Research Article

In-Vitro Study of Anticancer Activity and Isolation of Active Constituent from *Achyranthes Aspera* Linn Leaf Extract

Miss. Pranali Patil[1]* Mrs. Bhavana U. Jain[2]

[1]*PG student, Appasaheb Birnale College of Pharmacy, South Shivaji Nagar, Sangli

[2]Assistant Professor, Appasaheb Birnale College of Pharmacy, South Shivaji Nagar, Sangli

---

ABSTRACT

Achyranthes aspera L. a tiny herb of to the Amaranthaceae family that has numerous industrial and therapeutic uses, making it extremely valuable economically in developing nations. *Achyranthes aspera* L. leaves were extracted with pet ether, chloroform, and ethanol using the Soxhlet extraction method. Globally, cancer is a problem for public health. The use of medicinal plants in cancer treatment has significantly increased cancer patients' chances of survival and quality of life. Natural drugs are largely responsible for the pharmaceutical cure, while manufactured drugs are more harmful to an animal's body. Numerous plants and their individual components have been demonstrated to have anticancer potential.

Achyranthes aspera L. leaf extract shows the presence of flavonoids, alkaloids, saponins, glycosides, proteins, amino acids & tannins. Isolation is done by TLC and Column Chromatography and characterization is done by spectroscopic methods UV and IR it shows presence of Betaine (Alkaloid). The activity was carried out using ethanolic leaf extract, and the in-vitro test was conducted against MCF7 (breast cancer cells). When compared to the common medication 5FU, the Ethanol Extract exhibits a higher percentage of inhibition against breast cancer cell lines at various concentrations. So, we can conclude that the *Achyranthes aspera* L. leaf extract samples exhibit good anticancer activity against breast cancer cell lines based on the percent of inhibition.

KEY WORDS: - *Achyranthes aspera* Linn, In-vitro anti-cancer activity, ethanol extract, Breast cancer, Phytochemical analysis, Spectroscopic methods.

INTRODUCTION

Uncontrollably growing and spreading aberrant cells is the hallmark of cancer sickness. Malignant tumors are the end result of several alterations in cell physiology that occur in cancer, an incredibly complicated disease. Natural products have been used for thousands of years in medical care and illness prevention. Evidence of the utilization of natural sources for disease cures can be found in the ancient cultures of North Africa, India, and China. (1)

Traditional medical systems include a large portion of natural medications. One success of popular therapeutic diversity is herbal medicine. Since the beginning of time, people have utilized plants and other products as medicine because they are readily available, affordable, and meet immediate needs. Over 82% of people worldwide receive their main healthcare from traditional herbal medicine, according to the World Health Organization. Many chemicals with potential therapeutic uses are produced by medicinal plants. Conventional chemotherapy medications cause cell death, but their ability to harm healthy cells limits their use. It is crucial to identify natural agents that successfully display apoptosis and cell cycle regulating characteristics while also exhibiting little toxicity to normal cells. These agents can be either plant extracts or bioactive compounds. (2)

Achyranthes aspera is a member of the Amaranthaceae family and is a ubiquitous wasteland herb found in tropical Asia, Africa, Australia, and America. It is a shrub that can reach a height of 1-2 meters. It has obovate or greenish spikes that are elliptic and opposite, bracteates and bracteolate flowers, simple leaves, oblong utricles for fruits, and solitary, inverse seeds. It is regarded as a therapeutic plant in several Indian medical systems. The *Achyranthes aspera* plant is bitter, pungent, stomachic, laxative, carminative, and has limited value in treating heart illness, bronchitis, vomiting, abdominal pain, piles, and itching, according to Ayurveda. (3)

The recent research work was undertaken to evaluate the anticancer activity from Ethanolic leaf extract of *Achyranthes aspera* against breast cancer cell lines.

MATERIALS AND METHODS

Plant material:

Fresh leaves of *Achyranthes aspera* L. were collectedin January 2024, from Sangli, Maharashtra, India. Before drying, wash all leaves to remove dirty, dusty leaves. The main drying process is the elimination of internal moisture. For 2 weeks, shade drying was done in an environment with natural air flow and a temperature of 25°C. The dried plant material can be stored for an extended period of time. the leaves dried completely; we used a mixer grinder to make them into a granular powder.

PREPARATION OF EXTRACTS

Soxhlet Extraction: -

Organic solvent extraction is carried out by using Soxhlet extraction. Extraction was performed with organic solvents such as pet ether, chloroform, and ethanol. About 30 g of dry leaves powder of *Achyranthes aspera* L. was extracted by pet ether by continuous Soxhlet extraction. The extraction was continued until the solvent became colorless. The pet ether extract was filtered, and the powder in the extraction apparatus was removed from the extractor, dried, and then used for extraction with chloroform and ethanol. These pet ether, chloroform, and ethanol extracts were stored in separate bottles and labelled. Ethanol extract was further used for anti-cancer activity. [4] [5]

CELL LINES AND CELL CULTURE

MCF7 (Breast Cancer Cells)

Media: DMEM with high glucose (Cat No-11965-092), FBS (Gibco, Invitrogen) Cat No -10270106Antibiotic – Antimycotic 100X solution (Thermo fisher Scientific)-Cat No-15240062.

PHYTOCHEMICAL ANALYSIS

The plant extracts were screened for phytochemicals for the detection of the flavonoids, alkaloids, steroids, tannins, saponins, glycosides, proteins and amino acids. [6]

PHARMACOLOGICAL SCREENING

IN VITRO STUDY OF ANTI-CANCER ACTIVITY BY MTT ASSAY

Procedure: -

For 24 hours, at 37 degrees Celsius and 5% CO2, cells were cultured in culture media at an amount of 1 × 104 cells/ml. In micro plates (tissue culture grade, with 96 wells), cells were planted at a concentration of (100µl) 104cells/well) in 100µl culture media and 20, 40, 60, 80, and 100 µg/ml of samples, respectively. Cell line and Solvent (0.2% in PBS) were cultured in control wells. Each sample underwent three rounds of incubation. To find the proportion of cells that survived after culture and the survival rate of control cells, controls were kept in place. In a CO2 incubator, cell cultures were cultured for 24 hours at 37°C and 5% CO2. The medium was totally withdrawn after incubation, and 20µl of the MTT reagent (5 mg/min PBS) was added. Cells were incubated in a CO2 incubator at 37°C for 4 hours following the injection of MTT. Used a microscope to examine the holes for formazan crystal growth. Only live cells were able to convert the yellowish MTT to a dark-colored formazan. Following the full removal of the medium. added 200µl of DMSO, let it sit for ten minutes, and then covered with aluminum foil, incubate at 370C. A microplate reader operating at 550 nm was used to measure the absorption of each of the three samples that were tested. (7) (8) (9)

ISOLATION AND IDENTIFICATION OF PHYTOCONSTITUENTS

Thin Layer Chromatography: -

Chromatography is a process for breaking down mixtures of substances into their individual components. Silica gel G is an adsorbent used in TLC. A silica gel slurry was produced in distilled water. The slurry was applied to a glass plate measuring 7.5 by 2.5 cm to form a 0.2 mm-thin layer. Before activation at 110 ^{0}C for an hour, the Plates were air dried. The sample was applied via an applicator. The slide was kept for the creation of a solvent system. To increase component resolution, a range of solvent solutions were used.

Several solvent solutions were tested, but **Chloroform: Methanol (8:2)** produced the finest constituent resolution (10)

Calculation: -

The retention factor (Rf) value was determined using the formula below

$$\text{Rf value} = \frac{\text{The length of the solute front (cm)}}{\text{The length of the solvent front (cm)}}$$

Column Chromatography: -

The stationary phase was 150–200 grams of silica gel (100–200 mesh). For an hour, it was turned on in a hot air oven at 110°. For separation, a glass column three times longer than the total volume of adsorbent was utilized. A benzene-prepared slurry of activated silica gel was added to a column that had already dried. To keep the prepared column from drying out, a small amount of benzene was kept on top of it. The ethanolic extract of Achyratthes aspera Linn was added to the column without causing any disturbances after it had set. [(11)]

Elution of Mobile Phase:

From TLC, the mobile phase was chosen. **Chloroform: Methanol (8:2)** was saturated and then introduced into column. Column chromatography required a substantial amount of mobile phase, which was manufactured in accordance with TLC mobile phase. Several fractions were created by separating the active ingredients. By modifying the exit valve, the flow rate could be managed. The flow rate is kept constant until the active ingredient has divided into distinct portions. [(12)]

SPECTROSCOPIC ANALYSIS

Ultraviolet Spectroscopy:

UV-visible analysis was performed on the isolated chemical. The UV analysis was carried out at A.B.C.P., Sangli using a UV spectrophotometer (JASCO V- 730). By combining an isolated chemical with ethanol, a sample with a concentration of 10 g/ml was created.

Fig. no.1 – UV spectrophotometer (JASCO V- 730)

Infrared Spectroscopy:

One of the most potent analytical methods that permits the potential of identifying chemical structures is infrared spectroscopy. Functional groups in a compound's structure are easier to recognize via IR. Concentration and desiccation were used to dry the isolated chemical from the column chromatography. A pellet was made using a KBr press after the dry chemical had been triturated with KBr. The analysis was carried out using FTIR of Jasco company at Appasaheb Birnale College of Pharmacy, Sangli.

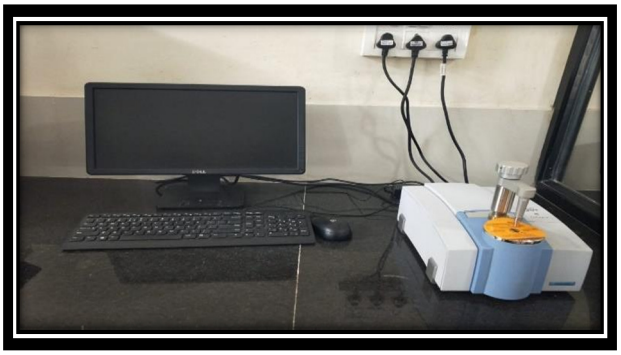


Fig. no.2 – IR instrument (JASCO FTIR- 410)

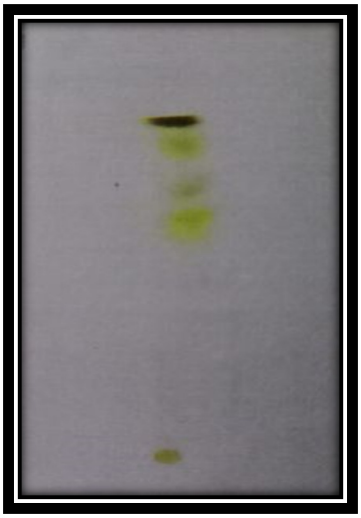
RESULTS AND DISCUSSION

Table No. 01 - Effects of compound against MCF7 Cell lines

Sr. No.	Sample Code	Conc. (µg/ml)	OD			Mean	% Of Inhibition	% Of Viability	IC50 (µg/ml)
1	Control		1.307			-	-	-	-
2	Standard	**20**	1.007	1.007	1.007	**1.007**	**22.95%**	**77.04%**	
	(5,Flurour -acyl)	**40**	0.923	0.920	0.923	**0.922**	**29.45%**	**70.54%**	
		60	0.639	0.638	0.639	**0.638**	**51.18%**	**48.81%**	**54.59**
		80	0.358	0.356	0.357	**0.357**	**72.68%**	**27.32%**	
		100	0.311	0.310	0.311	**0.311**	**76.20%**	**23.80%**	
3	EE	**20**	0.884	0.882	0.884	**0.883**	**32.44%**	**67.56%**	
		40	0.744	0.743	0.742	**0.743**	**43.15%**	**56.85%**	
		60	0.681	0.680	0.680	**0.680**	**47.97%**	**52.03%**	**65.25**
		80	0.557	0.556	0.558	**0.557**	**57.38%**	**42.62%**	
		100	0.452	0.450	0.451	**0.451**	**65.49%**	**34.51%**	

At the different Concentrations Sample Code EE (ethanol extract) shows the high percentage of inhibition and against breast cancer cell line as compared to standard drug 5FU. On the basis of percent of inhibition, we can conclude that the samples ethanol extract shows good anticancer activity against breast cancer cell lines.

Thin Layer Chromatography: -



Rf value for spot: -

Spot 1 = $\frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$

= $\frac{7.5}{8}$

= 0.93

Spot 2 = $\frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$

= $\frac{7.2}{8}$

= 0.90

Spot 3 = $\frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$

= $\frac{6.9}{8}$

= 0.86

Spot 4 = $\frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$

= $\frac{6.5}{8}$

= 0.81

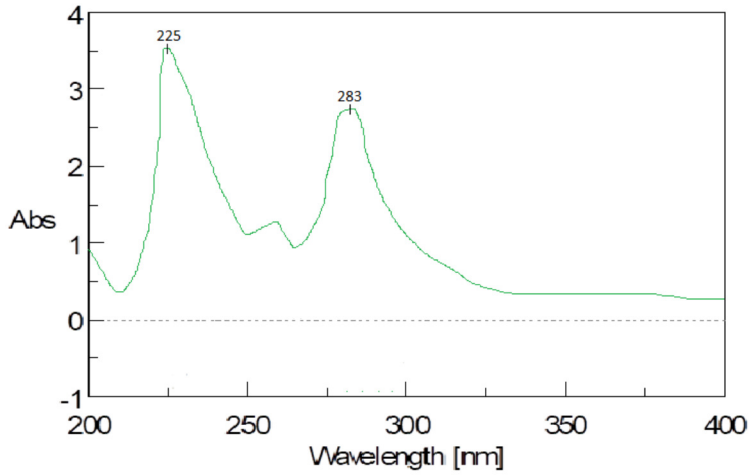
Column Chromatography for The Isolation of Active Components: -

Column chromatography was performed using a glass column. For column chromatography, there are two methods: the wet packing method and the dry packing method. The activated silica gel G slurry (100–120 mesh), which was created in a solvent system and was rendered bubble-free by constant stirring, was packed using the wet packing method. The glass column was loaded and packed with the slurry that had no air bubbles. The slurry was added with some solvent still sitting on top of the adsorbent. In ethanol, dried extract was dissolved before being introduced to the column's top. The silica column was then created by running a solvent solution over it, including Chloroform: Methanol (8:2)

Fig 4. Column Chromatography

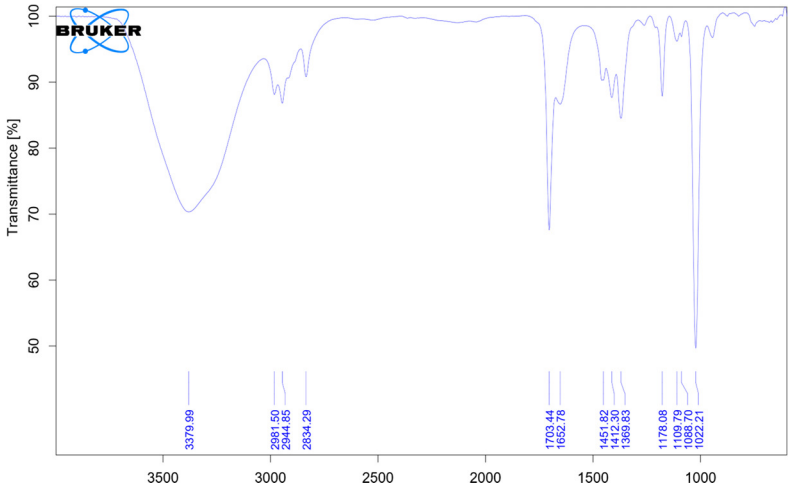
SPECTROSCOPIC ANALYSIS

Ultraviolet Spectroscopy: -



Because the λ max of standard are 220(3.54) and 281(2.81), respectively and the λ max of the sample was found to be 225nm and 283nm for an isolated component obtained from *Achyranthes aspera* these figures indicate the presence of Betaine alkaloid in the sample.

IR Spectroscopy:



Sr.no.	Peak value (cm^{-1})	Functional group	Assignment
1.	3379.99	Secondary amine	N-H stretch
2.	2981.50, 2944.85 & 2834.29	Aromatic C-H	C-H stretch
3.	1451.82 & 1412.30	Aromatic C=C	C=C stretch
4.	1369.83	Nitro group	N-O stretch
5.	1178.08 & 1109.79	Amine C-N	C-N stretch
6.	1088.70 & 1022.21	C-O	C-O stretch

According to the FTIR spectrum shown above, the functional group of isolated and standardcomponent is almost same. So standard compound may be present based on the IR spectra.

DISCUSSION

- The leaves of *Achyranthes aspera* linn. were subjected to phytochemical screening, which identified the presence of flavonoids, alkaloids, carbohydrates, saponins, glycosides, proteins, amino acids, and tannins.
- At the different Concentrations *Achyranthes aspera* L. leaf extract shows the high percentage of inhibition and against breast cancer cell line as compared to standard drug 5FU. On the basis of percent of inhibition, we can conclude that the samples ethanol extract shows good anticancer activity against breast cancer cell lines.
- Alkaloids were found by chemical isolation of ethanolic extract of *Achyranthes aspera.* These alkaloids were identified by thin layer chromatography and isolated by column chromatography using solvent system Chloroform: Methanol.
- Spectroscopic study is done by UV and IR It shows presence of Betaine alkaloid.

ACKNOWLEDGMENT

I would like to express my gratitude to my guide Mrs. Bhavana U. Jain and towards my college Appasaheb Birnale College of Pharmacy, Sangli. For the constant support to my Master's study and research.

REFERENCES

1. Anand, P. Kunnumakkara B. Sundaram, C. Harikumar, KB. Tharakan, ST. Lai OS; Sung, B. Aggarwal, BB. 2008. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res, 25: 2097-2116 DOI: 10.1007/s11095-008-9661-9
2. Arora, S. and Tandon, S. 2014. Achyranthes aspera Root Extracts Induce Human Colon Cancer Cell (COLO-205) Death by Triggering the Mitochondrial Apoptosis Pathway and S Phase Cell Cycle Arrest, The Scientific World Journal Volume (2014), Article ID 129697. https://doi.org/10.1155/2014/129697
3. Geetha, K. Narayanan, K. and Murugesan, A. 2010. Antimicrobial efficiency of *Achyranthes aspera* L. against selected pathogenic organisms. J Bio sci Res;1(3):187-90.
4. Arora, S. and Tandon, S. 2014. Achyranthes aspera Root Extracts Induce Human Colon Cancer Cell (COLO-205) Death by Triggering the Mitochondrial Apoptosis Pathway and S Phase Cell Cycle Arrest, The Scientific World Journal Volume (2014), Article ID 129697. https://doi.org/10.1155/2014/129697
5. Aziz, Md. Rahman, M. Mondal,A. Rahman, Md. 2005. 3-Acetoxy-6-benzoyloxyapangamide from Achyranthes aspera. J Pharm Sci;4(2):113-6
6. Hullatti, KK. and Uma, DM. 2010. Activity Guided Isolation of Cytotoxic Compounds from Indian Medicinal Plants Using BSL Bioassay. JCPR; 01:16- 18.
7. Basic Biology and Clinical Assessment, Stephen-2005, Pages 343-349 https://doi.org/10.1016/j.molstruc.2023.137351
8. MTT Assay Protocol for Cell Viability and Proliferation by Sigma pvt.Ltd. doi: 10.6026/97320630018289
9. Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods.* 65(1-2):55-63 https://doi.org/10.1016/0022-1759(83)90303-4
10. Hansen MB, Nielsen SE, Berg K. 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *Journal of Immunological Methods.* 119(2):203-210 https://doi.org/10.1016/0022-1759(89)90397-9
11. Sasidharan S, Chen Y, Saravanan D, et. al. "Extraction, isolation and characterization of bioactive compounds from plants' extracts." Afr J Tradit Complement Altern Med. 2011; 10.
12. Altemimi A, Lakhssassi N, Baharlouei A, et. al. "Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts." Plants. 2017; 6: 42.

13. Ujang ZB, Subramaniam T, Diah MM, et. al. “Bio guided fractionation and purification of natural bioactive obtained from Alpinia water extract with melanin inhibition activity.” J Biomatter Nanobiotechnology. 2013; 4: 265–72.
14. Harborne JB. “Phytochemical methods: A guide to modern techniques of plant analysis.” 3rded. New York, NY: London, UK Thomson Science; 1998. pg. 219.
15. Ahlam Rashed, Ameerah Shaeroun, Ahmed Belgasem Ahmed, et. al. “Thin Layer Chromatography (TLC) and Phytochemical Analysis of Moringa Oleifera Methanol, Ethanol, Water and Ethyl Acetate Extracts.” Saudi J Med Pharm Sci, Oct 2019; 5(10): 817-820.
16. Vivek K. Bajpai, Rajib Majumder and Jae Gyu Park. “Isolation and purification of plant secondary metabolites using column-chromatographic technique.” Bangladesh J Pharmocol. 2016; 11: 844-848.
17. Silverstein, Webster. Spectrometric identification of organic compounds. 6th ed; John Wiley Sons. Pg. 136-140.
18. Chatwal A. Instrumental methods of chemical analysis. 5th ed; Himalaya publishing housepg. 2.62-2.68, 2.213-2.225.
19. Kadimpati, Kishore Kumar & Naidu, P.V. & Kumar, C. & Gunesh, G. & Rao, M. (2006). Antimicrobial activity of Achyranthes aspera. Biosciences, Biotechnology Research Asia. 3. 171-174.
20. Londonkar, Ramesh & Reddy, C.V. & Kumar, A.K. (2011). Potential antibacterial and antifungal activity of Achyranthes aspera L. Recent Research in Science and Technology. 3. 53-57.