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RESEARCH ARTICLE

ANTICANCER ACTIVITY OF MEDICINAL PLANT *SWERTIA CHIRATA*

Mendes Sarah Vailanka, Kabita Nayak and *Sheeba, E.

Department of Microbiology, Brindavan College, Bangalore, Karnataka, 560094, India

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ABSTRACT

The study was carried out to check the anticancer activity of the plant *Swertia chirata*. The anticancer activity was studied on HCT 116 and Calu 6 cell lines by MTT assay using the methanol extract of leaves and stem of the plant separately at a concentration ranging from 10-320 µg/ml. It showed that methanol extracts of both leaves and stem exhibited anticancer activity on HCT 116 cell lines in which the methanol extract of leaves exhibited more anticancer activity compared to stem. It was observed that the stem exhibited anticancer activity at higher concentrations whereas, no effect was observed against Calu 6 cell line.

INTRODUCTION

Swertia chirata is a bitter medicinal plant which belongs to the family Gentianaceae. Gentianaceae is a family of flowering plants containing a wide range of colours and floral patterns. *Swertia chirata* is annual, biennial or perennial herb of seasonal growth. It has an elongated stem, the size of which ranges from 60 cm to 150 cm. The stem is cylindrical at base and quadrangular upward. The colour of stem is greenish-brown when the plant is young and changes from light brown to light violet as the plant attains maturity. The root are generally small, 5-10 cm in length, light brown, somewhat twisted and gradually tapering.

It has leaves in opposite pair about 10 cm long, without stalks, pointed at the tip. Flowers are greenish yellow, tinged with purple and the seeds are small light brown to dark brown in colour. *Swertia chirata* is known for its medical and pharmaceutical importance. It promotes digestion, particularly of fats, and aid in regulating blood sugar levels. It is an effective medication for leishmaniasis, diabetes, nausea, bloating, hiccups, malaria, tuberculosis, dyspepsia, asthma, liver disorders, chronic fevers, anaemia, constipation, indigestion, common cold, diarrhea, leprosy, hysteria, convulsion and intestinal worms. It has large number of chemical constituents some of them are swertinin, swerchirin,

mangiferin, amarogentin, amaroswerin, gentianine, swertiamarin, xanthones, lignan, triterpenoids, pentacyclic triterpenoids, etc. In Ayurveda, the plant is used as stomachic, febrifuge, antihelminthic, diuretic as well as for treatment of some types of mental disorders. Anticancer activity of this medicinal plant was essential to cure the disease.

MATERIALS AND METHODS

Sample collection

Swertia chirata plants were collected from Vasco – Goa and identified.

Processing of sample

The leaves and stem of the plant sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. After washing, both leaves and stem samples were shade dried for 5 days and then ground into fine powder. The powdered samples were stored in clean, dry and sterile container for further use.

Sample extraction

30 grams of powdered sample of both leaf and stem was extracted in 50 ml of methanol separately using Soxhlet apparatus at 60°C for 48 hrs.

*Corresponding author: Sheeba, E.

Department of Microbiology, Brindavan College, Bangalore, Karnataka, 560094, India.

The methanol extract of each sample was then cooled at room temperature, filtered through Whatmann No. 1 filter paper and the filtrate was evaporated in flash evaporator to complete dryness. The dried extracts were then scraped and stored in clean sterile container for further use.

Anticancer Activity by MTT Assay

70-80% confluent cell lines (HCT116 and Calu6 cells) were trypsinized. Then the cells were checked for viability and centrifuged. 50,000 cells/well of HCT116 and Calu6 cells were seeded in a 96 well plate and incubated for 24 hrs at 37°C, 5% CO₂ incubator. Compounds to be tested from 0-320 µg/ml (two fold variations) in RPMI media without FBS and antibiotics were to be incubated for 24 hrs. After incubation with compounds, the media was removed from the wells and 100 µl/well (50 µg/well) of the MTT (5 mg/10ml of MTT in 1X PBS, the solution was filtered through a 0.2 µm filter and stored at 2-8°C for frequent use or frozen for extended period) working solution was added and incubated for 3-4 hrs.

After incubation with MTT reagent, the media was removed from the wells and 100 µl of DMSO was added to rapidly solubilise the formazan. The absorbance was measured at 540 nm.

$$\% \text{ of Inhibition} = 100 - (\text{sample/control}) \times 100$$

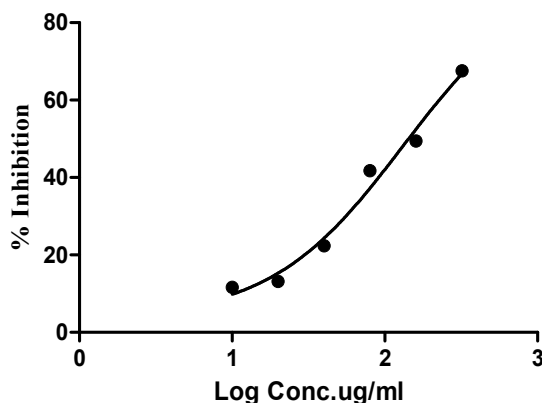
RESULTS

The anticancer activity of methanol extract of leaf and stem of *Swertia chirata* was tested against HCT 116 and Calu 6 cell lines by MTT Assay. Among the two cell lines, no cytotoxic effect was observed against Calu 6 cell line whereas the leaf extract of the plant showed cytotoxic effect against HCT 116 cell line at concentration ranging from 10-320 µg/ml with IC₅₀ value of 131.5 µg/ml. IC₅₀ is the concentration at which 50% of the cells are inhibited. The stem extract of *Swertia chirata* showed anticancer activity against HCT 116 cell line but since due to one of the value in % inhibition being negative, concentration vs % inhibition graph could not be plotted and hence IC₅₀ value could not be calculated.

Table 1. MTT assay using Colorectal Cancer HCT 116 cells Absorbance values of effect of plant samples on HCT 116 cells by MTT assay

Plant's Name	Conc. µg/ml	OD at 540 nm	% Inhibition	IC ₅₀ µg/ml
Stem <i>Swertia chirata</i>	Control	0.747	0.00	Not active
	10	0.74	0.92	
	20	0.743	0.52	
	40	0.748	-0.13	
	80	0.694	7.10	
	160	0.645	13.62	
	320	0.537	28.16	
Leaf <i>Swertia chirata</i>	0		0.00	131.5
	10	0.660	11.62	
	20	0.649	13.15	
	40	0.580	22.39	
	80	0.435	41.75	
	160	0.378	49.44	
	320	0.242	67.55	

MTT assay -HCT116 cells



	S. chirata Leaf
log(inhibitor) vs. response	
Best-fit values	
BOTTOM	92.74
TOP	3.514
LOGIC50	2.119
IC50	131.5

Figure 1. Anticancer activity

So methanol extract of stem was considered as not effective. Hence, it is considered that among stem and leaves, leaves showed dose dependent cytotoxic effect on HCT 116 Colorectal cancer cells and its IC₅₀ values are determined as shown in the Table 1 and Figure 1.

DISCUSSION

Present study carried out by us regarding anticancer activity of methanol extract of leaf and stem against HCT 116 and Calu 6 cell lines at a concentration ranging from 10-320 µg/ml, methanol extract of leaf showed cytotoxic effect on HCT 116 cell lines at all concentrations while stem showed only little activity at all concentrations. It was concluded that stem if tried with higher concentrations can have cytotoxic effect. Calu 6 cell line was not inhibited by the stem extract. The results obtained by us are not similar to Syed *et al.* 2013 who reported that the activity against MDBK cancer cell lines was not encouraging. But our result is in agreement with Prosenjit and Sukta, 2010 who reported that the anticancer activity was observed when the crude extract of the plant was tested against DBMA induced mouse skin carcinogenesis model.

Conclusion

Since, cancer is a dreadful disease and the treatment like surgery, radiation therapy can only provide temporary relief, the study conducted can be helpful in discovering the drugs and new methods of treatment to cure cancer. The present study helps to understand the presence of bioactive compounds in the plants in the pharmaceutical industry in future. As the search for new drugs are in demand, plant extracts may provide an attractive alternative source against various infections and chronic diseases. Many of the existing synthetic drugs cause various side effects; hence development of drug based on plant compounds could be useful in meeting the demand for newer drug with minimal side effects. The present study provides the evidence of anticancer properties of the medicinal plant *Swertia chirata*. The plant extracts can be used for discovering several drugs for cancer after purification. Since the crude extracts are found to be effective, there is a possibility that the plant extracts will be useful for treatment of illness after proper analysis and process. Further study and analysis are necessary to determine the specific activities of the plant.

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