AMERICAN JOURNAL OF PHARMTECH RESEARCH
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In-Vitro Anticancer Activity of *Abutilon Indicum* Against Human Breast and Lung Cancer Cell-Lines

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ABSTRACT

Plants have been used as medicines for thousands of years. They have always been used as a rich source of biologically active drugs. According to WHO (World Health Organization) report, about 80% of the population, mostly in developing countries still depends on traditional medicinal system for their primary health care. The present investigation is focused on the phytochemical investigation of abutilon indicum species for anticancer activity. The *Abutilon* L. genus of the Malvaceae family comprises about 150 annual or perennial herbs, shrubs or even small trees widely distributed in the tropical and subtropical countries. The aqueous extract of the *A. indicum L.* showed significant cytotoxic activity against both the selected cancer cell lines *viz.* human breast cancer cell line MCF 7 and human lung cancer cell line A 549.

Keyword: WHO, Abutilon indicum, Anticancer activities, MTT assay.

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Received 09 July 2017, Accepted 8 July 2017

Please cite this article as: Patil VM *et al*., *In-Vitro* Anticancer Activity of Abutilon Indicum Against Human Breast and Lung Cancer Cell-Lines. American Journal of PharmTech Research 2017.

INTRODUCTION

Plants have been used as medicines for thousands of years. People depend on plants for several purposes like for wood, timber, non-timber forest products, food, medicine etc. They have always been used as a rich source of biologically active drugs and have numerous traditional uses to serve mankind for many thousand years. Now days, they are used widely because of growing awareness of people towards unwanted side effects and high cost of the allopathic medicines which makes them beyond the reach of common people. According to WHO (World Health Organization) report, about 80% of the population, mostly in developing countries still depends on traditional medicinal system for their primary health care. The different systems of medicinal usage like Ayurveda, homeopathy and Unani which are the local health traditions, focuses on the use of plant products for the treatment of human and animal diseases. Medicinal plants contain numerous biologically active compounds which are helpful in the treatment of various diseases and improving the life. India has very rich plant diversity and houses about 47,000 plant species but traditional healers' uses only 2500 plant species out of which about 100 species of plants serve as natural principles source of medicine. A large number of plants still remain unexplored with regard to their medicinal properties and they can also be sources of potentially active compounds for the development of new drugs to treat various diseases.

The *Abutilon indicum* L. genus of the Malvaceae family comprises about 150 annual or perennial herbs, erect, shrubs or even small trees widely distributed in the tropical and subtropical countries of America, Africa, Asia and Australia [1,2]. It is known as "Atibala" in Hindi and found in the outer Himalayan tracts from Jammu to Bhutan up to an altitude of 1500m and extending through the whole of northern and central India. Various plants of *Abutilon* species are traditionally claimed for their varied pharmacological and medicinal activities. Furthermore, different plant parts contain specific phytoconstituent responsible for their biological activity [3]. Cancer is a major cause of morbidity and mortality in developed and developing countries alike. Population ageing is often assumed to be the main factor driving increases in cancer incidence, death rates, and health-care costs [7].

Breast cancer is the most common cancer in women in India and accounts for 27% of all cancers in women. As per Globocan data, new cases registered were 1,44,937 out of which deaths reported are 70,218. The incidence rates in India begin to rise in the early thirties and peak at ages 50-64 years. Overall, 1 in 28 women is likely to develop breast cancer during her lifetime. In urban areas, 1 in 22 women develops breast cancer during her lifetime as compared to rural areas where 1 in 60

women develops breast cancer in her lifetime. Agewise and typewise incidence of cancer is shown in Figure 1 and Figure 2

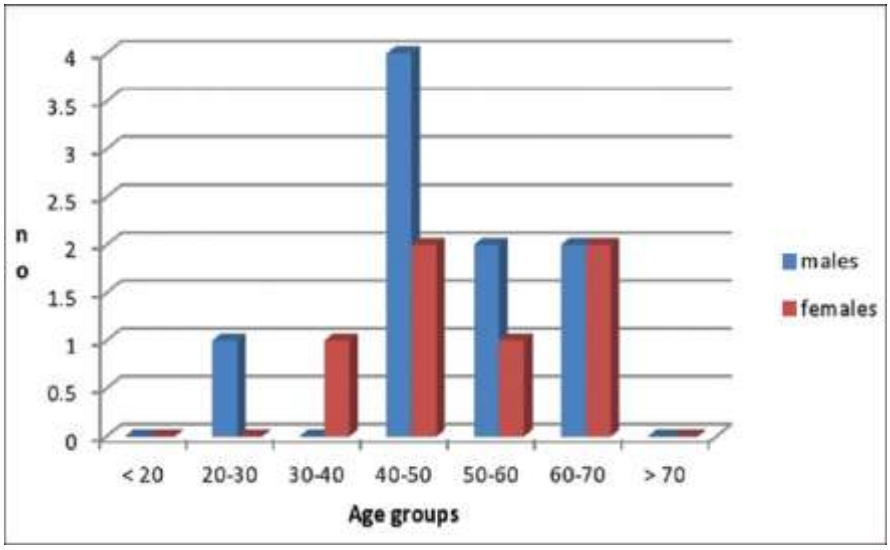

Figure 1: Age wise incidence of cancer

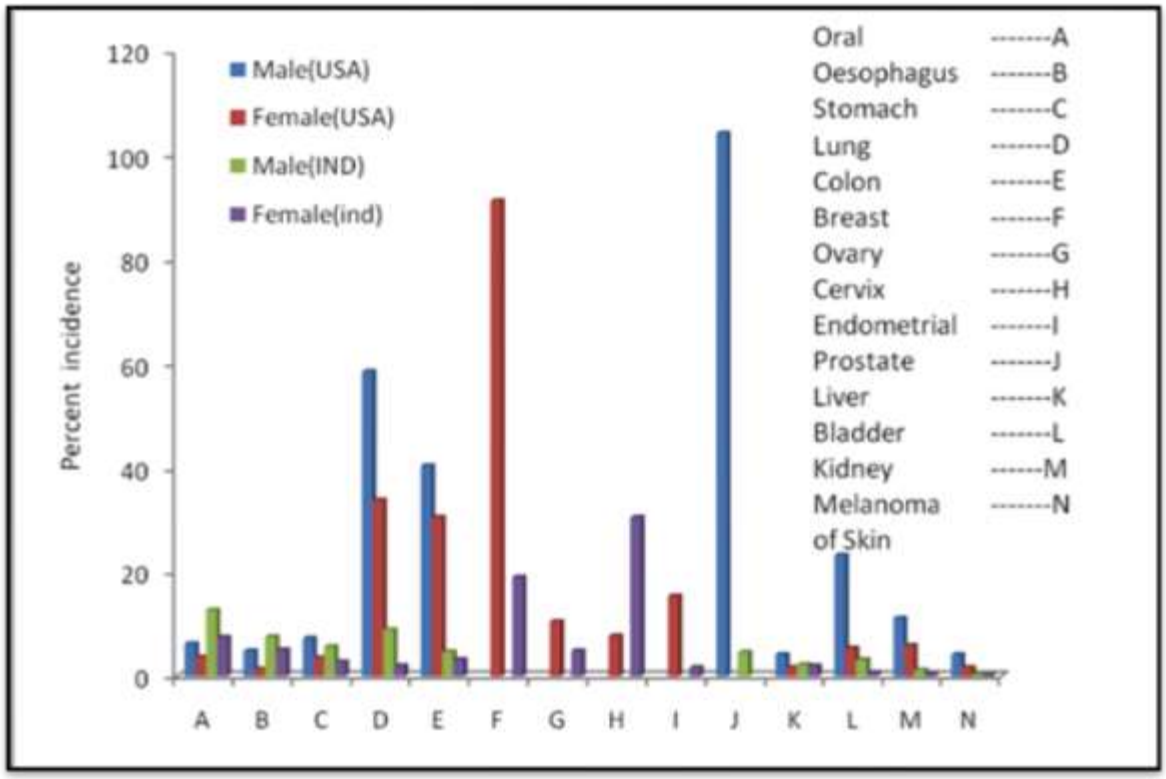

Figure 2: Type wise incidence of cancer

In 2015, an estimated 231,840 new cases of invasive breast cancer were diagnosed among women, as well as an estimated 60,290 additional cases of in situ breast cancer. In 2015, approximately 40,290 women were died from breast cancer [8].

Lung cancer is the second most commonly diagnosed cancer in both male and female. Lung cancer continues to be the leading cause of cancer related deaths worldwide. Despite improvements in survival for many other types of cancer in recent years, 5-year survival for lung cancer has remained relatively poor, mainly because by the time a diagnosis is made, lung cancer is often well advanced and treatment options are limited.

Plant profile:

The present investigation is focused on the phytochemical investigation of abutilon plant species for *in-vitro* anticancer activity. The plant species was collected from the forest area near Khanapur, Dist- Belguam, Karnataka, India (Figure 3).

Figure 3: Whole plant of Abutilon indicum

Botanical Name : ***Abutilon indicum*** L.

Family : Malvaceae

Synonyms : Rishyaprokta, Kankatika, Balika, Rishagadha, Bhuribala.

Common name : Abutilon, Indian mallow.

Habitat : Common along road sides.

Description:

Greyish to mentose undershrubs, reaching 1 m or more in height. The Leaves of Abutilon indicum are up to 12cm long, cordate, ovate, acuminate, toothed rarely sub-trilobate, petioles 7.5cm long. Stipules 9mm long linear acute, deflexed pedicles often 2.5-5 long auxiliary solitary jointed very near the top. Calyx 12.8mm long divided to the middle lobes ovate, apisculate. Corolla 2.5cm in diameter, opening in the evening. Staminal tube hairy of the base filaments long carpals usually 15-20, longer than the calyx with a distinct small acute point hairy ultimate shining dark brown

seed brown black densely minutely scrobiculate (Figure 4 and 5). It is fairly common road side weed which grown in hotters part of the India as weed [7].

Figure 4: Fruits of Abutilon indicum

Figure 5: Leaves and flower of Abutilon indicum

Plants are an essential and integral component in the world of prescription medicine and have the ability to make various chemical constituents like flavonoids, proteins, alkaloids, and steroids, glycosides, phyto sterols, and phenolic compounds, Carbohydrates, amino acids, Saponins, glycosides are isolated from these plants .

Traditional Uses:

In traditional medicine, various parts of the plant *Abutilon indicum* are used as a demulcent, aphrodisiac, laxative, sedative, astringent, expectorant, tonic, anti-inflammatory and analgesic and to treat leprosy, ulcers, headaches, gonorrhea, and bladder infection, gonorrhea treatment and as an

immune stimulant. In ancient days, maidens were made to consume a spoonful of this powder with a spoonful of honey, once in a day, for 6 months until the day of marriage, for safe and quick pregnancy. The plant is very much used in Siddha medicines. The root, bark, flowers, leaves and seeds are all used for medicinal purposes by Tamils. Root and bark are used as aphrodisiac, anti diabetic, nervine tonic, diuretic, anthelmintic, pulmonary sedative and in fever. The bark is used as febrifuge, analgesic, alexeteric, astringent. [4]. The seeds from this plant are considered to be aphrodisiac and can be used as a laxative for those having hemorrhoids and in the treatment of coughs, puerperal disease, chronic dysentery. The leaves are used as adjunct to medicines used for pile complaints. The flowers are used to increase semen in men. The juice from its leaves has been used to formulate into an ointment for quick ulcer healing.In addition, it is used in cleaning wounds and ulcers; treating vaginal infections, and hemorrhoids; and can also be used as an enema. It is also effective in the treatment of leprosy. [5, 6]. The decoction of the leaves is used in toothache, tender gums and internally for inflammation of bladder [9,10].

MATERIALS AND METHOD

Chemical and reagent:

All the chemicals and regents were procured from Merck, Loba chemie, Mumbai and Hi-media, Mumbai.

Cell culture:

Cell lines of different tissue origin such as MCF-7 (human breast tumor) and A 549 (human lung cancer) obtained from the National Center for Cell Science, Pune, India. Cells were cultured in minimum essential medium (MEM) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin and 50 µg/ml streptomycin in a humidified atmosphere of 5% CO_2 in air at 37^{o}C in a CO_2 incubator.

Preparation of extract:

A whole plant *Abutilon Indicum Linn* was collected in mid of winter from the forest area near Khanapur, Dist- Belguam, Karnataka, India. It was washed, shade dried, powdered and stored in airtight container for future use. 30 g of powdered crudedrug were extracted in a soxhlet extractor using petroleum ether, chloroform, ethanol and aqueous. Petroleum ether extraction was carried out for defatting of the crude drug at (60-80^{0}C) for 8 hr. The marc left after the petroleum ether extract was taken and subsequently extracted with chloroform, ethanol and aqueous. Extracts were concentrated by rotary vacuum evaporator and the residue obtained was dried, weighed.

Qualitative phytochemical analysis:

Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test:

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Test for phenol (Ferric Chloride Test):

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for saponins:

Crude extract was mixed with 5 mL of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides (Salkowski's test):

Crude extract was mixed with 2 mL of chloroform. Then 2 mL of concentrated sulphuric acid (H_2SO_4) was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring,

Test for proteins (Ninhydrin test):

Crude extract when boiled with 2 mL of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Test for carbohydrates (Benedict's test):

Crude extract when mixed with 2 mL of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Test for terpenoids:

Crude extract was dissolved in 2 mL of chloroform and evaporated to dryness. To this, 2 mL of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

Detection of flavonoids (Alkaline Reagent Test):

Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids.

Test for steroids:

Crude extract was mixed with 2 mL of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

Another test was performed by mixing crude extract with 2 mL of chloroform. Then 2 mL of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Thin layer chromatography:

The extracts were analyzed by thin layer chromatography (TLC) on analytical plates over silica gel G (TLC grade). The solvent system used for separation of petroleum ether extract was chloroform and methanol (7:3); chloroform extract with Chloroform, ethyl acetate and ethanol (2:4:4), ethanol extract with chloroform and methanol (9:1) and aqueous extract with chloroform and ethanol (8:2). The TLC plates were spotted using the glass capillary and developed by spraying with ninhydrin reagent resulted in the formation of bright yellow spot indicating the presence of flavonoids in this extract. The plates were also visualized under ultra violet light at 365nm.

Anticancer activity [11, 12]:

Cell treatment

The monolayer cells were detached and single cell suspensions were made using trypsin-ethylenediaminetetraacetic acid (EDTA). A hemocytometer was used to count the viable cells and the cell suspension was diluted with a medium containing 5% FBS in order to obtain final density of $1x10^5$ cells/ml. 96-well plates at plating density of 10,000 cells/well were seeded with one hundred microlitres per well of cell suspension and incubated for cell attachment at 37° C, 5% CO_2, 95% air and 100% relative humidity. The cells were treated with serial concentrations of the test samples after 24 hr. Serial dilution method was used for preparing test samples of different concentrations. Cells were initially dissolved in dimethylsulfoxide (DMSO) and further diluted with serum free medium to obtain twice the desired final maximum test concentration. The required final drug concentrations of 6.25, 12.5, 25, 50, 100 µg/ml were obtained by adding aliquots of 100 µl of the different drug dilutions to the appropriate wells already containing 100 µl of medium. After addition of the drug the plates were incubated for an additional 48 hr at 37° C, 5% CO_2, 95% air and 100% relative humidity. The medium without samples served as control and triplicate was maintained for all concentrations.

MTT assay

After 48 h of incubation, to each well 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added and incubated at 37° C for 4 h. The medium with MTT was flicked off and the formed formazan crystals were solubilized in 100µl of DMSO. Using micro plate reader the absorbance was measured at 570 nm. The % cell inhibition was determined using the following formula,

% Cytotoxicity = {(Control absorbance – Test absorbance)/Control absorbance} x 100.

RESULTS AND DISCUSSION:

The leaf extracts were dried using a rotary vacuum evaporator and the residue obtained was weighed (table1).

Table 1: Residue obtained from various extracts of *Abutilon indicum Linn.*

Sr. No.	Solvent	Residue (g)
1.	Petroleum ether	5.95
2.	Chloroform	5.60
3.	Ethanol	4.50
4.	Aqueous	3.60

The presence of various phytoconstituents of the extracts was detected by phytochemical screening. The Petroleum ether extract was found to contain phenolic compound, saponin, carbohydrate and sterols. The chloroform extract found to contain phenolic compound, carbohydrates, glycoside, terpenoid, sterols and flavonoids. The ethanol extract was found to contain alkaloid, phenolic compound, carbohydrates, proteins, saponin glycosides and flavonoids whereas aqueous extract contains alkaloid, phenolic compound, carbohydrates, saponin, sterols, glycosides and flavonoids (table 2).

Table 2: Phytochemical investigation of the whole plant extracts of *Abutilon indicum Linn.*

Extracts	Alkaloid	Phenolic compounds	Saponin	Glycosides	Protein	Carbohydrate	Terpenoids	Flavonoids	Sterols
Pet. ether	-	+	+	-	-	+	-	-	+
Chloroform	-	+	-	+	-	+	+	+	+
Ethanol	+	+	+	+	+	+	+	+	-
Aqueous	+	+	+	+	-	+	-	+	+

(+) indicates presence and (-) indicates absence of constituents

The extracts were subjected to thin layer chromatography. The solvents were selected based on the phytochemical separations; each extract was separated in a different solvent system, in different ratios. The spots well separated intensely with chloroform extract with the solvent system chloroform and methanol (7:3). The TLC plates were spotted using the glass capillary and developed by spraying with ninhydrin reagent resulted in the formation of bright yellow spot indicating the presence of flavonoids in this extract. The plates were also visualized under ultra violet at 365nm. The naked eyes observed spot in bright yellow colored and in UV chamber spot observed in fluorescent yellow colored. Rf (× 100) value for the chloroform extract was found to be as 72 and 58 which is the near the Rf value for flavanol and flavones.

Anticancer activity:

MTT Assay:

The aqueous extract of the *A. indicum L* showed significant cytotoxic activity against human breast cancer cell line MCF 7 at concentration 50 and 100 µg/ml (table 3). At the lower concentrations it showed diminished or slight activity. Whereas chloroform and ethanol extracts showed significant activity only at the concentration 100 µg/ml. Figure 6 shows cytotoxic activity of various extracts against human breast cancer cell line MCF 7.

Table 3: Percent cytotoxicity of cancer cells treated with extracts of *Abutilon indicum Linn.*

Extracts	Concentration of the extract (µg/ml)	Absorbance		% Cytotoxicity	
		MCF 7	A 549	MCF 7	A 549
Control	-	0.492±0.0041	0.469±0.0040	0	0
AICH	6.25	0.568±0.0042	0.498±0.0023	-2.4500	-2.2315
	12.5	0.498±0.0013	0.420±0.0009	-2.1300	1.0380
	25	0.350±0.0034	0.385±0.0031	5.3568	4.8967
	50	0.315±0.0032	0.295±0.0020	23.4180	26.4508
	100	0.280±0.0045	0.250±0.0012	34.8756	36.5690
AIET	6.25	0.530±0.0042	0.550±0.0012	-2.8360	-2.6855
	12.5	0.515±0.0023	0.375±0.0023	-2.4432	4.6540
	25	0.480±0.0033	0.325±0.0008	4.3670	6.8450
	50	0.385±0.0043	0.290±0.0036	4.9078	27.1125
	100	0.325±0.0039	0.248±0.0014	21.9900	36.7856
AIAQ	6.25	0.511±0.0032	0.485±0.0035	-2.3250	-2.1467
	12.5	0.389±0.0011	0.460±0.0006	4.7650	0.4550
	25	0.378±0.0053	0.397±0.0036	5.0234	3.9078
	50	0.305±0.0048	0.337±0.0028	25.9867	6.1230
	100	0.260±0.0049	0.276±0.0018	36.5543	32.1557

n=3; AICH= chloroform extract of *Abutilon indicum*, AIET= ethanol extract of *Abutilon indicum*, AIAQ= aqueous extract of *Abutilon indicum.*

The chloroform and ethanol extracts of the *A. indicum L* showed significant cytotoxic activity against human lung cancer cell line A 549 at concentration 50 and 100 µg/ml. At the lower concentrations it showed diminished or slight activity. Whereas aqueous extract showed significant activity only at the concentration 100 µg/ml. Figure 7 shows cytotoxic activity of various extracts against human lung cancer cell line A 549.

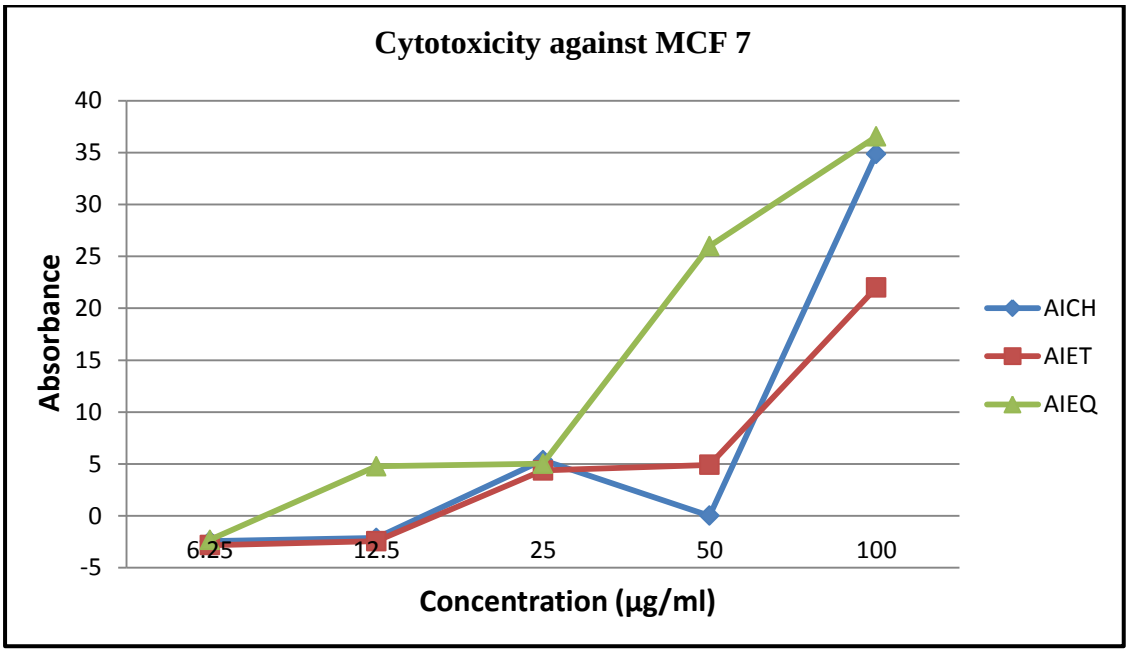

Figure 6: Cytotoxicity of various extracts of *Abutilon indicum Linn.* on MCF 7

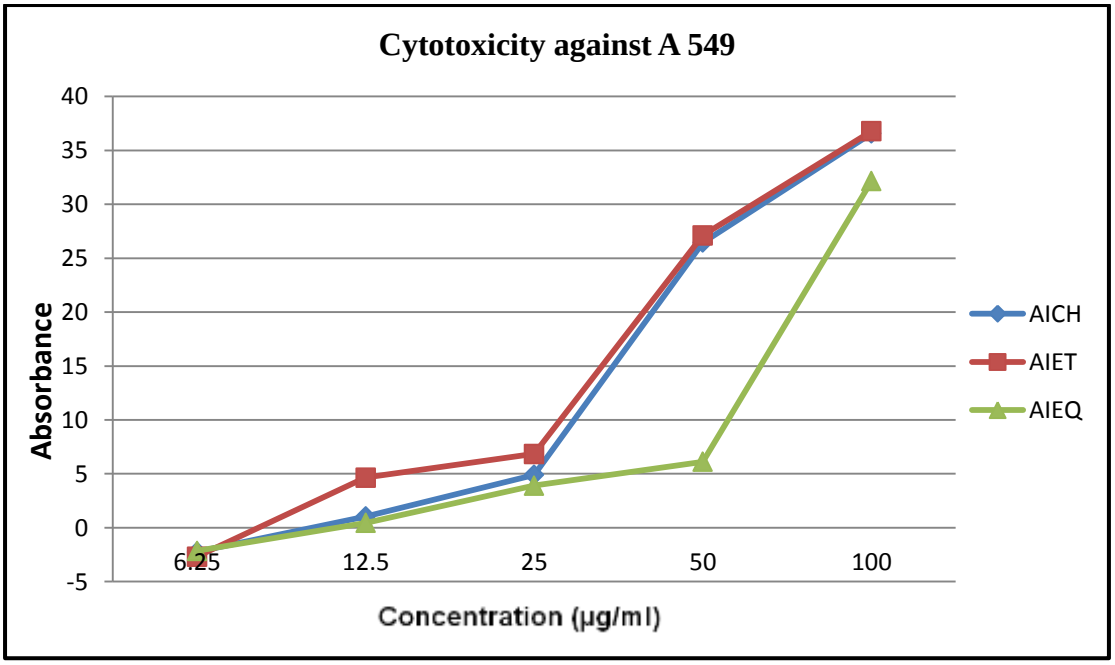

Figure 7: Cytotoxicity of various extracts of *Abutilon indicum Linn.* on A 549

CONCLUSION:

The plant "*Abutilon Indicum*" belongs to family *Malvaceae* was taken for our studies to screen and give a report on analgesic and anti inflammatory studies. The plant is subjected to phytochemical and colour reaction. This gives the valuable information about the plant for the future workers. On the basis of the results of this study, it is possible to conclude that the aqueous extract of the *Abutilon indicum L.* showed significant cytotoxic activity against both the selected cancer cell lines *viz.* human breast cancer cell line MCF 7 and human lung cancer cell line A 549. It seems safe, however to conclude that these parts do possess biological activities following oral administration.

ACKNOWLEDGEMENTS:

Authors are thankful to Shivaji University, Kolhapur for funding the proposal through 'Research Grant' also thankful to NCCL, Pune for providing cancer cell lines.

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