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Anticancer activity of Fruits of *Momordica Dioica* by using MTT assay

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Abstract

To evaluate the anticancer activity of fruits of *Momordica dioica* by using MTT assay. The aqueous extract of fruits of *Momordica dioica* was tested against ovarian carcinoma of PA-I cell lines and human cervical cancer of Hela cell line at various concentrations. The anticancer effect of the aqueous extracts on cell inhibition was studied using MTT assay. The results showed that aqueous extract of fruits of *Momordica dioica* inhibited growth of PA-I and Hela cells at IC_{50} concentration is 40 µg/mL respectively. Alterations in the cell morphology were also observed after treatment of the cell lines with fruit extract. The present work revealed that *Momordica dioica* contains some important chemical constituents extracted using aqueous as solvent that can be used further in the management of cancer treatment.

Keywords: Anticancer activity; *Momordica dioica*; Cucurbitaceae; MTT assay.

Introduction

Cancer is one of the dangerous diseases of the 20th century occur in humans, spreading fastly in 21st century and presently there is lots of new anticancer agents was discovered from natural products or plants [1,2]. There are millions of plants available in the world with greater importance. The compounds isolated from various parts of plants play a vital role in treatment of various diseases and have received good attention in recent years due to their different pharmacological properties including cytotoxic and anticancer activity [3]. Plants play a vital place in the treatment of cancer. It is estimated that plant derived compounds one or the other way constitute more than 50% of anticancer agents [4,5].

Momordica dioica is a perennial, dioecious climber belonging to *Cucurbitaceae* family, which is commonly known as spiny gourd, teasel gourd or small bitter gourd worldwide whereas in India, it is known as Kankro, Kartoli, Kantola, Ban karola or Janglee karela. *Momordica dioica* has been known to have many medicinal properties namely anti-tumorogenic, analgesic, anti-diabetic, anti-inflammatory and anti-allergic activity [6-9].

There are five active constituents isolated from the dichloromethane extract of roots of *Momordica dioica* which were found to possess anticancer activity in pharmacologic testing on cancer cell (L1210). The growth inhibitory index (%) was shown to be 50%, at the dose of 4 µg/mL [10]. The methanol extract of seed of *Borreria hispidia* and *Momordica dioica* shows good anticancer activity [11]. Constituents derived from plants are going to be promising and hundreds of literatures are available to explain role of plants in treatment of cancer. Based on survey of literature, there is no work has been carried out on the evaluation of anticancer property of aqueous extract of fruits of *Momordica dioica*. Hence in present study, anticancer potential of aqueous extract of fruits of *Momordica dioica* was

assessed by investigating the inhibition of cell growth of PA-I and Hela cancer cells after treatment with extract. Morphological changes of cancer cell lines treated with the fruit extracts were also observed in this study.

Materials and Methods

Procurement of plant material

The fruits of *Momordica dioica* were collected from the local area of Nandurbar (Maharashtra) and authenticated by Dr. Santosh K. Tayade, Head, Dept. of Botany, Art's, Science and Commerce College, Lonkheda, Shahada, Dist-Nandurbar, Maharashtra, India. All the reagents and chemicals were purchased from Rajesh Chemicals, Mumbai.

Preparation of fruit extract

Collected fruits were washed with distilled water, shade dried and crushed to a coarse powder and extracted with water by maceration method. Extract was dried over anhydrous sodium sulphate and solvent was removed in vacuum at 40°C by sing rotary evaporator (Rotavapour Buchii, Switzerland) [12].

Cell culture

Cultured cancer cells are valuable reagents for rapid screening of potential anticancer agents as well as for lucidation of mechanism of their activity. Human ovarian cancer cell lines (PA-I) and human cervical cancer cell lines (Hela) used in this study, were obtained from National Center for Cell Science (NCCS), Pune, India. The cell lines were maintained in 96 wells micro titer plate containing DMEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamicin (10 µg), Penicillin (100 Units/ml) and Streptomycin (100 µg/ml) in presence of 5% CO_2 at 37°C for 48-72 hours.

Cytotoxicity assay on cancer cell lines [13]

In vitro growth inhibition effect of test compound was assessed by calorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells. Remove the supernatant from the plate and adds fresh DMEM solution and treat with different concentrations of extract or compound appropriately diluted with DMSO. Control group contains only DMSO. In your study, 10, 20, 40 and 80 µl of the stock solution (10 mg/ml prepared in DMSO) were added to respective wells containing 100 µl of the medium. So, the final concentrations were 10, 20, 40 and 80 µg/ml. After 48 hrs incubation at 37°C in a humidified atmosphere of 5% CO_2, stock solution of MTT was added to each well (20 µl, 5 mg per ml in sterile PBS) for further 4 hr incubation. The supernatant carefully aspirated, the precipitated crystals of "Formazan blue' were solubilized by adding DMSO (100 µl) and optical density was measured at wavelength of 570 nm by using LISA plus. Wells containing cells without the aqueous fruit extract served as blank. Cisplatin was used as positive control. The concentration required for a 50% inhibition of cell viability (IC_{50}) was determined by using the formulae.

Surviving cells (%)=Mean OD of test compound/Mean OD at control × 100

Morphological studies

Cells were photographed after 48 h under inverted light microscope at 40x magnifications to examine the morphological changes of PA-I and Hela cell lines treated with the aqueous extract of fruits of *Momordica dioica*.

Statistical analysis [14]

The experiments were carried out in triplicates and the data were expressed as mean ± SEM. The significance of difference among the various treated cells and control cells were analyzed by means of one-way ANOVA.

Results

There are millions of plant-based compounds are available and have been playing an important role in the development of several clinically useful anticancer agents. The predominant aims of analyzing anticancer activity of the aqueous extract of fruits of *Momordica dioica* is either to isolate bioactive agents for direct use as anticancer drugs or to identify bioactive compounds that can be used as lead substance in the preparation of semi synthetic drugs to treat cancer. In the present investigation, aqueous extract of fruits of *Momordica dioica* is prepared. In this study, we demonstrate the anticancer potential of the aqueous extract of fruits of *Momordica dioica* in well characterized PA-I and Hela cell lines. Among the different concentrations of the aqueous extract of fruits of *Momordica dioica*, 50% cell viability was determined at the concentration of 40 µg/ml in PA-I and 40 µg/ml in Hela cell lines (Tables 1 and 2). Cisplatin served as a positive control which showed 50% cell viability at the concentration of 2.5 µg/mL on both the cancer cell line (Tables 1 and 2).

Figures 1 and 2 shows photographs of normal PA-I and Hela cancer cell line respectively. Figure 3 and 4 shows the morphological changes in PA-I and Hela cancer cells treated with aqueous extract of fruits of *Momordica dioica*, indicating the formation of spherical shaped cells which is the onset of apoptosis occurring in the concentration dependent manner. As the cell's intrinsic cell death program, apoptosis plays a key role in growth control of cells and tissue homeostasis. Therefore, the induction and recovery of the apoptotic response in tumor cells are relevant steps in anticancer treatment.

Table 1. Percentage inhibition of PA-I cells treated with different concentrations of aqueous extract of fruits of *Momordica dioica*.

Concentration µg/ml	*Momordica dioica*	Cisplatin	IC_{50} (µg)
80	37.79 ± 0.69****		40 µg
40	51.03 ± 0.75****		
20	60.51 ± 0.63****		
10	65.13 ± 0.40****		
2.5		51.17 ± 0.28****	2.5 µg
Cell control	0	0	0

Values expressed as mean ± SEM, ****p<0.0001, ***p<0.001, **p<0.01 and *p<0.05 indicates significant difference compared to the cell control and analyzed using Dennett's Multiple Comparison Test.

Table 2. Percentage inhibition of Hela cells treated with different concentrations of aqueous extract of fruits of *Momordica dioica*.

Concentration µg/ml	*Momordica dioica*	Cisplatin	IC_{50} (µg)
80	48.95 ± 0.14****		40 µg
40	49.72 ± 0.36****		
20	53.28 ± 0.235****		
10	53.77 ± 0.12****		
2.5		49.82±0.03****	2.5 µg
Cell control	0	0	0

Values expressed as mean ± SEM, ****p<0.0001, ***p<0.001, **p<0.01 and *p<0.05 indicates significant difference compared to the cell control and analyzed using Dennett's Multiple Comparison Test.

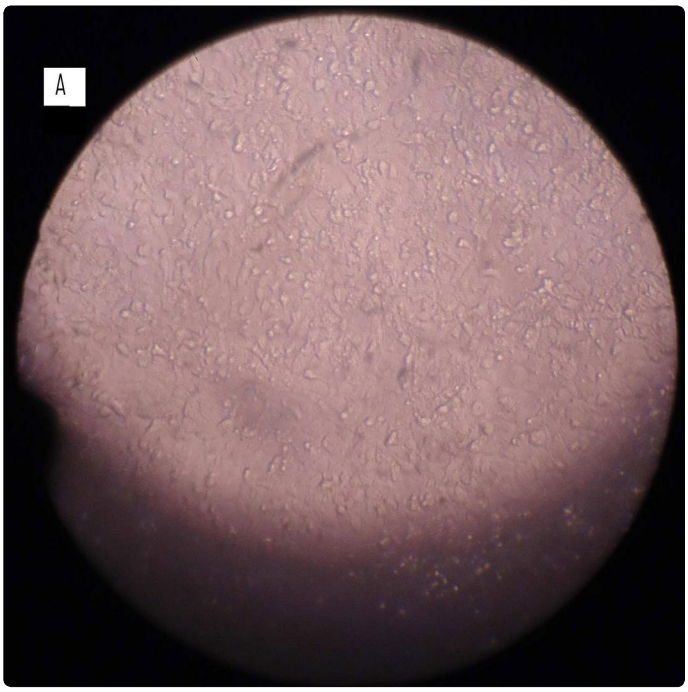

Figure 1. Photomicrographs of PA-I Ovarian cell lines A) normal PA-I cells.

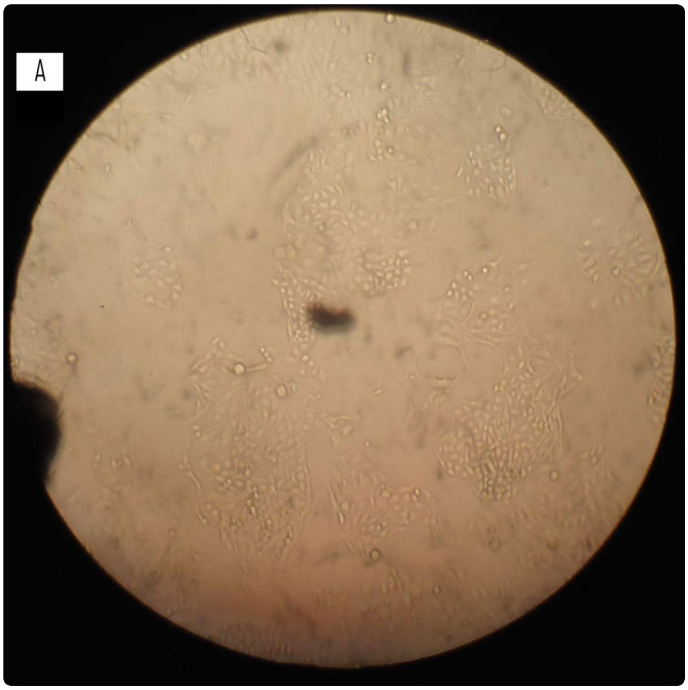

Figure 2. Photomicrographs of cervical cancer of Hela cell lines A) normal Hela cells.

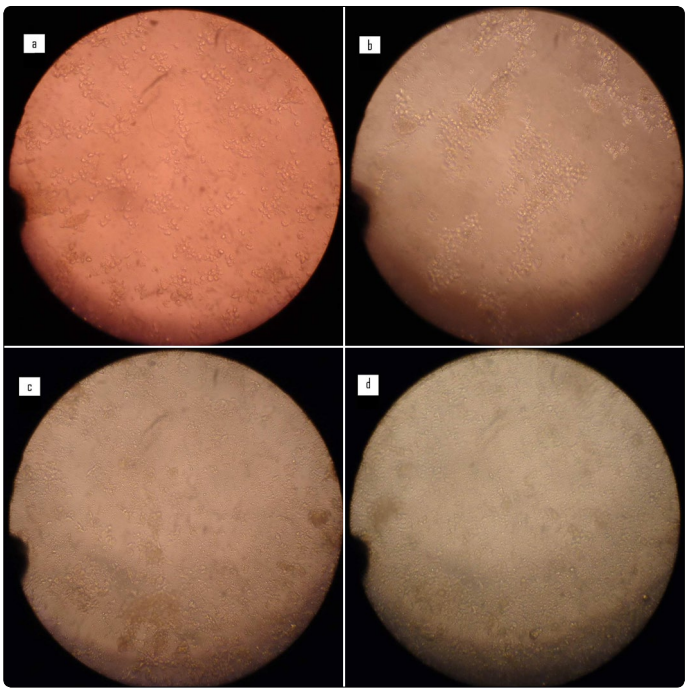

Figure 3. Photomicrographs of PA-I Ovarian cell lines for treated with aqueous extract of fruits of *Momordica dioica* at: a) 10 µg/ml, b) 20 µg/ml, c) 40 µg/ml d) 80 µg/ml.

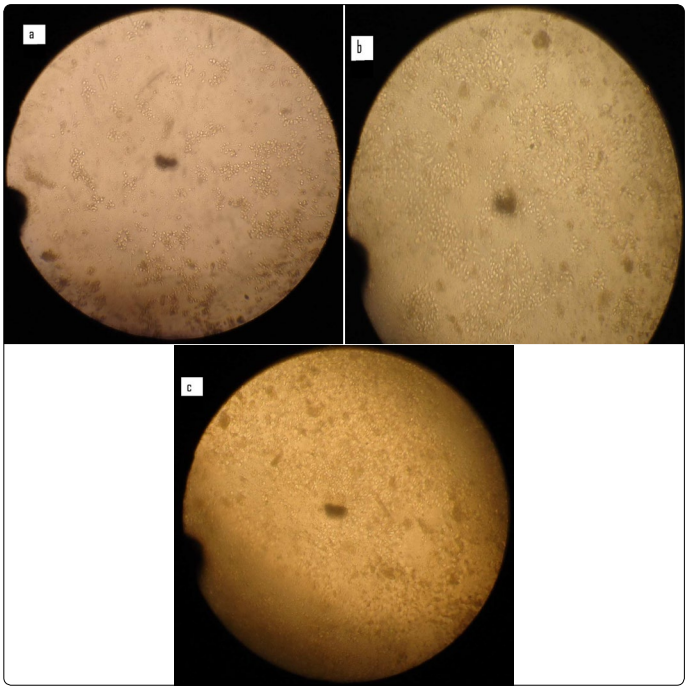

Figure 4. Photomicrographs of cervical cancer of Hela cell lines for treated with aqueous extract of fruits of *Momordica dioica* at: a) 10 µg/ml, b) 20 µg/ml, c) 40 µg/ml, d) 80 µg/ml.

Discussion

The anticancer potential of cucurbitaceae plant species has been recorded, which includes the cell growth inhibiting effects of methanolic leaf extract of *Luffa acutangula* (Linn.) Roxb. (Cucurbitaceae) on HL-60, Jurkat and RAW 264.7 cell lines [15]. The plant *Luffa aegyptiaca* Miller (cucurbitaceae) shows good anticancer activity [16]. The plant of various extracts of *Luffa cylindrica* Linn. (Cucurbitaceae) shows good anticancer activity [17]. The plant *Momordica charantia* L. (cucurbitaceae) shows anticancer activity on ovarian cancer cell lines. The methanolic

extract of seeds of *Momordica dioica* shows good anticancer activity by causing 50% inhibition of A549 cells at IC_{50} value of 3.125 µg/mL and MCF-7 cells at IC_{50} value of 1.56 µg/mL [11]. According to the above mentioned discussions, it can be stated that family of cucurbitaceae plants may contain potential compounds or active principles which can act as effective sources of anticancer agents.

Conclusion

The result of this study indicate that the aqueous fruit extract of *Momordica dioica* showed anticancer activity by causing 50% inhibition of PA-I cells at IC_{50} value of 40 µg/mL and Hela cells at IC_{50} value of 40 µg/mL. In essence, the present work revealed that *Momordica dioica* contains some important phytoconstituents extracted using aqueous as solvent that can be used further in the management of cancer treatment.

Disclosure of Statement

The authors declare no conflict of interest.

Acknowledgements

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