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In vitro cytotoxic activity of *Mesua ferrea* L. (Seeds) Extracts

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Abstract
The *In vitro* cytotoxicity tests on the extracts of *Mesua ferrea L. against* Hek (Human embryonic kidney cell lines), IMR-32 (Human neuroblastoma) and C6 (Rat Glioblastoma) were achieved using MTT assay. The absorbance of each well was determined using a microplate reader at 550nm. A graph of cell viability versus concentrations was plotted for each extract. The half maximal inhibitory concentration (IC_{50}) values were obtained from the plotted graph. Futher dilutions will only be performed on the extracts with IC_{50} values less than 15 µg/ml. Three independent experiments were conducted to assure the accuracy of the results. The crude extracts of M. ferrea L. (Seeds) showed significantly anticancer activities against Hek (Human embryonic kidney cell lines), IMR-32 (Human neuroblastoma) and C6 (Rat Glioblastoma). Among the two extracts Ethanol extract showed strong anticancer activity agains the three tested cancer cell lines while as hexane extract showed moderate anticancer activity.

Keywords: *mesua ferrea* L., *in vitro* cytotoxic activity

1. Introduction
Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value. Natural product compounds from plants provide biologically active compounds, many of which have been developed as new lead chemicals for pharmaceuticals [1]. Cancer which is the uncontrolled growth of abnormal cells in the body [2] is a serious health problem. Treatments include surgery, chemotherapy and radiation therapy. Many cancers have developed resistance to prolonged chemotherapy. Hence there is a need to develop more effective and safer medicines such as herbal medicines.
M. ferrea L. is a tree of tropical Asia and belongs to family Guttiferae. Various parts of the plant are used medicinally in India, China, Malaysia and Thailand [3, 4]. Its bark is given in treatment of cough, dysentery, vomiting, sore throat and fever. Their flowers are astringent and stomachic. The leaves and flowers in combination with other drugs are used for the treatment of snake bite and scorpion sting. The seed oil is used as an embrocation in rheumatism and found useful in the treatment of itch [5]. The aim of this study was to discover plants with promising bioactivities which can then be developed into drugs through preclinical and clinical developments. Our ongoing research is focused on the screening of the extracts of M. ferrea L. (Seeds) against a panel of human cancer cell lines by using MTT Assay.

2. Materials and methods
2.1 Chemicals
The organic solvents used in the experiments were of analytical grade and purchased from Qualigen Chemicals, India. The other chemicals used were of analytical grade and obtained from Merck, India.

2.2 Plant sample
Air-dried seeds of M. Ferrea L. were collected from the local spice market of District Ujjain, India. Plant samples were duly authenticated by department of Botany, Vikram University, Ujjain, India.

2.3 Extraction procedure
The plant samples were washed several times with tap water and finally with distilled water to remove dust. The samples were dried under shade at room temperature. The seeds were separated from dried pods by crumbling and then screening. The shade dried seeds were further ground by means of a mechanical blender (Bajaj GX10, India) to fine powder. One hundred grams of the seed powder was sequentially extracted for 3 days with each solvent hexane (500 mL ×3) and ethanol (500 mL×3) using a Soxhlet apparatus over a water bath. The extracts obtained were filtered through Whatman No. 1filter paper and then evaporated to dryness by using a rotary evaporator (Buchi, Switzerland). The final crude extracts were collected in an airtight container and then refrigerated at 4 ± 2 ◦C until further use.

Cytotoxicity Assay (MTT Assay)
The anticancer activities of the crude extracts of M. ferrea L. (Seeds) against the three Cancerous cell lines were undertaken by using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetra zolium bromide) assay. The three tested cancer cell lines were Hek (Human embryonic kidney cell lines), IMR-32 (Human neuroblastoma) and C6 (Rat Glioblastoma). All the three cell lines were maintained in DMEM (Media) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic (Pencillin/ Streptomycin). All the cell lines were cultured in 75cm^2 T- flask and maintained at 37 ^{0}C in 5% carbon dioxide humidified incubator for 24 hours. The MTT Assay is based on the protocol illustrated by Mosmann [6] and was executed in 12- well flat bottom plates. The varying concentrations of 5,10,15,20,25,30,35,40,45,50 and 55 µg/ml were prepared by a serial dilution method. After 24 hours 300µl of MTT solution and PBS (Phosphate buffer saline) was added to all the wells and incubated for 3 hours in a 5% CO_2 humidified incubator.

300µl of DMSO (Solubilization buffer) were added to each well and incubated for 10 minutes.

The absorbance of each well was determined using a microplate reader at 550nm. A graph of cell viability versus concentrations was plotted for each extract. The half maximal inhibitory concentration (IC_{50}) values were obtained from the plotted graph. Futher dilutions will only be performed on the extracts with IC_{50} values less than 15 µg/ml. Three independent experiments were conducted to assure the accuracy of the results.

3. Results and Discussion

The crude extracts of M. ferrea L. (Seeds) showed significantly anticancer activities against Hek (Human embryonic kidney cell lines), IMR-32 (Human neuroblastoma) and C6 (Rat Glioblastoma). Among the two extracts Ethanol extract showed strong anticancer activity agains the three tested cancer cell lines while as hexane extract showed moderate anticancer activity.

C6 Glioblastoma)	
Ethanol	**Hexane**
0.498	0.395
0.472	0.368
0.455	0.357
0.421	0.329
0.402	0.313
0.386	0.298
0.349	0.283
0.332	0.273
0.315	0.254
0.278	0.244
0.269	0.239
0.263	0.231

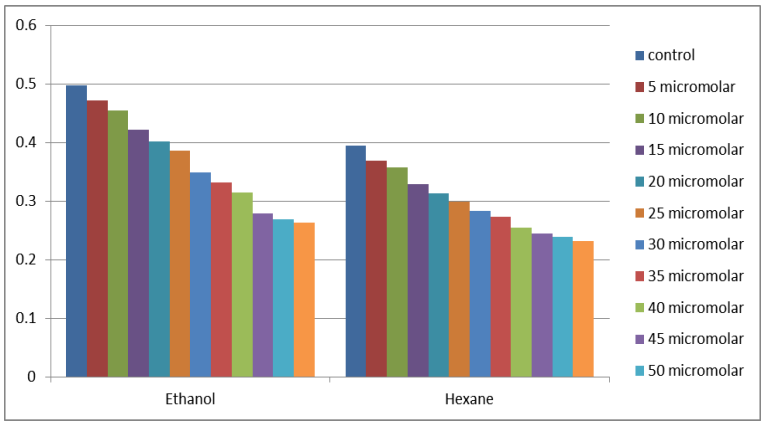

Fig 1

HEK-T	
Ethanol	**Hexane**
0.591	0.531
0.542	0.503
0.433	0.413
0.415	0.383
0.386	0.354
0.377	0.346
0.332	0.313
0.315	0.283
0.278	0.273
0.269	0.262
0.263	0.254
0.249	0.249

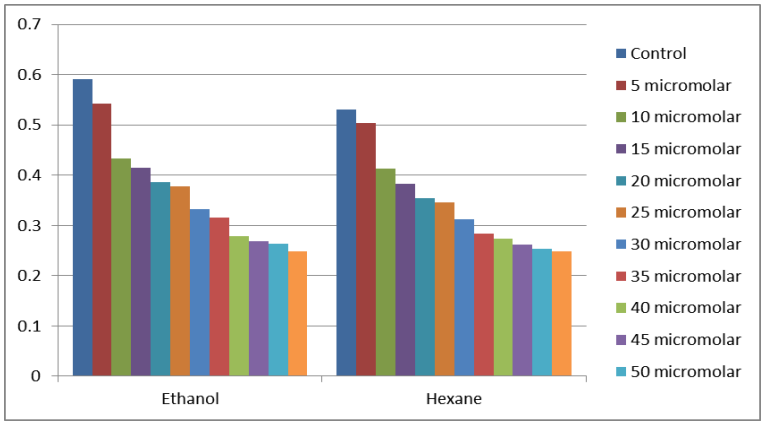

Fig 1

IMR32	
Ethanol	**Hexane**
0.573	0.531
0.561	0.503
0.491	0.413
0.473	0.383
0.452	0.354
0.441	0.346
0.412	0.313
0.392	0.283
0.362	0.273
0.354	0.262
0.349	0.254
0.335	0.249

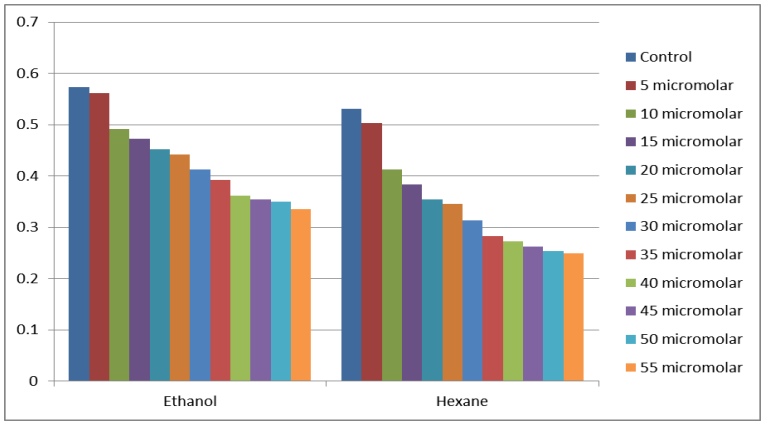

Fig 1

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5. Conclusion

Therefore according to these results, we suggest that the crude extracts of M. ferrea L. (Seeds) could be another potential source for the new drug development.

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