focused on DLA cell lines, without examining other carcinomas. Therefore, a more detailed investigation involving various types of cancer cells is warranted using the same plant extract. The extracts were prepared in methanol; however, exploring additional combinations in different solvents is essential to comprehend the broader potential effects of the plant on various carcinomas. The results obtained in the present investigation are highly significant, particularly given the limited exploration of specific cancer cells against *Plectranthus* leaf extracts. This information could prove invaluable for future studies, allowing for comparisons with other types of cancer cells. Notably, DLA and EAC, being important cancer cell types, have garnered increased attention and importance in ongoing research. The comprehensive exploration of this plant is imperative to uncover a multitude of remarkable compounds within it. One such example is the isolation of a novel abietanediterpene, 16-acetoxy-7-α,12-dihydroxy-8,12-abietadiene-11,14-dione, which has been successfully identified in the acetone extract of the root of Plectranthus hereroensis. This discovery underscores the potential for further in-depth investigations to reveal additional noteworthy compounds within the genus.[15] This study can serve as baseline data for future research aiming to discover drug compounds from *Plectranthus*. For this plant to be optimally beneficial in cancer research and treatment, it necessitates a more comprehensive investigation from various aspects.

CONCLUSION

Plants serve as reservoirs of potentially remarkable compounds, offering valuable insights for the

development of new drugs. Thorough investigation and validation of traditional medicine are crucial steps in comprehending the potential benefits of herbal drugs. The current study aimed to standardize the pharmacognosy, phytochemistry, and anticancer potential of the plant *Plectranthus amboinicus* (Lamiaceae). The data presented in this study will be instrumental in the synthesis of new anti-cancer drugs of pharmaceutical significance derived from *Plectranthus amboinicus*. *In vitro* human clinical trials are essential for comprehending the impact of the plant extract on human tumor cells. Traditionally, this plant has been effectively used to address various ailments, including the common cold. The elevated antioxidant activity of the plant could be attributed to the substantial presence of phytochemicals in the methanol extract. These findings not only validate the traditional medicinal uses but also underscore a promising potential for developing lead compounds from the plant, supported by scientific evidence. The plant extract demonstrated effectiveness against DLA-induced solid tumors and EAC-induced ascites tumors.

This discovery may be utilized to develop effective therapeutic approaches for the prevention or treatment of various types of cancer in human beings.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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