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## RESEARCH ARTICLE

# CELL DEATH INDUCED BY HYDROETHANOLIC EXTRACT OF *ARTOCARPUS HETROPHYLLUS* SEED IN HELA – CERVICAL CANCER CELL LINE

**[1]Thiruselvi, M. and [2,*]Brindha Durairaj**

[1]Assistant Professor, Department of Biochemistry, Dr NGP Arts and Science College, Coimbatore, Tamil Nadu
[2]Associate Professor and HOD, Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu

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**ABSTRACT**

*Artocarpus hetrophyllus* has been traditionally used for treatment of diverse diseases and it possesses antibacterial, anti-inflammatory, antidiabetic, antioxidant, and antipyretic properties. *Artocarpus hetrophyllus* has a potent cytotoxic effect on human cancer cells. In this study, we reveal the cytotoxic effect of hydroethanolic extract on HeLa cell line. The dose-dependent effect of extract was measured by MTT assay and nuclear changes during apoptosis was assessed by Ethidium bromide Staining. Phytochemical screening and individual components present in the extract were also identified by comparison of both mass spectrum and their GC retension data. The IC50 values of hydroethanolic extract was found to be 60.25 µg/ml. Most of the cells were observed for nuclear changes by Ethidium bromide staining.Preliminary phytochemical screening revealed the presence of possible role of secondary metabolite. The quantitative data of 25 volatile constituents was obtained by peak area and the retention time were calculated for all the compounds. The major volatile components identified in the extract of *Artocarpus hetrophyllus* were found to be 1-Dodecene(17.7 %) 1-Tetradecene(17.2%) and Hexadecene (11.2%) respectively. The overall result indicates the promising baseline information for the potential uses of the hydroethanolic extract of *Artocarpus heterophyllus* seed spermoderm as an anticancer agent.



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## INTRODUCTION

Cancer is one of the leading causes of human death which is estimated at 8.2 million and will likely rise to 13 million worldwide per year till 2030 (Mehta *et al.,* 2014). There are over 200 different known cancers that afflict humans. The global burden of cancer continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer-causing behaviours (Siegel *et al*., 2013). Cervical cancer is world's one of most deadly – but easily preventable cancers of women, responsible for more than 2, 70000 deaths annually, of which 85% occur in developing countries (Jemal *et* al., 2011). It is the fourth most commonly diagnosed cancer in women in 2012, with an estimated 527,600 new cases worldwide. With rising population and aging, number of cervical cancer cases is expected to increase 1.5-fold by 2030 (Naina, 2016). 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 (Chanda and Nagani, 2013).

****Corresponding author: Brindha Durairaj,***
Associate Professor and HOD, Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu.

Medicinal plants represent a vast potential source for anticancer compounds. These compounds are extremely complex molecular structures, which would be difficult to synthesize (or conceptualize) in the laboratory. A large volume of clinical studies have reported the beneficial effects of herbal medicines on the survival, immune modulation, and quality of life (QOL) of cancer patients, when these herbal medicines are used in combination with conventional therapeutics (Yin *et al.,* 2013). *Moracea* is large family comprising sixty genera and nearly 1400 species including important group such as *Artocarpus*, *Morus*, and *Ficus*. *Artocarpus heterophyllus* or Jackfruit (family of *Moraceae*) is a monoecious evergreen tree that is grown in several tropical countries. It produces a large pear or barrel-shaped fruit that can grow up to 90 cm long, 50 cm thick and having a weight of 20 kg. Several individual fruits, covered with fleshly and juicy perianths, are found under the spiny surface. The seed is large, long and has a slimy membranous test and a brown tegmen. *A.heterophyllus* is widely distributed in tropical region and has been used as traditional folk medicine against inflammation, malarial fever and so on. Traditionally *Artocarpus hetrophyllus* is used in treatment of ulcer, night blindness and bone loss. It possesses antibacterial, anti-inflammatory, anti-diabetic, antioxidant,

antipyretic property, it is also an immunity booster (Deepika *et al.,* 2011)The jackfruit seeds are consumed usually as roasted, boiled, steamed, and are consumed as a snack (Farha, 2013).Moraceae plants including *A. Heterophyllus* are rich sources of the isoprenylated phenolic compounds, including flavonoids (Jaikumar and Jasmine, 2016).The objective of this study is to investigate cytotoxic activity using hydroethanol extract of *Artocarpus heterophyllus* seed spermoderm on HeLa cell line by MTT assay and to assess the nuclear changes using EtBr Staining. Individual components present in the extract were also identified by GCMS.

## MATERIALS AND METHODS

### Plant Material

*Artocarpus heterophyllus* seeds were obtained from local market, Trivandrum, Kerala. The inner thin membranous brown tegmen of *Artocarpus heterophyllus* seeds (Spermoderm) were used for this study.

### Preparation of Seed extract

About 25 g of the inner thin membranous brown tegmen of the Jack fruit seed was dried and powdered. The powdered material of *Artocarpus heterophyllus* seeds was extracted with hydro ethanol using Soxhlet extractor exhaustively for 20-24 hours. The extracts were concentrated to dryness under reducedpressure and controlled temperature (40-50°C). The dried extractsobtained were used in this study.

### Preliminary Phytochemical analysis

Preliminary screening of *Artocarpus hetrophyllus* on hydroethanolic and aqueous extracts for various phytoconsituents were done and results are noted.

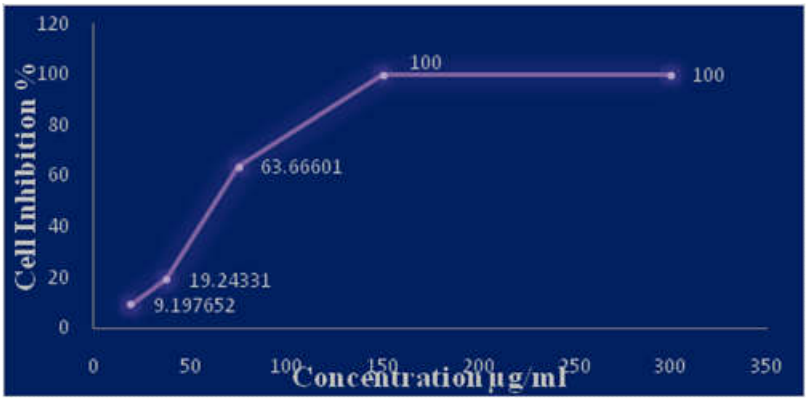
### MTT Assay

The extent of cell survival was quantified using MTT assay. The treated cells were incubated with 50µl of MTT at $37^0$C for three hours after removing the medium and serum. After incubation, 200µl of PBS was added to all the samples. The liquid was then carefully aspirated. Then 200µl of acid-propanol was added and left overnight in the dark. The absorbance was read at 650nm in a microtiter plate reader (Anthos2020, Austria). The optical density of the control cells were fixed to be 100% viability and the percent viability of the cells in the other treatment groups was calculated (Rena *et al* 2004).The percentagecytotoxicity was calculated by following formula:

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual Test Group}}{\text{Mean OD of control Group}} \times 100$$

### Fluorescence microscopy analysis of nuclear fragmentation Ethidium Bromide Staining

The treated cells were incubated for 5 minutes with 10ml of ethidium bromide and spread by placing a coverslip over it.




**Graph 1. Percentage of Cell inhibition for various concentration of hydroethanolic extract of *Artocarpus Hetrophyllus* by MTT assay**

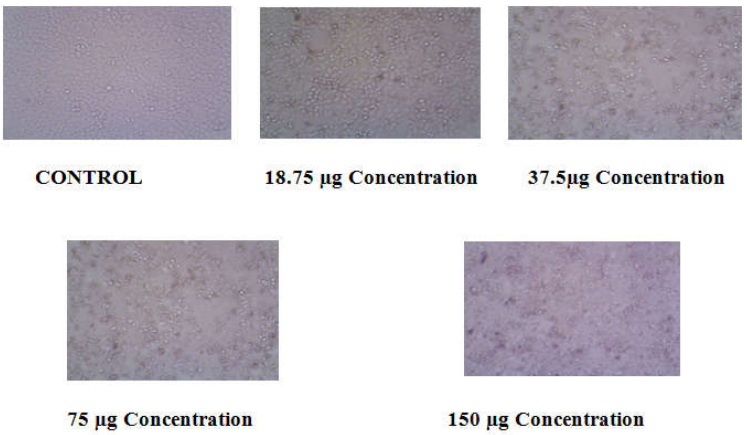



**Figure 2. Decrease in cell viability and increase in cytotoxicity**

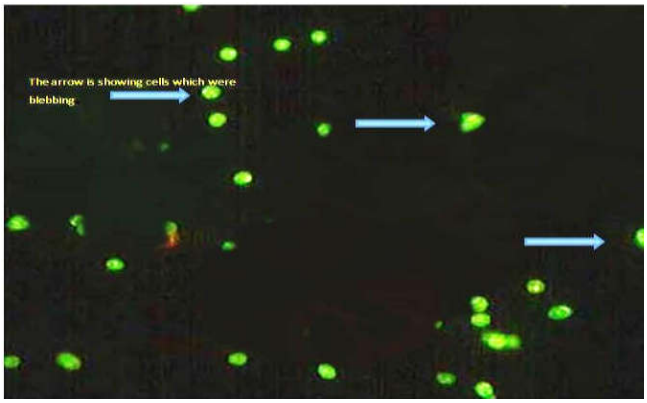



**Fig. 3. Qualitative characterization nuclear morphology by EtBr/AO staining using fluorescence microscopy**

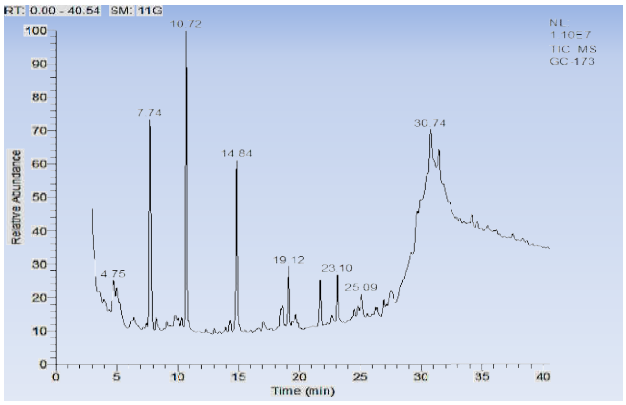



**Figure 4. GCMS Spectrum of Hydroethanolic extract of *Artocarpus Hetrophyllus***

The apoptotic cells were scored by counting the cells with condensed chromatin and fragmented nuclei under fluorescent microscope (Nikon, Japan) using UV2A filter at 400x magnification.

$$\text{Apoptotic Ratio} = \frac{\text{Number of apoptotic cells}}{\text{Number of normal cells}}$$

Apoptotic nuclei undergo typical changes including chromatin condensation, peripheral marginalization, nuclear shrinkage and subsequent fragmentation[15]. These changes were investigated in the cells in the presence and the absence of seed extract by ethidium bromide staining.

### GCMS Analysis

A Thermo GC-Trace Ultra Ver: 5.0 GC-MS system used for this study consisted of a model Thermo MS DSQ II gas chromatograph. A fused-DB35-MS Capillary standard Non-polar Column Dimension (30mts, ID: 0.25mm, FILM: 0.25µm) was also used. The GC temperature program was as follows: initial temperature was 100 °C, held for 1 min, increased to 130 °C at a rate of 2 °C/min, then to 200 °C at a rate of 3 °C/min, and finally to 280 °C at a rate of 6 °C/min and held for 10 min. The split ratio was 1:12, injection temperature was 250 °C, transfer line temperature was 270 °C, The mass spectrometer was operated at 70 eV in Run time 46.19(min).The quantitative data of volatile constituents was obtained by peak area normalization using a HP 5890 GC/FID instrument, operated under the same conditions, except for the carrier gas that was hydrogen produced by a Packard hydrogengenerator.

## RESULTS AND DISCUSSION

### Phytochemical analysis

Preliminary phytochemical analysis revealed the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. The observations made in the phytochemical screening are presented as follows: The preliminary phytochemical analysis of two different extracts revealed the presence of alkaloids, flavonoids, phenols tannins, steroids, glycosides and saponins. From the results it is evident that both the extracts were found to contain selected analysed phytochemicals. When compared with aqueous extract, hydroethanolic extract was found to contain all the active phytoconstituents screened in the spermoderm of *Artocarpus hetrophyllus* seeds.

**Table 2. Volatile constituents of Tegmen extract of *Artocarpus hetrophyllus***

| S.NO | CONSTITUENTS | RT | % |
|---|---|---|---|
| 1 | d-Nerolidol | 3.63 | 0.53 |
| 2 | Hexadecane, 1-bromo- (CAS) | 4.75 | 3.44 |
| 3 | à-PINENE, (-)- | 5.02 | 3.87 |
| 4 | Pregnane-3,11,20,21-tetrol, cyclic 20,21-(butyl boronate), (3à,5á,11á,20R) | 6.42 | - |
| 5 | 1-Dodecene (CAS) | 7.74 | 17.72 |
| 6 | Benzaldehyde diethylacetal | 8.25 | 1.15 |
| 7 | 1-TETRADECENE | 10.72 | 17.21 |
| 8 | 1-Hexadecanol (CAS | 14.31 | 0.95 |
| 9 | Hexadecene | 14.84 | 11.28 |
| 10 | 1-Nonadecene (CAS) | 19.12 | 3.55 |
| 11 | Heptanedioic acid, 4-(ethoxycarbonylmethylene)-, diethyl ester | 19.69 | - |
| 12 | Hexadecanoic acid, methyl ester (CAS) | 21.70 | 3.41 |
| 13 | Hexadecanoic acid, 2,3-dihydroxypropyl ester (CAS) | 22.65 | 0.69 |
| 14 | Hexadecanoic acid, ethyl ester (CAS) | 23.10 | 2.96 |
| 15 | 1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS) | 24.48 | 0.57 |
| 16 | 9-Octadecenoic acid, methyl ester (CAS) | 25.09 | 2.77 |
| 17 | 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)- (CAS) | 26.35 | 1.39 |
| 18 | 3-Acetoxypentadecane | 26.90 | 0.76 |
| 19 | Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydroxyphosphinyl]oxy]methyl]-1,2-ethanediyl ester (CAS) | 27.51 | 2.06 |
| 20 | Hexadecanoic acid, 1-(hydroxymethyl)-3.751,2-ethanediyl ester (CAS) | 29.13 | 0.74 |
| 21 | Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (CAS) | 29.62 | 3.75 |
| 22 | HEXADECANOIC ACID, 2-HYDROXY-1,3-PROPANEDIYL ESTER | 30.74 | 7.8 |
| 23 | 1,2-Benzenedicarboxylic acid, diisooctyl ester (CAS) | 31.43 | 2.12 |
| 24 | 9,12-Octadecadienoic acid (Z,Z)-, 2,3-bis[(trimethylsilyl)oxy]propyl ester (CAS) | 31.86 | 1.39 |
| 25 | 6,7-Dimethoxy-1-methyl-3-[4-N-benzylcarboxamido)]phenylvinyl-2(1H)-quinoxalinone | 34.19 | - |

### *In vitro* cytotoxic activity of hydroethanolic extract of *Artocarpus heterophyllus* by MTT assay

The cytotoxicity study was carried out for seed extract of *Artocarpus heterophyllus*. These extract was screened for its cytotoxicity against HeLa cell lines at different concentrations to determine the $IC_{50}$ (50% growth inhibition) by MTT assay.MTT assay, after treatment with various concentrations of *Artocarpus hetrophyllus* cell viability and cytotoxicity were tested.The anticancer activity of the extract is presented in Figure 2. Decrease in cell viability and increase in cytotoxicity by the extract was observed on HeLa cell lines in a dose dependent manner.The $IC_{50}$ value of *Artocarpus hetrophyllus* seed extract was found to be 60.25µg/ml and is presented in the Table II. The results of our study are in accordance with the results obtained by Prema *et al.*, 2014.

### Fluorescence microscopy analysis of nuclear fragmentation Ethidium Bromide Staining

The Ethidium Bromide staining showed that the seed extract induced apoptosis in HeLa cervical cancer cells, nuclear changes such as chromatin condensation around the nuclear membrane were noticed Figure 3. ethidium bromide staining on *Euphorbia antiquorum* latex imparts complete protection to the primary cells which was exposed to etoposide and also latex does not induce apoptosis in normal cells but it modulates the apoptotic effects produced by etoposide.

### GCMS

Individual components were identified by comparison of both mass spectrum and their GC retention data. The identified volatile constituents and their percentage are listed in the table II. Figure 4 shows the spectrum analysis of the extract by GCMS. The quantitative data of 25 volatile constituents was obtained by peak area and the retention time were calculated for all the compounds.The major volatile components identified in the extract of *Artocarpus Hetrophyllus* were 1-Dodecene(17.7 %) followed by1-Tetradecene(17.2%) and Hexadecene (11.2%)

### Conclusion

This study showed that *Artocarpus Hetrophyllus* extract inhibits the proliferation of HeLa cells by apoptosis. Phytochemical analysis reveals that the maximum classes of phytoconstituents are present in the extract. The compounds responsible for cytotoxic activity was analysed by GCMS method. The overall results indicate the promising potential of the hydroethanolic extract of *Artocarpus heterophyllus* seed spermoderm as an anticancer agent.

## REFERENCES


Chanda S and Nagani 2013. In vitro and in vivo Methods for Anticancer Activity Evaluation and Some Indian Medicinal Plants Possessing Anticancer Properties: An Overview, Journal of Pharmacognosy and Phytochemistry, Volume 2 Issue 2, 140 – 152.

Deepika, G., Sonia, M., Avijit, S. and Gupta, K. R. 2011. Phytochemical, nutritional and antioxidant activity evaluation of seeds of jackfruit (*artocarpousheterolphyllus* lam.).,2, 336-337.

Farha, A.K. 2013. Apoptosis mediated cytotoxicity induced by isodeoxyelephantopin on nasopharyngeal carcinoma cells: Asian journal of pharmaceutical and clinical research vol 6, suppl 2, issn - 0974-2441.

Jaikumar, B., Jasmine, R. 2016. A Review on a few medicinal plants possessing anticancer activity against human breast cancer, International Journal of PharmTech Research, Vol.9, No.3, pp 333-365.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E. and Forman, D. 2011. Global cancer statistics.CA Cancer J Clin 61(2):69–90

Mehta, K., Gandhi, V., Pathak, S., Aggarwal, B.B. and Grover, R.K. 2014. Multi-Targeted Approach to Cancer Treatment: An International Translational Cancer Research Symposium. Anticancer Research , 34, 6791-6795.

Naina, K. 2016. Cervical Cancer; a Nightmare for Womanhood: Review of Recent Advances, Women's Health & Gynecology, Volume 2 • Issue 2, 2-9

Prema, D., Kamaraj, M., Achiraman, S., Udayakumar, R. 2014. *In vitro* antioxidant and cytotoxicity studies of *Curcuma amada*Roxb. (Mango ginger),*International Journal of Scientific and Research Publications*, 4 (4).

Siegel, R., Naishadham, D., Jemal, A. 2013. Cancer statistics, 2013. CA Cancer J Clin 63:11–30

Sumathi, S., Malathy, N., Dharani, B., Sivaprabha, J., Hamsa, D., Radha, P. and. Padma, P.R. 2011. Cytotoxic studies of latex of Euphorbia antiquorum in in vitro models, Journal of Medicinal Plants Research, 5(19), 4715-4720.

Tejpal, A. and Amrita, P. 2016. jackfruit:A Health boon, Int.J.Res.Ayurveda Pharm7(3): 59- 64

Yin, Y. S. Y., Wen-Chi Wei, Feng-Yin Jian, and Ning-Sun Yang 2013. Therapeutic Applications of Herbal Medicines for Cancer Patients, Evidence-Based Complementary and Alternative Medicine, Volume 2013, 1-15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