

**ANTICANCEROS ACTIVITY OF ANNONA MURICATA L LEAVES ON CERVICAL
CANCER CELL LINES**Gayathri Krishna K. U.^{1*} and Dr. Sr. G. S. Mary Fabiola²¹Scholar, Department of Zoology, Nirmala College for Women, (Autonomous), Coimbatore, Tamilnadu.²Assistant Professor, Department of Zoology, Nirmala College for Women, (Autonomous), Coimbatore, Tamilnadu.***Corresponding Author: Gayathri Krishna K. U.**

Scholar, Department of Zoology, Nirmala College for Women, (Autonomous), Coimbatore, Tamilnadu.

Article Received on 22/09/2020

Article Revised on 12/10/2020

Article Accepted on 01/11/2020

ABSTRACT

In this study the anti-cancerous activity of *Annona muricata* on cervical cancer cell lines was examined. The cytotoxicity was evaluated by direct microscopic analysis and MTT assay. MTT assay reveals that the extraction of *A. muricata* inhibit the growth of human cervical cancer cell lines. The inverted phase contrast tissue culture microscope (Labomed TCM-400 with MICAPSTM HD camera) displayed cellular shrinkage, granulation, and vacuolization. In summary, The *A. muricata* has displayed growth inhibitory activity against L6 cells and which induce cell death predominantly via apoptosis and could be exploited as cervical cancer treatment after evaluation.

KEYWORDS: *Annona muricata*, cervical cancer, L6 cells.**INTRODUCTION**

Plant used in treating diseases is as old as civilization and traditional medicines are still a major part of habitual treatments of different diseases (Wadmidh, 2013.^[1] Fabricant *et al.*, 2001;^[2] Alvino and Alvino, 2009).^[3] Many plant materials used in traditional medicines are readily available in rural areas. A lot of species investigated are selected from developing countries in Africa and Asia where herbal therapies are practiced and medicinal plants are relied upon for primary treatment (Ochwang *et al.*, 2014^[4]; Freiburghaus *et al.*, 1996^[5]; Kamatou *et al.*, 2008^[6]). Among this one of the important medicinal plants which show many medicinal properties is *A. muricata* (In Tamil, Mullu Seetha).

The health problems of greatest significance today are the chronic diseases. Cancers are the second cause of death and have the highest burden of diseases in 21st Century (Boyle and Levin, 2008).^[7] Cancers are the second cause of death and have the highest burden of diseases in 21st century (Boyle and Levin, 2008). Cancers, including breast, cervical and ovarian cancer, lead to hundreds of thousands of premature deaths among women. Cervical cancer is the second most common cancer in women worldwide. Many cancer patients use herbal medicine as alternative medicines including phytochemicals in addition to, or following the failure of standard cancer therapy (Eisenberg *et al.*, 1998).^[8] Currently, several plant-derived compounds have been successfully employed in treatment. With successful clinical trials drug being developed from plant origin are popular for clinical development. Their non-toxic effect on normal cells and their cytotoxic effect on

cancer cells put them in high demand. Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. Several synthetic agents are used to cure the disease but they have their toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents.

MATERIALS AND METHODS**Collection of plant material**

A. muricata leaves were collected from Palakkad district, Kerala.

Identification of plant materials

Authenticated by Dr. M. U. Sharief, Scientist 'E' and Head of office, Botanical survey of India, Southern regional center, Coimbatore.

Extraction

The freshly collected samples were washed and cleaned thoroughly under running water. For the preparation of different extract, the samples were shade dried for 15 days and then pulverized into fine powder using willemill grinder. The extraction was done by using Soxhlet extraction. Selected solvent (Ethanol) was carried for the extraction. After extraction the solvent is removed typically by means of a rotary evaporator yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded after extraction running with all the selected solvent. % yield is calculated using following formula.

Yield (g) = $W1/W2 \times 100$

W1- weight of extract residue after solvent removal

W2- Weight of powdered sample taken

Invitro cytotoxicity determination by MTT assay

L6 cell line were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium Himedia). The cell line was cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator (Galaxy® 170 Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

Fifteen mg of MTT (Himedia, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilisation Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilise the formazan crystals. The absorbance values were measured by using micro plate reader at a wavelength of 570 nm (Laura B. Talarico *et al.*, 2004).

Cytotoxicity effect by direct microscopic observation

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Labomed TCM-400 with MICAPSTM HD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

RESULT AND DISCUSSION

Cancer which is a dreadful disease, increasing with changing modern life style, nutrition balance and global warming, Cancer treatment do not have potent medicine as the currently available drug are causing side effect under certain instances. The natural product derived from medicinal plant have gained significant in the treatment of cancer.

Women's cancers, including breast, cervical and ovarian cancer, lead to the hundreds and thousands of premature deaths among women. Cervical cancer is the second most common cancer in women worldwide. Yet, because of poor access to screening and treatment services, the vast majority of deaths occur in women living in low- and middle-income countries. Effective methods for early detection of precancerous lesions using cytology (Pap smear) exist and have been shown to be successful in high income countries. However, competing health care priorities, insufficient financial resources, weak health systems, and limited numbers of trained providers have made high coverage for cervical cancer screening in most low- and middle-income countries.

Soheil *et al.*, (2015)^[9] reported that *Annona* species are the most important therapeutic compounds for human. All portions of the *A. muricata* tree, similar to other *Annona* species, including *A. squamosa* and *A. reticulata* are extensively used as traditional medicine against an array of human ailments and diseases, especially cancer and parasitic infections.

Different classes of constituent "annonaceous" metabolites such as acetogenins are believed to play a major role in the anti-cancer properties of graviola on mammalian cells, in addition to many other constituents such as alkaloids, flavonoids, sterols and others.

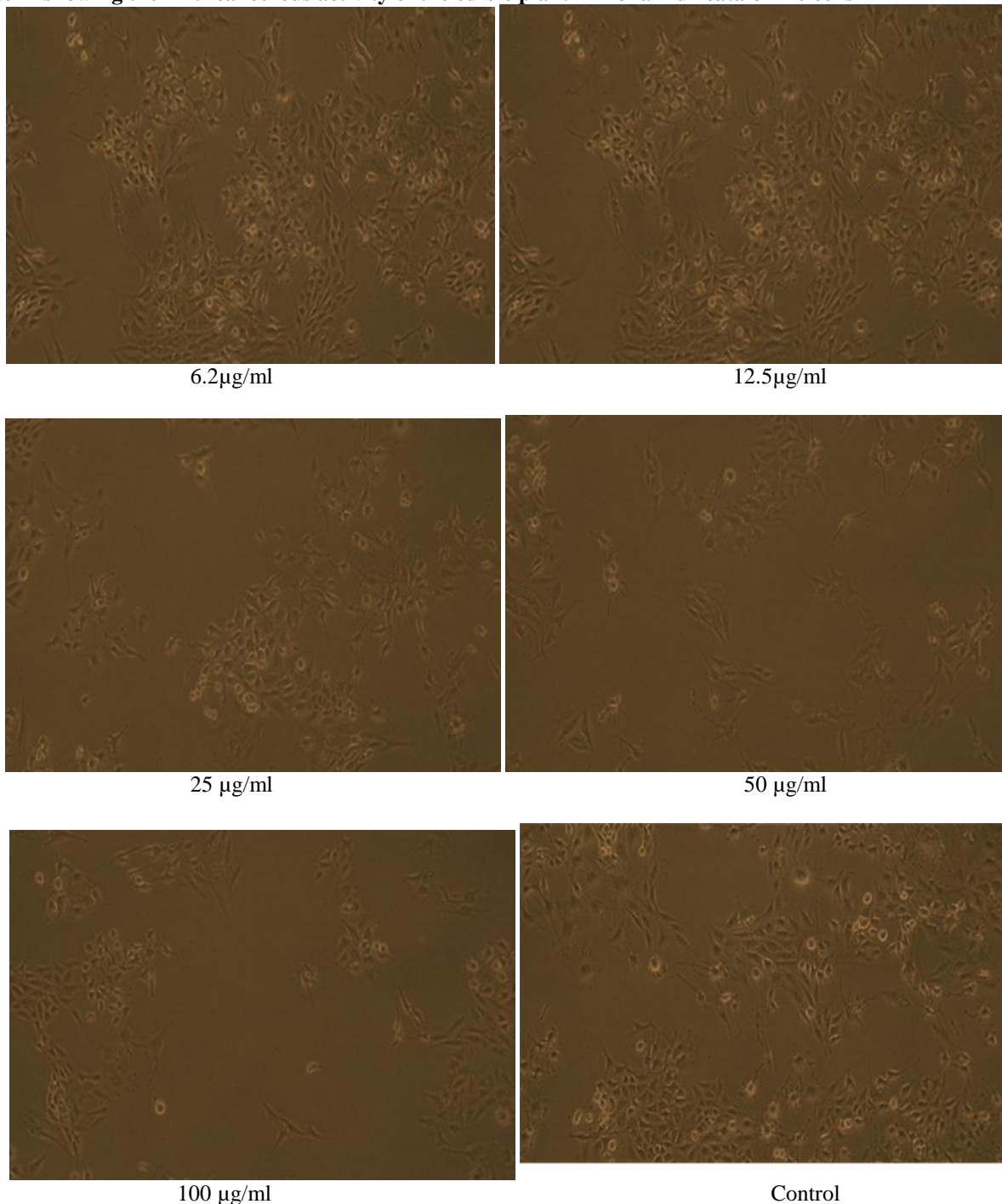
Syed *et al.*, (2016)^[10] reported that the crude extract of *A. muricata* exhibited the anti-cancerous activity as they inhibited the proliferation of breast cancer cell lines.

In the present study the cytotoxic effect of leaf extract of selected sample on human cervical cancer cell lines(L6) were tested by using MTT assay (cell viability), this assay is used for the evaluation of ant cancerous activity. The medium without sample were served as control and triplicate were maintained at different concentration.

The *A. muricata* leaf extract was treated under several concentration ranging from 6.25µl,12.5µl,25µl,50µl and 100µl against (L6) cancer cells. The present study reveals that in 25µl,51.52% of cell the viability occurs. At 50µl, 37% and at 100µl, 22.16% of cell viability. Thus, the percentage of cell viability was reduced based on dose dependent. More level of concentration showed less amount of cell viability. Thus *A. muricata* showed maximum cell viability in high concentration compared to low concentration. Hence from the present study *A. muricata* is seen to have more bioactive compounds that are capable to treat human cervical cancer.

Table 1: Showing the percentage of cell viability.

Sl.No	Concentration	Viability
1	6.25	89.3550
2	12.5	75
3	25	51.52
4	50	37
5	100	22.1607

Plate -1 showing the Anti-cancerous activity of the edible plant *Annona muricata* on L6 cells**CONCLUSION**

The annonaceae is very primitive family comprising about 120 genera and more than 2000 species on the basis of morphology and habitat. It is very homogenous as source of edible fruit and oil.

From the present study it reveals that in *A. muricata* the anti-cancerous activity against human cervical cancer. The cell viability decreases with the different level of concentration. Thus, it become a way to use *A. muricata* to treat cancer. The drug available for cancer are few and are also more expensive. This edible plant that are

growing from the natural condition and environment and the drug that are obtained from the plant has much adverse effect when given as medicine.

ACKNOWLEDGEMENT

The authors appreciate the cooperation of Athmic Biotech solutions Ltd, Thiruvananthapuram, Kerala where the cytotoxicity analysis of sample was done.

REFERENCES

1. Wamidh, H.T. Anticancer and antimicrobial potential of plant derived natural products.

- Phytochemicals bioactive and impact on health, 2013.
2. Fabricant, D.S. and Frans worth, N.R. The value of plants used in traditional medicine for drug discovery. *environment health perspect*, 2001; 109: 69-75.
 3. Alviano, D.S. and Alviano, D.S. Plant extracts: Search for new alternatives to treat microbial diseases *pharm biotechnol*. 10: 106-121. ovarian cancer properties of *Annona muricata* Linn. (Annonaceae) seed ethanol extract. *Int J Pharm Front Res*, 2009; 2: 9-17.
 4. Ochwing, D.O., Kimwela, C.N., Oduma, J.A., Gathambi, P.K., Mharja, J.M. and Kiama, S.G. Medicinal plant used in the treatment and management of cancer Kakamega country Kenya. *Journal of Ethanopharmacology*, 2014; 151: 1040-1055.
 5. Frieburghaus, F., Kaminsky, R., Nkunya, M.S.D.N. Bran, R. Evaluation of African plants for their in-vitro tripanocidal activity. *Journal of Ethanopharmacology*, 1996; 55: 1-11.
 6. Kamatou, G.P.P., Vanzyl, R.L., Davids, H., Van, H.F.R., Lourens, A.C.U. antijoin, A.M. Anti-malarial and anti-cancer activities of selected South African *Salvia* species and isolated compounds from *S. radula*. *South African journal of botany*, 2008; 74: 238-243.
 7. Boyle, P. and Levin, B. World cancer report. International Agency for Research on Cancer Press, 2008; 42: 51.
 8. Eisenberg, D.M., Davis, R.B. and Ettner, S.L. *et al.*, Trends in alternative medicine use in United States. *AM Med Assoc*, 1998; 1569-75.
 9. Soheil, Z.M. Hamed, K., Elham, R. and Mohammadiavad. *Annona muricata* leaves induce G1 cell cycle arrest and apoptosis through mitochondria mediated pathway in human HCT-116 and HT-29 colon cancer cells. *Journal of ethanopharmacology*, 2014; 156: 277-289.
 10. Syed, U.F.S.N., Muhammad, F.R., Muhajir, H., Noorjahan, B.A. and Nik Mohd, A.N.A.R. Anti-cancer effect of *Annona muricata* Linn. Leaves crude extract (AMCE) on breast cancer cell line. *BMC complementary and alternative medicine*, 2016; 16: 31.