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Cytotoxicity effect of Ethanol extract of *Kedrostis Foetidissima(Jacq.)Cogn.* in Human Osteosarcoma Cell Lines (MG-63)

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Abstract: The ethanol extract of *Kedrostis foetidissima (Jacq.) Cogn*. was analysed for its cytotoxicity against Human Osteosarcoma Cell Line (MG-63) using MTT assay. The IC50 value (154.9 µg/ml) of the dose dependant analysis implies ethanol extract of *Kedrostis* to show good cytotoxicity, inhibit cell growth and induce cell death and to act as potent natural source in cancer treatment.

Keywords: Kedrostis, Osteosarcoma Cell Line, Anticancer

Introduction: Millennium together, plants have been a resource of drugs and their constituents play a vital part in medicine. The constituents derived from various parts of medicinal plants may contain bioactive metabolites [1].In ancient times, plant extracts has been used for treating various diseases; this practice was widely increased in this decade. Traditionally, for long time, herbal medicines are being used in the healing of cancer [2].Plant derived constituents and their derivatives were effective in treating a variety of cancers [3, 4, 5,]. Anticancer property is effectively shown by few bioactive compounds derived from natural sources. Certain plant derived compounds used in conventional medicine are general dietary elements considered to be safe [6].

The genus Kedrostis consists of 58 species. Among this 58 species, *Kedrostis foetidissima* has traditionally been used in South India, Sri Lanka and Ethiopia in the treatment of various diseases. Recent findings shows that this medicinal plant posses anti bacterial [7], antioxidant [8], wound healing [9], anti-diabetic [10] activity. This medicinal plant shows anti proliferative activity against lung cancer [11] and breast cancer [12]. The above findings intended us to analyse Cytotoxicity effect of Ethanol extract of *K.foetidissima* in human osteosarcoma cell lines (MG-63).

Materials and Methods

Collection and Identification of Plant Materials

Kedrostis foetidissima (jacq.)cogn., was collected from Aliyar hills, Pollachi, Tamilnadu. The plant specimen was authorized by Dr.P.Satyanarayanan, Scientist 'D', Botanical survey of India, S.R.C., TN Agri University, Coimbatore, Tamilnadu (BSI/SRC/5/23/2010- 11/Tech. - 1309). The collected whole plant material was cleaned well and shade dried.

Preparation of plant Extract

The dried plant parts of K. *foetidissima* were grounded into fine particles. Calculated quantity of plant material (25 g) was refluxed for 6 h with 1L distilled ethanol. The supernatant ethanol extract was evaporated to crude extract and refrigerated for further use.

Cytotoxicity Analysis

Cytotoxicity study of ethanol extract of *Kedrostis foetidissima(Jacq.)Cogn.* in human osteosarcoma cell lines (MG63) was performed according to standard procedure[13,14].

Cell lines

The human osteosarcoma cell lines (MG63) were got from NCCS(National Centre for Cell Science), Pune and developed in Eagles Minimum Essential Medium consists of 10% FBS (fetal bovine serum). The cells were preserved at 5% CO2, 95% air, 37^{0}C and 100% virtual moisture.

MTT Assay

The monolayer cells were separated with Trypsin- EDTA (ethylenediaminetetraacetic acid) to create single cell suspensions. Hemocytometer is used to count the viable cells and the concentration was reduced using medium includes 5% FBS to get ultimate density of 1x10^5 cells/ml. Cell suspensions were seeded into 96-well plates with the concentration of 100µl/well having the density of 10,000 cells/well. Cell suspensions were incubated at 5% CO2, 95% air, 37^{0}C and 100% virtual moisture for cell attachment. After twenty four hours, the cells were made to

react with different concentrations of extracts. The extracts were first dissolved in DMSO (dimethylsulfoxide) to get an aliquot. It was diluted to twice the required ultimate utmost concentration using serum free medium. Total of five different concentrations were obtained by four further serial dilutions. One hundred micro litres of each sample were mixed to the wells which previously have 100 µl of medium, ensuring in the essential final concentrations. Then the plates with sample and medium were incubated at 5% CO2, 95% air, 37^{0}C and 100% virtual moisture for further 48 h. A control was prepared simultaneously without samples. For accurate results, triplicate was made for test samples as well as for control. 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide (MTT) is reacted upon by mitochondrial enzyme in living cells, succinate-dehydrogenase. This cleaves the tetrazolium ring, converting the yellow MTT to an insoluble purple formazan. The amount of formazan formed was directly proportional to the number of viable cells.

After incubation (48 h), 15µl MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated for 4h at 37^{0}C. The medium containing MTT was then removed off and the formed formazan crystals were dissolved in 100µl of DMSO. The absorbance was measured at 570 nm using micro plate reader.

% Cell Inhibition = 100- Absorbance of sample /Absorbance of control x100.

The above formula was used to calculate the % cell inhibition. Nonlinear regression graph was drawn using Log concentration and % Cell inhibition and **IC$_{50}$** was resolved by means of the software Graph Pad Prism.

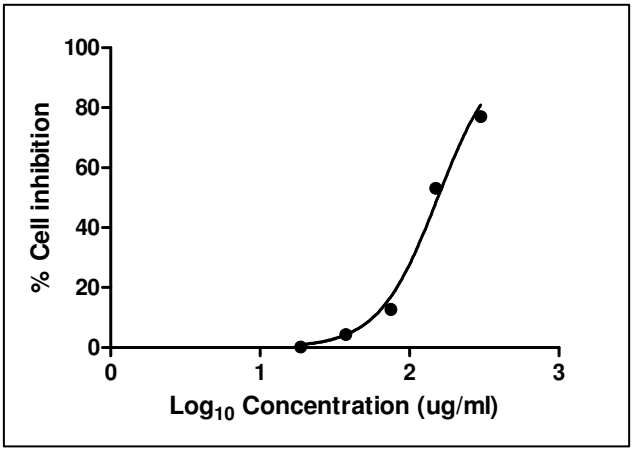
Results and Discussion

The ethanol extract of K. *Foetidissima* was analysed for its cytotoxicity efficacy against human osteosarcoma cell lines (MG-63) in a dose dependant manner by MTT assay. The results obtained and the **IC$_{50}$** values calculated are tabulated (**Table-1**). The observations were plotted as nonlinear regression graph between % Cell inhibition and Log concentration **(Fig-1)**.Figures 2-6 shows the cell inhibition at various concentrations. Fig-7 is the cell inhibition of control. The IC$_{50}$ (154.9 µg/ml) value shows *K. foetidissima* to be a potent natural source against Human Osteosarcoma Cell Lines MG-63. This reveals *K. foetidissima* to be a promising source in treating bone cancer. Phytochemical analysis confirms the presence of metabolites such as alkaloids, Phenols, Terpenoids, Steroids in *K. foetidissima* which may be responsible for this appreciable anticancer activity.

Table-1. Cytotoxicity of K. *foetidissima* on Human Osteosarcoma Cell Lines (MG-63)

S.NO	CONC (µG/ML)	% CELL INHIBITION
1.	18.75	0.236035
2.	37.5	4.40598
3.	75.0	12.66719
4.	150.0	53.10779
5.	300.0	77.02596

Fig-1 Nonlinear regression graph between % Cell inhibition and Log concentration (µg/ml)



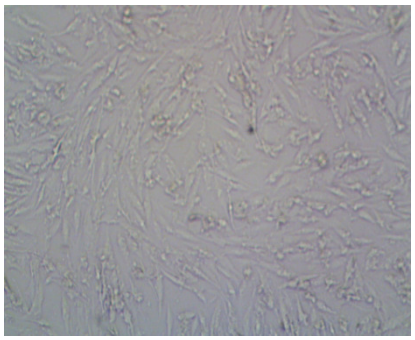


Fig-2 Cell Inhibition at 18.75 (µg/ml)

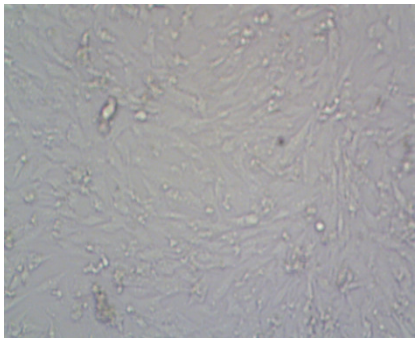


Fig-3 Cell Inhibition at 37.5 (µg/ml)

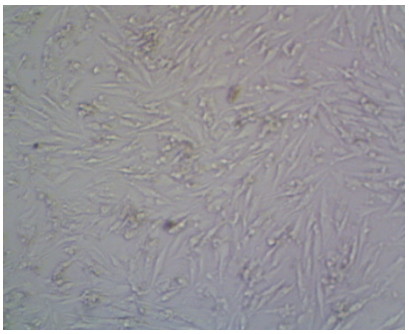


Fig-4 Cell Inhibition at 75 (µg/ml)

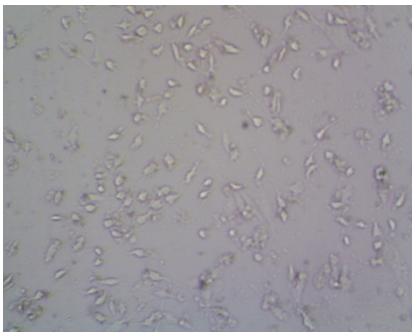


Fig-5 Cell Inhibition at 150 (µg/ml)

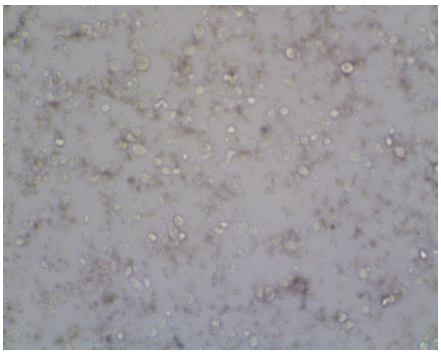


Fig-6 Cell Inhibition at 300 (µg/ml)

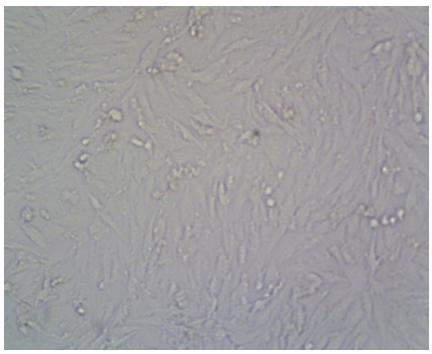


Fig-7 Cell Inhibition Control

K. *foetidissima*, a medicinal plant of cucurbitacae family, which can be easily domesticated, has immense therapeutical value. Various extracts of K. *foetidissima* are reported to possess anti cancer activity against Human Lung cancer (A-549), Breast cancer
(MCF-7) and also recommended for pharmacological tests on HIV cases. K. *foetidissima* was proved to posses anti bacterial, anti fungal, antioxidant property and also wound healing efficiency. Search of literature shows minimum research work on this species K. *foetidissima*. In our laboratory, column chromatographic isolation of medicinally active compounds from the extracts of this plant is in progress. The IC50 value 154.9 (µg/ml) of this study prominently reveals the potency of the plant to induce cell death and hinder cell growth. The results of this research promises a potent natural source for herbal based cancer drug without any side effects.

Conclusion

Natural therapies may reduce the risk of side effects caused by chemotherapy in cancer treatment. Now-a-days some plant derived bioactive compounds are being used in the treatment of cancer. Moreover several *in vitro* studies have proved various plant derived substances to

show promising anticancer efficacy. The result of our present study also reveals K.*foetidissima to* show promising cytotoxicity against Human Osteosarcoma Cell Lines (MG-63) due to the presence of medicinally effective secondary metabolites in its extracts. Further isolation of compounds and their efficacy as anti-cancer drug is warranted.

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