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By

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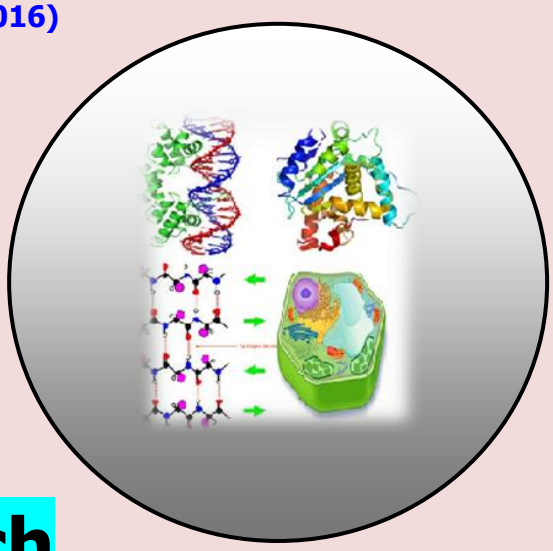

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RESEARCH PAPER

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Anticancer activity of *Butea monosperma* Linn.

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ABSTRACT

Traditional knowledge is valid and necessary. Butea monosperma represents astronomical Moon, a feminine aspect in Indian Traditional Knowledge. The cervical cancer is the fourth most common cancer in women. With an intention of benefit to them, an in-vitro efficacy of cold macerated aqueous extract of stem bark of Butea monosperma against HeLa cancer cell lines was studied through MTT assay, as such aqueous extract was not reported earlier against HeLa cancer cell lines. As concentration of extract increased viability of cell lines decreased. At 50 µg/100 µL concentration 77.88 % and at 450 µg/100 µL only 20.57 % of cell viability was observed. IC 50 value was 269 µg/100 µL. 350 µg/100 µL and 450 µg/100 µL concentration of extract showed less viability percentage than Oxaliplatin (Control) and indicated the probable presence of potent bioactive phytochemicals. Further purification of crude extract, identification of compounds using chromotagraphic techniques and searching identified compounds in plant based anticancer compound databases may lead to further progress in anticancer research.

Key words: Indian Traditional Knowledge, Butea monosperma, crude aqueous stem bark extract, MTT assay HeLa cancer cell lines and Oxaliplatin.

Abbreviations: HeLa=*Henrietta Lacks or "Helen Lane" or "Helen Larson"* ; MTT=*3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide*; IC=*Inhibitory concentration*;µg=*microgram*; µL=*microliter*; µmol=*micromoles*; DMSO= *Dimethyl sulphoxide*; DMEM= *Dulbecco's Modified Eagle's Medium*; D-PBS=*Dulbecco's phosphate buffered saline*; ELISA= *Enzyme linked immune Sorbent Assay*; GCMS=*Gas Chromatography- Mass spectrometry*; LCMS = *Liquid Chromotagraphy -Mass Spectrometry*; IEC=*Ion Exchange Chromatography*; HPLC=*High Performance Liquid Chromatograpy;* BM = *Butea monosperma*

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INTRODUCTION

The Cervical cancer remains the most common cancer in women in Eastern and Middle Africa. Around 85 percent of global burden occurs in the less developed regions (Globacan.iarc.fr). Cancer is the leading cause of the morbidity and mortality worldwide. Currently over a hundred types of cancers are known differentiated by aetiology and natural history (Jamal et al., 2016). The global burden of cancer cases may rise to 21.4 million by 2030 (Aslam et al., 2017). Natural products play a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases (Newman and Cragg, 2017). It has been a predominant practice that as new chemical entities become hard to find, scientists across the world, often go back to earlier substances to find novel properties (Shravan, 2012). Traditional knowledge is valid and necessary. Indian Traditional Knowledge quotes '*Palashagam Somaya*' (Chandrakanth et al., 1990) means *Butea monosperma* represents astronomical entity Moon which is recognized as a feminine aspect in Vedic culture. *Butea monosperma* (Linn.) (Fabaceae) commonly known as Flame of the forest, is a medium-sized dry season-deciduous tree, growing to 15 m (49 ft) tall (Amit KD, 2017) and it is used in traditional system of medicine for the treatment of various diseases. Medicinal uses of the *Butea monosperma* are mentioned in the ancient scriptures of Ayurveda, Siddha, Unani and Homeopathy. The present study is aimed to examine the *in-vitro* cytotoxicity effect of cold macerated crude aqueous extract of stem bark of *Butea monosperma* on HeLa cervical cancer cell line, which was not reported earlier.

MATERIALS AND METHODS

Chemicals, Reagents and Equipment

DMSO (PHR1309, Sigma), DMEM- High Glucose - (AL111, Himedia), Fetal Bovine Serum (RM10432, Himedia), MTT Reagent (5 mg/ml) (4060 Himedia), D-PBS (TL1006, Himedia), Oxaliplatin (O9512, Sigma), 96-well plate for culturing the cells (Corning, USA), T25 flask (12556009, Biolite - Thermo), 50 ml centrifuge tubes (546043 Torson), 1.5 ml centrifuge tubes (Torson), 10 ml serological pipettes (Torson), Adjustable multichannel pipettes and a pipettor (Benchtop, USA), 10 to 1000 ul tips (Torson), 96-well ELISA plate reader or spectrophotometer capable of measuring the absorbance (ELX-800 Biotek), Inverted microscope (Biolink), 37°C incubator with humidified atmosphere of 5% CO2 (Healforce, China).

Plant identification

Through field check survey (www.fs.fed.us/r6/sfpnw/issssp/.../inv-sp-tesp-survey-field-guide-2005-03.doc pp.16), at around Geo-coordinates 17˚24'21.45''N 78˚31'27.41''E*, Butea monosperma* was identified in May 2012 using standard keys and descriptions near Department of Genetics and Biotechnology, Osmania University, Hyderabad, India.

Extraction

10-15 grams of dried coarse bark powder was transferred to conical flask and 100 mL of distilled water was added. A magnetic bead was placed inside the conical flask and placed on running magnetic stirrer, for 6-8 hours, after which whole contents were transferred to sterile petriplates which were placed in clean surface area, to dry for 2 days. Pasty extracts were scraped and collected into vials, now known as crude extract. *Stock preparation*: 1 mg crude extract was dissolved in 200 µl of pure DMSO, and to that 800 µl of DMEM- High Glucose was added to get 1 mg /ml stock. This stock was further diluted to required concentrations as shown in *table 1.*

In-vitro cytotoxicity assay

Cell line and culture

Human cervical carcinoma HeLa cell lines were procured from National Centre for Cell Science, Pune. Cells were cultured and maintained in DMEM – High glucose media.

In-vitro MTT assay

The 3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) dye reduction assay was performed to determine the cytotoxic effect of aqueous bark extract of *Butea monosperma* at various concentrations.

The assay depends on the reduction of MTT by mitochondrial dehydrogenase, an enzyme present in the mitochondria of viable cells, to a blue formazan product. 200 µL cell suspension was seeded in a 96-well plate (20,000 cells per well), without the test agent. Cells were allowed to grow for about 12 hours and treated with various concentrations (50, 150, 250,350 and 450 µg/100 µL) of aqueous extract of bark of *Butea monosperma*. The wells were incubated for 24 hours at 37 ˚C in a 5% CO2 atmosphere in a humidified incubator. After 24 hours of incubation, 10 µL of MTT (0.5 mg/ml in PBS) was added to each well, and plate was wrapped with aluminium foil to avoid exposure to light and further incubated for three hours at 37 °C. The resulting formazan was dissolved in 100 µL of dissolving buffer (DMSO) and gently stirred in a gyratory shaker to enhance dissolution. Absorbance of the solution was then read at 570 nm using ELISA reader. 630 nm was used as reference wavelength. All determinations were carried out in duplicates. Concentration of aqueous bark extract of *Butea monosperma* showing 50% reduction in cell viability (IC 50 value) was then calculated using linear regression equation i.e. y=mx+c, where y = 50, 'm' and 'c' values were derived from the viability graph.

Assay controls:
(i) Medium control (medium without cells)
(ii) Negative control (medium with cells but without the experimental drug/compound)
(iii) Positive control (medium with cells and with 50 µ mol of Oxaliplatin)

RESULTS

In-vitro cytotoxic effect

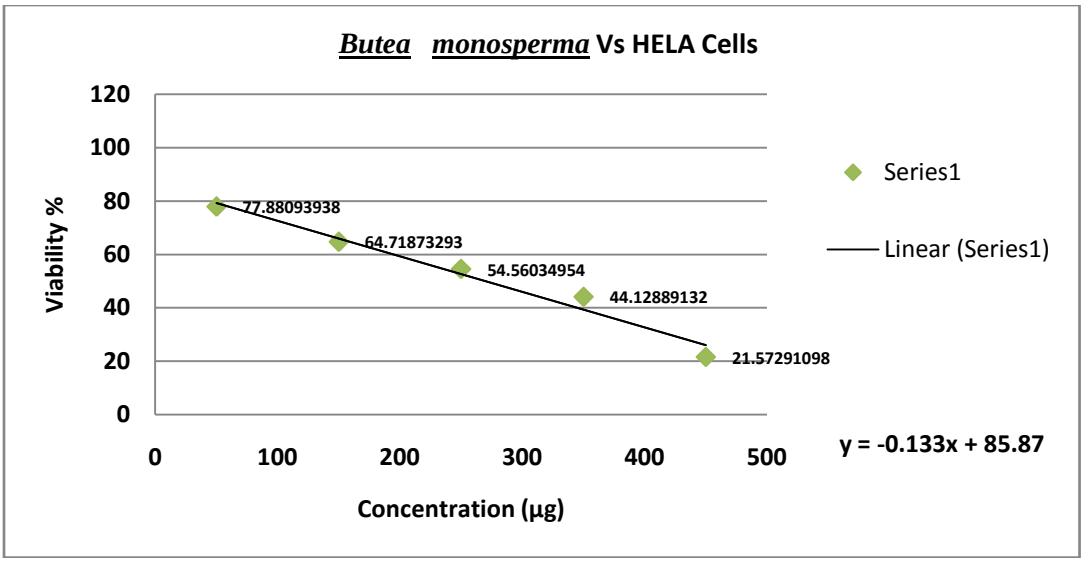
The cytotoxicity improved with increase in concentration of the extract as shown in *Graph 1, Table 2* and *Fig. 1.0* to *7.1.* At 50 µg/100 µL 77.88 % of cell viability and at a high concentration of 450 µg/100 µL only 20.57 % of cell viability was observed. IC 50 value was of 269 µg/100 µL. 350 µg/100 µL and 450 µg/100 µL of aqueous extract of bark of *Butea monosperma* showed less cell viability than Oxaliptalin (50 µmol).

Table 1. Dilution of Stock of plant extract.

Sl No	Stock Concentration	Drug/Extract	DMEM Media	Total (µL)	In ELISA Plate we added after cell seeding
1	50 µg /ml	50 µL	450 µL	500 µL	150 µL
2	150 µg /ml	150 µL	350 µL	500 µL	150 µL
3	250 µg /ml	250 µL	250 µL	500 µL	150 µL
4	350 µg /ml	350 µL	150 µL	500 µL	150 µL
5	450 µg /ml	450 µL	50 µL	500 µL	150 µL

Table 2. Results of *In-vitro* MTT assay.

Parameter	Blank	Untreated (UT)	OXP (50 µ mol)	50 µg	150 µg	250 µg	350 µg	450 µg
Reading 1	0.011	0.937	0.491	0.743	0.611	0.524	0.421	0.222
Reading 2	0.017	0.922	0.483	0.711	0.602	0.503	0.415	0.201
Mean	0.014	0.9295	0.487	0.727	0.6065	0.5135	0.418	0.2115
Mean OD-Mean B	NA	0.9155	0.473	0.713	0.5925	0.4995	0.404	0.1975
Standard deviation	-	0.01060 6602	0.005656854	0.022627	0.006364	0.014849	0.004243	0.014849
Standard error	-	0.00750 1133	0.004000604	0.016002	0.004501	0.010502	0.003	0.010502
Viability %	NA	100	51.66575642	77.88094	64.71873	54.56035	44.12889	21.57291
IC50= 269								



Graph 1. *Butea monosperma* and HELA cells.

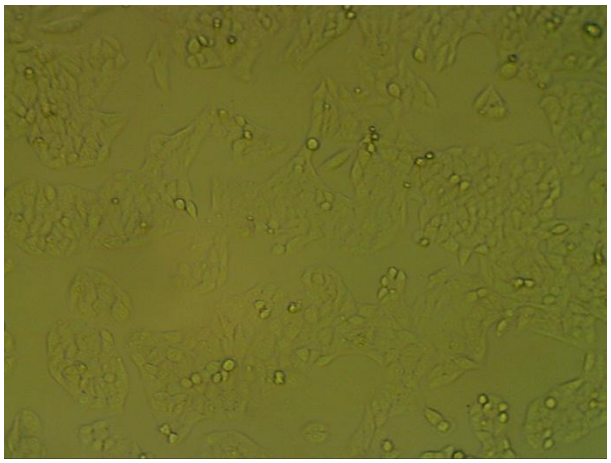


Figure 1.0 Untreated HELA cells – 1.

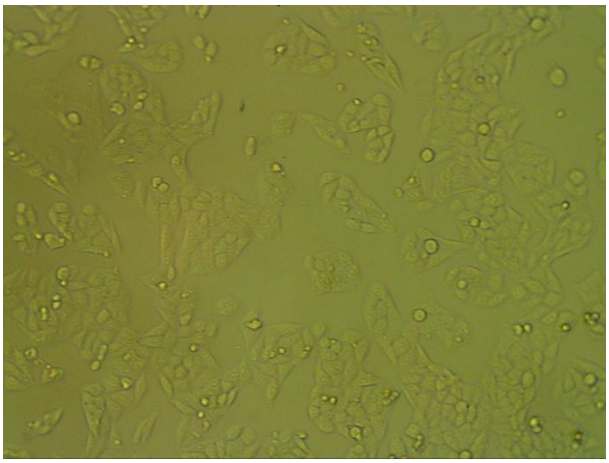


Figure 1.1 Untreated HELA cells – 2.

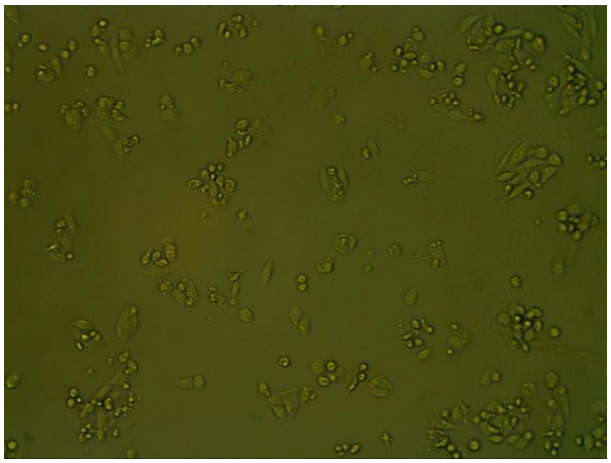


Figure 2.0 Oxaliptalin (50 µ mol) treated HELA cells – 1.

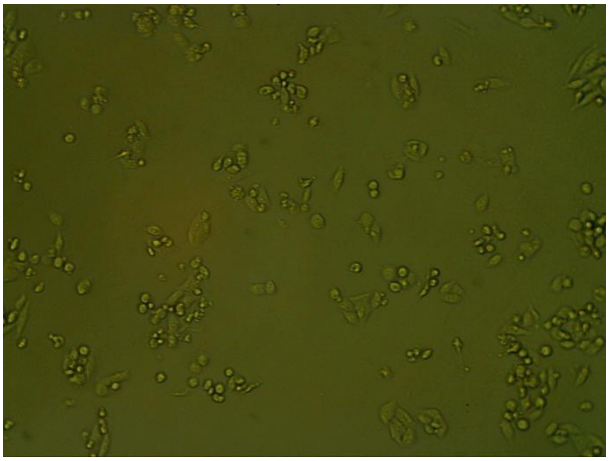


Figure 2.1 Oxaliptalin (50 µ mol) treated HELA cells -2.

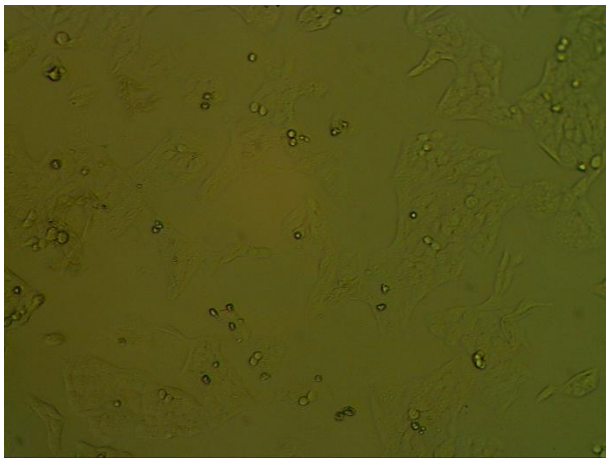


Figure 3.0 *Butea monosperma (BM)* (50 μ g) treated HELA cells – 1.

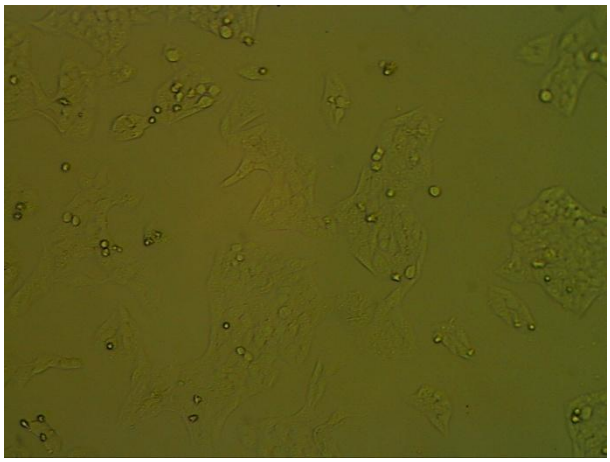


Figure 3.1 BM (50 μg) treated HELA cells – 2.

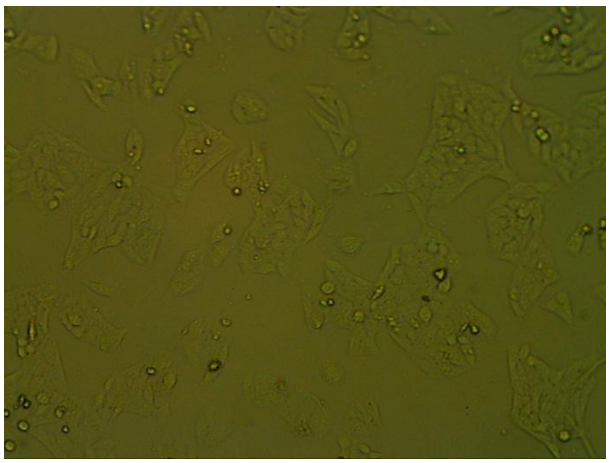


Figure 4.0 BM (150 μg) treated HELA cells -1.

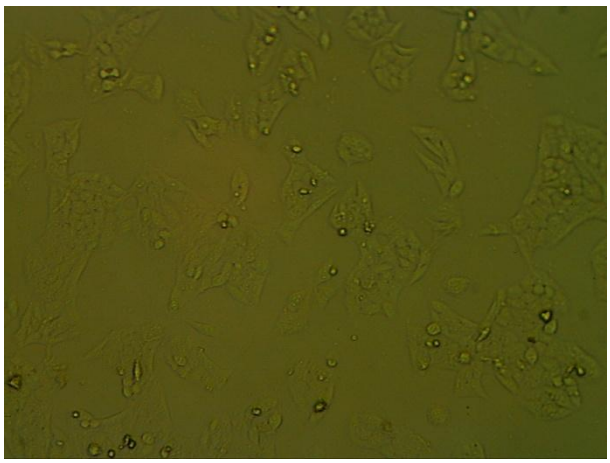


Figure 4.1 BM (150 μ g) treated HELA cells – 2.

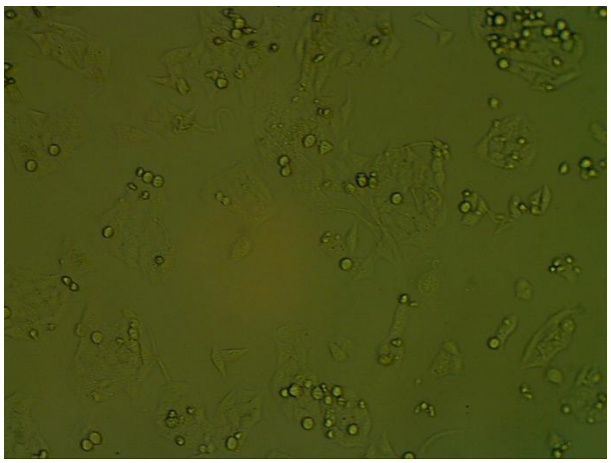


Figure 5.0 BM (250 μg) treated HELA cells -1.

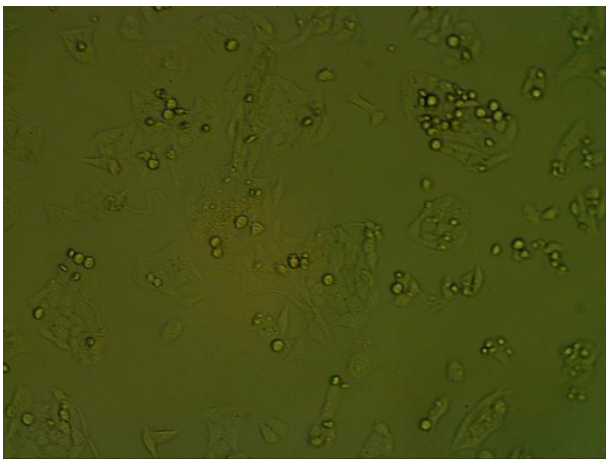


Figure 5.1 BM (250 μg) treated HELA cells -2.

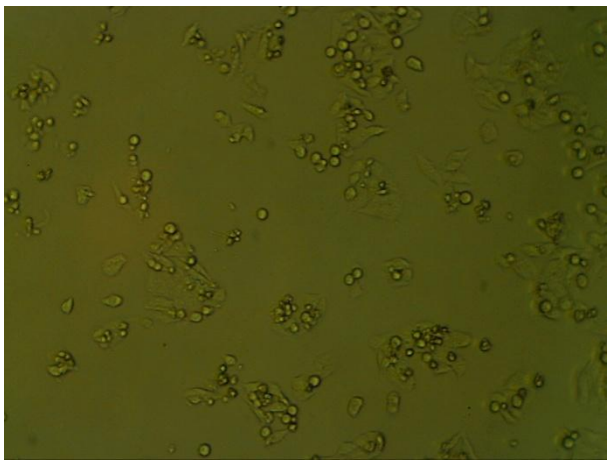


Figure 6.0 BM (350 μ g) treated HELA cells – 1.

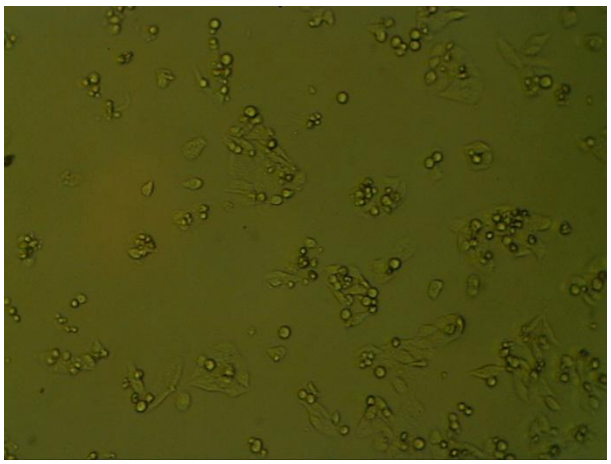


Figure 6.1 BM (350 μg) treated HELA cells – 2.

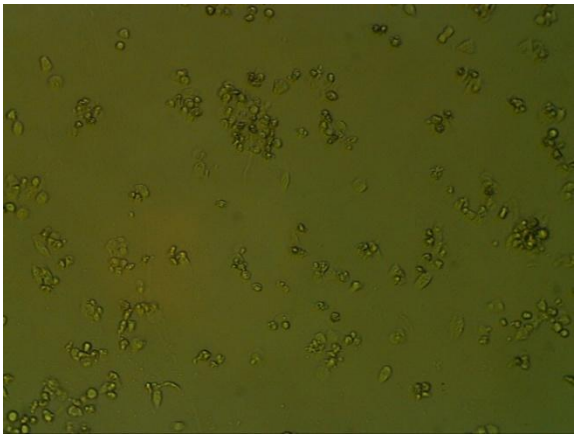


Figure 7.0 BM (450 μg) treated HELA cells – 1.

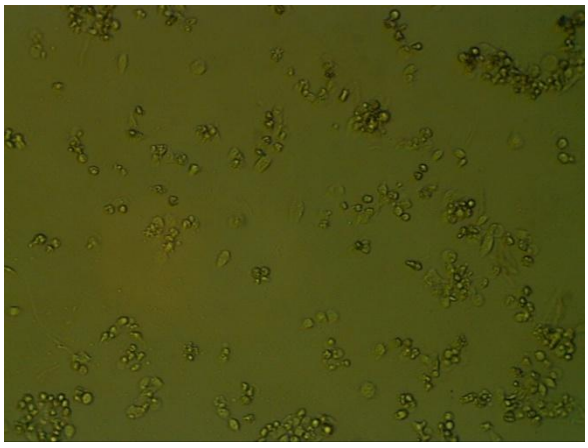


Figure 7.1 BM (450 μg) treated HELA cells – 2.

DISCUSSION

As many of the current treatments are limited for anti-cancer activity, it is necessary to identify other anticancer drugs, which are more potent, selective and less toxic than conventional treatment. From results of this research, it is clear that aqueous crude bark extract of *Butea monosperma* may do contain comparatively potent bioactive anticancer phytochemicals than Oxaliplatin against HeLa cervical cells. Extracts has to be further purified and analyzed through chromotagraphic techniques like GCMS, LCMS, IEC,HPLC etc. to identify the compounds. Presence and absence of anticancer potency of individual compounds present in a crude extract/purified fraction can be known through InPACdb(Indian Plant Anticancer Compound database), npact(Naturally occurring Plant base Ant cancerous Activity-compound-target database), TIPdb (Taiwan Indigenous Plant Database), NPCare(Natural products and fractional extracts for cancer regulation database), CancerHSP (Herbs database of Systems Pharmacology) etc., databases. Once an anticancer potency of a compound is found recorded in a database, further research can be focused on its isolation, purification and *in-vitro* and *in-vivo* studies, for future humane benefit.

CONCLUSION

Traditional knowledge awaits its currently relevant wider application for human benefit. Therefore the traditional spiritual virtues and medicinal values of *Butea monosperma* recorded in ancient Indian literature has to be further explored to find a novel solution to global burning issues like Cancer, AIDS and other similar ailments, for the welfare and wellbeing of humankind and nature.

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