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Anti-mitotic activity of aqueous leaf extracts of *Azadirachta indica* A. Juss. and *Simarouba glauca* DC. On *Allium cepa* L. root tips

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Abstract
Antimitotic activity is referred as the arrest of cell multiplication in mitosis. The present study deals with the evaluation of antimitotic activity of leaf extracts of *Azadirachta indica* A. Juss. and *Simarouba glauca* DC. using *Allium cepa* root tip cells. 50% and 25% of leaf extracts of both the plants were prepared and onion root tips were exposed to each concentration separately. The root tips were then squashed on microscope slides and mitotic indexes were calculated. The results indicated that the average mitotic index of *Allium cepa* were considerably decreased with higher concentrations of both plant extracts in comparison with the control. The chromosomal aberrations observed during this study are revealing the cytotoxic effect of extracts of the selected leaves for preventing cell proliferation. The results of present study provides information about how effective these plants in terms of their medicinal value and also to understand why these plants can be used for developing a new anti-cancer drug.

Keywords: Antimitotic, *Azadirachta indica, Simarouba glauca, Allium cepa*

Introduction
The anticancer properties of plants have been recognized for centuries. The National Cancer Institute (NCI) has screened approximately 35,000 plant species for potential anticancer activities. Due to the advantages of medicinal plants they are of high demand. Several species of medicinal plants have been investigated and selected for the preparation of cancer medicines. Recently, there has been an increased scientific interest in the study of materials from plant source as an anticancer compound [1].

The antimitotics are considered as mitosis blockers and consequently, cell death inducers. Antimitotic agents widely used in chemotherapy, target exclusively proliferative cells and commonly induce a prolonged mitotic arrest followed by cell death via apoptosis. As of today Himalayan plants are a major contributor to the herbal pharmaceutical industry both of India and other countries. Considerable works have been done on these plants to treat cancer [2].

The general principle of the mechanisms of mitosis are best and most easily studied in the actively growing region of plants such as a shoot or root apex. Mitosis is the characteristic type of cell division which keep the genetic continuity and integrity of organisms and preserves their somatic chromosome value. It is also known as equational division. Since the daughter cells formed are genetically and morphologically identical. Mitosis involves two closely related processes, karyokinesis and cytokinesis. Karyokinesis include 5 sub stages; prophase, prometaphase, metaphase, anaphase and telophase [3].

Azadirachta indica A. Juss commonly known as neem, or Indian lilac. It is a tree in the family Meliaceae. It is typically grown in tropical and semitropical regions. Its fruits and seeds are the source of neem oil. Products made from neem trees have been used in India for over two millennia for their medicinal properties. The medicinal properties of neem have been known to Indians since time immortal. It has been used in Ayurvedic and Unani systems of medicine since ages. Neem products are believed to have anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative properties [4].

A. indica has complex of various constituents including nimbin, nimbidin, nimbolide and limonoids. Such types of ingredients play role in disease management through modulation of various genetic pathways and other activities [5]. Accumulating evidences indicates that the anticancer effects of neem limonoids are mediated through the inhibition of hallmark capabilities of cancer such as cell proliferation, apoptosis evasion, inflammation invasion and angiogenesis [6]. Neem leaves, natural sources of flavonoids, polyphenols, isoprenoids, sulphurous and polysaccharides, play important role in scavenging the free radical and subsequently arresting disease pathogenesis [7].

Simarouba glauca DC. is commonly known as paradise tree, dysentery bark and lakshmitaru. Its seeds produce an edible oil. The tree is well suited for warm, humid, tropical regions. It is effective in treating certain diseases like diarrhea, malaria, edema, fever and stomach upset. *S. glauca* has been carried out to explore the molecular mechanisms leading to cancer cell death [8]. The leaves and bark have a long history of medicinal use, right from the early centuries. It is mainly used in treatment of malaria, fevers, dysentery and as an astringent to stop bleeding etc. The main active compounds are a group of triterpenes called quassinoids. Several of the quassinoids found in *Simarouba glauca* such as ailanthinone, glaucarubinone and holacanthone are considered the plant's main therapeutic constituents and are the ones documented to be antiprotozoal, antiamoebic, antimalarial and even toxic to cancer and leukemia cells [9].

Among the plant species, *Allium cepa* has been used to evaluate DNA damages, such as chromosome aberrations and disturbances in the mitotic cycle [10]. The ease to handle, stable karyotype, fast response, clear mitotic phases, large chromosome and small chromosome number are some of the reasons that make *Allium cepa* suitable in cytotoxic, antimitotic and genotoxic evaluations.

The major objective of this study is to evaluate and compare the antimitotic activity of leaf extracts of *Azadirachta indica* A.Juss. and *Simarouba glauca* DC. on *Allium cepa* root tip cells.

Materials and Methods

Plant Collection

The leaves of both plants were collected and washed in tap water to remove the impurities and then allowed to shade dry under room temperature. After proper drying the leaves were powdered separately and stored in air tight containers.

Preparation of Leaf Extracts

30 grams of powdered leaves of both plants were weighed and soaked with 100ml distilled water separately and continuously stirred for 1 hour. Then it was placed in boiling water bath for 30 minutes to get the aqueous extract. The extract obtained was then kept for 72hrs. At the end, each leaf extracts were filtered using filter paper, and the filtrate obtained was collected in a beaker and stored in refrigerator separately. 50% and 25% of aqueous extracts of both the plants were prepared by taking 10ml and 5ml aqueous extracts respectively making up to 20ml using distilled water.

Antimitotic Activity-*allium cepa* L. Root Assay

The *Allium* test was performed according to the method described by Fiskesjo (1985) [11] and Grant (1982) [12]. Locally available *Allium cepa* bulbs were washed, unscaled and grown on sand trays for 72 hours. It was watered daily. The bulbs whose roots have grown to approximately 2-3cm were selected and washed. The clean bulbs were placed on test tubes containing aqueous extracts such that the roots were immersed in the extracts. The duration of the treatment was 2hrs. The sprouted roots were also treated with well water, which served as control. After the treatment duration the roots were taken out and the root tips were hydrolyzed in 1N HCl, stained using acetocarmine and were then squashed on microscope slide covered with a cover slip. The slides were then examined through a light microscope and photographs were taken. The numbers of dividing and non-dividing cells were recorded and mitotic indexes were calculated using following equation:

$$Mitotic\ Index\ (MI) = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells}\ X\ 100$$

Chromosomal morphology, their changes and aberrations were also observed and recorded. Changes in cellular and chromosomal morphology were photographed under a light microscope.

Fig 1: Treatment of onion root tips with leaf extracts.

Results and Discussion

The evaluation of anti-mitotic activity of aqueous leaf extracts of *Azadirachta indica* A.Juss. and *Simarouba glauca* DC. in onion root tips revealed that, the extracts of both leaves showed a significant reduction in mitotic index when compared to that of control, and also the mitotic suppression increases with the concentration of extracts (*table 1*). The treatment also induced a range of chromosomal aberrations in the root tip cells of onion like nuclear lesions, prolonged prophase, stickiness between chromosomes, laggards etc. (*Figure 3*). When comparing the effect of both extracts, the extract of *Simarouba glauca* DC.

leaves showed more antimitotic activity on *Allium cepa* root tip cells than *Azadirachta indica* A. Juss. leaf extracts in both the concentrations (*figure 4*). Hence the present study provides information about how effective the selected plants in terms of their medicinal value and also to understand why these plants can be used for developing a new anti-cancer drug. Similar works was done in the evaluation of antimitotic activity of *Aplotaxis auriculata* rhizomes [13] and *Mukia maderaspatana* L. leaf extracts, [14] using *Allium cepa* root meristematic cells and got similar results. Similar anti-mitotic study along with phytochemical studies were conducted with polyherbal extracts by using onion root model and the results showed a significant reduction in mitotic index in polyherbal extract in high concentration in comparison to standard vincristine as well as in comparison to individual plant extracts [15].

Table 1: Effect of aqueous extracts of *Azadirachta indica* A. Juss. and *Simarouba glauca* DC. on mitotic index of *Allium cepa* root tip cells.

Concentration	Fields	Mitotic Index (MI)	Average Mitotic Index (MI)
Control	1	87.80	
	2	75.92	80.33
	3	77.27	
Simarouba glauca DC. (25%)	1	42.18	
	2	61.36	50.19
	3	47.05	
Simarouba glauca DC. (50%)	1	21.33	
	2	23.33	22.84
	3	23.88	
Azadirachta indica A. Juss. (25%)	1	63.88	
	2	58.88	58.97
	3	54.16	
Azadirachta indica A. Juss. (50%)	1	25.75	
	2	28.35	26.65
	3	25.86	

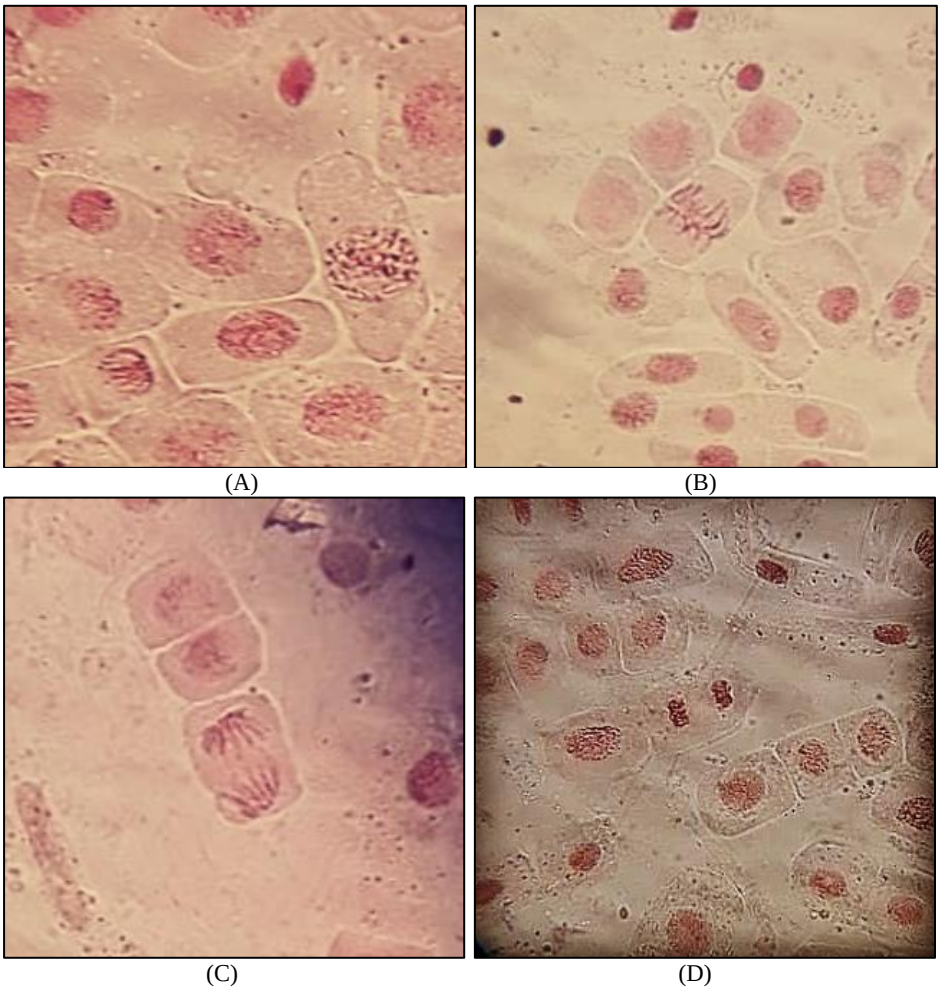

Fig 2: Various stages of mitotic cell divisions in *Allium cepa* root tip cells, (a) Prophase, (b) Metaphase, (c) Anaphase, (4) Telophase

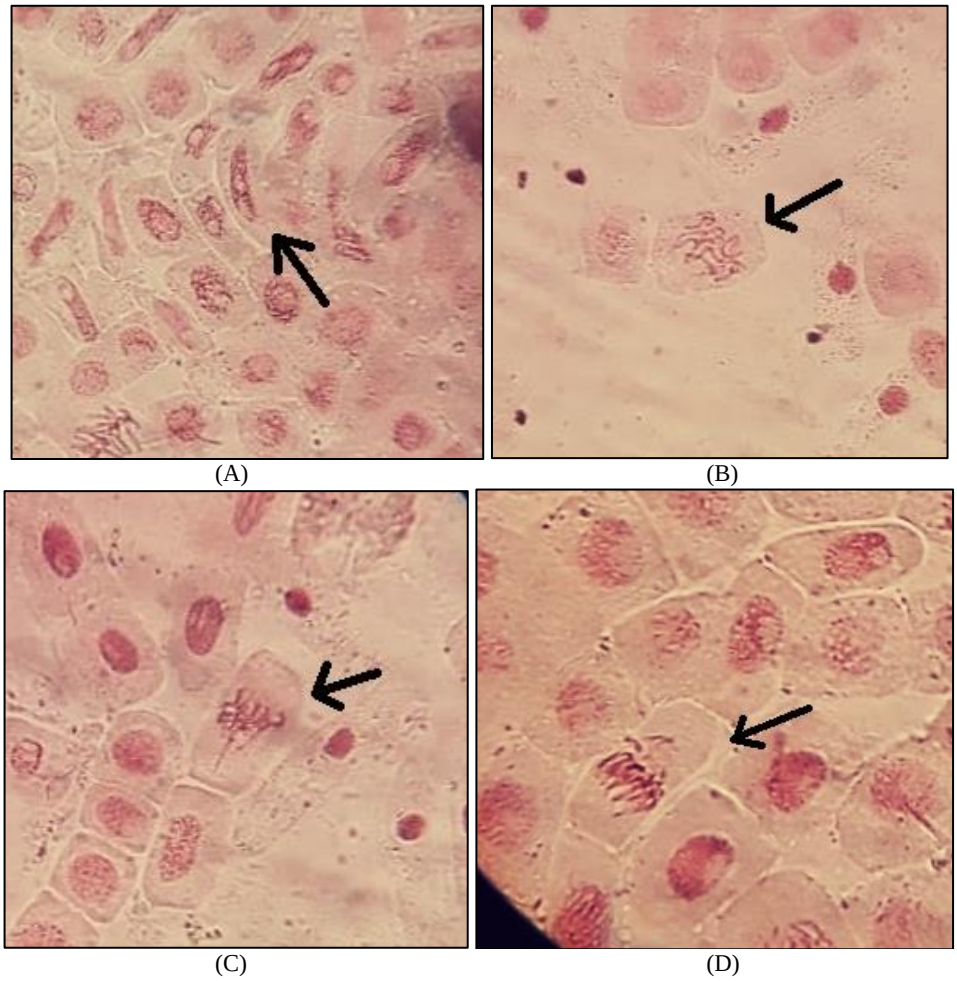

Fig 3: Chromosomal aberrations observed in *Allium cepa* root meristem cells treated with aqueous extracts of *Azadirachta indica* and *Simarouba glauca*. (a) Nuclear lesions, (b) Prolonged prophase, (c) Stickiness between chromosomes, (d) Laggard.

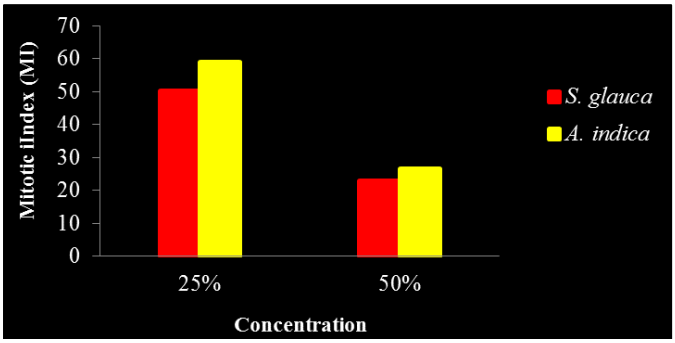

Fig 4: Antimitotic activity of leaf extract of *Azadirachta indica A.Juss. and Simarouba glauca* DC. *on mitotic index of onion root tips.*

Conclusion

The present study was an attempt to evaluate the antimitotic activity of leaf extracts of *Azadirachta indica* A. Juss. and *Simarouba glauca* DC. on *Allium cepa* L. root tip cells. The results revealed that, the extracts of both leaves showed a significant reduction in mitotic index of root tips when compared to control, and also the mitotic suppression increases with the concentration of extracts. On comparison of antimitotic activity of two selected plants, the leaf extracts of *Simarouba glauca* DC showed marked antimitotic property than that of *Azadirachta indica* A. Juss. Certain chromosomal aberrations were also observed in the higher concentrations (50%) of both plant leaf extracts. Thus the present study suggested that the aqueous extracts of leaves of *Azadirachta indica* A. Juss. and *Simarouba glauca* DC. has significant inhibitory and mitodepressive effects on the cell division of *Allium cepa* L. meristems. Hence these plants can be used for their anti-cancer properties. Further studies have to be conducted to obtain more detailed mechanism of action of plant material in view of its antimitotic activity.

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