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EVALUATION OF ANTI - CANCER ACTIVITY OF BARK OF CRATAEVA *NURVALA* BUCH. HAM AGAINST THREE CELL LINES

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Keywords:

Crataeva nurvala, MTT assay, A549 cell lines, Hela cell lines, MDA-MB cell lines, Anti-cancer activity

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ABSTRACT: The present study was aimed to evaluate *in-vitro* anti-cancer activity of ethanolic extract of *Crataeva nurvala* Buch. Ham. The method used for determining the anticancer activity was the MTT bio-assay using A549(Human Lung Carcinoma) cell line, Hela (Human Cervix)cell line and MDA-MB(Human Adenocarcinoma, Mammary Gland)cell line. The ethanolic extract of *Crataeva nurvala* bark at different concentration (10µg, 20 µg and 30 µg) was used as test for this bioassay. Vincristine30µg/ml (anticancer drug) was used as reference standard and blank as control. Optical density and % cell lysis was estimated at the end of the study. Extract showed promising anticancer effect. % Cell lysis was found to be in the range nearer to the reference standard for all the cell lines and optical density at 492nm is (0.575to 1.191) almost equal to that of reference standard i.e. 1.151 and always more than blank. The IC_{50} values were found to be less than 10µg against A549 cell line, the activities against HELA cell line were found to be 13µg and IC_{50} values were found to be 20µg against MDA-MB cell lines. These finding support that *Crataeva nurvala* bark extract was found to have a good anti-cancer activity.

INTRODUCTION: Cancer is a group of diseases characterized by abnormal, excessive, uncoordinated, autonomously controlled and purposeless proliferation of cells due to local tissue invasion and distant metastasis [1]. Cancer is the second to the cardiovascular disease in causes of mortality in world. More than 1.2 million cases of cancer are diagnosed annually and more than 500,000 lives in United State each year. According to Kidwai Memorial Institute of Oncology, the crude incidence of cancer in India is about 1000 per 100,000 populations i.e. approximately one third the rate of developed countries and continuously increasing per day due to stressful life style [2].

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In recent years, the increase in the number of cancer cases has motivated the growth of cancer research. A large number of natural products and dietary components have been evaluated as potential chemo preventive agents, and herbal remedies used in traditional folk medicine provide a largely unexplored source of potential novel drugs[3]. Chemotherapy is an effective treatment against various types of cancer either singly or in combination with surgery and/or radiotherapy.In spite of a number of new anti-cancer drugs the prevalence of cancer has been increased now-a-days.

However, chemotherapeutic effects of most of the drugs showed limited efficacies due to the development of various side effects[4]. It has become a major cause of death and there is an urgent need for its control. The most important drawback of the current cancer therapeutic practices such as chemotherapy and radiation therapy is the

suppression of immune system. Plants have been a prime source of natural products for the treatment of various diseases and they are highly effective conventional drugs for the treatment of many forms of cancer[5-9]. A traditional and folklore medicine plays an important role in the health services around the globe. About three quarters of the world population relies on the plants and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity. Several plants have been used folklore medicine[10].

Crataeva nurvala was yet not scientifically reported having anti-cancer activity. However plant is frequently preferred in the treatment of Urinary disorders, Prostate enlargement, Bladder sensitivity and Kidney stones. Plant also showed Anti-arthritic, Hepatoprotective, and Cardio-protective actions[11]. Lupeol is a naturally occurring triterpene isolated from *Crataeva nurvala* stem bark, various *In-vitro* and Preclinical animal studies suggested that lupeol has a potential to act as an Anti-inflammatory, Anti-microbial, Anti-protozoal, Anti-proliferative, Anti-invasive, Anti-angiogenic and cholesterol lowering agent. It is noteworthy that lupeol has been reported to selectively target diseased and unhealthy human cells, while sparing normal and healthy cells[12-13]. The objective of the present work is to evaluate the anti-cancer activity of the ethanolic extract of *Crataeva nurvala* bark.

MATERIALS AND METHODS:

Plant Material: *Crataeva nurvala* Buch-Ham (Family: Capparidaceae) commonly known as Varuna, is an evergreen tree indigenous to India [14]. It is a medium sized branched deciduous plant distributed throughout the river banks of southern India and other tropical, sub-tropical countries of the world, wild or cultivated[15]. It requires dry, hot climate and shady places to grow effectively. Vedic literatures described its potentiality as blood purifier and to maintain homeostasis.

Its bark is hot, bitter at first and then sweets harp taste, easy to digest, Stomachic, Laxative, Anthelmintic, Expectorant and Anti-pyretic. Plant of *Crataeva nurvala* collected from the forest region nearby Amravati, Maharashtra, India. The collected plant was authenticated from Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur; the voucher specimen has been deposited in the office (specimen No.9802)

FIGURE 1: AUTHENTICATED HERBERIUM OF *CRATAEVA NURVALA* FROM RASHTRASANT TUKADOJI MAHARAJ NAGPUR UNIVERSITY, NAGPUR

Preparation of Extract: Dried coarsely powdered bark of *Crataeva nurvala* (500 g) were defatted with petroleum ether at 50^0 - 60^0 C for 72 h. using Soxhlet apparatus. The marc left subsequently extracted with ethanol (95%, 60-70^{o}C) for 72 h. The crude brown residue mass of extract were concentrated, stored and preserved (2-8^{o}C). The Percentage yield of extract (5 % w/w) was found on dry wet basis.[16].

Maintenance of Cell culture: The method used was the MTT assay for evaluation. The A549 cell lines, Hela cell lines and MDA-MB cell lines were maintained at The Maratha Mandal Dental College, Belgaum, Karnataka. Cell lines were grown in Minimal Essential Medium (MEM) supplemented with 4.5 g/L Glucose, 2 ml L-Glutamine and 5% Fetal Bovine Serum (FBS) (growth medium) at 37 ^{0}C in 5% CO_2 incubator.

MTT Assay[17-18]:

Materials and Equipment: 5mg/ml MTT (3-{4, 5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromine solution in PBS. Sterilized by filtration, Stored in dark at 4°C, Stable a month, 96-well plates, Multichannel pipettes, Micro-plate reader equipped with a 490 nm filter.

Experimental procedure: After treatment of cells with the xenobiotics, the cell monolayers were getting washed with warm PBS using a multichannel pipette.100 µL of culture medium containing MTT solution (10:1) was added to each well. It was recommended to have some wells in the plate without cells but incubated with MTT solution in order to have blanks of the readings. Cells were kept for 3h in the cell incubator. Incubation medium was carefully removed.100µL of Dimethyl sulphoxide was added and the plate is gently shaken to re-suspend formed for reason and waited until a homogenized color a formed.

Reading of results and calculations: Absorbance was measured at 490 nm of wells containing cells and blanks. The mean of the absorbance of wells was calculated with the all treatments after subtracting of blank absorbance. The results were normalized considering control wells as 100% (maximum absorbance obtained), expressing then the results as percentage of controls. The O.D. of control was taken to be negative. Increase in O.D. indicates increased cell lysis. The O.D. increases due to increased turbidity which was due to increased cell lysis. Three concentrations (10, 20 and 30µl) of extract was used. The IC_{50} value and % lysis cell at every concentration was also calculated. Three cell lines were used for performing anticancer activity i.e A549, HELA, MDA-MB.

The percentage inhibition of cancer cell lines was calculated by the following equation

$$100-(At-Ab) / (Ac-Ab) \times 100$$

Where At: Absorbance of test,
Ab: Absorbance of blank,
Ac: Absorbance of control.

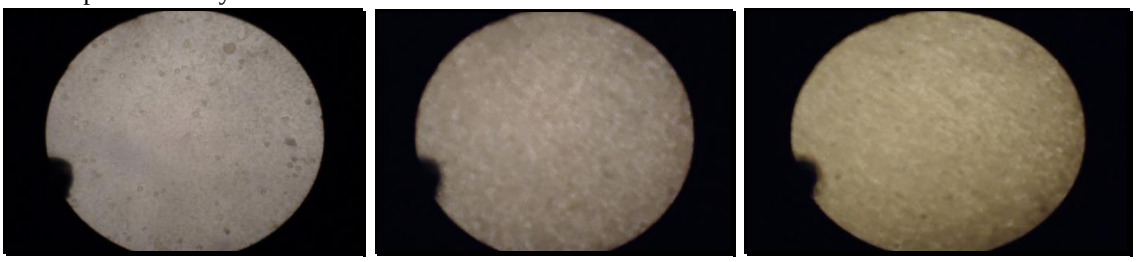
RESULT: The effect of ethanolic extracts of *Crataeva nurvala* (Et*Cn*) was studied by using MTT assay.

Against A549-Human lung carcinoma cell line: The IC_{50} value of *Crataeva nurvala* against A549-Human lung carcinoma cell line was found to be 10µg and the results are mentioned in **Table 1** and **Figure 2** and **Figure 3**.

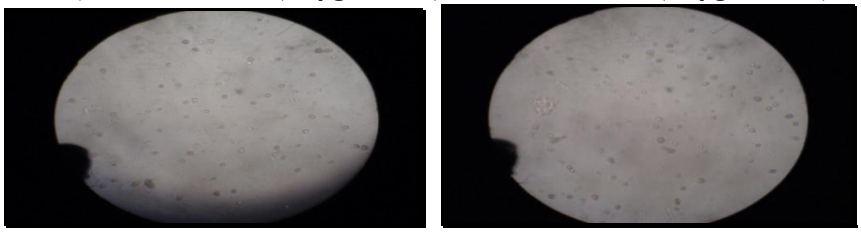
TABLE 1: *IN-VITRO* CYTOTOXICITY EFFECT AGAINSTA549 CELL LINES

S.No.	Name of drug	Concentration (µg)	O.D. at 492nm	%of cell lysis	IC_{50}
1.	*Crataeva*	10	1.454	70%	
2.	*nurvala*ethanolicextract	20	1.588	87%	10µG
3.	(Et*Cn)*	30	1.659	100%	
4.	Vincristine	30	1.151	90%	
5.	Control	00	0.379	No lysis	

Where, O.D. –Optical Density



(10µg of Et*Cn*) **(20 µg ofEt*Cn*)** **(30 µg of Et*Cn*)**



(Vincristine 30µg) **(Control)**

FIGURE 2: MTT BIOASSY MICRO PLATES FOR THE CELL LINE A549 - HUMAN LUNG CARCINOMA AT VARIOUS CONCENTRATIONS

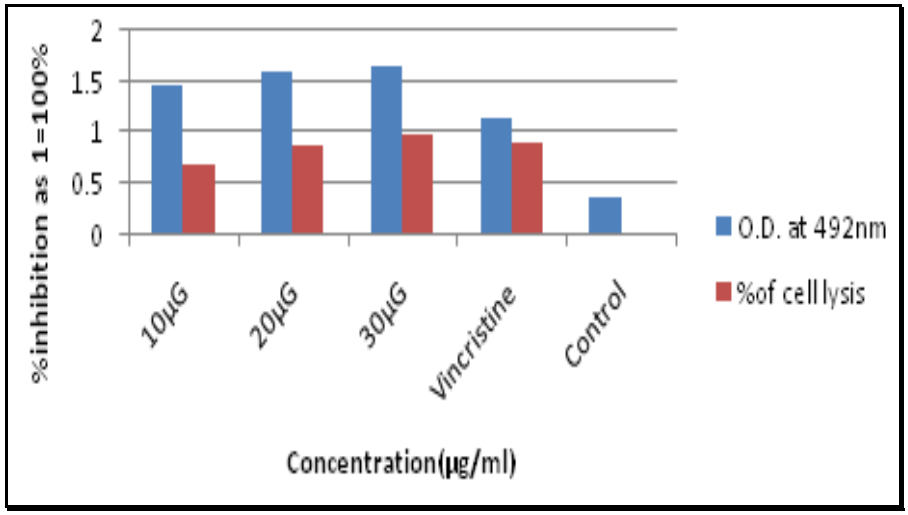


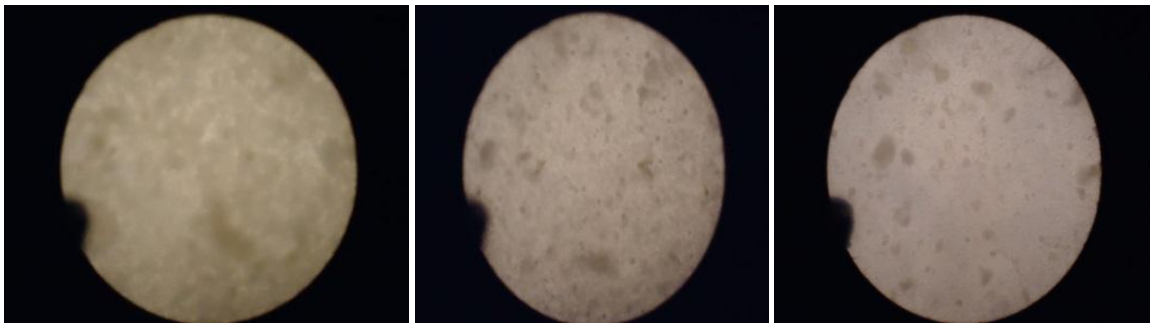
FIGURE 3: GRAPHICAL PRESENTATION OF OPTICAL DENSITY AND % CELL LYSIS AGAINST THE CELL LINE A549 - HUMAN LUNG CARCINOMA AT VARIOUS CONCENTRATIONS

Against HELA-Human cervix carcinoma cell line: The IC_{50} value of *Crataeva nurvala* against HELA cell line was found to be 13µg and the results are mentioned in **Table 2**. and **Figure 4** and **Figure 5**

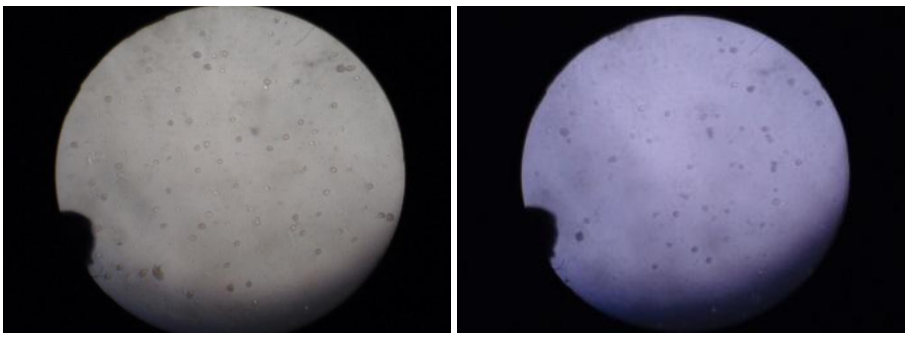
TABLE 2: *IN-VITRO* CYTOTOXICITY EFFECT AGAINST HELA CELL LINE

S.No.	Name of drug	Concentration (µg)	O.D. at 492nm	%of cell lysis	IC_{50}
1.	*Crataeva*	10	0.575	50%	
2.	*nurvala*ethanolicextract	20	0.958	75%	13 µG
3.	(Et*Cn*)	30	1.111	100%	
4.	Vincristine	30	1.151	90%	-
5.	Control		0.379	No lysis	

Where, O.D. –Optical Density



10µg of Et*Cn* **20µg of Et*Cn*** **30µg of Et*Cn***



Vincristine (30µg) **Control**

FIGURE 4: MTT BIOASSY MICROPLATE FOR THE CELL LINE HELA - HUMAN CERVIX CARCINOMA AT VARIOUS CONCENTRATIONS

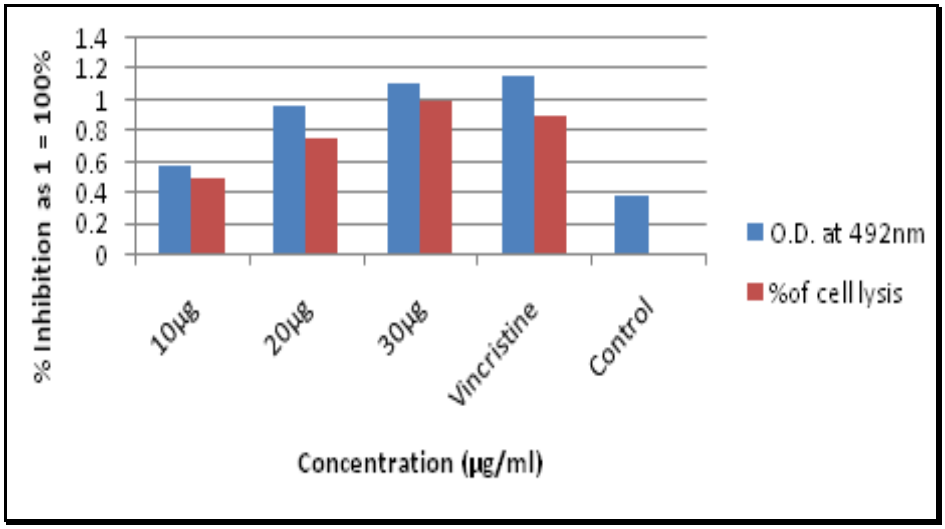


FIGURE 5: GRAPHICAL PRESENTATIONOF OPTICAL DENSITY AND % CELL LYSIS AGAINST THE CELL LINE HELA - HUMAN CERVIX CARCINOMA AT VARIOUS CONCENTRATIONS

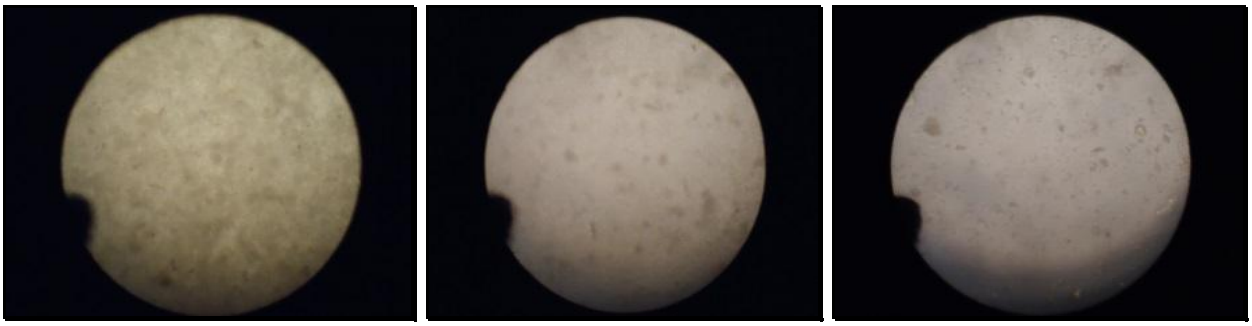
Against MDA-MB-Human Adenocarcinoma, Mammary Gland Carcinoma cell line:

The IC_{50} value of *Crataeva nurvala* against MDA-MB cell line was found to be 20µg and the results are mentioned in **Table 3** and **Figure 6** and **Figure 7.**

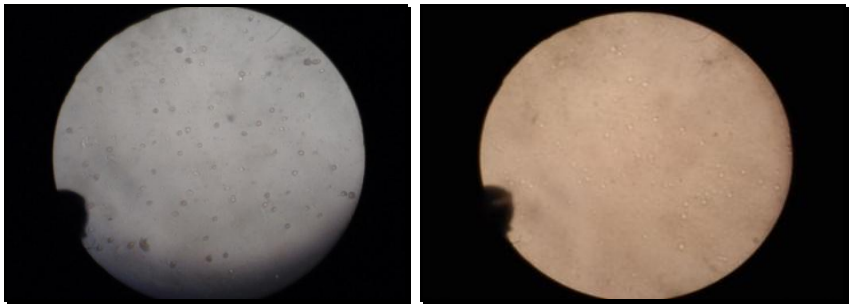
TABLE 3: *IN-VITRO* CYTOTOXICITY EFFECT AGAINST MDA-MB CELL LINE

S.No.	Name of drug	Concentration (µg)	O.D. at 492nm	%of cell lysis	IC_{50}
1.	*Crataeva*	10	0.558	25%	
2.	*nurvala*ethanolicextract	20	0.970	50%	20 µG
3.	Et*Cn*	30	1.199	75%	
4.	Vincristine	30	1.787	90%	
5.	Control		0.379	No lysis	

Where, O.D. – Optical Density



10µg of Et*Cn* **20µg of Et*Cn*** **30µg of Et*Cn***



Vincristine 30µg **control**

FIGURE 6: MTT BIOASSY MICROPLATE FOR THE CELL LINE MDA-MB - HUMAN ADENO CARCINOMA, MAMMARY GLAND CARCINOMA AT VARIOUS CONCENTRATIONS

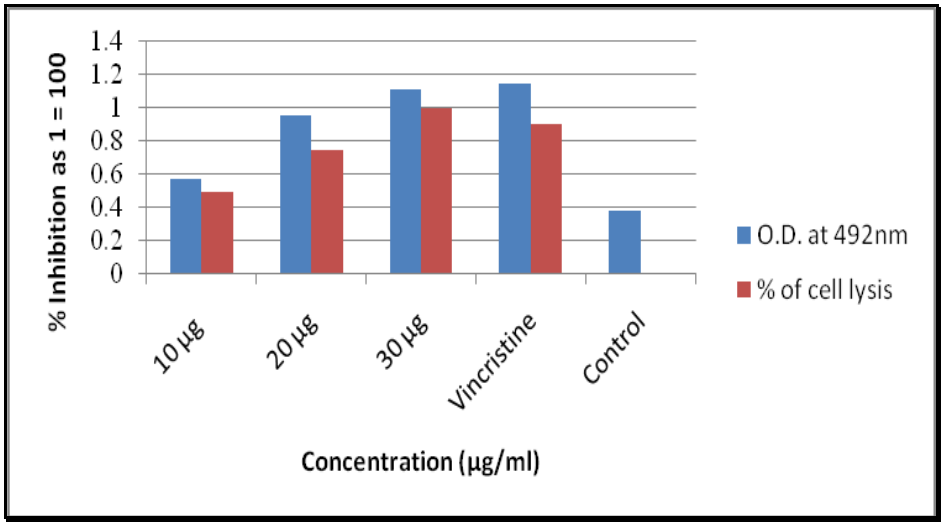


FIGURE 7. GRAPHICAL PRESENTATION OF OPTICAL DENSITY AND % CELL LYSIS AGAINST THE CELL LINE MDA-MB - HUMAN ADENO CARCINOMA, MAMMARY GLAND CARCINOMA AT VARIOUS CONCENTRATIONS.

DISCUSSION: Table 1 recorded the anticancer effect of *Crataeva nurvala* using MTT assay represented by optical density and % cell Lysis against different cell lines. Also the inhibitory concentration (IC_{50}) of each extract is recorded to determine the potency. The cell lines used is A549-Human lung carcinoma, the extract recorded cell lysis upto 100% at highest conc. It also recorded good value of the inhibitory concentration i.e.IC_{50} (i.e.10 µg) indicating the potential of the ethanolic extract. Data of cell line-A549 indicate that optical density of the samples plate was increase with increase in the concentration which was more than the value for the control at lowest concentration and at the highest concentration it reached near the value of the standard drug, indicating that extracts possesses the anticancer activity. Graphical representation of the data is given in the **Figure 3** and Images of micro plates represented the effect of the drug on the cell in comparison to the control and the standard. Number of dead cells in the plate can be seen easily in **Figure 2**.

Table 2 recorded anticancer effect of the extract using cell line HELA- Human Cervix Carcinoma, the extract recorded cell lysis upto 100% at highest conc. It also recorded good value of the inhibitory concentration i.e.IC_{50} (i.e.13µg) indicating the potential of the ethanolic extract. Data of cell line-HELA indicate that optical density of the samples plate was increase in the same manner that of the A549 which was more than the value for the control at lowest concentration and at the highest concentration it reached near the value of the standard drug, indicating that extracts possesses the anticancer activity. Graphical representation of the data is given in the **Figure 5** and Images of microplates represented the effect of the drug on the cell in comparison to the control and the standard. Number of dead cells in the plate can be seen easily in **Figure 4**.

Table 3 recorded anticancer effect of the extract using cell line MDA-MB- Human adenocarcinoma, mammary gland carcinoma, and the extract recorded cell lysis upto 75% at highest conc. It also recorded good value of the inhibitory concentration IC_{50} (i.e.20µg) indicating the potential of the ethanolic extract. Data of cell line-MDA-MB indicate that optical density of the samples plate was also increased with the increase in the concentration. Graphical representation of the data is given in the **Figure 7** and Images of microplates represented the effect of the drug on the cell in comparison to the control and the standard. Number of dead cells in the plate can be seen easily in **Figure 6**.

CONCLUSION: In the present investigation the different concentration of the ethanolic extracts of *Crataeva nurvala* with three different cell lines, Vincristine as standard anticancer drug were used. The parameter like % Cell Lysis, Optical Density and IC_{50} value were evaluated for the anticancer activity. The result of Cell line- A549 - Human lung carcinoma showed that optical density of the

samples plate was increase with increase in the concentration which was more than the value for the control at lowest concentration and at the highest concentration it reached near the value of the standard drug, indicating that extract posses the anticancer activity. The parameter of % Cell lysis value for the test sample was found to be always more than that of the control i.e. ranging from 70 to 100 % and equal to the standard, which support the data for anticancer potential of the extract. The IC_{50} value of 10µG is indicative of good anticancer potential of the extract.

In order to conform reproducibility of the result, more work is needed with the cell line Hela - Human cervix and cell line MDA-MB - Human adenocarcinoma, mammary gland. The Results shown in **Table 2, 3** and **Figure 4, 5, 6, 7** respectively were found to be identical and indicative of anticancer potential of the extract.

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