Original Article

# *In vitro* Cytotoxic Activity of *Abrus precatorius* Seed Extracts Against MCF-7 Cell Lines

**Pusapati Madan Ranjit*[1], V. Manasa[1], Y. A. Chowdary[1], k. Prabhu Das Kiran[1], K. Phani Kumar[2], P. Venkateswara Rao[1] and Girijasankar Guntuku[3]**

[1]NRI College of Pharmacy, Pothavarappadu (V), Agiripalli Mandal, Krishna District, A.P, India
[2]ANU College of Pharmaceutical Sciences, Nagarjuna Nagar, Nagarjuna University, Guntur (Dist.), A. P, India
[3]University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, AP, India

**Address for Correspondence**

NRI College of Pharmacy
Pothavarappadu (V)
Agiripalli Mandal,
Krishna District
A.P, India
**E-mail:** madanranjit@gmail.com

## ABSTRACT

*Abrus precatorius linn* (Fabaceae) is a climbing shrub widely distributed in India, tropical and subtropical regions of the world. The objective of this research work is to evaluate the *in vitro* cytotoxic effects of the ethanolic extract (AHE) and ethyl acetate (AEE) against MCF-7 cell lines by using MTT assay. Tamoxifen was used as a standard to compare the cytotoxic activity of the extracts. The preliminary phytochemical evaluation of, the ethanolic extract of seed showed the presence of alkaloids, flavonoids, phenols, tannins, and saponins. Ethyl acetate extract contained steroids and terpenes. Both the extracts are tested on MCF-7 cell lines to observe the *in vitro* cytotoxic activity.The $IC_{50}$ concentration of tamoxifen, ethanolic extract (AHE) and ethyl acetate (AEE) are 37.79 µg/ml, 60.89µg/ml, and 143.8µg/ml respectively. The $IC_{50}$ concentration of ethanolic and ethyl acetate extract showed lower activity when compared with standard tamoxifen, this may be due to its crude nature. Based on the previous reports on cytotoxic properties of *Abrus precatorius* seeds with that of the present results it clearly indicates that, A*brus precatorius* seeds has potential cytotoxic properties and can be used as a source of antitumor agents.

**Keywords**: *Abrus precatorius*, MTT assay, MCF-7, Cytotoxic activity.

## INTRODUCTION

*Abrus precatorius Linn* (Fabaceae) is distributed throughout India and it is called as Indian Wild Liquorice, Jequirity, Crab's Eye and Precatory Bean in English. Many authors explained the use of the seeds in baldness. Seeds contain abrin, a toxalbumin,

indole derivatives, anthocyanins, sterols, terpenes. Abrin causes agglutination of erythrocytes, haemolysis and enlargement of lymph glands. A nontoxic dose of abrin (1.25mcg/kg bodyweight), isolated from the seeds of red variety, exhibited a noticeable increase in antibody-forming cells, bone marrow cellularity and alpha-esterase-positive bone marrow cells. Oral administration of agglutinins, isolated from the seeds, is useful in the treatment of hepatitis and AIDS[1]. The plant is traditionally used for the treatment of sore tongue also has diaphoretic action. Seeds of *Abrus precatorius* are commonly used as purgative, emetic, aphrodisiac and for treating nervous disorder in traditional medicine[2]. Previous studies are reported that preliminary phytochemical analysis of ethanolic extracts of seed showed the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids like phyto constituents[3]. Other reports are confirmed that, seeds showed *in vitro* cytotoxic properties on various cell line[4]. Based on the review of literature, the objective of this research was to evaluate the potentiality of cytotoxic effects of *Abrus precatorius* on MCF- 7 tumour cell lines.

## MATERIALS AND METHODS

### Collection of plant material

*Abrus precatorius linn.* seeds were purchased from Brahma herbal products, Vijayawada (Voucher no.1124). The purchased seeds were authenticated by Dr. K. Madhava Chetty, Asst. Professor, Dept. of Botany, Sri Venkateswara University, Tirupati. Herbarium specimen was deposited in the department of pharmacognosy with specimen No: 007.NRI/COL/P.COG/ (Seeds).

### Preparation of extracts

*Abrus precatorius* seeds were subjected to soxhlet extraction with 70% ethanol and ethyl acetate for 48 hours, the extracts were collected and evaporated to dryness and stored at 4°C until use. The percentage yield of ethanolic extracts was 12.5% and ethyl acetate was 5.5%.

### Preliminary phytochemical screening

Preliminary phytochemical screening of ethanolic extract (AHE) and ethyl acetate (AEE) extract by using standard methods for identification of reducing sugars[5], protein[6], fata[6], resins[7], tannins[7] flavonoids[8] alkaloids[7], saponins[5] and phenols[9].

## IN VITRO CYTOTOXIC ACTIVITY

### Cell culture

Carcinoma of breast cancer [Michigan Cancer Foundation (MCF-7)], cell lines used in this study were procured from National Centre for Cell Science, Pune. This cell line was maintained in Dulbecco's modified essential medium (DMEM) supplemented with in minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine, antibiotics (50U/mL of Benzyl pencillin, 50µg/mL of streptomycin and 50µg/ml of amphotericin-B) and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% $CO_2$ incubator.

### MTT assay

The MTT assay developed by Mosmann[10] used to determine the inhibitory effect of test compounds on cell growth *in vitro*. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate not the same concentration but minimum of 5000 cells per well were seeded l in growth medium and cultured at 37°C in 5% $CO_2$ to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of extract (12.5, 25, 50, 100, and 200 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hours. The compound was

prepared as 1.0 mg/ml concentration stock solutions in PBS. Each well then received 5 µl of fresh MTT (0.5mg/ml in PBS) followed by incubation for 2hr at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilize the colored formazan product. Tamoxifen is taken as positive control in order to compare $IC_{50}$ of extract against the standard drug used. Culture medium and solvent used as negative controls. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 570 nm on an ELISA reader, Anthos 2020 spectrophotometer. The percent cell viability was determined with respect to control, is calculated using the formula.

% Viability = corrected OD of sample /Control OD * 100 and percentage of inhibition was determined by using formula, % Inhibition = 100-%viability.

## STATISTICAL ANALYSIS

The data was represented as Mean ± SEM and $IC_{50}$ values were calculated using Graph pad prism 5 version.

## RESULTS & DISCUSSION

Preliminary phytochemical evaluation (Table.1) reported that ethanolic extract of the seeds showed the presence of alkaloids, flavonoids, phenols, tannins, and saponins. Ethyl acetate extracts consist of steroids and terpenes. Both the extracts were tested on MCF-7 cell lines to observe *in vitro* cytotoxic activity. Table 2 showed percentage inhibition of cancer cell with tamoxifen (ST), test ethanolic extract (AHE) and ethyl acetate extracts (AEE). Figure 1 showed the comparison of percentage inhibition of cancer cell against tamoxifen. Table 3 showed the comparison of $IC_{50}$ values of standard tamoxifen (ST), ethanolic extract (AHE) and ethyl acetate extracts (AEE). The above results indicate that, ethanolic extract (AHE) showed good activity on MCF-7 cell lines, when compared with ethyl acetate extract. This may due to variations in composition of phytochemical constituents. Ethanolic extracts contain presences of alkaloids and flavonoids. Cytotoxic activity may due to flavonoidal or alkaloid content. Phenols and its congeners are known to cytotoxicity on various cancer cell lines and induce caspase-mediated apoptosis activity[11, 12]. The $IC_{50}$ concentration of ethanolic and ethyl acetate extract showed lower activity when compared with standard tamoxifen; this may be due to its crude nature. Fig 2.showed the comparison of $IC_{50}$ values of standard tamoxifen (ST), test extracts ethanolic extract (AHE) and ethyl acetate extracts (AEE). V. V. Subba Reddy and M. Sirsi reported and confirmed that ethanolic extract seed showed *in vitro* on different tumor cell lines, like, Yoshida Ascites Sarcoma (YAS), Yoshida Sarcoma (YS) and Mouse Fibrosarcoma (MFS). The tumor cells incubated with the extract showed cellular pathology, decreased viable cell count, and prolongation of survival period in tumor-transplanted animals[4].

## CONCLUSION

From the above results it can be concluded that, *Abrus precatorius* seeds have potential cytotoxic properties and can be used as a source of antitumor agent. Further studies are required to identify and isolate the active components present in the extracts responsible for its cytotoxic activity.

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**Table 1.** Showed the Preliminary phytochemical constituents of ethanolic extract (AHE) and ethyl acetate extracts (AEE)

| Test for | Ethanolic extract (AHE) | Ethyl acetate extract (AEE) |
|---|---|---|
| Alkaloids | + | - |
| Glycosides | - | - |
| Flavonoids | + | - |
| Steroids | - | + |
| Phenols | + | - |
| Saponins | + | - |
| Terpenes | - | + |
| Carbohydrates | - | - |
| Proteins | - | - |
| Tannins | + | - |

**Table 2.** Showed mean % inhibition ± SEM of MCF-7 Cell line against standard tamoxifen (ST), ethanolic extract (AHE) and ethyl acetate extract (AEE)

| conc (ug/ml) | Mean % inhibition ± SEM | | |
|---|---|---|---|
| | Tamoxifen (ST) | AEE | AHE |
| 12.5 | 10.06± 0.28 | 3.8±0.36 | 10±0.23 |
| 25 | 33.6± 0.27 | 15±0.07 | 19.3±0.08 |
| 50 | 66± 0.18 | 28.6±0.24 | 50.5±0.19 |
| 100 | 81.9± 0.4 | 32.5±0.22 | 68.46±0.30 |
| 200 | 84.9± 0.19 | 62.5±0.22 | 71.93±0.21 |

**Table 3.** Showed that $IC_{50}$ values of standard tamoxifen (ST), ethanolic extract (AHE) and ethyl acetate extract (AEE)

| Cell line | $IC_{50}$ Values | | |
|---|---|---|---|
| | Standard | AHE | AEE |
| MCF-7 cells | 37.79 | 60.89 | 143.8 |

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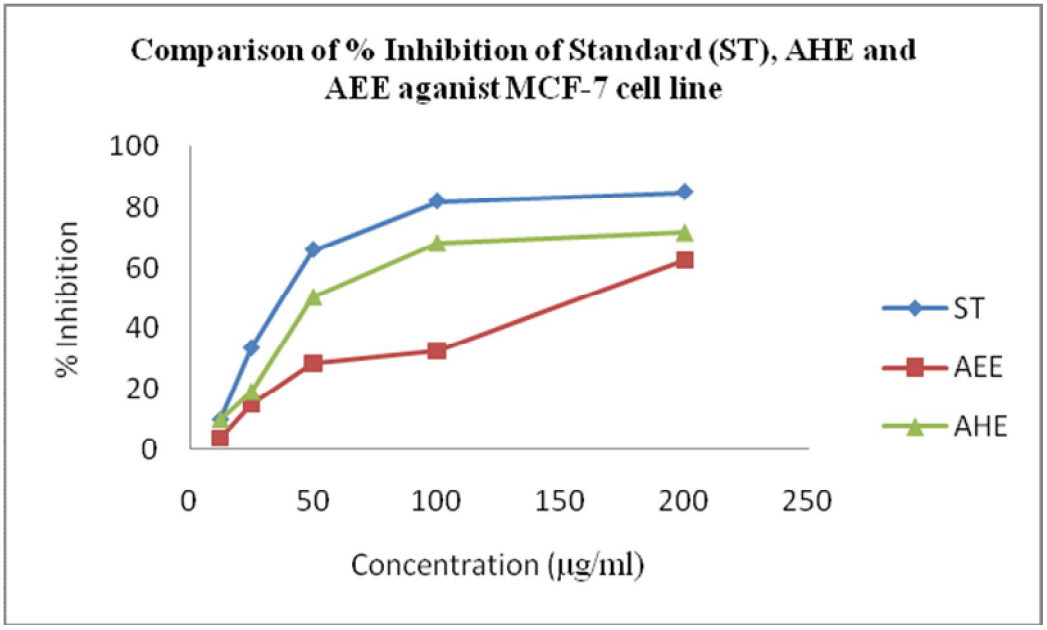


**Figure 1.** Showed comparison of % inhibitions of Standard tamoxifen (ST), ethanolic extract (AHE) and ethyl acetate extract (AEE)

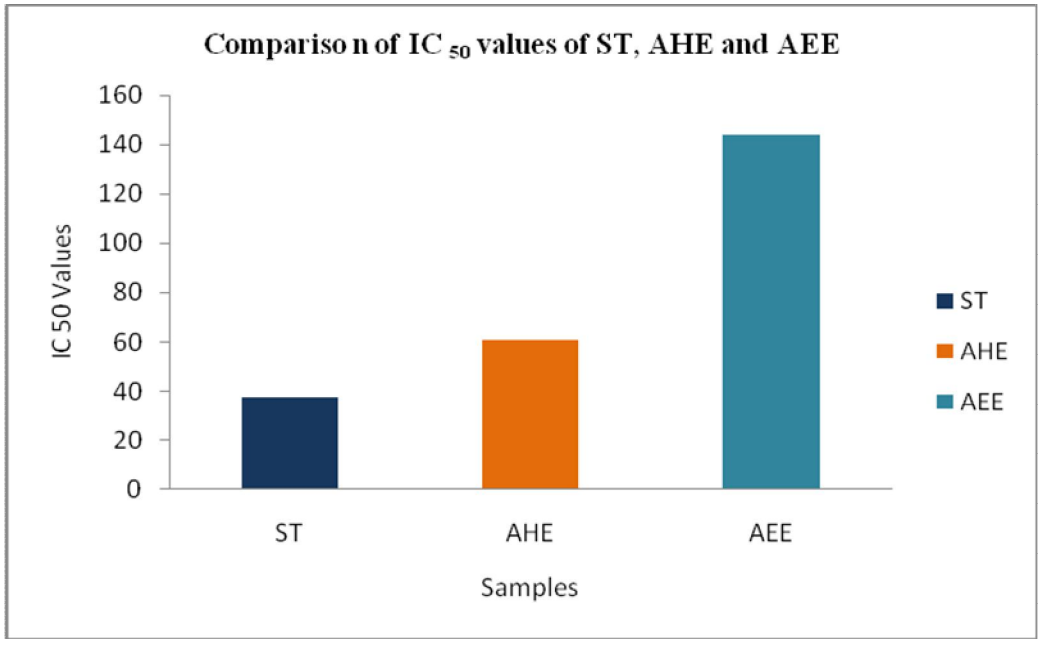


**Figure 2.** Showed the comparison of IC50 values of standard tamoxifen (ST), ethanolic extract (AHE) and ethyl acetate extract (AEE)