European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 08, 2020

Evaluation of Anticancer effect of Ethanolic extract of Lepidagathis pungens Nees., Whole Plant By MTT Assay- An In Vitro Study

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ABSTRACT: Extracts from natural products, especially plants, have served as a valuable source of diverse molecules in many drug discovery efforts and led to the discovery of several important drugs. Lepidagathis pungens Nees., (L.pungens) is a herb of tropical Asia belonging to the family Acanthaceae. The present study deals with the in vitro anticancer activity of ethanolic extract of L.pungens against L6, Ehrlich Ascites Carcinoma (EAC), human breast cancer cells (MCF 7), human cervical cells (He La) and human hepatocellular carcinoma cell lines (Hep G2). MTT assay was used to assess the in vitro anticancer activity. The extract at various doses were treated against all the cell lines. The parameters analyzed were percentage of cytotoxicity, percentage of cell viability and IC_{50} values. In results the ethanolic extract of L.pungens showed more potent activity against MCF 7 and EAC cell lines but moderate activity against He La and Hep G2 cell lines. The anticancer nature of the ethanolic extract is due to the presence of phytoconstituents like flavanoids, alkaloids, phenolic compounds, terpenoids, etc. However, further indepth studies are required before final conclusion on the mechanisms involved could be drawn to explain the observed activity. In conclusion, the ethanolic extract of L.pungens possess anticancer activity against various types of cancer cells, correlated to their total phenolic content.

Keywords : MTT Assay, Anticancer, Carcinoma, Phytoconstituents, Cell line

1. INTRODUCTION

Natural products and related drugs are used to treat 87 percent of all categorized human diseases including bacterial infection, cancer and immunological disorders [1]. About 25 percent of prescribed drugs in the world originate from plants [2] and over 3000 species of plants have been reported to have anticancer properties [3]. About 80 percent of the populations in developing countries rely on traditional plant based medicines for their primary health care needs [4]. Internationally, about one in six passings is because of malignancy [5]. This growing trend indicates the deficiency in the current cancer therapies, which include surgery, radiotherapy, and chemotherapy [6,7]. There is a basic requirement for hostile to tumor operators with higher viability and less symptoms that can be obtained at a reasonable

cost [8,9,10]. In such manner, the hunt and advancement of new medications have expanded, and plant turned into a pertinent asset for the revelation of anticancer compounds [11,12]. Normally inferred against proliferative medications, for example, doxorubicin, bleomycin, daunorubicin, vincristine, mitomycin C and vinblastine assume an imperative part in therapeutic growth chemotherapy in various strong tumors, and hematological malignancies [12,13]. Herbal products are worldwide accepted as a source of complementary and alternative medicine in [14] various diseases especially in cancer [15]. They provide us relatively safe, effective, and economical therapeutic options, particularly in case of cancer where treatment is long term and cost is excruciatingly high [16]. A vast amount of plant species globally is tested and bio assayed for the drug discovery of multiple illnesses globally [17]. Medicinal plants from systematic screening programs or by serendipity possess an important position in the drug discovery and many modern drugs have their origin in traditional medicine of different cultures.

Many natural compounds such as terpenoids, phenolic acids, lignans, tannins, flavonoids, quinones, coumarins, and alkaloids have been found from plant sources that have significant antioxidant activity and play an important part in the therapy of cancer [18]. Several studies have shown that antioxidant compounds demonstrate anti-inflammatory, antitumor, antimutagenic, and anticarcinogenic activity [19]. Natural antioxidant compounds can directly prevent the proliferation of cells and boost the immune system [20]. The cancer and infectious disease study regions have a leading role on the use of medicinal plants as a drug discovery source. The FDA endorsed extremely validated anticancer and anti-infectious drugs for 60-75 percent of natural drugs such as medicinal crops [21]. These drugs have been regarded from the discovery of natural drugs in chemoprevention for chemotherapeutics in cancer studies [22,23]. Hence, the present work is an attempt to study the anticancer activity of *L.pungens* whole plant belonging to the family; Acanthaceae using cell line cultures.

2. MATERIALS AND METHODS

Plant collection and authentication

The mature and healthy whole plant of *L.pungens* was collected from Tirunelveli district. The specimen was authenticated by Dr.V.Chelladurai, M.Sc., Ph.D., Research Officer-Botany, Central Council for Research in Ayurveda & Siddha, Govt. of India. Tirunelveli, Tamil Nadu and specimen have been deposited in the Department of Pharmacognosy, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal District, Tamil Nadu.

Preparation of crude extracts

The whole plant of *L.pungens* was precisely washed with tap water and dried underneath dimness in room temperature for two weeks. Then they were crushed into powder and stored in room temperature. The pulverized materials were passed through sieve no. 40 and 80. The crushed materials of identical size present between those two sieves were collected and packed in an airtight container for supplementary use. About 1 kg of shaded and dried whole plant *L. pungens* was extracted in soxhlet successively with petroleum ether for defatting and ethanol. Extract was evaporated using rotary vacuum evaporator. The ethanolic extract

obtained was weighed and stored for further use. All the solvents used for this work were of analytical grade.

Preliminary Phytochemical Analysis of L.pungens Extracts

The ethanolic extract was screened for the presence of phytochemicals as described in the textbook by Harborne A J [24]. Freshly prepared extracts were tested for flavonoids (Alkaline reagent test, Shinoda's test, Lead acetate test and Sulphuric acid test), alkaloids (Dragendorff's test, Hager's test, Mayer's test, Wagner's test and Tannic acid test), steroids (Salkowsky's test and Libermann - Burchard's test), glycosides (Baljet test, Legal's test, Keller- killiani test and Borntrager's test), saponins (Lead acetate and Foam test), tannins (Ferric chloride test ,Gelatin test), Vanillin hydrochloride test, Lead acetate test, Ammonia test and Potassium dichromate test.), phenolic components, fixed oils (Spot test and Saponification test),carbohydrates (Fehling's test, Molisch'stest, Tollen'stest, Benedict's test, Seliwanoff's test and Bromine water test), proteins (Ninhydrin test, Biuret test, Xanthoproteic test and Millon's test) and terpenoids (Knoller'stest).

In Vitro Cytotoxic Activity

Principle

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Cell proliferation kit

MTT (Roche applied sciences, Cat. No. 11465 007 001).

Media

DMEM (Dulbecoo's Modified Eagels medium, high glucose), DMEM (Dulbecco's Modified Eagels medium, low glucose), FBS (Fetal Bovine Serum) (Bioclot, Lot No: 07310).

Glasswares and plastic wares

96-well micro titer plate, Tissue culture flasks, Falcon tubes, Reagent bottles

Equipments

Fluorescence inverted microscope (Leica DM IL), Biosafety cabinet class II (Esco), cytotoxic safety cabinet (Esco), CO_2 incubator (RS Biotech, mini galaxy A), Sciences; Veer Narmad South Gujarat University, Surat by Dr. Minoobhai Parabia, Dr. Ritesh Vaidh.

Cell line used for cytotoxicity screening

Rat skeletal muscle cell line (L6), Ehrlich's Ascites Carcinoma cell line (EAC), Human Breast cancer cells(MCF 7), human cervical cells (He La) and human hepatocellular carcinoma cell line (Hep G2), the cell lines brought from Amala Cancer Institute, Thrissur, Deep freezer, ELISA plate reader (Thermo), Micropipettes (Eppendorff), RO water system (Millipore).

Procedure[25]

The monolayer cell culture was trypsinized and the cell count was adjusted to 3-lakh cells/ml using medium containing 10 percent newborn calf serum. To each well of 96 well microtitre plates, 0.1 ml of diluted cell suspension was added. After 24 hours, when the monolayer

formed the supernatant was flicked off and 100 µl of different test compounds were added to the cells in microtitre plates and kept for incubation at 37º C in 5% CO_2 incubator for 72 hours and cells were periodically checked for granularity, shrinkage, swelling. After 72 hours, the sample solution in wells was flicked off and 50 µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37º C in 5% CO_2 incubator. The supernatant was removed, 50 µl of iso-propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 490 nm.

The percentage growth inhibition was calculated using following formula,

% Cell Inhibition = 100-{(At-Ab) / (Ac-Ab)} x100

Where,

At= Absorbance value of test compound.

Ab= Absorbance value of blank.

Ac=Absorbance value of control.

Data interpretation

Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

% Cell Survival= {(At-Ab) / (Ac-Ab)} x100

% Cell Inhibition= 100 - Cell Survival

3. RESULTS AND DISCUSSION

Ethanolic extract of the whole plant of *Lepidagathis pungens* was screened for cytotoxic properties on L6, EAC, MCF, He La and Hep G2 cells lines by MTT Assay. The ethanolic extract not exhibited any cytotoxicity against the normal L6 cell line. Among EAC, MCF, He La and Hep G2 cell lines showed significant cytotoxicity with the IC_{50} value of 177.19 µg/ml, 160.97 µg/ml, 309.12 µg/ml and 497.50 µg/ ml. Among the cell lines the ethanolic extract showed significant cytotoxicity against MCF 7 cell line followed by moderate activity against EAC , He La and Hep G2 cell lines as shown in Tables and Figures. Preliminary phytochemical studies of ethanolic extract demonstrated the presence of flavonoids, alkaloids, phenols and triterpenoids. Flavonoids, tannins, saponins and triterpenes have all been reported to possess antitumor activity [26,27,28,29]. Flavonoids anticancer activity has been associated with various mechanisms such as the modulation of cell cycle arrest at the G1/S phase,induction of cyclin-dependent kinase inhibitors, downregulation of anti-apoptotic gene products, inhibition of cell-survival kinase and inhibition of inflammatory transcription factors and induction of Ca2+dependent apoptotic mechanism. Saponins have been reported to induce apoptosis by causing permeabilization of the mitochondrial membranes [28] or necrotic cell death depending on the types of cancer cells. Triterpenes were also found to cause cell cycle disruption by decreasing the number of cells in G0/G1 phase, with initial increases in S and G2/M [29] or by inhibiting nuclear factor-kappa B (NF-κB) [30]. Flavonoids have been found to possess antimutagenic and antimalignant effect [31,32] . Moreover, they have a chemopreventive role in cancer through their effects on signal

transduction in cell proliferation and inhibition of neovascularization [33,34]. However, further indepth studies are required before final conclusion on the mechanisms involved could be drawn to explain the observed activity. In conclusion, the ethanolic extract of *Lepidagathis pungens* possess anticancer activity against various types of cancer cells, to be correlated to their total phenolic content.

Table No:1. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens Nees*., whole plant on L6 cell line using MTT assay

S.No	**Conc.(µg/ml)**	**% cell viability**	**% Cytotoxicity**	**IC_{50} Value (µg/ml)**
1.	control	99.45±0.54	0.55±0.45	1972.11 ± 4.58
2.	15	94.36±2.26	5.64±0.52	
3.	30	91.53±1.56	8.47±2.14	
4.	60	86.15±2.33	13.85±2.14	
5.	120	80.64±1.21	19.36±1.25	
6.	240	78.25±2.15	21.75±2.04	
7.	500	74.89±1.59	25.11±1.44	
8.	1000	65.28±2.65	34.72±2.74	
9.	1500	56.49±1.48	43.51±1.78	
10.	2000	51.26±4.16	48.74±4.22	

Values are expressed as mean ± SEM, n=3

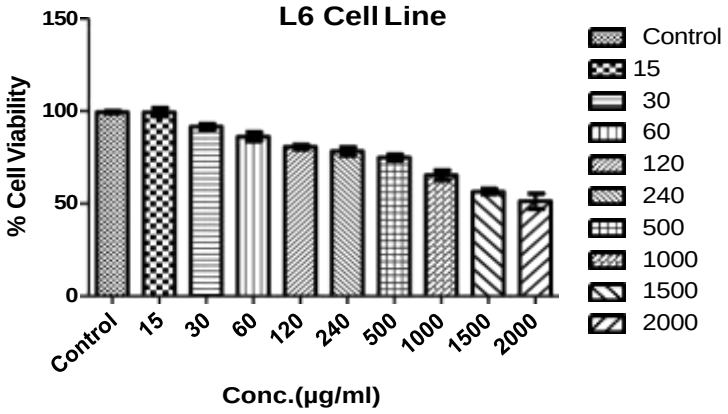

Figure No:1. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens Nees*., whole plant on L6 cell line

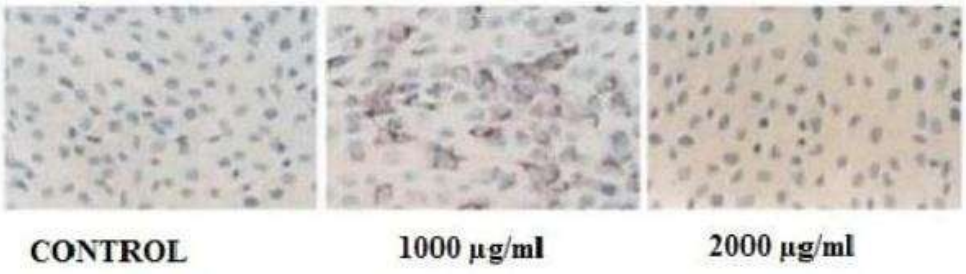

Figure No: 2. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens Nees*., whole plant on L6 cell line

Table No:2. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens Nees*., whole plant on EAC cell line using MTT assay

S.No	**Conc.(µg/ml)**	**% cell viability**	**% Cytotoxicity**	**IC $_{50}$ Value (µg/ml)**
1.	Control	98.27±1.51	1.73±1.52	546.06 ± 8.47
2.	15	84.32±2.66	15.68±2.65	
3.	30	76.54±3.41	23.46±3.51	
4.	60	67.33±2.16	32.67±2.63	
5.	120	59.88±0.89	40.12±0.79	
6.	240	49.97±2.04	50.03±2.10	
7.	500	35.48±2.73	64.52±2.64	
8.	1000	27.63±2.58	72.37±2.85	
9.	1500	19.17±1.84	80.83±1.75	
10.	2000	7.95±1.41	92.05±1.24	

Values are expressed as mean ± SEM, n=3

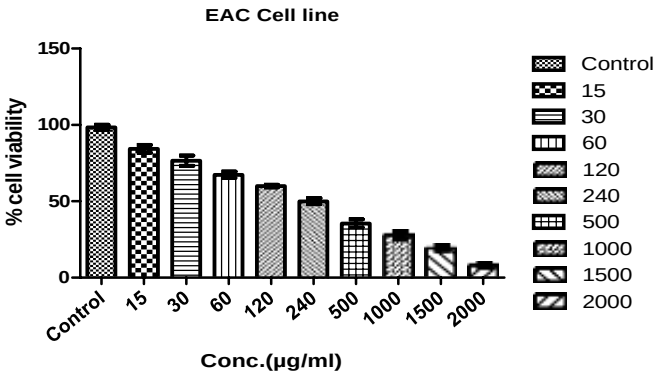

Figure No: 3. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens Nees*., whole plant on EAC cell line

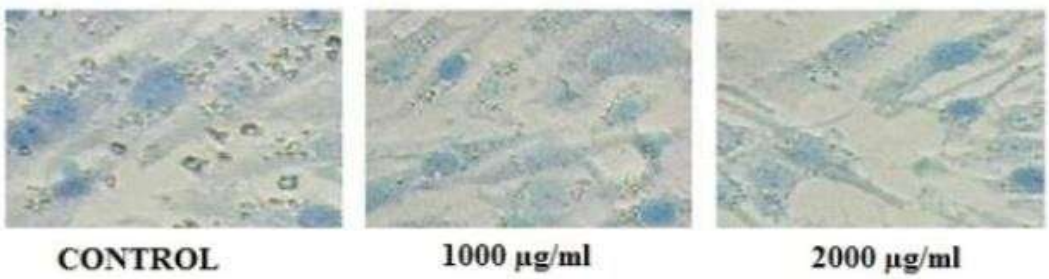

Figure No:4. Effect of ethanolic extract of *Lepidagathis pungens Nees*., whole plant on EAC cell line

Table No:3. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens Nees*., whole plant on MCF-7 cell line using MTT assay

S.No	Conc.(µg/ml)	% cell viability	% Cytotoxicity	IC $_{50}$ Value (µg/ml)
1.	Control	98.53±1.25	1.47±1.051	160.97 ± 2.87
2.	15	78.85±2.16	21.15±1.49	
3.	30	65.54±1.56	34.46±0.85	
4.	60	53.26±2.65	46.74±0.76	
5.	120	43.55±3.25	56.45±2.64	
6.	240	31.46±2.36	68.54±3.48	
7.	500	24.24±2.15	75.76±2.84	
8.	1000	17.54±1.75	82.46±2.54	
9.	1500	7.57±2.28	92.43±2.42	
10.	2000	1.10±0.61	98.90±0.51	

Values are expressed as mean ± SEM, n=3

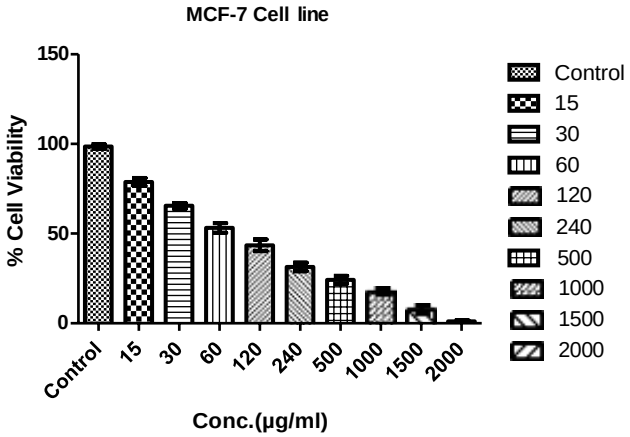

Figure No: 5. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens Nees*., whole plant on MCF - 7 cell line

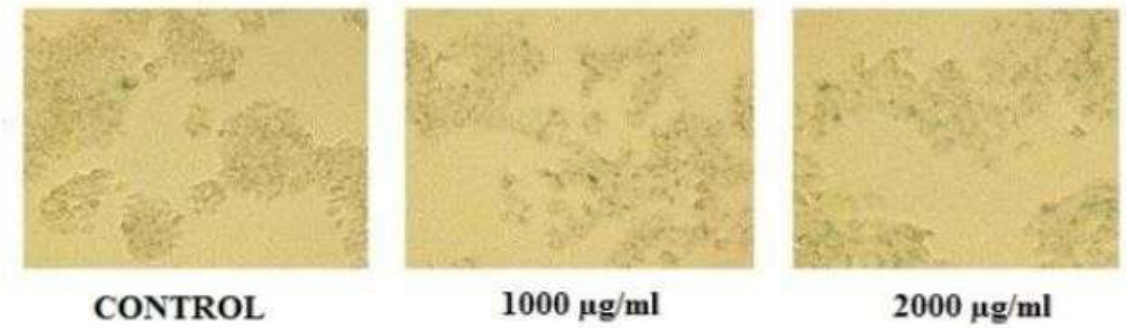

Figure No: 6. Effect of ethanolic extract of *Lepidagathis pungens Nees*., whole plant on MCF - 7 cell line

Table No:4. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens* Nees., whole plant on HeLa cell line using MTT assay

S.No	Conc.(µg/ml)	% cell viability	% Cytotoxicity	IC $_{50}$ Value (µg/ml)
1.	Control	98.34±1.21	1.66±1.84	309.12 ± 2.45
2.	15	83.0±2.61	17.0±2.86	
3.	30	73.84±2.48	26.16±1.87	
4.	60	61.50±3.02	38.50±1.38	
5.	120	48.17±2.32	51.83±0.94	
6.	240	34.84±2.79	65.16±2.88	
7.	500	26.34±2.66	73.66±2.32	
8.	1000	16.34±2.73	83.66±4.86	
9.	1500	8.5±1.87	91.5±3.82	
10.	2000	1.5±0.55	98.5±1.84	

Values are expressed as mean ± SEM, n=3

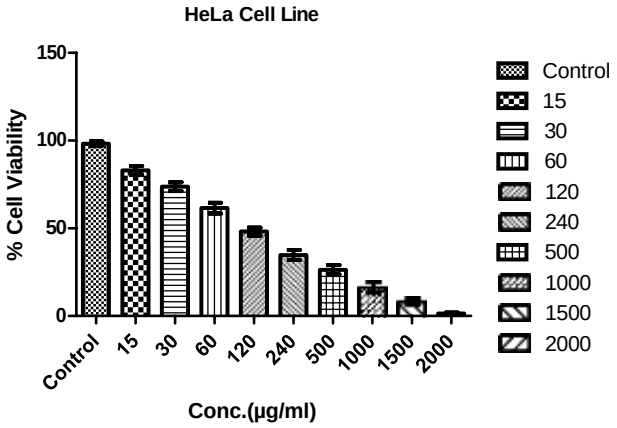

Figure No: 7. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens* Nees., whole plant on He La cell line

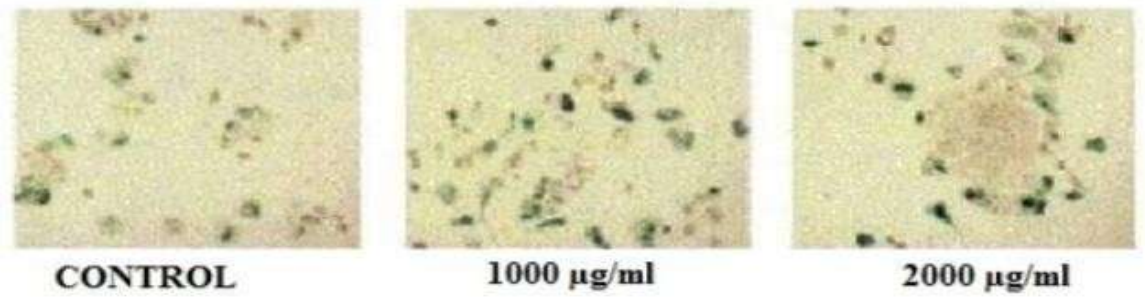

Figure No: 8. Effect of ethanolic extract of *Lepidagathis pungens* Nees., whole plant on He La cell line

Table No:5. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens* Nees., whole plant on HEP G2 cell line using MTT assay

S. No.	Conc.(µg/ml)	% cell viability	% Cytotoxicity	IC $_{50}$ Value (µg/ml)
1.	Control	98.84±0.75	1.16±1.86	497.50 ± 2.49
2.	15	97.17±1.72	2.83±2.85	
3.	30	77.34±1.63	22.66±0.24	
4.	60	67.5±2.88	32.50±4.23	
5.	120	59.17±2.99	40.83±0.62	
6.	240	38.00±1.79	62.00±1.59	
7.	500	30.00±3.03	70.00±0.95	
8.	1000	23.34±1.64	76.66±1.56	
9.	1500	12.67±2.66	87.33±0.88	
10.	2000	6.84±2.14	93.16±0.90	

Values are expressed as mean ± SEM, n=3

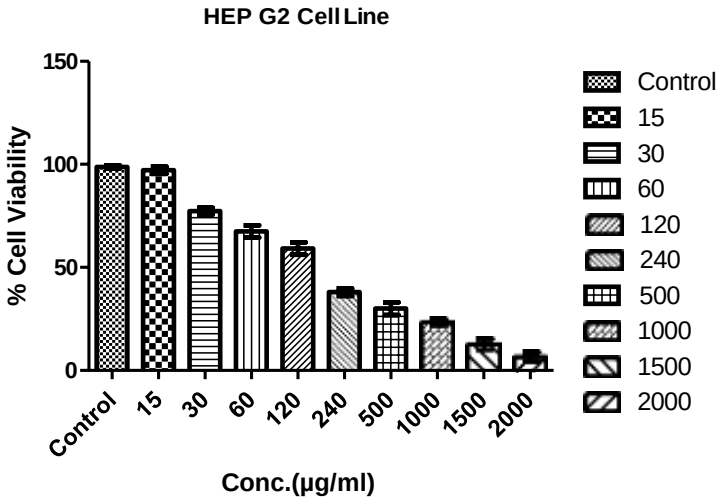

Figure No: 9. Evaluation of cytotoxicity and cell viability for ethanolic extract of *Lepidagathis pungens* Nees., whole plant on Hep G2 cell line

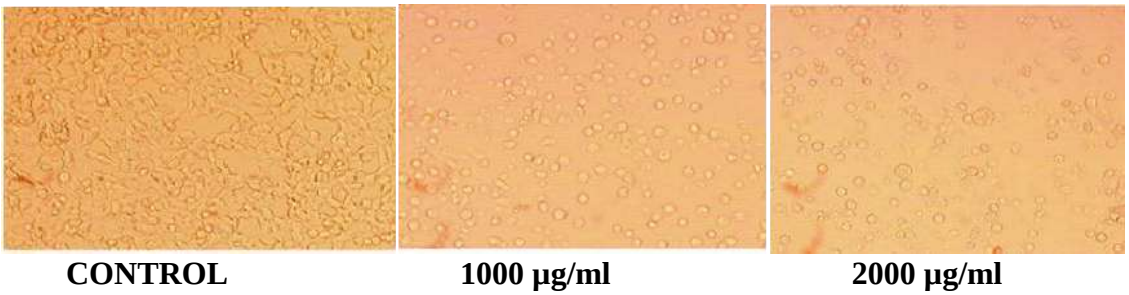

Figure No: 10. Effect of ethanolic extract of *Lepidagathis pungens* Nees., whole plant on Hep G2 cell line

4. CONCLUSION

The ethanolic extract of *Lepidagathis pungens* Nees., has more significant anticancer activity, it is mainly because of the phytoconstituents present in the extract. The phytochemical study revealed the presence of various phytoconstituents like flavanoids, phenolic compounds, alkaloids, triterpenoids, etc. The further study is planned to isolate the active constituent responsible for the anticancer nature of the ethanolic extract of *Lepidagathis pungens* Nees., and to elucidate the mechanism of action are in progress.

Acknowledgement

The authors are thankful to Research centre-Department of Pharmaceutics, Swamy Vivekanandha College of Pharmacy, Namakkal District for providing all the facilities to carry out this research work.

5. DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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