# IN VITRO CYTOTOXIC AND ANTITUMORACTIVITIES OF THREE INDIAN MEDICINAL PLANTS

**Raaman N[1], Selvarajan S[2], Gunasekaran CP[3], Varalakshmi Ilango[3], Md Iliyas[3] and Balamurugan G[4]**

[1] Centre for Advanced studies in Botany, University of Madras, Guindy Campus, Chennai-25.
[2] Central Research Institute for Siddha, Arumbakkam, Chennai-106.
[3] King's Institute of Preventive Medicine, Guindy, Chennai-25.
[4] Dept. of Pharmacology, C. L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai-97

## Summary

The cytotoxic and anticancer activities of the combined methanolic extract of *Asparagus racemosus, Curculigo orchioides* and *Tinospora cordifolia* was carried out using Vero cell line and $Hep_2$ cell line respectively, at various doses ranging from 10 to 800 µg in the confluent culture. The combined methanol extracts of *A. racemosus, C. orchioides* and *T. cordifolia* showed cytotoxic activity only from 700 µg concentration and exhibited the anticancer activity even from 100 µg concentration, which indicate the safety on the normal cells and efficacy on the cancer cells which may be due to the possibility of synergistic effects.

**Key words:** *Asparagus racemosus, Curculigo orchioides, Tinospora cordifolia,* Anticancer, Cytotoxic, Vero cell, $Hep_2$Cell

## Introduction

Traditional medicinal plants and practices have remained as a component of health care system of many societies in spite of the availability of well established alternatives. Apoptosis plays a central role in tumor development and it has been hypothesized that lack/failure of apoptosis leads to the development of tumors, including colon tumors. Thus induction of apoptosis in tumor cells is an effective approach to the regulation of tumor growth [1]. Epidemiological and experimental studies indicate that the risk of developing cancer may be attributable to combined actions of environmental factors and endogenous promoting agents [2, 3]. Apoptosis is a common protective mechanism by which individual cells containing unrepairable genetic lesions can be removed from the organism [4, 5]. Several studies suggest that failure of apoptosis of cells with potentially malignant mutations plays a significant role in progression of normal epithelium to a malignant tumor [6, 7].

*Asparagus racemosus* Wild, belonging to the family Asparagaceae is a well known Ayurvedic rasayana, and its root paste or root juice has been advocated in various ailments including the treatment of peptic ulcer [8], gastro duodenal ulcer [9], cough [10], immune adjuvant potential [11]. Phytoecdysteroids are reported to be present in *A. racemosus* [12].

*Curculigo orchioides* Gaerten, belonging to the family Amaryllidaceae is a shrub found all over India, especially in sandy situations of hotter regions and in Ceylon. The rhizomes of this plant are extensively used as a demulcent, diuretic and aromatic tonic, aphrodisiac, in the treatment of leprosy and nervous disease [13]. The rhizomes also possess immune stimulant potential [14], hepato protective [15], antioxidant [16] and platelet regeneration effect [17].

*Tinospora cordifolia* Miers, belonging to the family Menispermaceae, is available through out India as a climbing shrub and the stem is widely used in Siddha, Ayurveda for its properties like general tonic, anti allergic and anti diabetic [18]. Various other uses are reported such as anti-hyperglycemic [19, 20], anti-angiogenic [21], immune stimulator [22], anti-ulcerogenic [23], anti-stress [24], preventive role in brain neurotransmitter [25] and treatment of allergenic rhinitis [26]. The aim of the present study is to evaluate in vitro Cytotoxic and anticancer activities of the combined methanolic extracts of all the three Indian Medicinal plants.

## Materials and Methods

### Plant materials and extract preparation

The roots of *Asparagus racemosus*, rhizomes of *Curculigo orchioides* and stems of *Tinospora cordifolia* were collected from the outskirts of Chennai during April 2008 and identified and authenticated by Dr. Sasikala Ethirajulu, Research Officer (Pharmacognosy), Central Research Institute for Siddha, Chennai, Tamil Nadu, India. The plant parts were washed in running water to remove the adherent soil particles and dried in shade. The powdered plant parts were extracted with methanol. The extract was reduced to a dark colored molten mass by removing the solvent in a rotary vacuum evaporator (Yield: 26.16 %).

### Cytotoxic Activity [27]

### Cell line culture medium

Vero cells (African green monkey kidney cells) obtained from the Dept. of Virology, King's Institute of Preventive Medicine, Chennai, India were used for the study. Vero cells were cultured in Minimum Essential Medium (MEM) supplemented with 10 % inactivated Foetal Calf Serum (FCS), Penicillin (100 IU / ml), Streptomycin (100 IU / ml) and Ampotericin –B (5µg / ml) in a humidified atmosphere of 5% $CO_2$ at 37$^{o}$C until to reach the confluent monolayer.

### Preparation of 24 well plates in Vero cells with monolayer

The culture of Vero cells was taken after 48 h to attain the complete confluency in the tissue culture flask. Thereafter the cells were taken separately and the medium was aspirated. Five ml of Phosphate buffer saline (PBS) was added to both of the cells for washing and the PBS was discarded. Trypsin Phosphate Versine Glucose (TPVG – 5 ml) was added to both and after 4 min TPVG was completely discarded.

Thereafter, 5 ml of MEM (10%) was added. The cell suspension was mixed well with the help of pipette. To find out the cell count, 0.2 ml of the cell suspension was thoroughly mixed with 0.2 ml of trypan blue in the eppendroff. The cell count was done in the Naeubar counting chamber. Based on the cell count, the suspension was diluted to get 24 lakh cells/ml. One ml of the cell suspension was added to each well in the 24 well plates. After adding the cells, the plate was incubated at 37$^{o}$C for 48 h in 5% $CO_2$ incubator. After 48 h all the wells were screened under the inverted microscope. Care was taken to obtain the monolayer and the medium was removed. The stock drugs were prepared from the test drug by dissolving in MEM W/O FCS individually (10 g in 10 ml of MEM W/O FCS) and in combination of equal concentration (each 10 g in 30 ml of MEM W/O FCS). From the stock solution, the serial dilutions were made (10 µg, 50 µg, 100 µg…..800 µg).

For the cell control purpose, the MEM W/O FCS was added separately into 2 wells (1 ml cell with 1 ml MEM). For the drug control purpose, 1 ml of drug was added separately into 2 wells (1ml cell with 1 ml drug), which were labeled as neat. The culture preparation was performed in 24 well plates with confluent monolayer. After 24 h, the cells were screened under the inverted microscope to find out the cellular morphology.

## Anticancer Activity [28]

### Cell line culture medium

$Hep_2$ (Human epithelial carcinoma cell line of larynx) cells were obtained from the Dept. of Virology, King's Institute of Preventive Medicine, Chennai, India were used for the study. $Hep_2$cells were cultured in Minimum Essential Medium (MEM) supplemented with 10 % inactivated Foetal Calf Serum (FCS), Penicillin (100 IU / ml), Streptomycin (100 IU / ml) and Ampotericin –B (5µg / ml) in a humidified atmosphere of 5% $CO_2$ at 37$^{o}$C until to reach the confluent monolayer.

### Preparation of 24 well plates in $Hep_2$ cells with monolayer

The culture of $Hep_2$ cells was taken after 48 h to attain the complete confluency in the tissue culture flask. Thereafter the cells were taken separately and the medium was aspirated. Five ml of Phosphate buffer saline (PBS) was added to both of the cells for washing and the PBS was discarded. Trypsin Phosphate Versine Glucose (TPVG – 5 ml) was added to both and after 4 min TPVG was completely discarded. Thereafter, 5 ml of MEM (10%) was added. The cell suspension was mixed well with the help of pipette. To find out the cell count, 0.2 ml of the cell suspension was thoroughly mixed with 0.2 ml of trypan blue in the eppendroff. The cell count was done in the Naeubar counting chamber. Based on the cell count, the suspension was diluted to get 24 lakh cells / ml. One ml of the cell suspension was added to each well in the 24 well plates. After adding the cells, the plate was incubated at 37$^{o}$C for 48 h in 5% $CO_2$ incubator. After 48 h all the wells were screened under the inverted microscope. Care was taken to obtain the monolayer and the medium was removed. The stock drugs were prepared from the test drug by dissolving in MEM W/O FCS individually (10 g in 10 ml of MEM W/O FCS) and in combination of equal concentration (each 10 g in 30 ml of MEM W/O FCS). From the stock solution, the serial dilutions were made (10 µg, 50 µg, 100 µg…..800 µg).

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## Results

### Effect of the extract on cytotoxic activity

The methanolic extract of the plants was found to be nontoxic up to a concentration of 600 µg / ml. Toxicity was exhibited thereafter the concentration ranging from 700 µg / ml onwards in the Vero cell line (Table 1).

### Effect of the extract on anticancer activity

The methanolic extract of the plants on Hep2 cell line indicated non toxicity up to 50 µg / ml and thereafter exhibited toxicity (Table 2).

**Table 1: In Vitro Cytotoxic effect of Methanolic extract of Plants on Vero cell line**

| | | | | | |
|---|---|---|---|---|---|
| 10<br>NT | 10<br>NT | 50<br>NT | 50<br>NT | 100<br>NT | 100<br>NT |
| 400<br>NT | 400<br>NT | 300<br>NT | 300<br>NT | 200<br>NT | 200<br>NT |
| 500<br>NT | 500<br>NT | 600<br>NT | 600<br>NT | 700<br>T | 700<br>T |
| CC | CC | Neat<br>T | Neat<br>T | 800<br>T | 800<br>T |

**Table 2: In Vitro Anticancer effect of Methanolic extract of Plants on Hep2 cell line**

| | | | | | |
|---|---|---|---|---|---|
| 10<br>NT | 10<br>NT | 50<br>NT | 50<br>NT | 100<br>T | 100<br>T |
| 400<br>T | 400<br>T | 300<br>T | 300<br>T | 200<br>T | 200<br>T |
| 500<br>T | 500<br>T | 600<br>T | 600<br>T | 700<br>T | 700<br>T |
| CC | CC | Neat<br>T | Neat<br>T | 800<br>T | 800<br>T |

## Discussion

Antitumor activity of herbal medicine sho-saiko-to and its mechanism of action upon a murine malignant melanoma cell line (Mel-ret) have been studied and it was recognized that various naturally occurring substances from vegetables and herbs exert chemopreventive properties against cancer; the authors reviewed two such compounds isolated from garlic and *Rhizoma zedoariae* [29, 30]. Chloroform extract of *A. racemosus* have yielded a new antioxidant activity compound named racemofuran along with two known compounds asparagamine and racemosol and the structures have been fully characterized by spectroscopic data (UV, MS, NMR etc) [31]. Active constituents have been isolated from the dried root stock of *A. racemosus*. Their activity was examined against hepatocellular carcinoma induced by DEN in a rat model. Their ability to suppress the formation of mutant p53 protein and to combat oxidative stress was also evaluated. Anticarcinogenic property of active principles has been determined by developing a cancer model for hepatocellular carcinoma in Wistar rats [32]. The crude saponins obtained from *A. racemosus* shoots were found to have the antitumor activity. It inhibited the growth of human leukemia HL-60 cells in culture and macromolecular synthesis in a dose and time dependent manner [33]. A novel polysaccharide (RR1) is a α-glucan, exhibiting unique immune boosting properties. RR1 has been isolated from *T. cordifolia*, which is a well known in TIM for its anti-inflammatory, antiallergic, antiarthritic, antioxidant and immune stimulating properties. Treatment with *A. racemosus, T. cordifolia, W. somnifera* and *Picrorhiza kurro* significantly inhibited the ochratoxin A-induced suppression of chemotactic activity with the production of inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-alpha by macrophages [34]. Administration of the polysaccharide fraction from T. cardifolia was found to be very effective in reducing the metastatic potential of B16F 10 melanoma cells. There was a 72% inhibition in the metastases formation in the lungs of syngenic C57BL/6 mice, when the drug was administered simultaneously with tumor challenge [21]. Usage of medicinal plants for immunomodulator activities in cancer therapy has got the biphasic effects. Many of them are useful in the host defense mechanisms, which are normal or activated and some of them are tend to stimulate the individuals immune system [21]. Till today there is no exact treatment for cancer in synthetic medicine without cytotoxic effects, medicinal plant products are the only source to find out the effective anticancer drugs with reduced cytotoxicity.

## Conclusion

The combined methanol extracts of *A. racemosus, C. orchioides* and *T. cordifolia* showed cytotoxic activity only from 700 µg concentration and exhibited the anticancer activity even from 100 µg concentration, which indicate the safety on the normal cells and efficacy on the cancer cells which may be due to the possibility of synergistic effects.

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