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Research Article

# *INVITRO* CYTOTOXIC EVALUATION OF SOME MEDICINAL PLANTS BY MTT ASSAY

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**ABSTRACT**

Objective: The aim of the study was to evaluate the cytotoxic effect of the plants *Gyrocarpus asiaticus* and*Sophora interrupta*.

Methods: The method used was the MTT assay. For evaluation A431 cell lines and MCF 7 cell lines were used. Various concentration of the methanolic extract of these plants was used.

Results: The results showed promising effects with both these plants. The $IC_{50}$ values were found to be 60µg and 720µg for *G.asiaticus* and *S.interrupta* respectively against A431 cell lines. The activities against MCF 7 cell lines were found to be 530µg and 723µg for*G.asiaticus* and *S.interrupta*respectively.

Conclusion: The two medicinal plants were found to have a good cytotoxic effect andIn vivo studies are in process which will give a clear data about their cytotoxic effects.

**Keywords**: *G.asiaticus,S.interrupta,* MTT assay, A431 cell lines, MCF 7 cell lines, Cytotoxicity

## INTRODUCTION

Cancer is the abnormal growth of cells with uncontrolled division resulting in increased number of cells[1].Inspite of a number of new anti cancer drugs the prevalence of cancer has been increased now-a-days. The plants are showing a promising effect against cancer. In this study, two medicinal plants were taken to study its cytotoxic effects.[2]

*Gyrocarpus asiaticus* is one of the species in the genus *Gyrocarpus* belonging to the family Hernandiacea with the class Magnoliopside. The plant has lot of pharmacological activities such as anti-cancer, anti-inflammatory, anti-bacterial. The preliminary phytochemical studies showed high amount of phenolics, steroids, terpenoids, tannins and flavonoids. The phenolic content of the bark was also estimated[3]

*Sophora interrupta* belongs to the family Fabaceae (Leguminaceae, Papilonaceae) which is commonly called as Edwaria madarasapatna. The species belongs to this family have various pharmacological activities such as anti-cancer, anti-inflammatory, antispasmodic etc.[4]. The antioxidant effect of this plant was studied by various methods which showed a promising effect.

## MATERIALAND METHOD

### Plant materials

The whole plant of *Sophora interrupta* and bark of *Gyrocarpus asiaticus* were collected from Tirupathi, Andhra Pradesh in June 2010 and shade dried.

### Extraction

The dried parts were soxhelet extracted with 99% methanol. The extract was concentrated by evaporation to yield a concentrated extract.

### Chemicals and Cell Lines Used

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyltetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from Merck Ltd., Mumbai, India.

MCF7 and A431 cell lines were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% $CO_2$ at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 $cm^2$ culture flasks and all experiments were carried out in 96 microtitre plates.

### MTT Assay[5,6]

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x $10^5$cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% $CO_2$ atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% $CO_2$ atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilise the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% ($CTC_{50}$) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = \frac{100 - \text{Mean OD of individual test group} \times 100}{\text{Mean OD of control group}}$$

## RESULTS AND DISCUSSION

The effect of methanolic extracts of *S.interrupta* and *G.asiaticus* were studied by using MTT assay.

### Cytotoxic properties of the plant extracts against A431 cell lines

The $IC_{50}$ value of *G.asiaticus* against A431 cell lines was found to be 60µg and the $IC_{50}$ value of *S.interrupta* was found to be 720µg and the results were provided in Table 1 and Fig 1.

### Cytotoxic properties of the plant extract against MCF 7 cell lines

The $IC_{50}$ value of *G.asiaticus* against MCF 7 cell lines was found to be 530µg and the $IC_{50}$ value of *S.interrupta* was found to be 723 µg and the results were provided in Table 2 and Fig 2.

**Table 1: Cytotoxicity effect against A431 cell lines**

| S. No. | Name of the Drugs | Concentration(µg) | % cytotoxicity | $IC_{50}$ |
|---|---|---|---|---|
| 1. | *G.asiaticus* | 31.25 | 15.35 | 60µg |
| | | 62.5 | 53.7 | |
| | | 125 | 73.74 | |
| | | 250 | 77.66 | |
| | | 500 | 79.96 | |
| | | 1000 | 80.41 | |
| 2. | *S.interrupta* | 31.25 | 16.3 | 720µg |
| | | 62.5 | 22.36 | |
| | | 125 | 23.2 | |
| | | 250 | 26.49 | |
| | | 500 | 27.19 | |
| | | 1000 | 82.79 | |

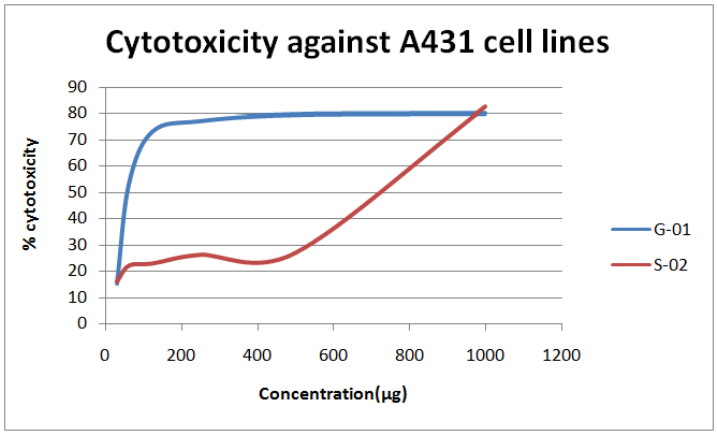


**Fig. 1: Cytotoxic activity against A431 cell line**

**Table 2: Cytotoxicity against MCF 7 cell lines**

| S. No. | Name of the Drugs | Concentration(µg) | % cytotoxicity | $IC_{50}$ |
|---|---|---|---|---|
| 1. | *G.asiaticus* | 31.25 | 4.26 | 530µg |
| | | 62.5 | 13.65 | |
| | | 125 | 18.52 | |
| | | 250 | 23.64 | |
| | | 500 | 48.76 | |
| | | 1000 | 75.04 | |
| 2. | *S.interrupta* | 31.25 | 3.4 | 723µg |
| | | 62.5 | 7.15 | |
| | | 125 | 17.45 | |
| | | 250 | 19.94 | |
| | | 500 | 22.07 | |
| | | 1000 | 84.42 | |

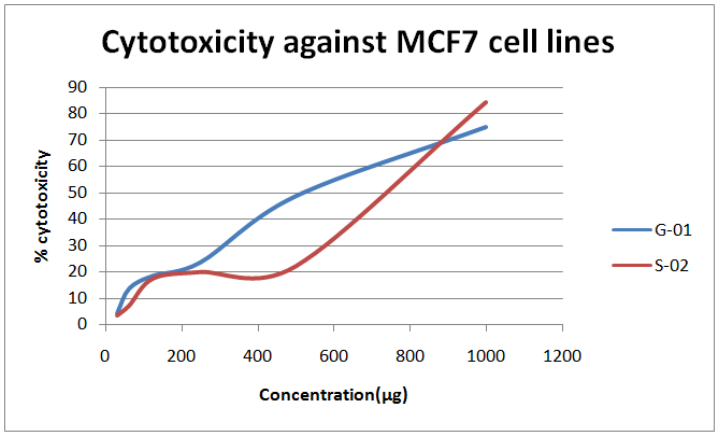


**Fig. 2: Cytotoxic activity against MCF 7 cell lines**

**CONCLUSION**

From the study it was evaluated that *G.asiaticus* was found to be more potent than *S.interrupta* and comparatively *G.asiaticus* is more potent against A431 cell lines than MCF 7 cell lines. In vivo studies are in process to evaluate its potency.

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