

EVALUATION OF ANTIMITOTIC EFFECT OF *C.CORIARIA* ON *ALLIUM CEPA* ROOTS

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Abstract

In the present study, an attempt has been made to evaluate the antimitotic activity of *C.coritaria*. The preliminary antimitotic screening was done using *Allium cepa* root tip assay. The mitotic index of the root tips markedly decrease with aqueous extract than the other fraction. The result obtained were compared with methotrexate a known drug available in the market as anti-cancer agent. The results obtained in the present study "Antimitotic effect of *C.coritaria* had excellent antimitotic activity that was comparable to the activity of methotrexate. The aqueous extracts of *C.coritaria* seem to prevent prophase stage in cell division where the DNA duplication occurs, Methotrexate was known as anticancer drug that inhibits DNA synthesis.

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Introduction

Cancer is essentially a problem of abnormal cell growth. Under the influence of chemicals, viruses and free radicals, normal cells are converted to tumor masses that divide in an uncontrolled manner. The cytotoxicity effect considering the ability of these natural polyphenols is shown to be mediated through apoptosis. Considering the ability of these natural polyphenols especially the tannin to absorb proteins and metal ions, these is possibility that they can elicit apoptosis signals through various receptors or proteins (Taraphdar, 2001). Apart from this, they are excellent antioxidants and they prevent the free radical attack on DNA by acting as scavengers of these free radicals (Ferguson, 2001).

Nevertheless natural polyphenols are mainly known for their antitumor, antimitotic, antiviral, anti-inflammatory activity and antioxidant properties. The involvement of free radicals in the pathology of human disease, atherosclerosis, cardiac disease, diabetes, cancer and ageing justified the use of natural antioxidants. Recent studies on tumor inhibitory compounds of plant

origin have yielded an impressive assay of novel structure. Besides epidemiological studies suggest that consumption of dyers containing fruits, vegetables, major sources of phytochemicals and micronutrients may reduce the risk of developing cancer (Renchean, 2001). Certain products from plants are known to induce apoptosis (Henderson, 1984).

The antimitotic activity was screened using *Allium cepa* root meristamatic cells which have been used extensively in screening of drugs with antimitotic activity. Cells of this region undergo repeated division. This region is called meristamatic region. This division is similar to the above mentioned cancer division in human. Hence these meristamatic cells can be used for preliminary screening of drugs with anticancer activity (Patil, 2004). The plant extracts of *C.corriaria* was carried out for the antimitotic activity in *Allium cepa* root tips. As this posses the antimitotic activity it was also carried in human cell lines.

Studies have shown that cytotoxic effect of polyphenol tannin against different tumour is mediated through apoptosis (Kauff, 2002) Polyphenol tannin induces cell death in various transformed cell lines such as PLC/PRF/5 (human hepatoma), HL-60 RG (Human promyelocytic leukemia), P-338 (mouse lymphoid neoplasma), and HeLa (human epithelian carcinoma). This plant polyphenolic which is a well known natural antioxidant induced DNA frangmentation of four different human myelogenous leukaemic cell clines (Spon, 1985). The aqueous extract of *C.corriaria* was used to evaluate the cytotoxicity effect on breast cancer cell line (MCF-7).

Caesalpinia coriaria (Jacq) Willd is small tree, stem without prickles, leaves with 6-8 pairs of pinnae. Flowers are small, light yellow or green, sweet scented in short dense particles. Pods are thick twisted not covered with pricks. Pods are astringent. The powder of pods is astringent, antiperiodic and tonic. A decoction is used for washing, bleeding piles. The bark is used in chronic fever. They carry very good antioxidant activity (Bate, 1954). Pods rich in tannin which are employed in medicine as astringents both in gastrointestinal tract and on skin abrasion. Recently interesting antiviral and anticancer properties have been contributed to certain tannins (Frank, 2002).

Material and Methods

The plant *C.corriaria* were collected from Captain Srinivasa Drug Research Institute, Arumbakkam Chennai, Tamil Nadu. *Allium cepa* bulbs were purchased from local market and stored for the entire study. Carmine stain was purchased from Qualigens. Other solvents used for extraction were of LR grade.

Preparation of plant extract

The pods of plant were air dried in the laboratory at room temperature. It was powdered and was extracted. 5 grams of powder was weighed and boiled in 100 ml water for 30 minutes to get the aqueous extract. 5 grams of powder was boiled with aqueous methanol (1:1) ratio 100 ml for 30 minutes at 60 ° C to get the methanol extract. 5 grams of powdered pod was weighed and mixed in aqueous methanol (4:1) and boiled at 60 ° C. Filter the solution , the filtrate was acidified with 2M sulphuric acid and then add chloroform thrice boiled at 60 ° C and then filter the extract. Extracts was concentrated and evaporated to dry extract. Extracts of desired concentration were prepared for further study by using these dried extracts.

Antimitotic activity

The antimitotic activity was evaluated using *A. cepa* root meristamatic cells. *A. cepa* bulbs were sprouted in tap water for 48 hours at room temperature. The bulb that developed uniform root was used for the experiment. These roots were used for the experiment. These roots were treated with above prepared extracts of 10 mg/ml concentration. The different fractions were mentioned in table 1. A blank with water was used as control. Methotrexate was used as a standard control. After 3 hour of treatment, the root tips were fixed in fixing solution of acetic acid and alcohol. Same preparation was made by staining in acetocarmine stain.

The mitotic index was calculated as

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

Results and Discussion

Effect of *C. coriaria* extracts on antimitotic activity

Antimitotic activity activity of *C. coriaria* was comparable to the activity of Methotrexate, which was listed in the below tables.

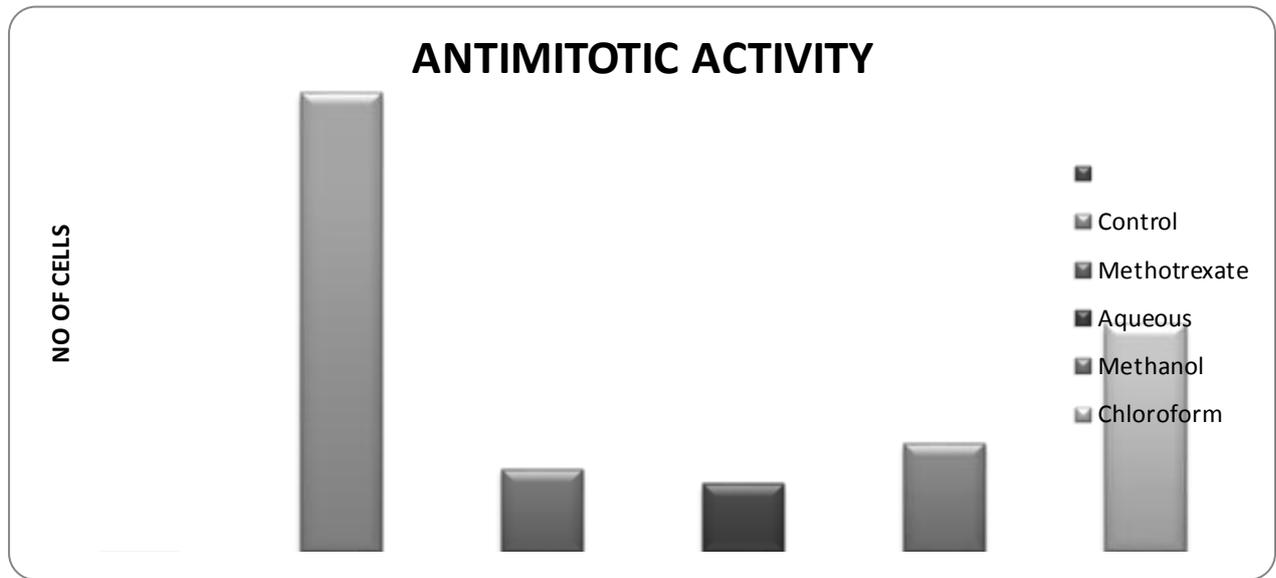
Table I – Antimitotic activity after treatment of *A. cepa* roots with extracts of *C. coriaria*

Extract	Conc./ml	6 ^o clock				10 ^o clock				2 ^o clock				Mitotic Index
		P	M	A	T	P	M	A	T	P	M	A	T	
Water	-	61	4	3	15	65	4	4	17	66	5	4	19	89
Methotrexate	0.10	18	1	-	1	14	1	-	-	12	1	-	-	16
Aqueous	10	14	1	-	1	12	1	-	1	10	-	-	-	13
Methanol	10	19	3	-	3	17	2	1	2	14	1	1	-	21
Chloroform	10	35	2	2	12	34	2	1	6	33	1	1	2	44

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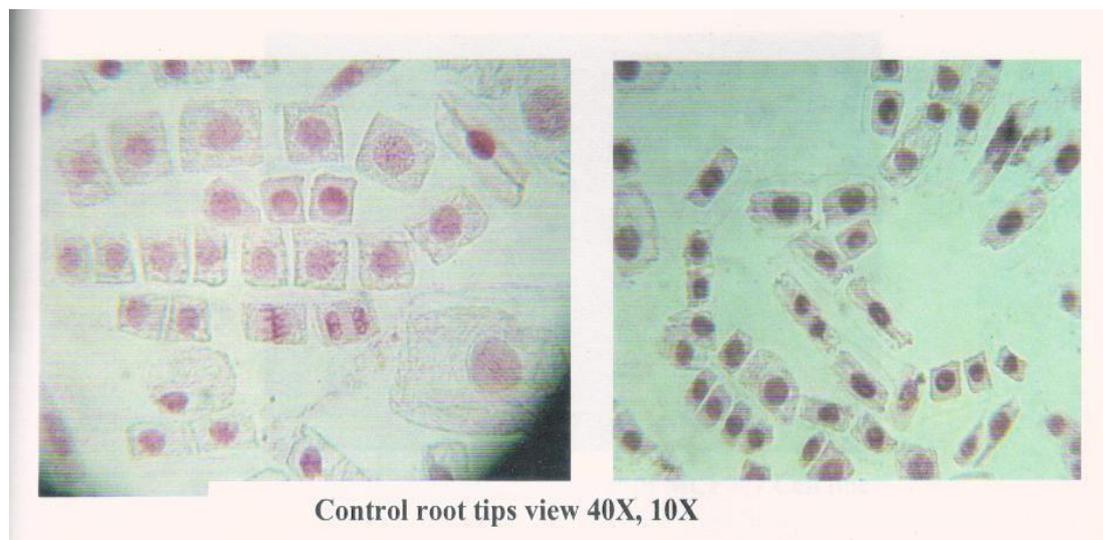
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Graph I – Antimitotic activity of *C.coriaria*

Table II – Antimitotic activity after treatment of *A.cepa* roots with aqueous, methanol, chloroform extract of *C.coriaria*.

Treatment (mg/ml)	Non-dividing cells	% of dividing cells			
Water	12	64	4	3	17
Methotrexate	83	15	1	0	1
Aqueous	86	12	1	0	1
Methanol	78	17	2	1	2
Chloroform	56	34	2	1	7



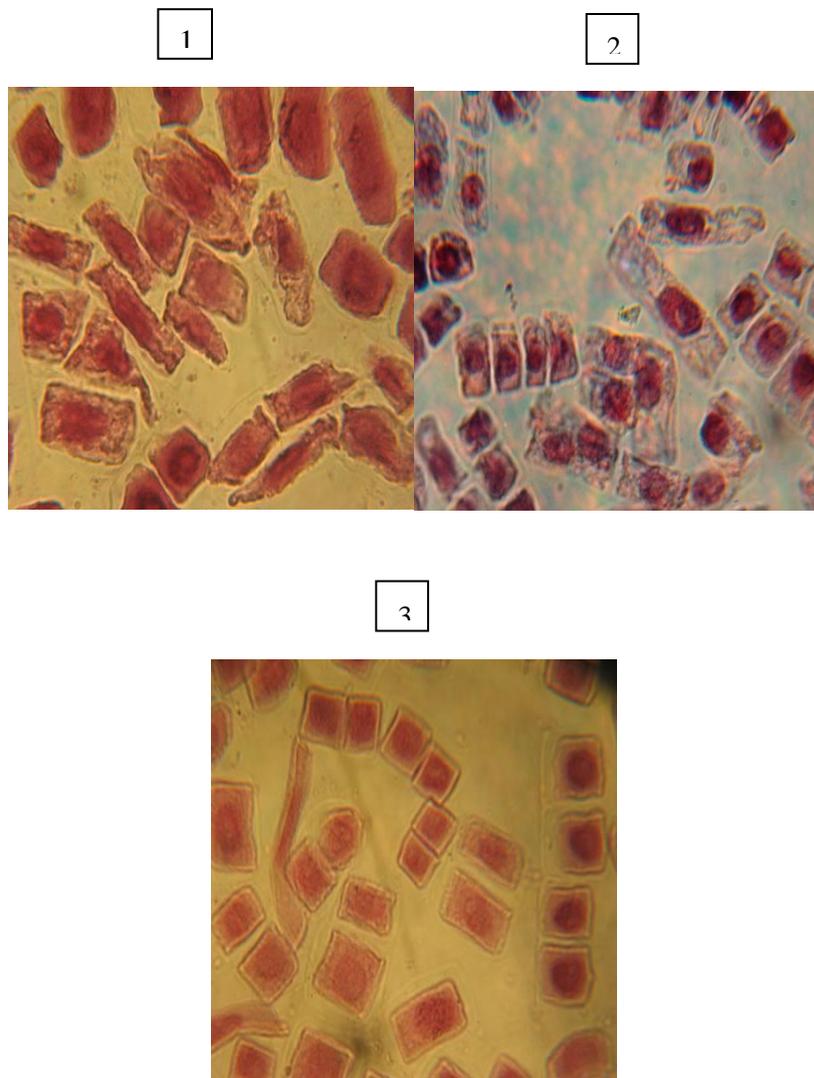


Figure 1 - 1. Aqueous extract treated roots tips view 40 x, 2. Methanol extract treated roots tips view 10 x, 3. Chloroform. Extract treated roots tips view 40 x

Effect of extracts of *C. coriaria* on mitotic activity

Antimitotic activity of the various extract was comparable to the activity of methotrexate (Table 1) the activity of aqueous extract was found to be higher than the remaining extracts. The aqueous extract showed lowest mitotic index i.e. highest activity (Table 1) amongst all the different fractionated extracts.

The inhibition produced by the *C. coriaria* was not significantly different to that of methotrexate. The cell division were differentiated and the number of cells in each phase of the cell division i.e. either prophase, metaphase, metaphase, anaphase or telophase were recorded and photographed (Figure 1). Thus the number of cells entering prophase decreased with various

extracts mainly aqueous extract. Since the cell do not enter prophase, further stages of cell division also decrease in aqueous extract. This assay may be used as an easy and inexpensive method to evaluate the antimitotic potential of agents that could be useful for treatment of cancer.

Summary and Conclusion:

The results obtained in the present study “*In vivo* antimitotic effects of *A.cepa* roots was screened which have been extensively in screening of the plant extracts with antimitotic activity (Abang, 1991).

The results from our study showed that the aqueous, methanol, chloroform extracts of *C.coritaria* had excellent antimitotic activity that was comparable to the activity of methotrexate. Biochemical analysis shows the above extracts contain tannin, which belongs to polyphenol group (condensed tannin). Hence the tannin from *C.coritaria* must be contributing to the antimitotic potential of the plant.

The aqueous extract of *C.coritaria* seems to prevent prophase stage in cell division where the DNA duplication occurs. Methotrexate was known as anticancer drug which inhibits DNA synthesis. The extract (Tannin) was binding with different cell proteins responsible for cell division. The extracts showed good antimitotic potential. Another possible mechanism of action reported for cytotoxicity effects was inhibition of DNA synthesis and thus the prevention was inhibition of DNA synthesis and thus prevention of cell division (Patil, 2004). Since tannin belongs to polyphenols this plant *C.coritaria* has very good content of tannin so tannin is probably the one of the compound in this plant, which exhibits the antimitotic effect.

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