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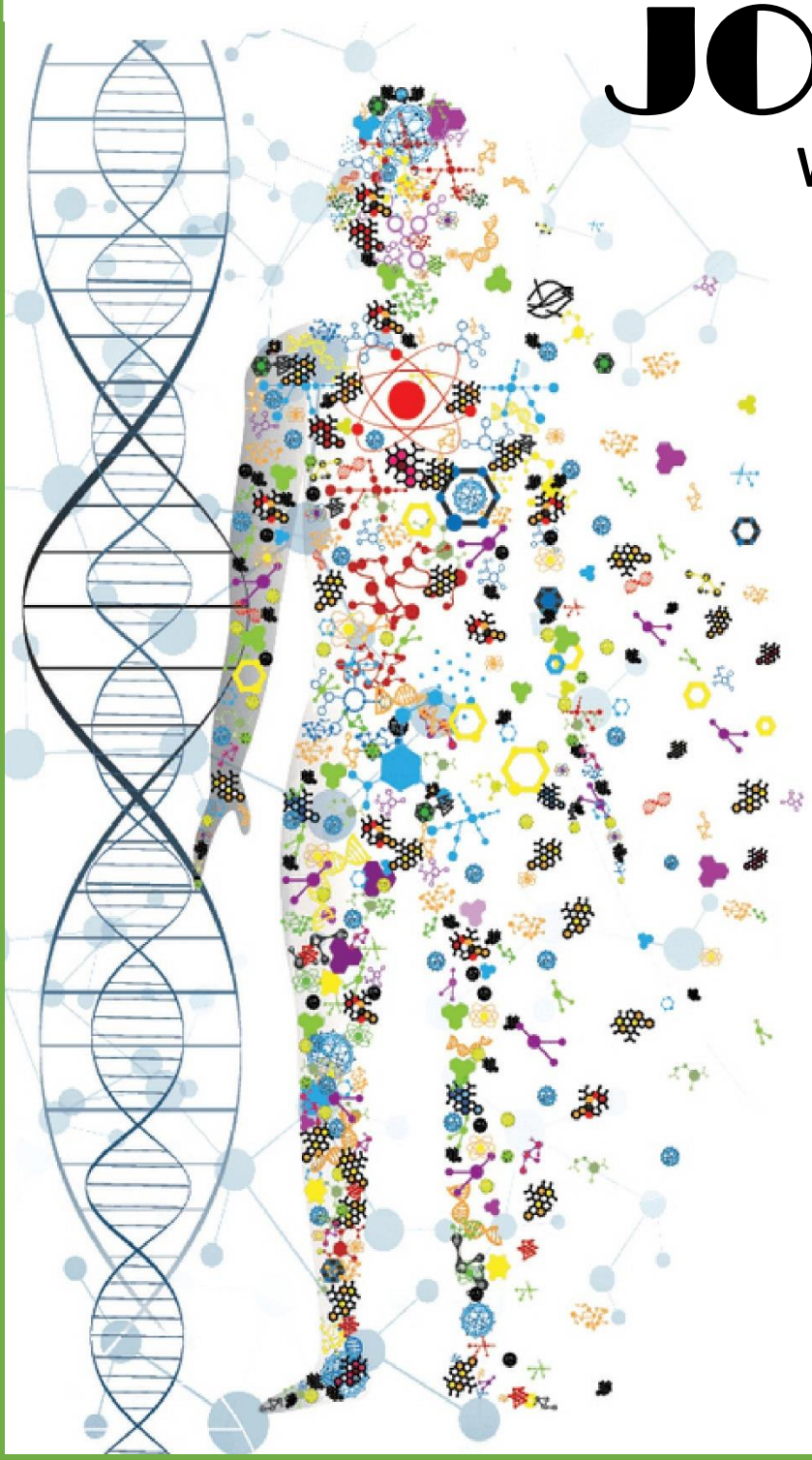

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PENTAS LANCEOLATA EXTRACT AND THEIR IN-VITRO COLON CANCER AGAINST HT-29 CELLINES

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ABSTRACT

Cancer of the colon, commonly referred to as colorectal cancer, is a form of the disease that may affect either the colon or the rectum, both of which are components of the large intestine. It is one of the malignancies that occurs most often all around the globe. The following is essential information and data on colon cancer. Cell lines are valuable tools for investigating various aspects of colorectal cancer, including its genetics, drug responses, metastatic potential, and molecular mechanisms [1]. They provide a controlled environment for researchers to conduct experiments and develop a better understanding of this disease. HT-29 is a well-known and widely used human colorectal cancer cell line. The shade dried flowers of Pentas Lanceolata leaves was subjected to successive solvent extraction method by using n-Hexane in soxhlet extraction.

The in-vitro assessment of anti-cancer activity was conducted using the HT-29 cell line, which is specific to breast cancer. The PL-2 compound, obtained from the n-Hexane extract, exhibited significant anti-cancer activity. A notable decrease in cell viability was observed, with the extent of reduction being dependent on both the duration of exposure and the dosage administered was observed [2].

INTRODUCTION:

Uncontrolled cell growth and dissemination are hallmarks of cancer. It is a complicated disease that may affect almost any tissue or organ and is a significant cause of mortality globally. The public and healthcare professionals must understand cancer basics. Cancer basics are covered in this introduction:

The Basic Nature of Cancer:

- **Cell Growth and Division**: In a healthy body, cells grow, divide, and replace old or damaged cells as part of a controlled and regulated process. However, cancer begins when cells lose this normal regulation.
- **Abnormal Cells**: Cancer starts with genetic mutations or changes in the DNA of a cell. These mutations can lead to the formation of abnormal cells that no longer respond to the body's signals to stop dividing and growing.
- **Tumor Formation**: As cancerous cells continue to divide and accumulate, they can form a mass of tissue known as a tumor. Not all tumors are cancerous; some are benign (non-cancerous), while others are malignant (cancerous).

Cancer Progression:

- **Local Invasion**: Malignant tumors have the ability to invade nearby tissues and organs. This can lead to damage and dysfunction of the affected tissues.
- **Metastasis**: A hallmark of cancer is its ability to spread to distant parts of the body. Cancer cells can break away from the primary tumor, enter the bloodstream or lymphatic system, and establish new tumors in other organs—a process known as metastasis.

Stage's of cancer:

- Stage 0 - Also called carcinoma in situ, this is the earliest stage where abnormal cells are found only in the layer of cells where they began.
- Stage I - Often called early-stage cancer, the cancer is small and localized to the original site. The cancer has not grown deeply into nearby tissues and hasn't spread to lymph nodes or other parts of the body.
- Stage II and III - These are considered locally advanced cancer. The cancer is larger than in Stage I and may have spread to nearby tissues and lymph nodes, but not to distant parts of the body.
- Stage IV - Known as metastatic or advanced cancer, this stage indicates that the cancer has spread from its original site to distant organs or tissues

Understanding these stages is vital because they help determining of Prognosis, Treatment options (surgery, chemotherapy, radiation, immunotherapy) [3-10].

Colon cancer is a significant public health concern, but early detection and advances in treatment have improved outcomes. Regular screenings and awareness of risk factors and the presence of symptoms is very necessary for the early identification and effective treatment of this illness. It is essential to confer with a medical expert in order to get individualized recommendations for screening and preventative measures based on one's particular set of risk factors. Colon cancer is staged to determine the extent and severity of the disease, which helps guide treatment decisions and provides information about prognosis. The staging of colon cancer is typically done using the TNM system, which considers three key factors: T (tumor), N (lymph nodes), and M (metastasis)[11].

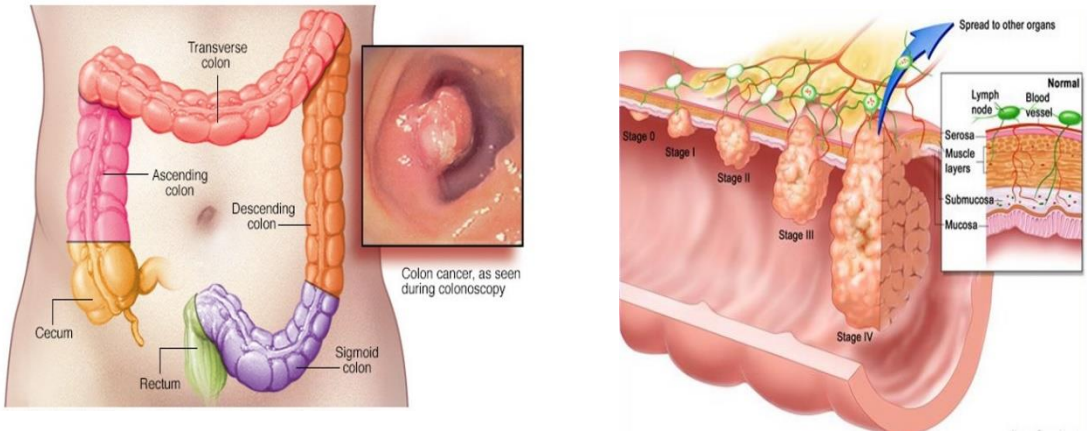


FIG-1. Colorectal cancer and its stages.

HT-29 is a well-known and widely used human colorectal cancer cell line. It has been a valuable tool in cancer research, particularly for studying colorectal cancer and related areas. Here is some key information about the HT-29 cell line:

- **Origin:** HT-29 cells were originally derived from a human colorectal adenocarcinoma tumor. They were isolated from the tumor tissue of a 44-year-old male patient and have since been cultured in laboratories for research purposes.
- **Characteristics:** HT-29 cells exhibit characteristics typical of colorectal cancer cells. They are epithelial in nature and have a moderate degree of differentiation. This cell line is considered moderately tumorigenic and can form tumors when injected into animals, making it useful for in vivo studies.
- **Research Use:** HT-29 cells have been extensively used in colorectal cancer research. Researchers use them to investigate various aspects of colorectal cancer biology, including cell growth, invasion, metastasis, drug responses, and molecular signalling pathways [12-15].

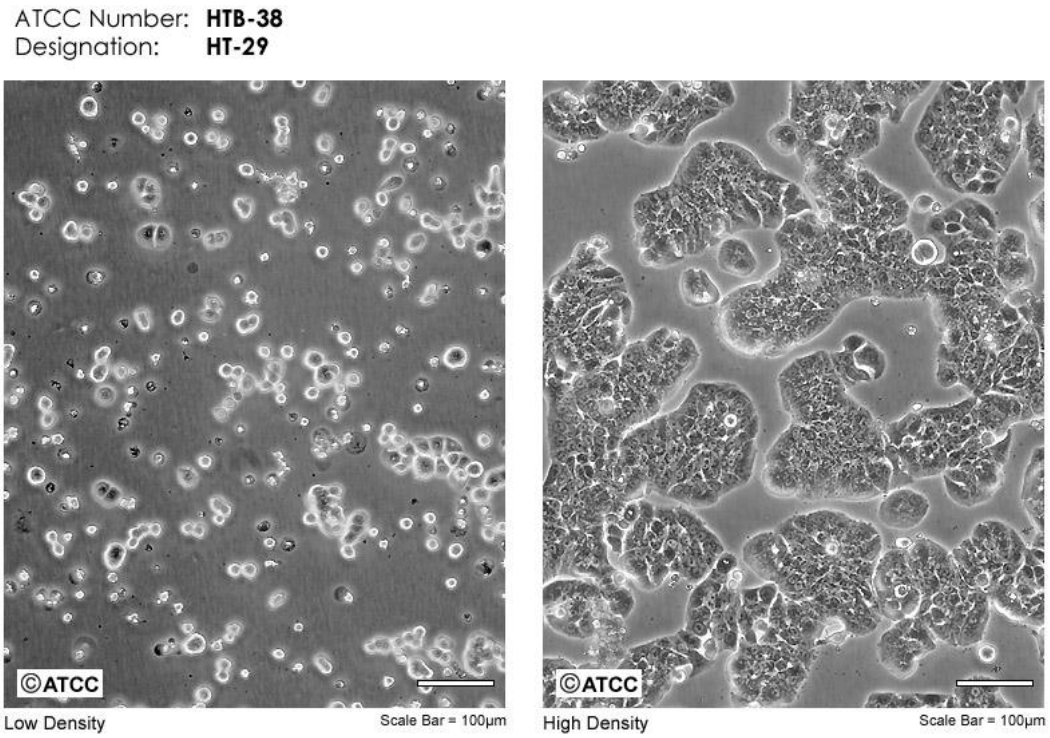


FIG-2. Cell morphology of HT-29 colon cancer cells.

Plant introduction:

1. Appearance: *Pentas lanceolata* is a perennial plant that typically grows to a height of 1 to 3 feet (30 to 90 centimeters). It has opposite lance-shaped leaves that are usually dark green. The most striking feature of *Pentas lanceolata* is its clusters of tubular flowers that form at the tips of stems. These flowers are arranged in rounded, star-shaped clusters, giving the plant its common name, "Egyptian starcluster". Flower colors can vary and include shades of pink, red, white, and lavender. Some varieties have multicolored flowers.

2. Bloom Period: *Pentas lanceolata* is known for its long bloom period, often producing flowers from spring to fall, and sometimes even year-round in suitable climates.

3. Habitat and Growing Conditions: In its native habitat, *Pentas lanceolata* is found in savannas and grasslands. It prefers warm, tropical to sub-tropical climates. It thrives in well-draining soil and full to partial sunlight. It is relatively drought-tolerant once established but benefits from regular watering in dry periods.

4. Use in Landscaping: *Pentas lanceolata* is a popular choice for landscaping due to its vibrant and long-lasting flowers. It is often used in flower beds, borders, and as a container plant. It attracts pollinators, including butterflies and hummingbirds, making it a favorite for butterfly gardens.

5. Varieties: There are several cultivated varieties and hybrids of *Pentas lanceolata* with different flower colors and growth habits, allowing for a range of landscaping possibilities.

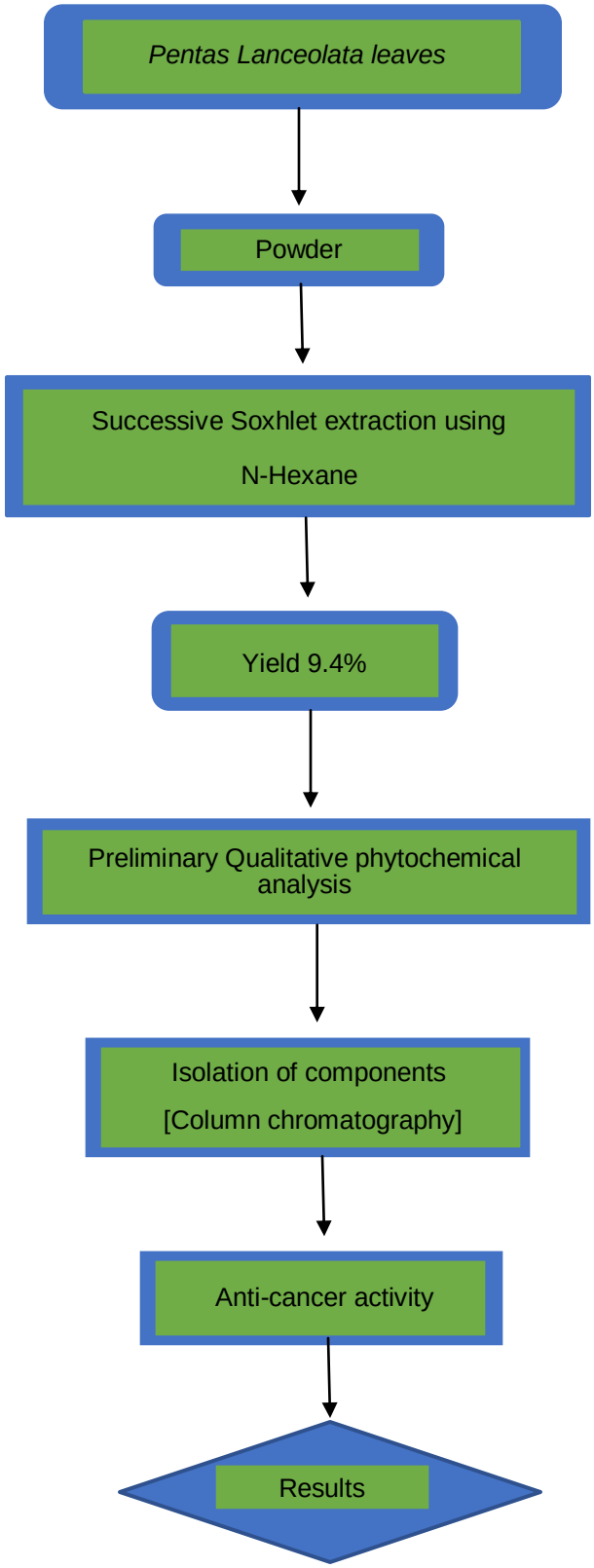
6. Maintenance: Maintenance of *Pentas lanceolata* typically involves deadheading (removing spent flowers) to encourage continuous blooming. Pruning can also help maintain a compact and bushy growth habit.

7. Other Names: Besides "Egyptian starcluster," *Pentas lanceolata* may also be known by other common names, including "starflower" and "African starflower."

8. Wildlife Attraction: This plant is known for its ability to attract various pollinators, which can help promote biodiversity in gardens and landscapes.

FIG-3. *Pentas lanceolata plant*.

METHODOLOGY:



The shade dried leaves powder of *Pentas Lanceolata* were subjected to successive solvent extraction method by using n-Hexane in Soxhlet extraction.

Phytochemical analysis was carried for the crude extract according to the method of Siddiqui and Edeoga. The phytochemicals analysed were Sterols, Triterpenoids, Flavonoids, Saponins, Carbohydrates, alkaloids, Tannins, Proteins, Glycosides. [16-18]

S.no	Chemical test	Reagents used (test performed)	Result
1.	Test for Sterols	Salkowaski Test	+
2	Test for Triterpenoids	Liberman-buchard Test	+
3	Test for Flavonoids	Shinoda Test	-
4	Test for Saponins	Foam test	+
5	Test for Carbohydrates	Molish's test	+
		Felhings Test	+
		Benedict's Test	+
6	Test for alkaloids	Mayer's Test	-
		Wagner's Test	-
		Hager's Test	-
		Dragendroff's test	-
7	Test for Tannins	Ferric Chloride Test	-
8	Test for Proteins	Biuret test	_
		Millon's test	_
9	Test for Glycosides	Legal Test	+
		Kellarkillyani Test	+

+Sign indicates presence; ++ sign indicates more Quantity; and – Sign indicates absence

Table 1. Phyto chemical Screening.

Quantitative analysis of crude extracts:

Solvent	Yield
N-Hexane	9.4 %

Table 2. Yield %.

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Isolation of phyto ingredients from N-Hexane extract: In a hot air oven at 110 degrees Celsius for one hour, 100 grams of LR-grade silica gel were warmed for column chromatography. Glass wool was used to build the floor. The column was gently tapped after each addition of activated silica gel to maintain packing throughout the experiment. While filling, little volumes of activated silica gel were put into the column. The column was tapped to remove air bubbles and uniformize the adsorbent bed. For column effectiveness, this was done. After dissolving the concentrated n-Hexane extract from Pentas lanceolata leaves in a little quantity of solvent, silica gel was put onto the column. 25 grams of extract was utilized. The chromatogram developed naturally overnight while the column's open end was covered with cotton to avoid drying. It was done to preserve the chromatogram. After the column was saturated with sample (PL-2), the solvent system began elution.

Biological activity:

Assay Principle:

The colorimetric MTT Cell Proliferation Assay quantifies and tracks cell proliferation. The package contains enough reagents for 960 96-well or 192 24-well tests. Plated cells may be treated with chemicals that affect proliferation. The proliferation reagent identifies the cells. Live cells convert yellow tetrazole MTT into purple formazan. Cell multiplication increases signal intensity, whereas a decrease may indicate harmful chemicals or poor culture conditions. The basic test concepts may be used to a broad variety of eukaryotic cell lines, including adherent and non-adherent cells and tissues. The cell proliferation reagent detects bacteria, yeast, fungus, protozoa, cultured mammalian, and piscine cells.[19-25]

Testing Procedure:

Plate and cultivate HT-29s separately on 96-well tissue culture plates with a clear flat bottom (100 µL per well). Cells that adhered were examined. Cell range should be between 5,000 and 8,000 cells in each well. HT-29 lines were tested in triplicate using n-Hexane extract of the sample at concentrations of 10, 50, 100, 150, 200, 250, and 300 µg/ml. For reference, control and negative controls were grown in triplicate. The culture times for each cell were 24, 48, and 72 hours. For four hours at 37°C, at the designated intervals, incubate all working wells of plates with 15 µL of MTT reagent solution per 100 µL cell culture. Make sure the volume of the reagent matches the volume of the cell culture. Using an orbital shaker, slowly mix 100 µL of DMSO in each well for an hour at room temperature. The DMSO volume should be adjusted to match the cell culture volume. Shake the container at 37°C or in a warm water bath to dissolve precipitates in the Solubilizer. An absorbance plate reader was used to measure the absorbance of each well at OD 570 nm. At 560–590 nm, formazan dye absorbs the most. If necessary, OD might be tested the next day. Evaporation is decreased by sealing the plate [26-28].

RESULTS:

Conc in µg/ml	% Viability at 24 hrs	% Viability at 48 hrs	% Viability at 72 hrs
10	91.26	88.36	87.12
50	71.18	69.82	67.36
100	62.84	60.37	58.02
150	53.68	51.38	48.39
200	41.53	39.32	38.27
250	33.08	31.28	29.54
300	21.35	20.28	19.49
IC50 in µg/ml	IC50 = 186.28 ± 1.02	IC50 = 184.73 ± 1.03	IC50 = 180.01 ± 1.09
Doxorubicin	IC50 = 52.37 ± 0.7 µM	IC50 = 49.13 ± 0.5 µM	IC50 = 48.62 ± 0.4 µM

Table 3. % Viability of HT-29s at 24, 48, 72 hrs on treatment of PL-2 sample extract.

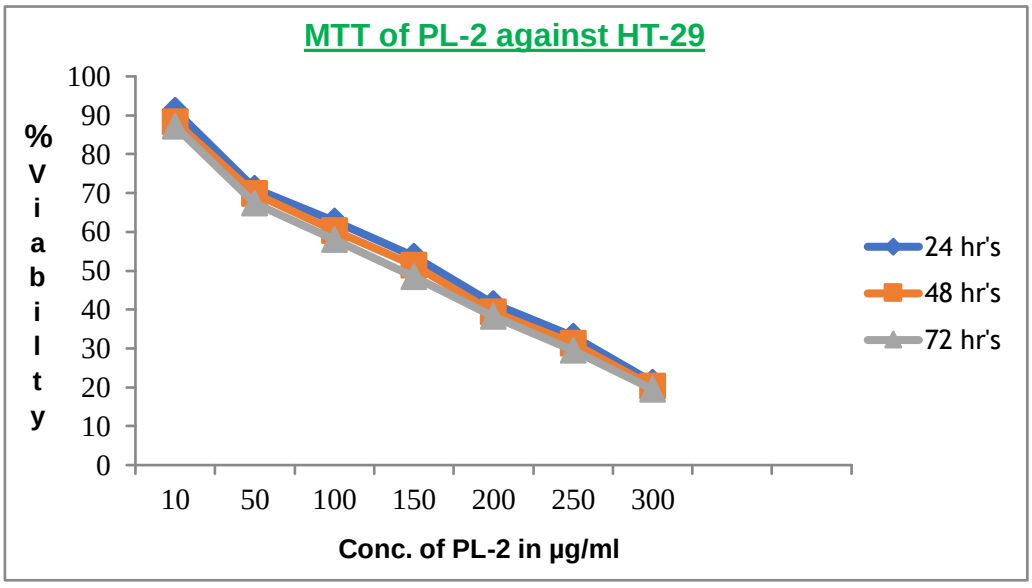

FIG-4. MTT results of n-Hexane isolated extract of *Pentas Lanceolata* leaves Sample on HT-29 cell lines at Three time points. IC_{50}: 186.28 µg/ml at 24 hr; 184.73 µg/ml at 48 hr; 180.01 µg/ml at 72 hr.

The MTT assay was conducted to evaluate the impact of the n-Hexane extract derived from the PL-2 sample on HT-29 cell lines. The cells were subjected to treatment with PL-2 extract at concentrations of 10, 50, 100, 150, 200, 250, and 300 µg/ml for durations of 24, 48, and 72 hours. A notable decrease in cell viability was observed, with the extent of reduction being dependent on both the duration of exposure and the dosage administered. The IC50 values for the isolated extract of PL-2 were determined as follows: 186.28 µg/ml at 24 hours, 184.73 µg/ml at 48 hours, and 180.01 µg/ml at 72 hours for HT-29. A graph depicting the percentage viability (%viability) versus concentration (µg/ml) has been generated based on the observed data. The graph illustrates different time points, namely 24 hours, 48 hours, and 72 hours, as shown in Figure 4 [29].

CONCLUSIONS:

The in-vitro assessment of anti-cancer activity was conducted using the HT-29 cell line, which is specific to breast cancer. The PL-2 compound, obtained from the n-Hexane extract, exhibited significant anti-cancer activity. The IC50 values for the isolated extract were determined to be as follows: 186.28 µg/ml at 24 hours, 184.73 µg/ml at 48 hours, and 180.01 µg/ml at 72 hours, respectively. The current study demonstrates that the n-hexane extract of PL-2 sample exhibits potent anti-cancer activity on HT-29 cell lines. [27-29].

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