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Comparative Study of Some Medicinal Plants for Their Phytotoxic-Antiproliferative Potentials

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Abstract:
This study was undertaken to carry out the screening of five plants (*Tecoma stans*, *Trema orientalis*, *Samanea saman*, *Alstonia scholaris*, and *Ampelocissus latifolia*) for their phytotoxic and antiproliferative effects using some basic bench-top assays. Aqueous extracts were prepared and comparative studies were done among these plants using wheat (*Triticum aestivum* L.) seedlings, onion (*Allium cepa* L.) bulbs, and lesser duckweed (*Lemna minor* L.) fronds as the experimental plant models. Among these plants, *Ampelocissus latifolia* and *Trema orientalis* showed superior antiproliferative activity in the primary screening test on wheat seedlings. Results of successive experiments revealed that *Ampelocissus latifolia*-aqueous extract showed the highest extent of phytotoxic and antiproliferative activities on all the bioassays. Thus, this study may open up a new arena to explore the scientific validation and justification of using the plants for therapeutic purposes.

Keywords: *Ampelocissus latifolia,* bioassay, lesser duckweed, onion, wheat

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Introduction

Since medieval times, plants have been the source of medicines for the treatment of different diseases. They act as a source of natural compounds for the discovery of drugs for diseases like cancer, diabetes, heart ailments, neurodegenerative diseases *etc*. (Balunas and Kinghorn, 2005; Kucuk, 2002; Newman *et. al*., 2003). Many plant species are screened and bioassayed for this purpose worldwide (Greenwald, 2002; Richardson, 2001). However, phytochemicals can be beneficial or harmful and a detailed investigation is needed for channeling them for therapeutic purposes. Bits of knowledge can be exploited to develop cheaper plant-derived therapeutically valuable drugs for disease management. Following this worldwide trend, the current study was undertaken to screen the aqueous extracts of five locally available plants and selection of a particular plant showing the highest antiproliferative and phytotoxic activities.

Methodologies

Collection of plants and aqueous extract preparation

Leaves of five plants (*Tecoma stans, Ampelocissus latifolia, Trema orientalis, Samanea saman, Alstonia scholaris*) were collected and processed using a standard protocol (Ray *et. al*., 2013). 50 g leaf dust was extracted with 500 mL distilled water for 6 h under low heating (60-70°C) in a water bath; every 2 h of extraction, it was filtered through No. 1 Whatman®. After 6 h of extraction, the filtrate was concentrated in a hot air oven at 50°C and stored at -20°C.

Root growth retardation and cytotoxicity assays

Experimental plants

Wheat (*Triticum aestivum* L.) seedlings, onion (*Allium cepa* L.) bulbs, and lesser duckweed (*Lemna minor* L.) fronds were taken for the experimental purpose.

Assays for antiproliferation and cytotoxicity

On wheat (*Triticum aestivum* L.) seeds

Similar sized wheat seeds were sterilized with 1% sodium hypochlorite. Three replicas of each with 30 seeds were set for the experimental purpose. Wheat seeds were placed on Petri dishes containing distilled water and then incubated at 25°C temperatures in dark in a BOD incubator for germination. After 24 h, seeds were treated with the aqueous extracts (4 mg/mL) of all five plants. Root lengths were measured after 96 h. The control groups were maintained in distilled water (Ray *et. al*., 2013).

On onion (*Allium cepa* L.) bulbs

Onions were washed well and allowed to sprout in distilled water. Sprouted onions were exposed to 2 mg/mL concentration of aqueous extracts of *A. latifolia* and *T. orientalis* for 2 h while maintaining the untreated control group in distilled water. Treated and untreated roots were cut and fixed in aceto-methanol (3:1) for 24 h, washed with distilled water, hydrolyzed in 1 N HCl at 60°C for 30 seconds, and stained with aceto-orcein (2%). Root tips were squashed and finally, the mitotic index (MI) % was scored.

On Lesser duckweed (*Lemna minor* L.) fronds

Twenty plants of *Lemna minor* having a rosette of two fronds were added to each flask containing 0.25, 1, and 2 mg/mL concentrations of aqueous extracts of *Trema orientalis* and *Ampelocissus latifolia* using Hoagland solution as the culture medium. The control group was maintained in Hoagland's solution. The experiment was done in quadruplet. The experimental setup was kept in a growth cabinet for 15 days. On the 15th day, frond numbers per flask were counted and percentages of growth inhibition were calculated (Azhar *et. al*., 2009). Additionally, toxicity percentage was calculated by calculating the percentages (%) of dead fronds.

Statistical Analysis

Root lengths were expressed as Mean±SEM and the statistical significance of the difference was analyzed with the Student's t-test. Mitotic index (MI) % was calculated as No. of cells in dividing phase/The Total No. of cells scored x 100. Here, the statistical significance of the difference was analyzed using 2x2 contingency χ^2-test. The level of statistical significance was considered at $p<0.05$, $p<0.01$, $p<0.001$.

Results

Root growth retardation of wheat seedlings

A preliminary comparative study was done among the five plants (*Ampelocissus latifolia, Trema orientalis, Tecoma stans, Samanea saman*, and *Alstonia scholaris*) using wheat seedlings. Root lengths were calculated after 96 h of extract exposure. Root growth inhibitions were 85.78, 63.43, 43.37, 57.11, and 55.76% after the exposures of aqueous extracts of *A. latifolia, T. orientalis*, *T. stans*, *S. saman*, and *A. scholaris* respectively. Here, the maximum effect was shown *A. latifolia* aqueous extract, followed by *T. orientalis* (Figure 1).

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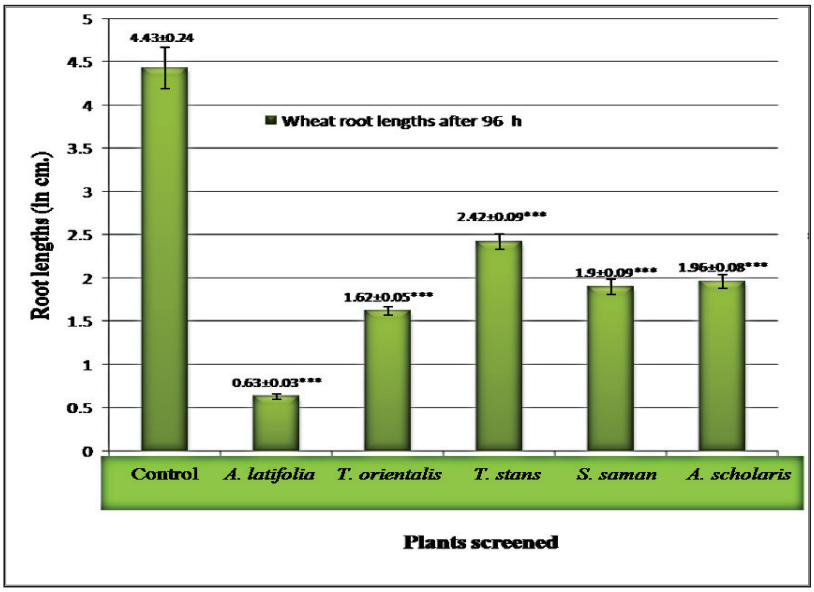

Fig. 1 Root lengths of wheat seedlings of untreated control and after treatment with leaf aqueous extracts of five plants screened. Experiments were done in triplicate and data were represented as Mean±SEM. ***Significant at $p<0.001$ by Student's t-test.

Mitodepression assay on onion

Based on the overall wheat-root growth inhibitory property of the five plants, two plants *A. latifolia* and *T. orientalis* were selected and further screening was done between them after treatment (2 mg/mL) of onion roots for 2 h. Data indicated mitotic index (MI) % reduction ($^{***}p<0.001$ by Student's t-test) in treated cells. A comparatively higher percentage (63.60%) of MI reduction occurred after *A. latifolia* aqueous extract treatment followed by *T. orientalis* (41.57%) (Figure 2).

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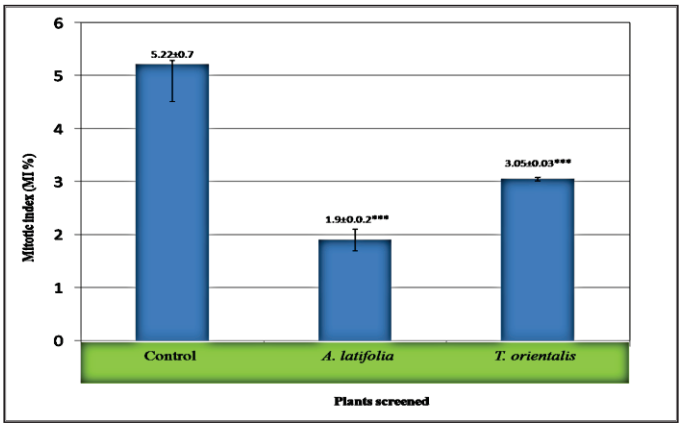

Fig. 2 Mitotic index (MI) % of onion (*Allium cepa*) root apical meristem cells. Experiments were done in triplicate and data were represented as Mean±SEM. ***Significant at $p<0.001$ by Student's t-test.

Lesser duckweed bioassay

The lesser duckweed bioassay was used to study the comparative phytotoxic activity of *A. latifolia* and *T. orientalis*. Data indicated trends of decrease of frond growth or budding after extract exposure. For the untreated control group, there was a 15% increase in the frond numbers, whereas, a concentration-dependent decrease in frond numbers was observed after exposure of the extracts for 15 days. Increase in frond numbers were 5%, 1.25%, 0% after exposure of 0.25, 1, and 2 mg/mL of aqueous extract *of A. latifolia*; 13.75%, 10%, and 3.75% after exposure of 0.25, 1, and 2 mg/mL of aqueous extract of *T. orientalis*. Simultaneously, the numbers of dead fronds were also calculated. The number of dead fronds increased from 7 to 18 to 37 and 5 to 7 to 8 after exposure of 0.25, 1, and 2 mg/mL concentrations of *A. latifolia* and *T. orientalis* extracts respectively (Table 1; Figure 3).

Table 1 Phytotoxicity of *A. latifolia* and *T. orientalis* on lesser duckweeds (*Lemna minor*) fronds

Treatment	Conc. (mg/mL)	Initial number of fronds	Number of fronds after 15 days		Number of dead fronds among the total number of fronds
			Total number	% Increase	
Control	00	80	92	15	1
***Ampelocissus latifolia* leaf aqueous extract**	0.25	80	84	5	7
	1	80	81	1.25	18
	2	80	80	0	37

***Trema orientalis* leaf aqueous extract**	0.25	80	91	13.75	5
	1	80	88	10	7
	2	80	83	3.75	8

conc.:concentration.

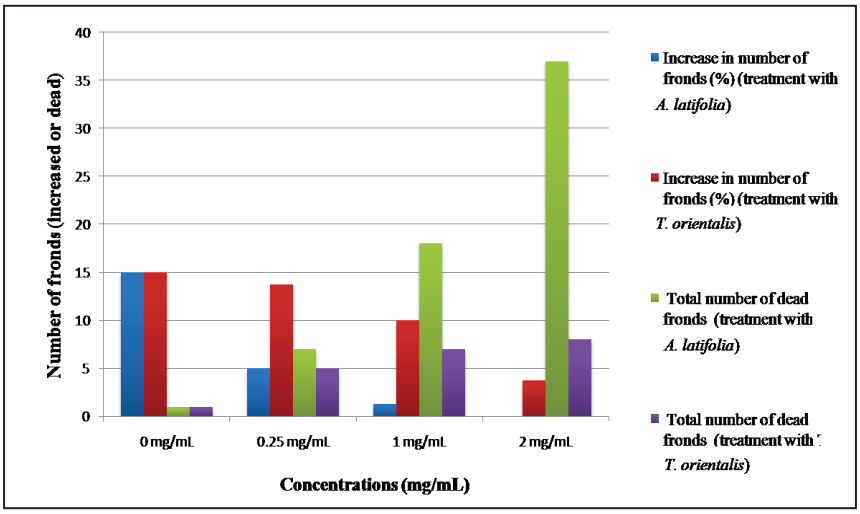

Fig. 3 Assessment of phytotoxic activity of aqueous extracts of *A. latifolia* and *T. orientalis* on *Lemna minor* fronds.

Discussion

Screening of plants or plant products for the assessment of antiproliferative and cytotoxic activities is the primary criteria for the selection of plants having significant growth inhibitory property (Levan, 1938). Initially, aqueous extracts of five different plants [*Ampelocissus latifolia* (wild grapes), *Trema orientalis* (pigeon wood), *Tecoma stans* (yellow bells), *Samanea saman* (rain tree), and *Alstonia scholaris* (devil's tree)] were screened using plant models for testing antiproliferative effects (Figure 1). Wheat root growths were recorded after 96 h of exposure. The maximum root length of wheat seedlings was recorded from the untreated control group and the minimum root length was recorded from the wheat seedlings, exposed to the aqueous extract of *A. latifolia*, followed by the treatment with *T. orientalis*. The lowest percentage of root growth retardation was calculated from *T. stans* extract-exposed seedlings. Here, all the five extracts showed statistically significant root growth retardation effects on wheat seedlings, though the degree of growth retardation effect was varied. In the next set of experiments, comparative studies were done between *A. latifolia* and *T. orientalis* using onion and lesser duckweed fronds (Table 1; Figure 2,

3). Data indicated trends of mitodepression ($^{***}p<0.001$ by Student's t-test) in treated groups. A comparatively higher percentage of mitotic index reduction occurred after *A. latifolia* aqueous extract treatment followed by *T. orientalis.* The maximum extent of phytotoxicity was observed after *A. latifolia* aqueous extract exposure in lesser duckweed bioassay. With increasing extract-concentrations (both *A. latifolia* and *T. stans*), percentages of *Lemna* budding decreased, where also *A. latifolia* aqueous extract-induced growth retardation was higher. Worldwide different research laboratories have discovered thousands of phytochemicals having growth inhibitory effect on different organisms through simple bench-top bioassays (Levan, 1938). Plant models, like *Vicia faba*, *Tradescantia paludosa*, *Pisum sativum*, *Hordeum vulgare*, *Crepis capillaries*, *Triticum aestivum*, *Vigna radiate, Allium cepa etc*. are widely used to analyze the antiproliferative property (Angayarkanni *et. al*., 2007; Levan, 1938). Thus using the ethnopharmacological approach, these screening programs are important in scientific validation of the traditionally used herbal remedies.

Conclusion

The preliminary studies are of immense value in the development of plant-based pharmaceuticals. This study depicts that *Ampelocissus latifolia* may hold a good prospect for its antiproliferative and phytotoxic values. Further studies are in progress to characterize and isolate the bio-active compounds present in this plant.

Conflict of Interest

Declared none.

Acknowledgment

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