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Cytotoxic potential of rhizome extracts of *Hedychium spicatum* L. in HepG2 cell line using MTT

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Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis. HCC is one of the most common cancers worldwide affecting 250,000 to 1,000,000 individuals annually. The incidence of HCC is highest in Asia and Africa, where the endemic high prevalence of hepatitis B and hepatitis C strongly predisposes to the development of chronic liver disease and subsequent development of HCC.

In view of that, herbal medicine may be used for the patients having HCC. India is the rich source of herbal medicine and endogenous herb are used for verities of the diseases.

Hedychium spicatum (Zingiberaceae) is an ayurvedic traditional medicinal plant found in entire Himalaya region at 1100–2500 m, East India and hills of South India (Anonymous 1994). Traditionally, the rhizome of the plant is used therapeutically because it possess various medicinal properties such as carminative, spasmolytic, hepatoprotective, anti-inflammatory, antiemetic, antidiarrhoeal, analgesic, expectorant, antiasthmatic emmenagogue, hypoglycaemic, hypotensive, antimicrobial, anthelmintic, insect repellent, antifungal, antioxidant, pediculicidal and cytotoxic activities (Asolkar *et al.* 1992).

The rhizome of *Hedychium spicatum* L. was collected from the Medicinal Research Development Centre, G B Pant University of Agriculture and Technology, Pantnagar. *In vitro* methods were used for assessing the cytotoxic activity and they were in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC/VPT/CVSC/193).

The rhizome was gathered from the plants, chopped like slice, shade dried for 15–20 days to dryness. Later, the chopped rhizomes were placed in fan equipped incubator at 37°C for 2–4 h, ground in electric grinder to fine homogenous powder. Dried and powered rhizome of *Hedychium spicatum* was soaked in aqueous, hydro ethanolic (1:1), hydro methanolic (1:1), methanolic and ethanolic solutions for 24 h. The solutions were filtered using muslin cloth, whatman filter paper No. 40 and then dried in rotary evaporator followed by final drying at 37°C. The dried extract was stored at 4°C until use.

Bismuth nitrate, nitric acid, potassium iodide, sodium carbonate, mercuric chloride, sulphuric acid, hydrochloric acid, sodium hydroxide, ferric chloride, alpha naphthol, copper sulphate, zinc chloride, 3-(4, 5dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), isopropanol, phosphate buffer saline (PBS), dimethyl sulfoxide (DMSO), calorimeter, potassium dihydrogen phosphate, CO_2 incubator, PBS, Spectrophotometer were purchased commercially. HepG2 cell lines were purchased from National Centre for Cell Sciences (NCCS), Pune, Maharashtra, India.

Each extract was taken into separate test tubes and shaken rapidly and vigorously and distributed for the test of the presence of alkaloid, reducing sugar, glycosides, saponins, flavonoids, tannin, flavanoid, phenolics, protein/amino acids, diterpenes, resins, fixed oils and fats and steroid (Fatope *et al.* 1993).

MTT test is a colorimetric assay that measures the reduction of yellow 3–(4, 5-dimethythiazol-2-yl)-2, 5diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT assay is based on the ability of mitochondrial dehydrogenase from viable cells to oxidise the tetrazolium rings of pale yellow MTT and form a dark blue formazan crystals. The cells are then solubilised with an organic solvent to release solubilized formazan reagent.

HepG2 human liver hepatocellular carcinoma cell lines were maintained in Minimal Essential Media with 10% FBS and grown to confluency at 37°C and 5% CO_2 in a humidified atmosphere in a CO_2 incubator. The cells were trypsinized (500 µl of 0.025% Trypsin in PBS solution) for 2 min and passaged to T flasks in complete aseptic conditions and incubated. Extracts were added to 80% confluent cells at a concentration of 100 µg, 500 µg and 1000 µg from a stock of 200 mg/ml along with the media to make the volume of well 200 µl and incubated for 24 h. The cell culture suspension was washed with 1× PBS and

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Table 1. Results of phytochemical analysis

Extract constituents	Etha-nolic	Metha-nolic	Hydro-ethanolic	Hydro-methanolic	Aqueous	Name of test	Colour developed
Alkaloids	+	+	+	+	+	Mayer's test	Cream white ppt
						Wagner' test	Redish brown ppt
Carbohydrates	+	+	+	+	+	Molish test	Violet ring
						Benedict's test	Red colour ppt
Glycosides	++	++	++	++	+	Borntrage's test	Pink colour
Phenols	++	++	++	++	++	Ferric chloride test	Dark green colour
Flavonoids	++	++	++	++			Pink to crimson colour
Protein and amino acids	+	+	+	+	+	Biuretts test	Violet or pink colour
Saponins	+	+	+	+	+	Froth test	A layer of foam
Diterpine	++	++	++	++	+	Copper acetate test	Bright green colour
Resins	+	+	+	+	+	Acetone-water test	Presence of turbidity
Fat and oils	+	++	+	+	+	Stain test	Oil stain
Phytosterols	++	++	++	++	++	Libermann- Burchard's test	Golden yellow colour
Tanins	+	+	+	+	+	Feric chloride test	Brownish green or blue black

then, MTT (20 µl) solution was added to the culture (MTT -5mg/volume dissolved in PBS). The plates were wrapped in aluminium foil and incubated 37°C for 4 h. The whole MTT was removed with 1× PBS and 200 µl DMSO were fed to each culture and incubated at room temperature for 15 min until the cell were lysed and colour was obtained. The plate was then read in ELISA reader to record the absorbance at 570 nm.

The extracts of the rhizome contains important bioactive constituent viz. alkaloids, tannins, flavonoids and phenolic compounds having medicinal value (Edeoga *et al.* 2005). The phytochemical screening of the different extracts of the rhizome sample revealed the presence of alkaloids, flavonoids and phytosterols etc. (Table 1) (Sofowora 1984). In the present study, these phytoconstituents may be responsible for various activities. Flavanoids are diverse family of compounds commonly found in fruits, vegetables and honey. Flavanoids are generally safe and associated with low toxicity, making them ideal candidates for cancer chemopreventive agents.

Per cent cell viability at different concentration of the extract of *H. spicatum* as obtained by MTT is shown in Table 2. The results indicate that the crude extract of *H. spicatum* on HepG2 cell lines had shown a dose-dependent anti-proliferative effect (Fig. 1A-D). MTT uptake assay results confirms dose-dependent anti-proliferative effect of various extract of *Hedychium spicatum* on HepG2 cell lines. As the dose of the extract increased, number of viable cells decreased which confirms the cytotoxic activity (Table 2). The extract have the potential power of cytotoxic activity, corroborated with the finding of Suresh *et al*. (2013) and Reddy *et al*. (2010). The IC_{50} value for methanolic extract was minimum and aqueous extract had maximum which indicated that the methanolic extract have more potential than aqueous (Table 3). MTT assay confirmed dose-dependent antiproliferative effect of crude extract of *H. spicatum* on HepG2 cell lines. It is concluded that the extract of *Hedychium spicatum* was found to possess dose-dependent cytotoxic activity on HepG2 cell lines.

Table 2. Cytotoxicity studies of different extract of *Hedychium spicatum* using MTT assay

Sample concentration (µg/ml)	% Cell viability				
	Etha-nolic	Metha-nolic	Hydro-ethanolic	Hydro-methanolic	Aqueous
25	95.856	98.076	92.440	93.579	91.313
50	87.676	81.401	85.748	87.904	85.079
100	70.650	70.427	71.772	71.039	70.293
250	60.986	63.487	63.941	60.187	57.115
500	50.677	53.993	55.584	54.415	52.727
1000	31.668	47.656	50.042	42.8	44.933
2000	27.128	36.236	40.815	36.361	31.166
3000	17.751	25.133	25.367	25.019	18.911

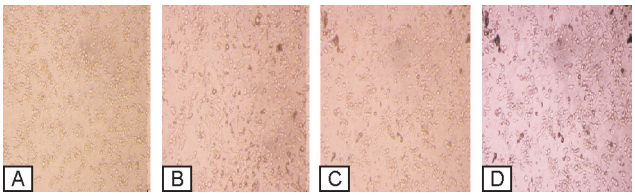

Fig. 1. A-D. Photograph of HepG2 cell line taken using inverted illuminating microscope. Control received vehicle (A), methanolic extract of *H. spicatum* (B), ethanolic extract of *H. spicatum* (C) and aqueous extract of *H. spicatum* (D) at their dose of their IC_{50} value.

Table 3. IC_{50} value of different extracts of *Hedychium spicatum*

H. spicatum extract	IC_{50}
Ethanolic	356.91
Methanolic	281.917
Aqueous	515.119
Hydromethanolic	391.57
Hydroethanolic	490.97

Hepatocellular carcinoma is associated with liver cancer which in most cases cannot be cured but the treatment and support may help for making life longer and better. Available treatment alternatives include chemotherapy, surgery, brachy therapy and ablation therapy or palliative therapy. Liver transplantation may also be used for the HCC but there are chances of rejection and also there may be the reoccurrence of HCC (Busuttil and Farmer 1996, Penn 1991). However, the severe side effects of chemotherapy and other available alternative therapy have remained a major problem. Therefore, present study was planned to identify endogenous herb that can prevent the development and recurrence of cancer. A wide variety of natural food and food products can induce apoptosis in various tumour cells. There is strong evidence supporting the positive role of medicinal plants in oncology with their ability to affect all phases of tumerogenic process. So, it is important to screen the natural endogenous products either as crude extracts or as isolated components for apoptotic properties to identify potential anti-cancer compounds. Presently, more than 55% used anti-cancer agents are derived from natural sources. The *Hedychium spicatum* plants are highly useful as they have good potential against many health ailments. Future studies must be undertaken to explore the anticancer effect of *Hedychium spicatum*.

SUMMARY

The present study was designed to evaluate the cytotoxic effects of extract of *Hedychium spicatum* rhizome extract on human liver hepatocellular carcinoma (HepG2) cell line using MTT assay. The crude extract of *H. spicatum* was prepared by cold maceration method, filtered, concentrated in different organic solution and tested for phytochemical evaluation and finally on HepG2 cell line. Dose-dependent cytotoxic activities were exhibited on HepG2 cell line. As the dose of the extract increased, the number of viable cells decreased. This confirms the cytotoxic potential of the rhizome of *H. spicatum.* The IC_{50} value of different extracts was determined and concluded that the methanolic extract had better potential than the other extracts.

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