IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
Volume 8 | Issue 1 | January, 2013 | 140-143

RESEARCH ARTICLE

Fruit part of *Momordica charantia* possesses remarkable *in vitro* anticancer efficiency against eight human cancer cells

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SUMMARY

In vitro assay for cytotoxic activity of fruits obtained from *Momordica charantia* has been carried out against eight human cancer cell lines from six different tissues *via* methanolic and aqueous extract at the concentration of 100 µg/ml. Results revealed that the 99 per cent methanolic extract suppressed the proliferation of seven human cancer cells. The most striking observations were 100 per cent growth inhibition against NCI-H322 (lung), 99 per cent growth inhibition against MCF-7 (breast) and 97 per cent growth inhibition on COLO-205 (colon) cancer cells. However, aqueous extract from the floral part of the plant showed activity (74%) against HCT-116 (colon). Based on the *in vitro* data, it is suggested that consumption of the components of this plant or ingestion of methanolic extract as tea may impart anticancer effects especially in the lung, breast, colon and will be evaluated for the possible isolation of active antitumor compounds.

Key Words : *Momordica charantia*, Methanolic extract, *In vitro* cytotoxic, Cancer cells

How to cite this article : Hussain, Shabir, Sharma, Vikas and Saxena, Ajit Kumar (2013). Fruit part of *Momordica charantia* possesses remarkable *in vitro* anticancer efficiency against eight human cancer cells. *Internat. J. Plant Sci.*, **8** (1) : 140-143.

Article chronicle : Received : 23.08.2012; **Revised :** 06.10.2012; **Accepted :** 23.11.2012

Momordica charantia (Linn.) also known as bitter gourd and belongs to Cucurbitaceae family, is widely cultivated in Asia, Africa and South America and extensively used in folk medicines as a remedy for diabetes, specifically in India, China and Central America. The fruit is oblong and resembles a small cucumber, young fruit is emerald green that turns to orange-yellow when ripe (Grover *et al*., 2002).

Multiple types of extracts from bitter melon had *in vivo* (Nagasawa *et al*., 2002; Kohno *et al*., 2004) and *in vitro* (Yasui *et al*., 2005) anticancer activity. Methanolic extract as well as momordin of *Momordica charantia* showed cell toxicity against human cancer cell lines (Lee *et al*., 1998). Chronic treatment with hot water extract of *Momordica charantia* inhibited uterine adenomyosis and mammary tumor growth in mice (Nagasawa *et al.,* 2002). Fruit and leaf extracts (50% methanol) from *Momordica charantia* possess chemopreventive potential on dimethyl benz (a) nthracene (DMBA) induced skin tumorigenesis, melanoma tumor and cytogenicity (Agrawal and Beohar, 2010). Alcoholic extract from the leaves of bitter melon have an anti-metastatic effect against rat prostate cancer progression both *in vitro* and *in vivo* (Pitchakaran *et al*., 2010). *Momordica charantia* extract inhibited human breast cancer cells (MCF-7 and MDA-MB-231) proliferation by modulating cell cycle regulatory genes and promotes apoptosis (Ray *et al*., 2010). *Momordica charantia* was found effective on highly metastatic PC-3M prostate cancer cell line (Rao *et al*., 2004). Bitter melon is known to contain glycosides such as momordin, vitamin C, carotenoids, flavonoids and polyphenols (Anila and Vijayalakshmi, 2000; Raj *et al*., 2005). MCP30, a protein isolated from bitter melon seeds selectively induces prostate cancer

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apoptosis (Xiong *et al*., 2009). Cucurbitane–type triterpene glycosides isolated from a methanol extract of the fruits of Japanese *Momordica charantia* exhibited marked inhibitory effect on induced mouse skin carcinogenesis (Akihisa *et al*., 2007). Based on an analysis of published literature, the fruit part of *Momordica charantia* was selected to evaluate its *in vitro* anticancer potential against eight human cancer cells by using SRB assay.

MATERIALS AND METHODS

Preparation of extracts :

The fruits of *Momordica charantia* were collected in the month of July from Baisht village, Udhampur, J&K, India and were authenticated at site by Dr. Satesh Kumar, Assistant Professor, Division of Vegetable Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-J). The freshly collected fruits were chopped, shade dried and ground into powdered form. The powdered dried fruit material was then extracted with different solvents at room temperature to obtain extracts for bioevaluation. The methanolic extract was prepared by percolating the dried ground fruit material (100 g) with 99 per cent methanol and then concentrating it to dryness under reduced pressure. The aqueous extract was obtained by boiling dried ground plant material (100 g) for 30 min. in distilled water (300 ml).

Preparation of stock / working solutions :

Stock solutions of 20 mg/ml were prepared by dissolving methanolic extract in DMSO and aqueous extract in sterile water. Stock solutions were prepared atleast one day in advance and were not filtered, but the microbial contamination was controlled by addition of 1per cent gentamycin in complete growth medium *i.e*. used for dilution of stock solutions to make working test solutions of 200 µg/ml.

Preparation of positive controls :

5-flurouracil and adriamycin were prepared in distilled water while paclitaxel was prepared in DMSO. Positive controls were diluted in gentamycin medium to obtain the concentration of 2×10^{-5}M, 1 x 10^{-6}M and 1 x 10^{-6}M.

***In vitro* assay for cytotoxic activity :**

Test material was subjected to *in vitro* anticancer activity against various human cancer cell lines (Monks *et al*., 1991). The cells were grown in tissue culture flasks in RPMI 1640 / MEM growth medium at 37°C in an atmosphere of 5 per cent CO_2 and 90 per cent relative humidity in a CO_2 incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with more than 97per cent viability were used for determination of cytotoxicity. An aliquot of 100µl of cells (10^5cells/ml) was transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for 24 h. Test material was then added to the wells and cells were further allowed to grow for another 48 h.

The antiproliferative SRB assay was performed to assess growth inhibition which estimates cell number indirectly by staining total cellular protein with the dye SRB (Skehan *et al*., 1990). The cell growth was stopped by gently layering 50µl of 50 per cent TCA on the top of growth medium in all the wells. The plates were incubated at 4°C for an hour to fix the cells attached to the bottom of the wells. Liquids of all the wells were then gently pipetted out and discarded. The plates were washed five times with distilled water and were air-dried. Sulphorhodamine B 100µl (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min. The unbound SRB was quickly removed by washing the cells five times with 1per cent acetic acid. Plates were air-dried, tris buffer (100µl, 0.01M, pH 10.5) was added to all the wells to solubilise the dye. Plates were gently stirred for 20 min. on a mechanical stirrer. The optical density was recorded on ELSIA reader at 540nm.

Suitable blanks and positive controls were also included. Each test was done in triplicate and the values reported herein are mean values of three experiments.

Calculation :

The cell growth was determined by subtracting average absorbance value of respective blank from the average absorbance value of experimental set. Per cent growth in presence of test material was calculated as under:

OD change in presence of control = Mean OD of control – Mean OD of blank

OD change in presence of test sample = Mean OD of test sample – Mean OD of blank

% Growth in presence of control = 100/OD change in presence of control

% Growth in presence of test sample = % Growth in presence of control × OD change in presence of test sample

% Inhibition by test sample = 100 – % Growth in presence of test sample

The growth inhibition of 70 per cent or above was considered active while testing extracts, but in testing of active ingredients at different molar concentrations, the growth inhibition of 50 per cent or above was our criteria of activity.

RESULTS AND DISCUSSION

For evaluating *in vitro* anticancer activity, the floral part of *Momordica charantia* was selected because of its known medicinal properties. The observations produced by the extracts (methanolic and aqueous) prepared from the fruits of the plant are summarised in Table 1 and the data represented in Fig. 1and 2.

Most significant results, that is strong antiproliferative effect on a range of human cancer cell lines was displayed by the methanolic extract as this particular extract was observed to be cytotoxic to a wide spectrum of seven human cancer cell

Table 1: Growth inhibitory effect of *Momordica charantia* along with positive controls against human cancer cell lines

Plant part used	Extract	Conc. (µg/ml)	Human cancer cell lines from six different tissues							
			Lung	Colon	Colon	Breast	Lung	Prostate	Leukemia	Glioblastoma
			A-549	COLO-205	HCT-116	MCF-7	NCI-H322	PC-3	THP-1	U-87MG
			Growth inhibition (%)							
Fruit	Methanolic	100	75	97	37	99	100	82	91	76
	Aqueous	100	16	34	74	42	5	14	0	13
Positive controls		Conc. (molar)								
5-Flurouracil		2×10–5	-	51	68	-	-	-	73	60
Paclitaxel		1×10–6	79	-	-	-	52	-	-	-
Adriamycin		1×10–6	-	-	-	60	-	59	-	-

Growth inhibition of 70% or above has been indicated in bold numbers

The mark (-) indicates that particular human cancer cell line was not treated with that particular positive control

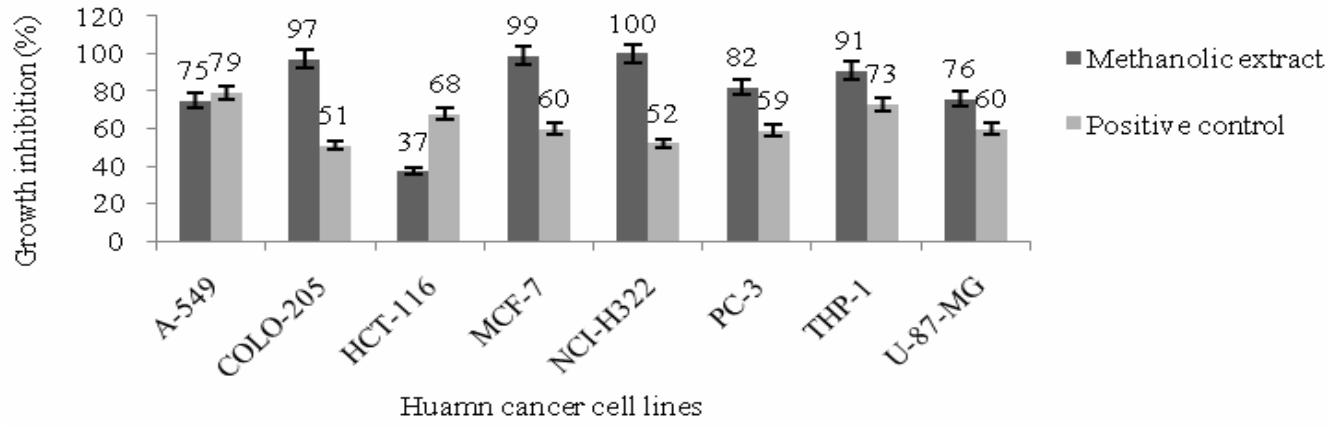

Fig. 1: *In vitro* cytotoxic effect of methanolic extract from *Momordica charantia* against human cancer cell lines

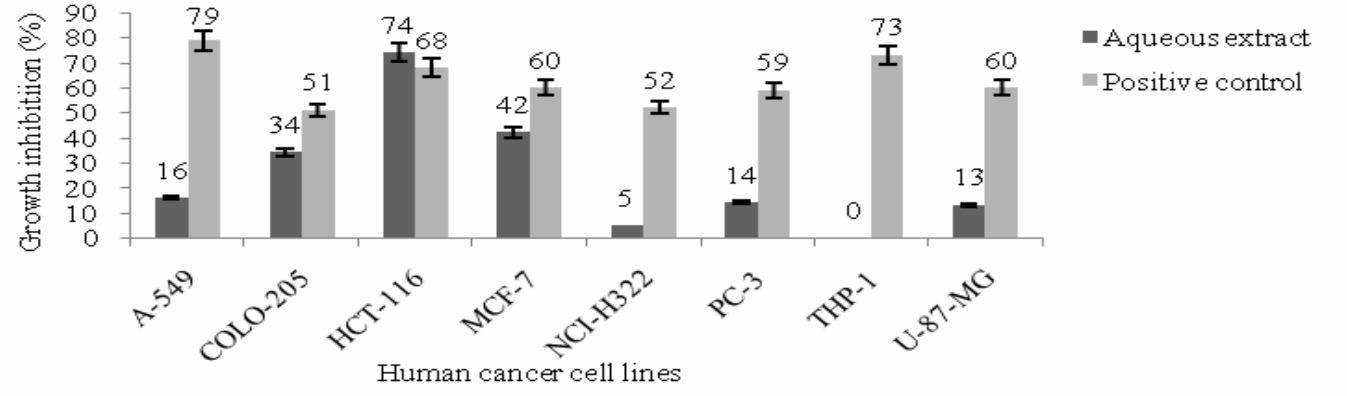

Fig. 2 : *In vitro* cytotoxic effect of aqueous extract from *Momordica charantia* against human cancer cell lines

lines and the growth inhibition range shown by this extract was between 75-100 per cent. The extract showed a high degree of growth inhibition against lung, breast and colon cancer cells as 100 per cent growth inhibition was observed against NCI-H322 (lung), 99 per cent growth inhibition was observed against MCF-7 (breast) and 97 per cent growth inhibition was observed against COLO-205 (colon). Significant results were also produced by the extract in case leukemia cancer cells as 91per cent growth inhibition was observed against THP-1 (leukemia). The extract showed 82 per cent growth inhibition on prostate cancer cells (PC-3), 76 per cent growth inhibition on glioblastoma cancer cells (U-87MG) and 75 per cent growth inhibition on lung cancer cells (A-549). What is quite remarkable in these observations is that the cytotoxic effect shown by the extract was much stronger than that shown by 5-flurouracil, adriamycin and paclitaxel (serving as positive

controls in present investigation). Surprisingly, this extract did not exhibit any significant cytotoxic effect against HCT-116, a human cancer cell line also from colon origin. However, aqueous extract from the floral part of the plant showed activity (74%) against only HCT-116.

Therefore, the results confirmed the therapeutic potency of *Momordica charantia* and suggest that the plant possesses certain constituents with cytotoxic properties against human cancer cells. The data was compared with literature values and it was found that the data was in good agreement with the published data (Kobori *et al*., 2008; Pitchakarn *et al*., 2010; Ray *et al*., 2010; Rao *et al*., 2004). Cancer has remained a major cause of death and the number of individuals living with cancer is continuing to expand, but most of the drugs used in cancer chemotherapy exhibit cell toxicity and can induce genotoxic, carcinogenic and teratogenic effects in non tumor cells. Therefore, the research for alternative drugs of natural origin, which are less toxic, endowed with fewer side effects and more potent in their mechanism of action, is an important research line. Thus, on the basis of present analysis, it is suggested that methanolic extract can be subjected for the isolation of active ingredient(s) that will surely serve as lead molecule (s) in the development of anticancer drugs especially for lung, breast and colon carcinoma to provide a great promise and service to cancer patients.

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