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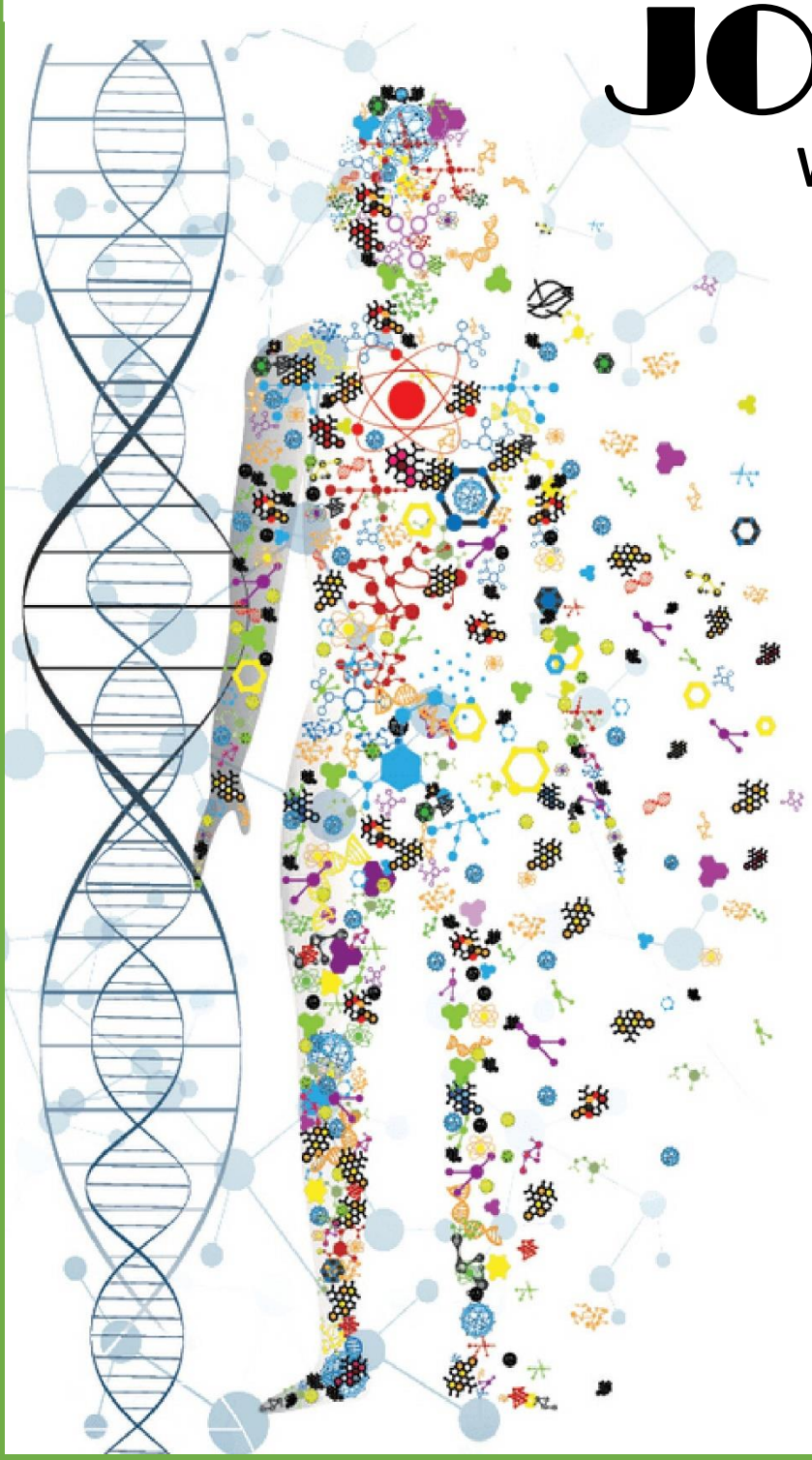

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IN-VITRO ANTI COLON CANCER ACTIVITY OF ARAUCARIA HETEROPHYLLA LEAVES EXTRACT AGAINST HT-29 CELLINES

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ABSTRACT

Colorectal cancer (CRC) is a significant global health concern, representing one of the most prevalent malignant neoplasms of the gastrointestinal tract. It typically develops from adenomatous polyps through a complex multistep process involving genetic and epigenetic alterations. The disease predominantly affects individuals over 50, though increasing cases are being reported in younger populations. Risk factors include family history, inflammatory bowel disease, obesity, sedentary lifestyle, high consumption of red meat, and low-fiber diet. The five-year survival rate varies significantly based on the stage at diagnosis, highlighting the critical importance of early detection and intervention. 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 leaves of *Araucaria heterophylla* 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 colon cancer. N-Hexane extract of *Araucaria heterophylla* Leaves, 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:

Cancer is a complex disease that occurs when cells in the body begin to grow uncontrollably due to genetic mutations. Think of it like a factory where the quality control system has broken down - cells that would normally be identified as faulty and eliminated are instead allowed to multiply rapidly and spread. These abnormal cells can invade nearby tissues and even travel to other parts of the body through the blood or lymphatic system. Symptoms may include changes in bowel habits, rectal bleeding, abdominal pain, and unexplained weight loss, though early stages often remain asymptomatic. Early detection through screening methods like colonoscopy, fecal occult blood testing, and sigmoidoscopy significantly improves survival rates. Treatment approaches vary based on stage and location, typically involving surgical resection as the primary intervention, often complemented by chemotherapy and/or radiation therapy. Recent advances in molecular targeting and immunotherapy have expanded treatment options. Prevention strategies emphasize lifestyle modifications, regular screening, and management of modifiable risk factors.

The staging of cancer is a crucial system developed to describe how much cancer is present in the body and where it is located. This information helps doctors determine the most appropriate treatment and predict outcomes.

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].

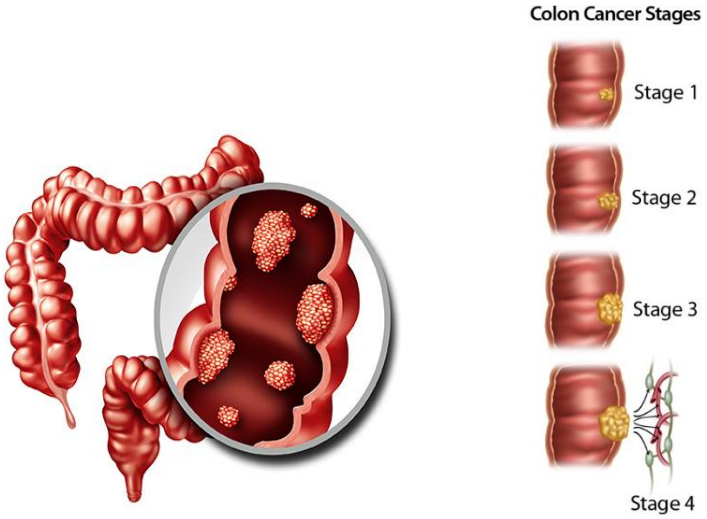

FIG-1. Colorectal cancer and its stages.

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].

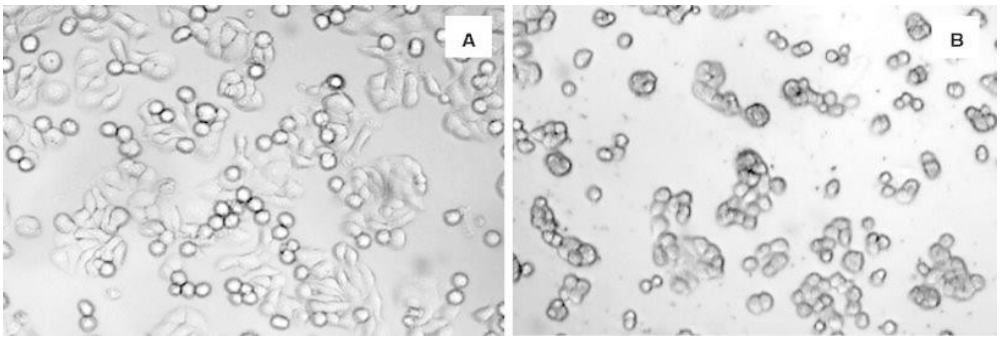

FIG-2. Cell morphology of HT-29 colon cancer cells.

Plant Profile:

Araucaria are mainly large trees with a massive erect stem, reaching a height of 30–80 metres (98–260 ft). The horizontal, spreading branches grow in whorls and are covered with leathery or needle-like leaves. In some species, the leaves are narrow awl-shaped and lanceolate, barely overlapping each other, in others they are broad and flat, and overlap broadly. The trees are mostly dioecious, with male and female cones found on separate trees, though occasional individuals are monoecious or change sex with time. The female cones, usually high on the top of the tree, are globose, and vary in size between species from 7 to 25 centimetres (2.8 to 9.8 in) diameter. They contain 80–200 large, edible seeds, similar to pine nuts though larger. The male cones are smaller, 4–10 cm (1.6–3.9 in) long, and narrow to broad cylindrical, 1.5–5.0 cm (0.6–2.0 in) broad. The genus is familiar to many people as the genus of the distinctive Chilean pine or monkey puzzle tree (Araucaria araucana). The genus is named after the Spanish exonym Araucano ("from Arauco") applied to the Mapuches of central Chile and south-west Argentina whose territory incorporates natural stands of this genus. The Mapuche people call it pehuén, and consider it sacred. Some Mapuches living in the Andes name themselves Pehuenches ("people of the pehuén") as they traditionally harvested the seeds extensively for food. No distinct vernacular name exists for the genus. Many are called "pine", although they are only distantly related to true pines, in the genus Pinus.[16-18]

FIG-3. Leaves of Araucaria heterophylla.

Taxonomical classification Kingdom:

Plantae Division: Pinophyta

Class: Pinopsida

Order: Pinales

Family: Araucariaceae

Genus: Araucaria

Species: A. heterophylla

Binomial name: Araucaria heterophylla (Salisb.) Franco

METHODOLOGY:

The shade dried leaves powder of *Araucaria Heterophylla* 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 alkaloids, amino acids and proteins, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, tannins, and terpenoids.

S.no	Phytochemical test	Reagents used (test performed)	observation	Result
1.	Test for Alkaloids	Mayer's Test	Formation of cream precipitate	++
		Hager's test	Formation of yellow colour	++
2	Test for Glycosides	Borntrager's test	No Formation of pink colour	_
3	Test for Tannins	Gelatin test	Formation of white colour ppt	+
4	Test for Saponins	Foam test	No Froth formation	_
5	Test for Flavonoids	Lead acetate test	Yellow ppt	+
		Alkaline reagent test	Yellow colour solution turns to colourless	+
6	Test for Phenols	Ferric chloride test	Formation of Blueish black colour	++
7	Test for Carbohydrates	Molisch's test	Formation of violet ring	++
		Fehling's test	Formation of red ppt	++
8	Test for Proteins and amino acids	Ninhydrin test	No Formation of blue colour	_
		Millon's test	No formation of white ppt	_

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

Table 1. Phyto chemical Screening.

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	92.61	89.98	87.78
50	78.81	70.28	67.36
100	65.46	63.7	58.64
150	53.86	51.73	48.42
200	41.35	38.74	36.48
250	38.08	35.64	29.57
300	23.39	20.19	19.15
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**

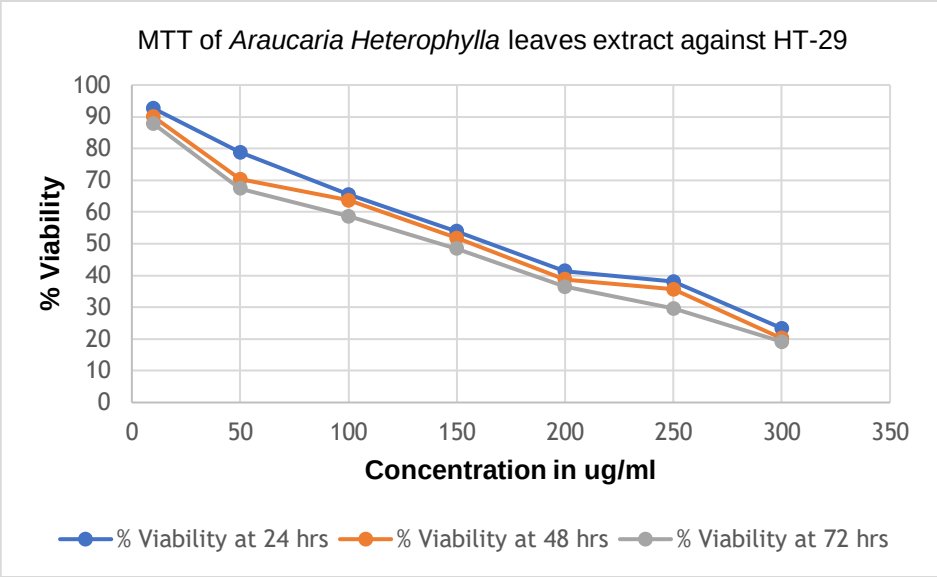

FIG-4. MTT results of n-Hexane isolated extract of *Araucaria Heterophylla* leaves Sample on HT-29 cell lines at Three time points. IC_{50}: 186.28 µg/ml at 24 hr; 184.73 µg/ml at 48 hr; 180.01 µg/ml at 72 hr.

The MTT assay was conducted to evaluate the impact of the n-Hexane extract derived from the extract of *Araucaria Heterophylla* leaves on HT-29 cell lines. The cells were subjected to treatment with extract of *Araucaria Heterophylla* leaves 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 extract of *Araucaria Heterophylla* leaves 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 FIG-3[29].

CONCLUSION:

The in-vitro assessment of anti-cancer activity was conducted using the HT-29 cell line, which is specific to colorectal cancer. Extract of *Araucaria Heterophylla* leaves, obtained from the n-Hexane, 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 *Araucaria Heterophylla* leaves exhibits potent anti-cancer activity on HT-29 cell lines[30-32].

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