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Effect of acetone fraction of leaf extract of *Simarouba glauca* DC. on mitotic cells of *Allium cepa* L.

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Abstract
The present study is an attempt to evaluate the effect of acetone fraction of *Simarauba glauca* DC. leaf extract on mitotic cells of *Allium cepa* L. The extract of *S. glauca* leaves decreased mitotic index of *A. cepa* L. cells at all the test concentrations. It was observed that extract induces mitotic aberrations, including stickiness, disorientation and tropokinesis. Tropokinesis at anaphase and telophase were frequent. The aberrations confirmed the presence of an active principle in the fraction and having significant influence on spindle apparatus. Thus there is a strong support in the light of the study towards the use of low concentration of this plant extract may reduce any ailment that involves uncontrolled cell division.

Keywords: Acetone fraction, *Simarauba glauca* DC., mitotic cells, *Allium cepa* L.

Introduction
Simarouba glauca DC. is gaining attention recently, due to its application in the treatment of various ailments including cancer. The effective application of the active principle of this plant as antimicrobial, antifungal, anti-inflammatory etc. were reported. The quantitative phytochemistry of the leaf, bark and seed extract of *S. glauca* DC. was studied by Bangar *et al*. [1]. Its utilisation in herbal medicine was reported by Manasi & Gaikwad [2]. Biological activities of members of Simaroubaceae were well described by Houel *et al*. [3]. Iasmine *et al*. [4] also reported that many species of the family Simaroubaceae, display prominent antitumor activity especially in the case of genera *Ailanthus*, *Brucea*, *Simarouba*, *Quassia*, *Picrolemma*, *Simaba* and *Picrasma*. The major metabolites related to the antitumoral activity of several species include quassinoids and alkaloids [5]. Santhana *et al*. [6]. reported the antibacterial, antioxidant, haemolytic and thrombolytic effect of the leaves of *Simarouba glauca*. Mikawlrawng *et al*. [7]. reported that the crude methanolic and ethanolic extracts of both fresh and dried leaves extracts of *Simarouba glauca* have strong inhibitory effect against *Aspergillus parasiticus* and *Fusarium oxysporum*. Umesh *et al*. [8]. investigated the total phenolic, flavonoids, and tannin contents of *S. glauca* leaves and evaluate the antioxidant potential and cytotoxic activity in different human cell lines. These findings suggests that *S. glauca* leaf extract contain bioactive molecules which exhibit antioxidant activity that could be synergisticaly influencing the cytotoxic activity in selected cancer cell lines. The present study is aimed to find out the effect of acetone fraction of leaf extract of *S. glauca* on mitotic cells of *A. cepa.*

Materials and methods
Healthy and disease free fresh leaves of *S.glauca* DC. were collected, properly shade dried and powdered. 1g of the powder was extracted using acetone. The extract is dried and diluted with water to obtain different concentrations in sequential gradation like 0.02, 0.04 and 0.06 per cent. Healthy and equal sized bulbs of *Allium cepa* L. was allow to germinate. A series of bulbs were placed on cavity blocks containing test solvent at room temperature along with the control for 24 hours. The root tips were squashed acetocarmine for preparing microscopic slides and these were observed under microscope for quantitative estimation of dividing cells and chromosomal abnormalities. Mitotic index, phase indices and total abnormality percentages were calculated. The mitotic index and phase index were calculated as the number of dividing cells per 1000 observed cells. The number of aberrant cells was noted per total cells of that division scored at each concentration.

Results and Discussion

Effect of *S. glauca* leaf extract on acetone at different concentrations on mitotic index, phase index and percentage of abnormalities on root meristem cells of *Allium cepa* was analysed during the study (Table-1). It was observed that mitotic index of the control was 8.35. In the case of treated population the mitotic index was found to be reduced than control. After treatment with 0.02, 0.04 and 0.06 per cent of acetone fraction of leaf extract, the mitotic index was found to be 4.79, 3.12 and 2.46 respectively. Smaka-Kinel *et al*. [9] reported that mitotic index is an acceptable measure of cytotoxicity for all living organisms. Similarly Panda & Sahu [10] also reported that the cytotoxicity level can be determined by the decreased rate of mitotic index. In the present study 0.04 percent of test concentration reduced the mitotic index more than 50 percent when compared to the control, A decrease of mitotic index below 50% usually has lethal effects. Antonsie-Wiez, [11] reported that if mitotic index decreases below 22% of control, that it causes sub lethal effects on test organism. In the present study mitotic index was found to be reduced 70.18 per cent in the highest concentration tried (0.06 percent). The present study also analysed that the metaphase and anaphase index was found to be highly affected due to the treatment with acetone fraction of the extract.

It was also found that along with reduction in mitotic index the acetone fraction of *S. glauca* leaf extract induces a few mitotic aberrations. The increase of mitotic abnormalities was in proportion with increasing concentrations of acetone. The cytological abnormalities such as stickiness, disorientation, tropokinesis etc. are frequently met within dividing cell population. Stickiness of chromosome found to be a notable anomaly in the present study. Stickiness was associated in the prophase stage of mitosis (Fig.1). Unoriented metaphase chromosome was also observed among dividing cells (Fig.2, 3 & Fig.4) after the treatment with 0.04% of acetone fraction of leaf extract of *S. glauca*. Tropokinesis was also met among the treated dividing population (Fig-5 and Fig-6). Here aberrations indicated that the active fraction of *S. glauca* possess a profound effect on spindle. Earlier studies attributed several reasons for the occurrence of stickiness of chromosome. Stephen [12] mentioned that stickiness was a type of physical adhesion that involves mainly the proteinaceous matrix of the chromatin material. Mercykutty & Stephen [13] also reported that this stickiness might be interpreted as a result of depolymerisation of DNA, partial dissolution of nucleoproteins, breakage and exchanges of the basic folded fibre units of chromatids and stripping of the protein covering DNA in chromosomes. Gaulden [14] postulated that sticky chromosomes result from the defective functioning of one or two types of specific non-histone proteins, which are involving chromosome organization and it is needed for chromatid separation and segregation. Ostergren [15] and Fiskesjo [16] was reported, sticky chromosomes indicated the presence of a highly toxic substance, inducing irreversible effects in the physical state of the chromatin. Khanna *et al.* [17] reported that stickiness of chromosomes has resulted from increased chromosomal contraction and condensation or might from the depolymerisation of DNA and partial dissolution of nucleoproteins. Chromosome stickiness reflects toxic effects, usually of an irreversible type and probably leading to cell death. Stickiness and clumping may be caused by genetic and environmental factors. Oyenike [18] reported that sticky chromosomes are indicative of a highly toxic, usually irreversible effect, probably leading to cell death. The result in general indicated that the extract of the *S. glauca* had a profound effect on the mitotic cells. It reduces the mitotic index and induces aberrations. The aberrations observed in the present study thrown light to the influence of the extract of *S. glauca* on the normal spindle activity and it is associated with 0.04 percent concentration onwards. Thus it was concluded that the lower concentration of the acetone fraction of the leaf extract of *S.glauca* reduced mitotic index but higher concentration induces chromosomal aberrations also.

Table 1: Mitotic index, phase index and percentage of abnormal cells of *Allium cepa* root tip meristem treated in acetone extract of leaves of *S. glauca* along with control.

Concentrations (%)	Mitotic index (%)		Phase index				Percentage of abnormal cells at various phases of mitosis			
	Range	Mean ± SD	P	M	A	T	P	M	A	T
0	3.69±10.29	8.35±1.87	32.16	24.57	21.94	20.93	0.7	0.67	0.17	0.25
0.02	1.10±7.59	4.79±3.43	43.39	30.28	8.30	23.03	8.13	47.92	41.67	11.11
0.04	1.02±5.67	3.12±1.38	57.46	6.42	12.61	23.52	8.89	50	62.5	33.33
0.06	1.19±4.04	2.49±0.87	52	23.69	11.01	13.30	40	66.67	75	44.44

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

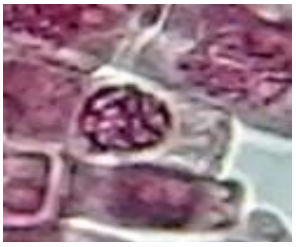

Fig.1.

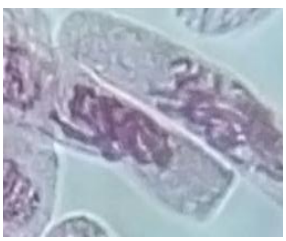

Fig.2.

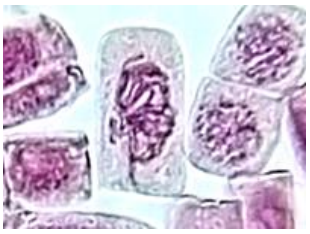

Fig.3.

Fig.4.

Fig.5:

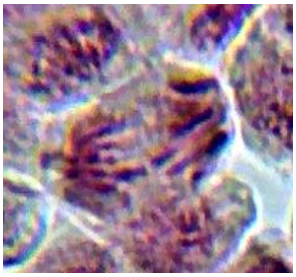

Fig.6.

Fg.1. Stickiness of chromosome at prophase stage of *A. cepa* root tip cell after treatment with 0.04 per cent concentration of acetone fraction of leaf extract of *S. glauca*

Fig.2, 3 & 4. Stickiness of chromosome at metaphase stage of *A. cepa* root tip cell after treatment with 0.04 concentration of acetone fraction of leaf extract of *S. glauca*

Fig. 5 & 6. Tropokinesis of chromosome at anaphase stage of *A. cepa* root tip cell after treatment with 0.06 concentration of acetone fraction of leaf extract of *S. Glauca*

Conclusion

The present study was observed that the acetone fraction of *Simarauba glauca* DC. leaf extract on mitotic cells of *Allium cepa* L. The extract of *S. glauca* leaves decreased mitotic index of *A. cepa* L. cells at all the test concentrations. It was observed that extract induces mitotic aberrations, including stickiness, disorientation and tropokinesis. More over in the case of treated population the mitotic index was found to be reduced than control. The cytological abnormalities such as stickiness, disorientation, tropokinesis etc. are frequently met within dividing cell population. Stickiness of chromosome found to be a notable anomaly in the present study. The present study highlights the value of herbal extracts for curing various ailments, in addition to that, It also gives some clues to reveal hidden phytochemical constituents or active principles of these herbs with respect different diseases.

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