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STUDY OF ANTIMITOTIC ACTIVITY OF MURRAYA KOENIGII BY USING *ALLIUM* CEPA ROOT TIP ASSAY

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ABSTRACT

Objective: Cancer is a dreadful disease and combating this disease is of great importance to public health. Phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of plausible anticancer compounds. In the present study we have utilized the Allium cepa root tip meristem model to evaluate antimitotic activities of leaves *Murraya koenigii* commonly known as Curry leaves belongs to family rutaceae. **Methods:** Preliminary antimitotic screening was done using Allium cepa root tip assay. The herbal powder obtained from plant part-dry leaves of *Murraya koenigii* were extracted with aqueous solvents by maceration methods. The antimitotic activity was analysed using Allium cepa root and Methotrexate was used as a standard drugs. **Result and Discussion:** In Allium assay, aqueous extract of leaves of Murraya koenigii (10 mg/ml) and Methotrexate shows showed significant concentration dependant inhibitory influence against the dividing cells of Allium roots and decreased root growth and mitotic index as compared to control distilled water. The results indicated the cytotoxic potential of extract due to antimitotic effects. The effect may be attributed to the presence of flavonoids, alkaloids and phenolic compounds in Curry leaves extract. **Conclusion:** The present work revealed that leaves of *Murraya koenigii* contains some important chemical constituents extracted using aqueous as solvent that can be used further in the management of cancer treatment.

KEYWORDS: *Allium cepa*, *Murraya koenigii*, rutaceae, maceration, flavonoids, alkaloids and phenolic compounds.

1. INTRODUCTION

Curry leaf (Murraya koenigii), belonging to the Rutaceae family, is native to India, Sri Lanka, Bangladesh and other South Asian countries. It is currently used a cooking spice and has been commonly used in traditional Indian medicine for thousands of years.[1] Studies report curry leaves to have hypoglycemic, antidiabetic[2,3], hepatoprotective[4], antibacterial[5], anti-inflammatory[6] and antioxidative properties.[7] The bioactivity of curry leaf has been attributed to the presence of phytochemicals including alkaloids, essential oils, phenolic acids[8], terepenoids, tocopherol, β-carotene, lutein[9], as well as minerals, proteins and fats.[8] Furthermore, Mani et al. suggest the alkaloids of curry leaf as a useful remedy for dementia and Alzheimer's disease.[10]

2. MATERIALS AND METHODS

2.1 Plant material & reagents

Curry leaf (Murraya koenigii) 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 (MS). All the reagents and chemicals were purchased from Rajesh Chemicals, Mumbai.

2.2 Preparation of aqueous extract of leaves

Collected leaves 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)**.** The aqueous extract was subjected to preliminary phytochemical testing for the presence of different chemical classes of compounds.[11,12]

2.3 Determination of Mitotic Index[13,14]

The red variety of Allium cepa (onions) were procured from the local market and stored for the entire study. The bulbs of Allium cepa were sprouted in tap water for 48 hours at room temperature. The roots thus developed were treated by dipping these in the aqueous solutions of leaves *Murraya koenigii* (10mg/ml) for 2 hours. Treatment of the roots with the distilled water and Methotrexate (GSK, India 2.5 mg) at concentration 0.1mg/ml served as negative and positive control

respectively. The roots thus treated with the above solutions were then cut to separate root tips and the root tips were transferred to the fixing solution {acetic acid (45% v/v) ethanol (95% v/v) in ratio of 1:3 v/v} for 10-15 hrs. After 10-15 hours, these were treated with 1 N hydrochloric acid and warmed in an oven at 50°C for 15 min. These root tips were then washed with distilled water and were stained with 2-3 drops of Carmine stain (LR Central Drug House Pvt Ltd). The slide was then squashed and observed under microscope. The numbers of cells in each stage of cell division were counted in four fields for each group.

Mitotic Index was calculated using the formula

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

3. RESULTS

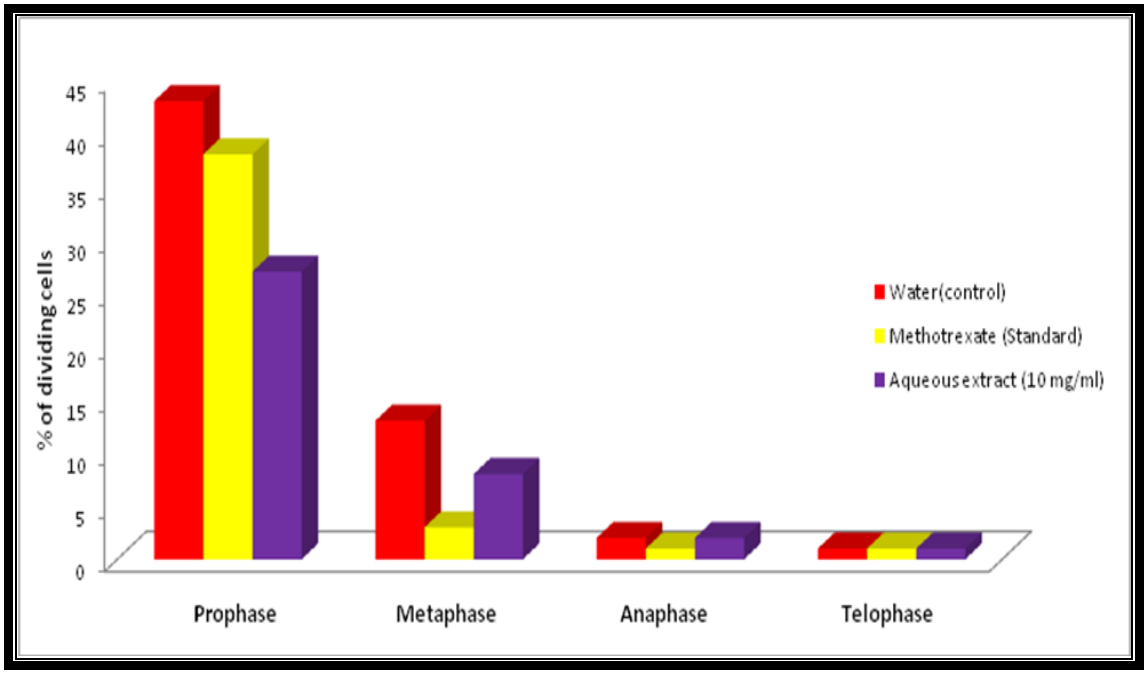
Table No.1 showed the Antimitotic activity after treatment of Allium cepa roots with aqueous extracts of Curry leaves (Murraya koenigii) and Methotrexate. The percentage of non dividing cells in water control, aqueous extract (10 mg/ml) and standard group (Methotrexate) was found to be 41%, 57%, and 62% respectively.

In water (Control), t he percentage of dividing cells in prophase, metaphase, anaphase and telophase was found to be 43, 13, 2 and 1 respectively. In Methotrexate (standard) drugs, the percentage of dividing cells in prophase, metaphase, anaphase and telophase was found to be 38, 3, 1 and 1 respectively. In 10 mg concentration of aqueous extracts, the percentage of dividing cells in prophase, metaphase, anaphase and telophase was found to be 27, 8, 2 and 1 respectively. The average mitotic index in water (Control), Methotrexate (standard) and 10 mg concentration of aqueous extracts of *Murraya koenigii* was found to be 58.6, 43.2 and 37.6 respectively.

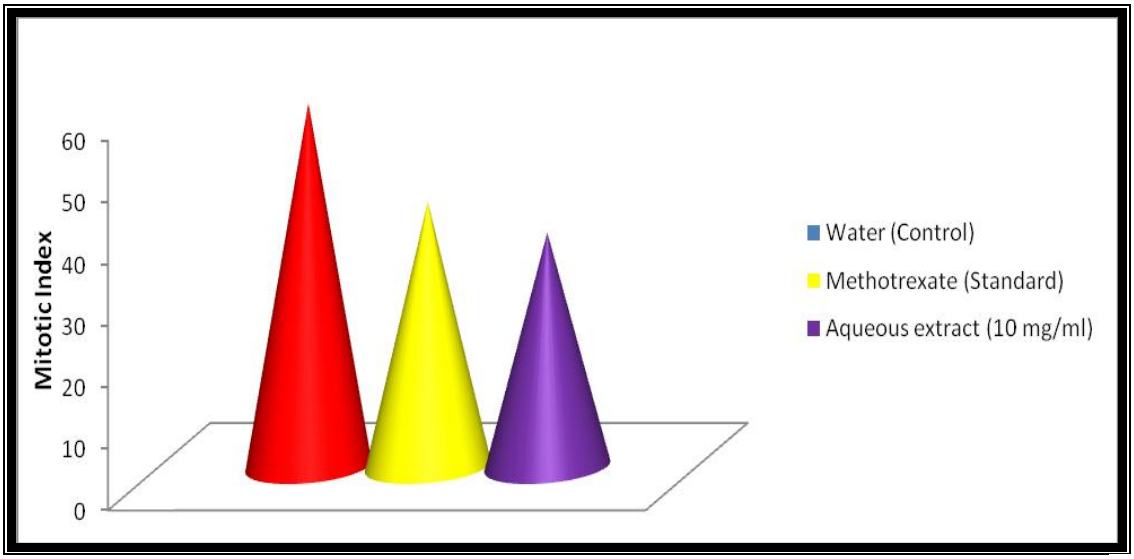
Table 1: Antimitotic activity after treatment of *Allium cepa roots* with aqueous extracts *of Curry leaves (Murraya koenigii)* and Methotrexate.

Sr. No.	Different solutions used for treatment	% of Non dividing Cells	% of dividing cells				Mitotic Index	Mitotic Index		
			P	M	A	T		Avg.	SD	SEM
1	Water(control)	41	43	13	2	1	59	58.6	1.221	0.804
2	Methotrexate (0.1 mg/ml positive control)	57	38	3	1	1	43	43.2	1.000	0.4472
3	Aqueous extract (10 mg/ml)	62	27	8	2	1	38	37.6	1.782	0.043

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



Graph No. 1% of dividing cells in after treatment of *A .cepa roots* with water (control), Methotrexate and aqueous extracts *of Curry leaves (Murraya koenigii).*



Graph No. 2: Mitotic index of water (control), Methotrexate and aqueous extracts *of Curry leaves (Murraya koenigii).*

4. DISCUSSION

The leaves of *Murraya koenigii* shows wide spectrum of medicinal activities. The plant contains various phytochemical compounds having diverse chemical structure and nature. The major constituents of the plant are flavanoids, alkaloids, saponins, sterols, tannins and phenolic compounds.

In the present study, attempts were made to investigate antimitotic activity of *Murraya koingii* by using Allium cepa roots tip assay. In *Allium cepa* root meristem model, commonly known as *Allium* assay, root meristematic cells are used for screening of drugs with anti-mitotic activity.[15,16] In meristematic region, the cell division is similar to cancer cell division in humans. Therefore, these meristematic cells can be evaluated for screening of drugs with potential anti-mitotic activity. Allium assay is considered a rapid, highly sensitive and reproducible bioassay for detecting cytotoxicity and genotoxicity. The root growth inhibition and antimitotic effects provide the indication of genotoxicity. The good genotoxic assay performance of *Allium cepa* as a plant system has been attributed to the easily studied karyo type of plant [2n = 16] and the ability to correlate outcomes of assays with those of mammalian cells in the course toxic evaluations.[17] The *Allium cepa* species [common onion] being characterized by homogenous meristematic cells and very large sixteen numbers chromosomes is ideal for use in bioassays.[18]

In *Allium cepa* assay, aqueous extracts of *Murraya koingii* (10 mg/ml) was found to exhibit dose dependent antimitotic action on *A. cepa* root meristematic cells as indicated by inhibition of root growth and decreased mitotic index after treatment. This suggests that the aqueous extract of *Murraya koingii* has fair antimitotic potential in concentration 10 mg/ml). In *Allium cepa* assay, mitotic index is considered as an indicator of cell proliferation biomarkers which measure the proportion of cells in the M-phase of the cell cycle.[19,20] From result, it was conclude that 10 mg/ml concentration of aqueous extracts of *Murraya koingii* inhibits cell division in *Allium cepa* assays and suggests that the leaves may exhibit inhibitory influence on abnormal cell growth as like in cancer. Though the present study validates the traditional use of extract in the treatment of cancer, further studies in cancer cell lines is necessary.

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