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Research Article

Evaluation of Antimitotic Activity of *Mukia maderaspatana* L. Leaf Extract in *Allium cepa* Root Model

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ABSTRACT

Mukia maderaspatana L. (called as "musumusukkai" in Tamil) is an important medicinal plant used as a herbal drug in cough and cold by folklores. The present study was carried out to evaluate the cytotoxic effect of various leaf extract (hexane, chloroform, acetone, ethylacetate, ethanol and aqueous) of *M. Maderaspatana* on meristematic cells of root tips of *Allium cepa.* Locally available *Allium cepa* bulbs were grown in 50ml water for 48 hours for root sprouting and then they were exposed to 10mg/ml concentration of each of the extract separately for 48 hours. Tap water was used as control and the cytotoxic drug methotrexate (1mg/ml) served as standard. The results indicated that the mitotic index and root growth rate of *A.cepa* were considerably decreased in treated in comparison to the control. Also the most effective extract was found to be acetone which reduced the mitotic index significantly. Its EC50 was found to be 10 mg/ml. Further, it was able to induce a high DNA fragmentation followed by leaf ethanol extract in *Allium cepa* root tip cells.
Keywords: *Mukia maderaspatana*, *Allium cepa*, methotrexate, mitotic index, EC50, DNA fragmentation

INTRODUCTION

Cancer is the second leading cause of death worldwide, where one in four deaths is due to cancer (Jemal *et al.,* 2011). Although progress has been made in the early diagnosis of cancer and in improvement of cancer treatment, the ability to provide long term survival has been limited.A number of undesired side effects sometimes occur during chemotherapy that prevent their extensive usage. Also, the high cost of treatment has led to increased emphasis on the use of plant materials as anticancer drug more recently (Cragg *et al.,* 2003).The benefit of many of the phytochemical is that they are "well tolerated", hence could be taken on a long term basis to either prevent tumor formation or tumor recurrence (Pezzuto, 1997).As the hallmark of cancer revolves around cell deregulation, it is not surprising that "antimitotic therapies" are effective against the abnormal proliferation of transformed cells (Chan *et al.,* 2012). Hence, the present study was under taken with the aim to evaluate the efficacy of *Mukia maderaspatana* leaf extract in inhibiting mitosis in onion root tip meristematic cells. It has been reported that Allium test shows good correlation with mammalian test systems (Fiskesjo, 1997).

Mukia maderaspatana.L popularly called as "musumusuki" in Tamil (syn. *Melthoria maderaspatana,Mukia maderaspatana, Cucumis scabella*) belongs to the family cucurbitaceae and it is a tendril climber. It is traditionally used as a leafy vegetable and to cure several aliments in South India.The plant has expectorant properties and is prescribed against chronic diseases with cough as a predominant symptom. (The Wealth Of India,VOL VI,2003). The earlier reports show that it possesses anti-inflammatory, hepatoprotective and anti-diabetic activities (Wani *et al.,* 2011). The tender shoots and bitter leaves are used as gentle aperients and prescribed in vertigo and biliousness. The roots of the plant when masticated relieve toothaches. (Riyazullah *etal*., 2010).Since the antimitotic activity in this plant has not been reported, an attempt has been made in the present study.

MATERIALS AND METHODS

Plant collection

The plant was collected from Captain Srinivasa Moorthy Drug Research Institute and Ayurveda (CSMDRIA), Anna Nagar, shade dried at 37^0C for 10 days. The leaf parts of the dried plant material were made into coarse powder.

Extraction

30g of powdered leaf material were soaked separately in 100 ml of various solvents like water,ethanol,ethyl acetate, acetone,chloroform and hexane for 72 hrs. Each extract was stirred every 24 hrs by using sterile glass rods. At the end, each extract was passed through Whattman No.1 filter paper and the filtrate obtained was concentrated using a rotary evaporator at 50^0 C. Each extract was preserved in vials and kept at 4^oC before use.

Antimitotic activity - *Allium cepa* root tip assay

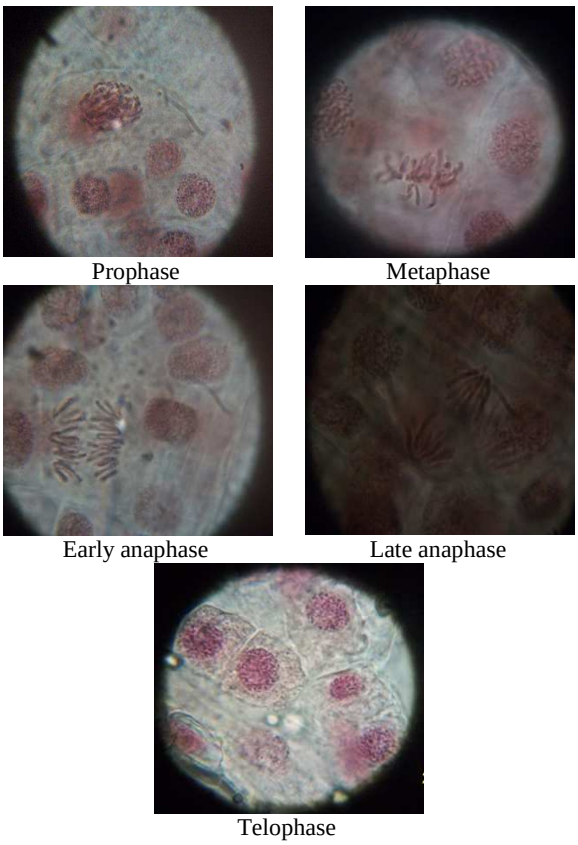
The allium test was performed according to the method described by Fiskesjo (1985) and Grant (1982).Locally available *Allium cepa* bulbs (30±10g) were washed, unscaled and grown over 50 ml tap water filled in plastic cups for 48hrs. The water was changed daily. The bulbs whose roots have grown to approximately 2 to 3cm were selected and treated with 10mg/ml concentration of various leaf extract of *Mukia maderaspatana* for next 48hrs. A control and a standard was also maintained for the same duration where selected bulbs were grown in 50ml plain water and 1mg/ml concentration of methotrexate respectively. At the end of the treated period for each group, the length of the roots and the number of roots were calculated. The roots tips from each group(control, standard and treated) were cut and fixed in ethanol: glacial acetic acid(3:1 v/v). Thereafter these were hydrolyzed in 1N HCL at 50^oC for 15 min after which they were washed with distilled water. The root tips were then squashed on a microscopic slide and stained with acetocarmine for 30 min. Excess stain was removed and cover slips were carefully placed on the smear. In each slide, the number of mitotic and total meristematic cells were counted in 5-8

field using high power (100X) light microscope and mitotic index was calculated. Chromosomal abnormalities were photographed.

Figure 1 *Allium cepa* bulbs showing the effect of various leaf extract of *Mukia maderasapatana* on root length following 48 hours incubation

Determinationof EC50

EC50 is defined as the concentration that produces a 50% decreases in root growth rate.The *Alli*um *cepa* root growth inhibition test was carried out for determining the EC50 value of *Mukia maderaspatana* leaf acetone extract (Rank, 2003). For the first 48hrs, the onion were grown in distilled water after which actively dividing root tip cells of *Allium cepa* were exposed to 0.1mg/ml, 1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml, 5mg/ml, 10mg/ml and 20mg/ml of *Mukia maderaspatana* leaf acetone extract for next 48hrs.Test solution and distilled water were changed every 24hrs.The best developed 10 roots of each onion in each group were measured and mean root length calculated.



Prophase Metaphase

Early anaphase Late anaphase

Telophase

Figure 1 The various stage of mitotic cell division in *Allium cepa* root tips (in control after 48h)

DNA fragmentation

DNA fragmentation is the separation or breaking of DNA strands into pieces. It was carried out by the method of Collins *et al.,* 1997. DNA was isolated from *Allium cepa* root tip cells treated with various leaf extract of *M.maderaspatana* for 48 hrs as well as from standard and control groups and they were subjected to agarose gel electrophoresis.

Statistical analysis

The values are given as mean±S.D and the data was analyzed by Student's t-test.

Table 1 The average root length and number in control and treated root tips after 48h

Groups	Root Number	Root Length(in cm)
Control	22±0.66	3.19±0.22
Standard	12±0.33	2.36±0.29
Treated		
LH	14±0.33	1.43±0.30
LC	11±0.91	1.53±0.10
LEA	13±1.06	1.60±0.09
LA	9±0.5	1.20±0.18
LE	10±0.6	2.00±0.29
LW	19±0.85	2.10±0.19

Mean value of 6 determinations (mean ± S.D)

RESULTS AND DISCUSSION

Microscopic observation revealed normal chromosomal phases of mitotic division in onion root tip cells in control (Figure 2).

Figure1 and Table 1shows that the leaf extracts from *Mukia madaraspatana* have inhibitory effect on root growth and length in *Allium cepa*.

Table 2 Mean mitotic index in *Allium cepa* meristem following 48 h incubation with leaf extract of *Mukia maderaspatana*

Groups	Mitotic index
Control-tap water	7.748±0.08
Standard-methotrexate	1.422±0.9
Treated-LC	2.3±0.6
LH	1.382±0.02
LW	2.632±0.07
LE	2.21±0.75
LA	1.242±0.5
LEA	2.688±0.84

In Table 2, the mitotic indexes are presented for control, standard and treated groups. It is evident from the results that in general the leaf extract under investigation reduces the Mitotic index significantly and in particular, LA (leaf acetone extract) shows good inhibition of meristematic cell in different stages of cell cycle. Its mitotic index is found to be 1.242 which is close to the standard's Mitotic Index i.e. 1.422.

Figure-3 Represents the percentage mitotic inhibition in standard and treated groups in comparison with control. Among the treated groups, the LA extract shows maximum mitotic inhibition (84%) in *Allium cepa* root tip cells followed by LC- 82% and LE-71%. Lowest percentage of mitotic inhibition occurred in LW and LEA treated group (66 % and 65.4 %) respectively.

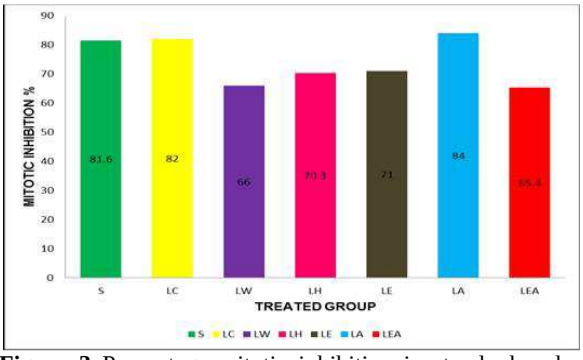


Figure 3 Percentage mitotic inhibition in standard and treated

Table 3 shows root growth inhibition of *Allium cepa* exposed to various concentration of *Mukia madaraspatana* leaf acetone extract. The EC50 value of leaf acetone extract was found to be 10 mg /ml concentration.

Table 3 Effect of M*ukia maderaspatana* leaf acetone extract on *Allium Cepa* L root growth

Applied concentration (mg/ml)	Mean root length (cm) ± S.D	Root length compared to control (%)	Decreased compared to control (%)
0.1	3.56±0.11	NS	-
1	3.1±0.17	NS	2.83
2	2.92±0.21	91.53	8.47
3	2.54±0.15	79.62	20.38
4	2.19±0.4	68.52	31.48
5	2.0±0.09	62.69	37.31
10	1.43±0.07	49.827	50.173
20	1±0.05	31.34	68.66

Mean value of 6 determination (mean ± S.D) P <0.05

Table-4 gives the percentage mitotic index and mitotic inhibition of *Allium Cepa* root tip cells following 48 h incubation with various concentration of *Mukia madaraspatana* leaf acetone extract. The % mitotic inhibition was found to be in a dose dependent manner.

Group/Applied Concentration(mg/ml)	**Mitotic Index %**	**% Inhibition**
Control	6.8±0.45	0.00
0.1	3.4±0.60	50
1	2.7±0.51	60
2	2.25±0.76	66
3	1.9±0.86	72
4	1.7±0.28	75
5	1.4±0.03	79
10	1.1±0.02	83
20	0.8±0.001	88

Mean value of 6 determination (mean ± S.D) P <0.05

Mitotic index is an acceptable measure of cytotoxicity for all living organism (Singh Shachi, 2012). The cytotoxicity level can be determined by the decreased rate of mitotic index. In our present study leaf acetone extract shows good inhibition of meristematic cell in different stages of cell cycle. Based on root growth inhibition studies,the EC50 value of our LA extract was found to be 10mg/ml concentration.

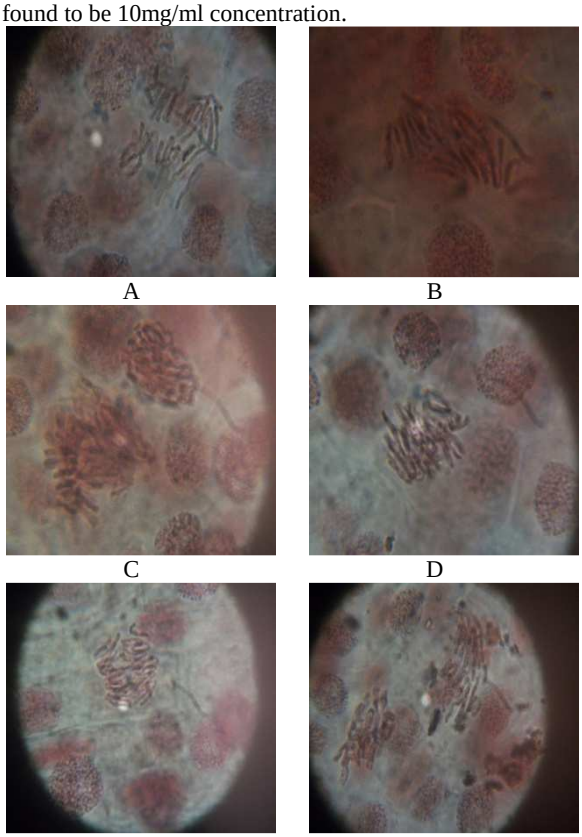


Figure 4 Chromosomal abnormalities induced in root tip cells of *Allium cepa* by leaf extract of *Mukia maderaspatana* following 48h incubation. A.Chromosomal bridge B. C-Mitosis C & D. Clumping of chromosome in the centre E. Pseudoanaphase F. Stickness between chromosomes

Reduction in mitotic activity could be due to inhibition of DNA synthesis or blocking in the G2 phase of the cell cycle preventing the cell from entering mitosis or due to disruption of microtubules (Kuras *et al.*, 2006). Changes in chromosomes were observed in treated groups like formation of chromosomal bridge, chromosomal fragmentation, clumping of chromosome in center, pseudoanaphase and stickness between chromosomes.In our earlier investigation with this plant, acetone and ethanol extract of leaves of *Mukia maderaspatana* had been found to possess phytochemical like phenol and tannins.Tannins and phenols which together constitute the polyphenolic group are known to have antioxidant, anticancer and antimicrobial activities (Nguji, 1988). This suggests that the antimitotic activity of LA extract of *Mukia maderaspatana* may be due to the presence of polyphenols in this plant.

A distinctive feature of apoptosis at the biochemical level is DNA fragmentation (Collins *et al.*, 1997).In treated group, DNA electrophoresis gave a “smear pattern” whereas control had normal DNA. The smearing is caused by the cleavage ofDNA into low molecular weight fragments that travel according to size on an agarose gel. Among the treated group, leaf acetone extract was able to induce a high DNA fragmentation followed by leaf ethanol extract in *Allium cepa* root tip cells.

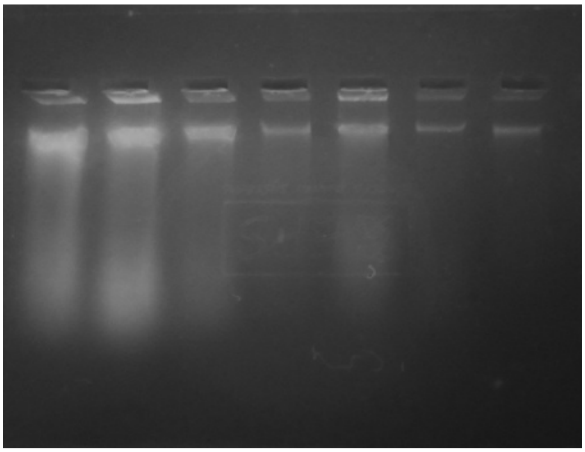


Figure 5 DNA Fragmentation Lane 1: DNA from sample LA, Lane 2: DNA from sample LE, Lane 3: DNA from sample S, Lane 4: DNA from sample LW, Lane 5: DNA from sample LH, Lane 6: DNA from sample LC, Lane 7: DNA from sample C

CONCLUSION

In respect of the above results we conclude that *Mukia madaraspatana* leaves contains antimitotic constituents that can stop mitosis in anywhere of the cell cycle. Further experiments are needed both *in vitro* and *in vivo* to obtain more detailed mechanism of action of the plant material in view of its antimitotic activity.

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