



## Evaluation of Antimitotic Activity of *Momordica Dioica* Fruits on *Allium Cepa* Root Meristamatic Cells

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### ABSTRACT

Natural occurring phenolic compounds play an important role in cancer prevention and shows antimitotic activity. Number of active constituents like phenolic acid, curcuminoids, coumarine, ligans, quinones, etc. is showing antimitotic activity of *Momordica dioica*. The present work is on phytochemical investigation and examines antimitotic activity of aqueous extract of fruits *Momordica dioica* at concentration of 15 mg/ml on *Allium cepa* root meristamatic cells.

The fruits are air dried and extracted with solvents like water by maceration method. The evaluation of antimitotic activity is done by using *Allium cepa* root meristamatic cells parameters where and methotrexate was used as a standard drugs.

In *Allium* assay, aqueous extract of fruits of *Momordica dioica* (15 mg/ml) and methotrexate act against cells of *allium* roots and lesser the growth of root and mitotic index when compared with distilled water as control group. The result indicated that cytotoxic property is due to presence of phenolic, alkaloids and flavonoids compounds in 15 mg/ml concentration of aqueous extract of *Momordica dioica* fruits extract.

On the basis of result, we concluded that, 15 mg/ml concentration of *Momordica dioica* fruits shows good antimitotic activity on the *Allium cepa* root tip assay.

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## 1. Introduction

In today's world, people are suffering from various fatal diseases. Cancer is one of that disease and second leading cause of death in the world. (Cragg and Newman, 2005) Now a days, various new anticancer agents are isolated from the natural sources of drugs. Natural sources of drugs are herbal based drugs consisting about 60-80 % of all other drugs and used as medicine from 1990. (Lee, 2010; Li and Vederas, 2009) The active constituent which is isolated from plants play a vital role in treatment of various diseases and gives special attention such herbal based plants due to its pharmacological activities. (Newman, et al., 2003) There are certain plants which are used in treatment of diseases like cancer. It is found that most of isolated compounds from plants show good anticancer activity (Gonzales and Valerio, 2006; Chopra, 1996). The *Momordica dioica* is recurring, having distinct male female plants consisting of family *curcurbitaceae*, which is commonly known as small bitter gourd or spiny gourd throughout the world where as in India it is called as jangle

Karela, Kantola or Karola. The plant *Momordica dioica* shows various pharmacological or medicinal properties like analgesics, anti-tumorigenic, anti-inflammatory and anti-diabetic activity (The useful plants of India, 1992; The wealth of India 1962; Kartika and Basu 1987).

The fruit of 10 mg/ml concentration of aqueous extract of *Momordica dioica* shows good antimitotic activity. (Patil, et al., 2018) From the dichloromethane extract of *Momordica dioica* roots, five active constituents are isolated which gives good anti-cancer activity on cancerous cell like L1210. At 4 mg/ml it shows good growth inhibitory index about 50 %. (Luo- L, et al., 1998) Based on review of literature that no work has to be done on antimitotic activity at concentration of 15 mg/ml of aqueous extracts of fruits of *Momordica dioica*. Hence in this study aqueous extracts of fruits of *Momordica dioica* in 15 mg/ml concentration was assessed by using *Allium cepa* root meristamatic cells. The 15 mg/ml concentration of aqueous extracts of fruits *Momordica dioica* was taken because to see the effect of fruits at 15 mg/ml concentration.

## 2. Materials and Methods

### 2.1. Plant Material & Reagents

Momordica diocia fruits were agglomerated from Nandurbar (MS) region and authenticated by Professor S. K. Tayade, Botany Department, College of Art's, Commerce and Science, Shahada, Lonkheda-425409, (MS). Chemicals and reagents were acquired from Rajesh Chemicals, Mumbai.

### 2.2. Preparation of Aqueous Extract of Fruits

Collected fruits were washed with distilled water, shade dried and pulverized to a rough form and extraction was done by maceration method using water-chloroform solvent. The filtrate was evaporated by using rotary-evaporator which removed water solvent in vacuum at 40°C. Small amount of water extract of *Momordica diocia* fruit was taken and dissolved in to small amount of distilled water to produce test solution. This test solution was used to study different chemical tests such as alkaloids, flavonoids, tannins, carbohydrates and proteins for detection of different chemical constituents present in the aqueous extract (Harborne, 1988; Kokate, et al., 1999).

### 2.3. Determination of Mitotic Index

(Abhang, et al., 1999; Willams and Omoh, 1996) Red qualities of onions (*Allium cepa*) were purchased from near market area and were stored for further study. The red onion bulbs were place in tap water at room temperature for 48 hours. On the other hand prepared different solutions at concentration of aqueous extract of *Momordica diocia* fruits at of 15 mg/ml, standard drug methotrexate (Zydu Cadila, Ahmedabad) at 0.1 mg/ml and distilled water which served as control respectively. The developed roots then dipped in to solutions of aqueous, methotrexate and distilled water for two hours. Then removed the roots then cut to separate tip part of roots and placed in to fixing solution consisting of acetic acid 45% v/v and ethanol 95% v/v in ratio of 1:3 v/v for near about 10-15 hours. Then roots are reacted with 1N HCL and place in to oven for 15 min at 50°C for warm of roots. Removed it then washed with distilled water and stained with staining agent like carmine. The roots were then placed on the slides and crushed and observed under microscope. Counted the number of cells present in each stages of mitosis and calculated mitotic index which is as **Mitotic Index** = Number of dividing cells × 100

### 2.4. Statistical Analysis

(Kulkarni, 1993) 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.

## 3. Results and Discussion

Table No. 1 showed the presence of phytoconstituents such as alkaloids, flavonoids, tannins, carbohydrates and proteins in aqueous extracts of fruits *Momordica diocia*.

**Table 1:** Preliminary Phytochemical screening of 15 mg/ml aqueous extracts of fruits *Momordica diocia*.

Sr. No.	Phytochemical Test	Test	Inference
1.	Test for carbohydrate	Molish test	+
		Fehling's test	+
2.	Test for proteins	Biuret test	+
3.	Test for flavonoids	Lead acetate test	+
		Shinoda test	+
4.	Test for alkaloids	Dragondroff's test:	+
		Wagner's test:	+
5.	Test for tannins and phenolic compounds	Lead Acetate test	+
		Ferric chloride test	-

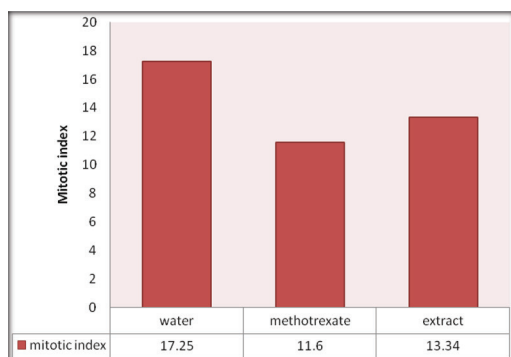
**Table 2:** Antimitotic activity after treatment of *Allium cepa* roots with 15 mg/ml aqueous extracts of fruits *Momordica diocia*, methotrexate and control water.

Sr. No.	Different Solutions used for treatment	Total No. of cells	No. of Dividing cells				No. of Non-dividing cells	Mitotic Index
			P	M	A	T		
1.	Water (Control)	145	15	4	2	4	120	17.25±1.15
2.	Methotrexate (0.1mg/ml Positive Control)	103	6	4	1	1	91	11.65±0.35**
3.	Aqueous Extracts (15mg/ml)	120	10	3	2	1	104	13.34±1.05*

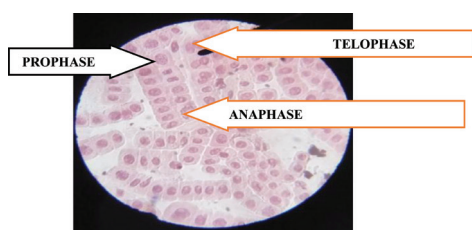
P-Prophase, M-Metaphase, A- Anaphase, T-Telophase

Table No. 2 showed that fruits *Momordica diocia* (15 mg/ml) and methotrexate gives antimitotic in *Allium cepa* root meristematic cells. The total number of non-dividing cells in controlled group (distilled water), standard drug group (methotrexate 0.1 mg/ml) and aqueous extract (15 mg/ml) was found to be 120, 91 and 104 respectively. The total number of dividing cells in different stages of mitosis such as prophase, metaphase, anaphase and telophase in controlled group (distilled water) was found to be 15, 4, 2 and 4, in

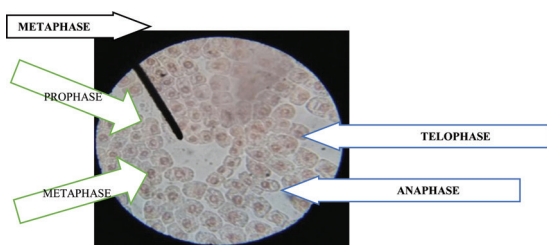
standard group (methotrexate) were found to be 6, 4, 1 and 1 and in 15 mg/ml concentration of aqueous extracts was found to be 10, 3, 2 and 1 respectively. The average mitotic index in water (control), methotrexate (standard) and 15 mg/ml concentration of aqueous extracts of *Momordica diocia* fruits was found to be 17, 11 and 13 respectively.



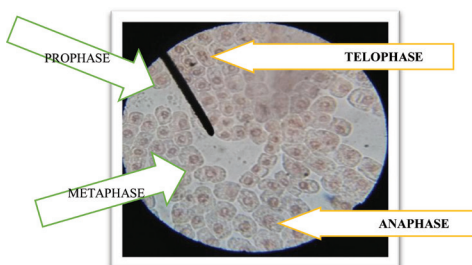
**Figure 1:** Mitotic index of water (control), methotrexate and aqueous extracts of fruits *Momordica diocia*.



**Figure 2:** Different stage of mitosis of *Allium cepa* roots after treatment with water (Control).



**Figure 3:** Different stage of mitosis of *Allium cepa* roots after treatment with standard drug Methotrexate.



**Figure 4:** Different stage of mitosis of *Allium cepa* roots after Treatment with 15 mg/ml concentration of aqueous extract of curry fruits.

The fruits of *Momordica diocia* shows wide spectrum of medicinal activities. The plant contains various phytochemical compounds having diverse chemical structure and nature. The main active constituents found in plants are phenolic, flavonoids and tannins compound. In existing study, *Allium cepa* root meristamatic cell model is used to study antimitotic property of fruits of *Momordica diocia* in 15 mg/ml concentration. (Abhang, et al., 1991; Latha, 1988) In this parameters, division of cells is same as that of division of cancerous cells in human being. Therefore these cells are used to study antimitotic activity in different parts of plants. *Allium cepa* meristamatic cells assay is fast, quick to detect toxicity in cells and toxicity in cells genetic material. The inhibition of root growth and antimitotic property gives indication of destruction of cells genetic material. The assay of destruction of cells of genetic material by *Allium cepa* as a plant is helpful in studying karyo type of plant and can be co-related to connect with antimitotic activity of mammalian cells in toxic evaluation.

The good genotoxic assay performance of *Allium cepa* as a plant system has been attributed to the easily studied karyo type of plant and the ability to correlate outcomes of assays with those of mammalian cells in the course toxic evaluations (Fiskesjo, 1985). In *Allium cepa* assay, aqueous extracts of fruits of *Momordica diocia* (15 mg/ml) was found to inhibit root growth in *Allium cepa* root meristamatic cells and decreased mitotic index after treatment. This suggests that the aqueous extract of fruits of *Momordica diocia* has fair antimitotic potential in concentration of 15 mg/ml. In *Allium cepa* assay, MI (mitotic index) is considered as an indication of biomarkers cells proliferation in metaphase cell cycle. (Bhattacharya and Halder, 2012; Periyanyagam, et al., 2013) Earlier it was proved that fruits of *Momordica diocia* shows good anticancer activity in different cell lines such as PA-I and HeLa cell line by using MTT assay (Ahirrao, 2019).

## Conclusion

Our finding support that 15 mg/ml concentration of aqueous extracts of fruits *Momordica diocia* inhibit cell division in *Allium cepa* meristamatic cells and may helpful in inhibiting abnormal cell growth like cancerous cells. Further experiments are needed, both *in vitro* and *in vivo* to obtain detail mechanisms of action. Positive outcomes from study introduce this plant as a new anticancer drug.

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