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Bioassay-guided screening of eight plant species for antiproliferative activity based on basic bench top assays

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Abstract:
This study revealed the screening of eight traditionally used medicinal plants (*Synedrella nodiflora, Holoptelea integrifolia, Polyalthia longifolia, Moringa oleifera, Schleichera oleosa, Cynodon dactylon, Aegle marmelos,* and *Litsea glutinosa* for their antiproliferative effects using some basic bench-top assays. Comparative studies were done with the aqueous extracts of these plants using wheat (*Triticum aestivum* L.) seedlings. Later, three plants showing the superior performances in the previous assay were subjected for further experimentation to study the mitotic index retardation using onion (*Allium cepa* L.) bulbs as the experimental plant model. Among these plants, *S. nodiflora, P. longifolia,* and *M. oleifera* showed greater antiproliferative activity in the initial screening test on wheat seedlings. Results of successive experiments indicated that the aqueous extract of *Synedrella nodiflora* showed the highest extent of antiproliferative activity on all the bioassays. Thus, this study may open up a new possibility to look at the scientific validation and justification of using the plants for therapeutic purposes.

Keywords: *Synedrella nodiflora,* antiproliferative, onion, wheat

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Introduction

Since ancient ages, plants are the richest resources of medicines to fight against diseases. Systematic screening of plants on the ground of their traditional and therapeutic values is a necessary footstep in the path of drug discovery (Newman *et. al*., 2003). A huge amount of plant species have been bio assayed for this screening purpose worldwide (Greenwald, 2002; Richardson, 2001). Most of the phytochemicals require proper scientific exploration regarding their therapeutic roles. Keeping a pace with the global trend, this study was done to monitor the aqueous extracts of eight locally available plant species having traditional and medicinal values and selection of a plant showing the highest antiproliferation.

In this study, eight different medicinal plants {*Synedrella nodiflora* (aerial parts), *Holoptelea integrifolia* (leaf and bark), *Polyalthia longifolia* (leaf), *Moringa oleifera* (leaf), *Schleichera oleosa* (leaf), *Litsea glutinosa* (leaf), *Aegle marmelos* (leaf), and *Cynodon dactylon* (leaf)} having traditional therapeutic values were collected. Aqueous extracts were compared based on their allelopathic and antiproliferative mode of actions.

Methods

Collection of plants

Fresh parts of eight different conventionally used medicinal plants (*Synedrella nodiflora* (aerial parts), *Holoptelea integrifolia* (leaf and bark), *Polyalthia longifolia* (leaf), *Moringa oleifera* (leaf), *Schleichera oleosa* (leaf), *Litsea glutinosa* (leaf), *Aegle marmelos* (leaf), and *Cynodon dactylon* (leaf) were collected from the Golapbag Campus, The University of Burdwan.

Preparation of aqueous extract

Collected plant parts were rinsed in tap water, dried up in shade, directly crushed into small pieces and crushed using an electric grinder (Philips Mixer Grinder HL1605, Kolkata, West Bengal, India) using standard protocol (Ray *et. al*., 2013 a, b). Dried powder of plant parts was kept in the sealed container for upcoming experiments. 50 g of desiccated powdered material was heated with 500 mL distilled water for 6 h, under low heating (60-70°C), in the water bath; every 2 h of extraction it was filtered by filter paper. After 6 h of extraction, the filtrate was concentrated in a

hot air oven at 50°C. The extracts were then stored at -20°C for further use. The extract concentrations were determined.

Root growth retardation for the initial screening
Experimental plants

Wheat (*Triticum aestivum* L.) seedlings and onion (*Allium cepa* L.) bulbs were used as models for the experimental purpose. In the first round of screening, wheat seeds were used for comparative screening among eight species [*Synedrella nodiflora* (aerial parts), *Holoptelea integrifolia* (leaf and bark), *Polyalthia longifolia* (leaf), *Moringa oleifera* (leaf), *Schleichera oleosa* (leaf), *Litsea glutinosa* (leaf), *Cynodon dactylon* (leaf), and *Aegle marmelos* (leaf)]. For the second round of screening, three plant extracts showing the superior results were selected for the *Allium cepa* test.

Antiproliferation assay
Wheat seeds

The wheat seeds were sterilized exteriorly with sodium hypochlorite solution (1%) (Ray *et. al*., 2013 a, b). Every extract treatment was done in three sets. Wheat seeds were allowed to germinate on wet filter paper in a Petri dish. Following 24 h of germination, seeds were treated with the aqueous extracts (2 mg/mL) of the plants. Root lengths were measured at 96 h of extract treatment and the percentages of growth retardation were calculated. The untreated control sets were maintained in distilled water throughout the experimental process (Ray *et. al*., 2013 a, b).

Onion bulbs

Onions were obtained from the local market. Before starting the experiment, the external dried scales of the bulbs and the desiccated bottom plates were removed without destroying the root primordia, washed well and kept for germination. Germinated onions were exposed to *S. nodiflora, M. oleifera,* and *P. longifolia*- aqueous extracts (4 mg/mL) for 24 h. The onion roots were cut and kept for fixation in aceto-methanol for 24 h, processed accordingly and stained with the aceto-orcein (2%). Individual cell phase frequencies were counted under a bright-field light microscope and the mitotic index was calculated (Ray *et. al*., 2013 b).

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Statistical analysis

Statistical significance of difference was done using Student's t-test (for wheat root-shoot growth retardation assay) and 2x2 contingency χ^2-test, d.f.= 1 (for cell cycle kinetics) considering the level of significance at $p<0.05$, $p<0.01$, $p<0.001$. Here data were expressed as Mean±SEM. Mitotic index Seedlings growth was measured and the percentages of growth retardation were calculated. Root lengths were expressed as Mean±SEM. Mitotic index % was calculated by the principle as follows: No. of cells scored in dividing phase/The Entire No. of cells considered for scoring x 100.

Results

Root growth retardation of wheat seedlings

Root lengths were measured after 96 h of extract exposure. Root growth inhibitions were calculated as 84.29, 55.07, 59.24, 75.15, 71.17, 64.02, 48.91, 63.42, and 60.24% as well as shoot growth inhibitions were calculated as 73.25, 51.81, 55.18, 66.51, 56.87, 62.65, 66.75, 73.98, and 69.40% after the exposures of aqueous extracts of *S. nodiflora* (aerial parts), *H. integrifolia* (leaf), *H. integrifolia* (bark), *P. longifolia* (leaf), *M. oleifera* (leaf), *S. oleosa* (leaf), *L. glutinosa* (leaf), *C. dactylon* (leaf), and *A. marmelos* (leaf) respectively. Data indicated that the minimum root growth occurred after *S. nodiflora* aqueous extract exposure (0.79±0.14 cm), followed by *P. longifolia* (1.25±0.15 cm), and *M. oleifera* (1.45±0.05 cm) in comparison to the untreated control (5.03±0.31 cm). Both *S. nodiflora* and *C. dactylon* exhibited nearly equal shoot lengths (1.11±0.15 and 1.08±0.19 cm respectively) which were the minimum values (Table 1; Figure 1).

Table 1. The growth retardation of wheat seedlings by nine aqueous extracts of eight plants

Treatment (Extraction parts)	Hours	Root lengths (cm)		Shoot lengths (cm)	
		Mean±SEM	%Decrease %)	Mean±SEM	%Decrease %)
Control	96	5.03±0.31	0.00	4.15±0.26	0.00
***Synedrella nodiflora* (aerial parts)**		**0.79±0.14*** **	**84.29**	**1.11±0.15*** **	**73.25**
Holoptelea integrifolia (leaf)		2.26±0.10***	55.07	2.00±0.13***	51.81
Holoptelea integrifolia (bark)		2.05±0.23***	59.24	1.86±0.23***	55.18
Polyalthia longifolia		**1.25±0.15*** **	**75.15**	1.39±0.15***	66.51

(leaf)				
Moringa oleifera (leaf)	**1.45±0.05*** **	**71.17**	1.79±0.12***	56.87
Schleichera oleosa (leaf)	1.81±0.17***	64.02	1.55±0.14***	62.65
Litsea glutinosa (leaf)	2.57±0.22***	48.91	1.38±0.12***	66.75
Cynodon dactylon (leaf)	1.84±0.25***	63.42	**1.08±0.19*** **	**73.98**
Aegle marmelos (leaf)	2.00±0.37***	60.24	1.27±0.30***	69.40

Experiments were done in three sets and data are represented as Mean±SEM (***Significant at $p<0.001$).

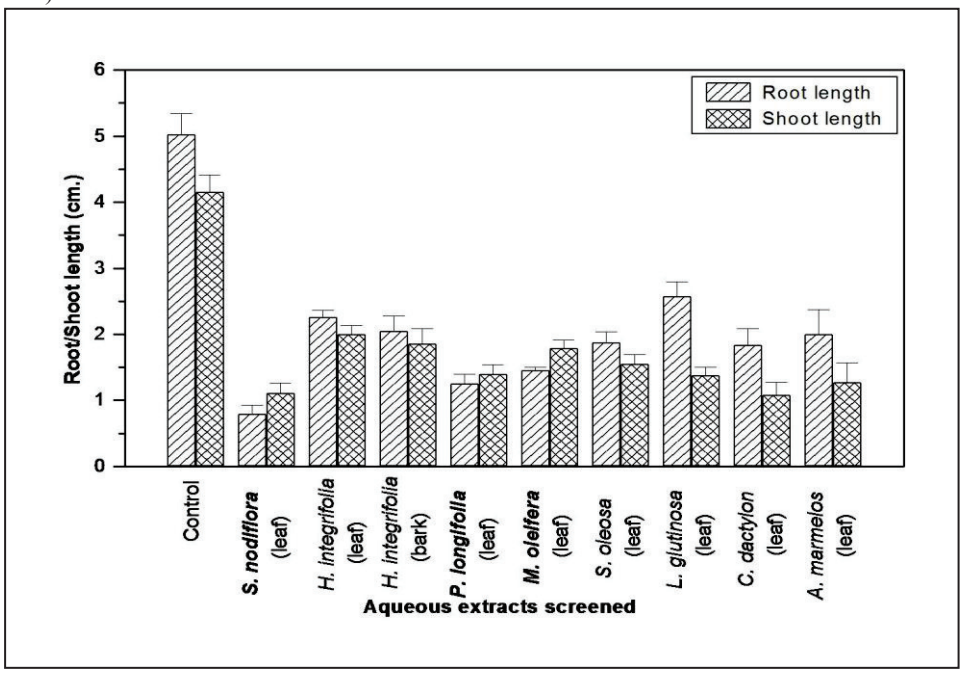

Figure 1. Root lengths of wheat seedlings (*Triticum aestivum*) of untreated control and after treatment with aqueous extracts of eight plants screened (2 mg/mL) for 48 h. Experiments were done in three sets and data are represented as Mean±SEM. ***Significant at $p<0.001$ as compared to the control by Student's t-test

Mito-depression assay using onion root apical meristem cells

Following the overall root growth inhibitory effect, three plants namely *S. nodiflora, P. longifolia,*

and *M. oleifera* were selected for further screening on onion bulbs. A comparatively higher percentage (85.09%) of mitotic index reduction occurred after *S. nodiflora* aqueous extract treatment followed by *M. oleifera* (83.33%), and *P. longifolia* (55.26%) (Table 2). Thus, based on the assessment of overall antiproliferation, *S. nodiflora* (Figure 2) was considered as the most potent one.

Table 2. Mitotic index reduction by *S. nodiflora, P. longifolia,* and *M. oleifera* aqueous extracts

Treatment	Conc. (mg/mL)	Treatment (h)	TCC	INC	DC	MI±SEM (Reduction %)
Control			1842	1758	84	4.56±0.19 (0.00)
Moringa oleifera	4	24	1309	1299	10	0.76±0.15*** (83.33)
Polyalthia longifolia			1226	1201	25	2.04±0.15*** (55.26)
Synedrella nodiflora			**2363**	**2347**	**16**	**0.68±0.08*** (85.09)**

Experiments were done in three sets and data are represented as Mean±SEM (***Significant at $p<0.001$) Conc.: concentration, h: treatment hour, TCC: total cells counted, INC: interphase cells, DC: dividing cells, MI: mitotic index.

Figure 2. *Synedrella nodiflora*: the aerial parts (including leaf, stem, and flower)

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Discussion

Screening of plants or plant products for the assessment of antiproliferative activity is essential for the selection of plants having significant growth inhibitory property. Initially, nine aqueous extracts of eight different plants [*S. nodiflora* (aerial parts), *H. integrifolia* (leaf and bark), *P. longifolia* (leaf), *M. oleifera* (leaf), *S. oleosa* (leaf), *L. glutinosa* (leaf), *A. marmelos* (leaf) and *C. dactylon* (leaf)] were screened using the wheat model for testing the antiproliferative effects (Table 1; Figure 1). In this study, the maximum wheat root length (5.03±0.31 cm) was from the untreated control group and the minimum length (0.79±0.14 cm) was from the *S. nodiflora* – treated group followed by the treatment with *P. longifolia* (1.25±0.15 cm) and *M. oleifera* (1.45±0.05 cm). The lowest percentage (48.91%) of root growth retardation was calculated from *L. glutinosa* leaf aqueous extract-exposed seedlings. Here, all the nine extracts reduced wheat root growth significantly, though the degree of growth retardation was diverse. In the next set of experiments, comparative studies were done among *S. nodiflora, P. longifolia,* and *M. oleifera* on onion model (Table 2). Data indicated trends of mito-depression in treated cells. A comparatively higher percentage of mitotic index reduction occurred after *S. nodiflora* aqueous extract (85.09%) treatment followed by *M. oleifera* (83.33%), and *P. longifolia* (55.26%). These differential activities of the aqueous extracts were due to the occurrence of different phytochemicals and also their relative abundance. Worldwide different research laboratories have discovered several phytochemicals having growth inhibitory effect on different types of organisms through the plain bench-top bioassays. Plant models, like *Vicia faba*, *Triticum aestivum*, *Pisum sativum*, *Vigna radiata, Allium cepa etc.,* are used to analyze the antiproliferative property of plant extracts (Angayarkanni *et. al*., 2007; Levan, 1938). Thus using the ethnopharmacological approach, these screening programmes are important in scientific validation of the traditionally used herbal remedies and to provide leads in the search for new active principles.

Conclusion

Therefore, based on the morphometric and antiproliferative actions of aqueous extracts of these plant products, *S. nodiflora* was observed to show the best result.

Conflict Of Interest

Declared none.

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