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Allelopathic effects of aerial parts aqueous extract of *Ampelocissus latifolia* (Roxb.) Planch. in apical meristem cells

Chaudhuri A., Kundu L. M., Dutta S., Chatterjee S., Goswami S., Roy G. C. and Ray S.*

Molecular Biology and Genetics Unit, Department of Zoology, The University of Burdwan, Golapbag, Burdwan, West Bengal, India

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ABSTRACT

This study was aimed to explore allelopathic effects of aerial parts aqueous extract of Ampelocissus latifolia (Roxb.) Planch. (AAEAL) in terms of cytological and morphological alterations in root apical meristems. Allelopathic effects of AAEAL was studied on green gram and onion root apical meristems by analysing growth retardation and /or by scoring mitotic index, colchicine like metaphase arrest and interphase nuclear condensation. AAEAL treatment showed significant allelopathic effects in terms of growth retardation in green gram and onion apical meristem cells in a dose dependent manner. In onion root tip cells mitotic index reduced after AAEAL treatment and could induce decreased nuclear volume, increased interphase chromatin condensation and various chromosomal abnormalities in onion root tip cells. In conclusion it may be said that aerial parts of A. latifolia contain water-soluble effective allelochemicals.

Keywords: Allelopathy, *Allium cepa, Ampelocissus latifolia*, Chromatin condensation, Herbicides, Phenolics.

INTRODUCTION

Allelopathy has been recognised as a vital ecological process that influences the primary and secondary plant succession and the structure, composition and dynamics of native and cultivated plant communities [1]. It is defined as any direct or indirect, stimulatory or inhibitory influence of plants on other plants due to the allelochemicals released into the environment [2]. Allelochemicals include phenolics, alkaloids, long-chain fatty acids, terpenoids, flavonoids etc. and play a significant role in agro-ecosystems and affects the seed germination and growth, quality and quantity of crop products [2]. Some of the allelochemicals exhibit biological activity and have been used in the pharmaceutical and agrochemical industries [3, 4]. The action of allelochemicals is governed by their presence as a single compound or mixtures. Imbibitions, seed germination, seedling growth and morphological alteration are widely considered for determining the allelopathic activity of plant products. Physiological effects of allelochemicals include inhibition of photosynthesis, respiration and enzymatic activities [2].

Ampelocissus latifolia (Roxb.) Planch. (Family: Vitaceae), native to Indian subcontinent is used extensively for its medicinal values [5-8]. Recently antibacterial, antioxidant [9, 10], cytotoxic and phytotoxic [11] and antiproliferative activities of *A. latifolia* have been reported [12]. This plant exhibits anti-inflammatory activity due to its inhibitory effect on histamine kinin and prostaglandin release [13]. Acetogenins like 22-epicalmistrin, uvaribonin and chalcone isolated from the root of Phillippine *Ampelocissus* showed significant cell growth inhibitory activity against a panel of human cancer cell lines [14].

The use of synthetic chemicals for controlling unwanted herbs or weeds is one of the most effective methods. Indiscriminate use of synthetic chemicals is continuously being phased out because of their adverse effects on the environment. As a result, the use of plant secondary metabolites as herbicide or weedicide is gaining renewed interest. At the present state of knowledge allelopathic activities of *A. latifolia* are not studied. Hence, the objective

of the present study was to evaluate the allelopathic effects of AAEAL through simple laboratory bioassays and to explore its cytological and chemical basis. Here, the phytotoxic, antiproliferative and cytogenotoxic effects were considered as the underlying allelopathic mode of actions and were correlated with its total phenolics.

MATERIALS AND METHODS

2.1 Chemicals
Glacial acetic acid, orcein and methanol were obtained from BDH chemicals Ltd., UK. Other chemicals used in the study were of analytical grade from reputed manufacturers.

2.2 Plant products collection, storage and extract preparation
Fresh aerial parts of *A. latifolia* were collected from Burdwan University campus, West Bengal, India. This plant species was taxonomically identified by Dr. Ambarish Mukherjee (Taxonomist), Professor, Department of Botany, the University of Burdwan. The voucher specimens (No.BUGBAC012) are maintained in the department for future reference.

Collected plant materials were washed in tap water, shade dried, directly crushed into small pieces and followed to pulverise using electric grinder (Philips Mixer Grinder HL1605). Ground powder was stored in air tight container for future use.

Twenty grams of dried powdered plant material was extracted in 400 ml of distilled water for 6 h at slow heat (50 °C) in water bath. At the end of 6 h extract was filtered through No. 1 Whatman® filter paper and stored at -20 °C for further use.

2.3 Experimental plants
Green gram (*Vigna radiata*) seedlings and onion (*Allium cepa*) roots were used as experimental plant models. Green gram seedlings were used for root growth retardation assay. Onion roots were used for cytometric, chromosomal aberrations and cell cycle kinetics analyses.

2.3.1 Culture and treatment of green gram seedlings
Green gram seeds were surface sterilized with 1 % sodium hypochlorite solution for 2 minutes and washed with distilled water vigorously for ten minutes and allowed for germination in dark at 25±2 °C on wet filter paper in glass Petri dishes, containing different concentrations (0.25, 0.5, 1, 2, 4 and 5 mg/ml) of AAEAL, covered with another Petri dish. Seedling lengths were recorded at 96 h. Only distilled water was used as culture medium for untreated control seedlings.

2.3.2 Culture and treatment of onion roots for cytological analysis
Onion bulbs were collected from local market and similar sized bulbs were allowed for root sprouting in test tubes. The 48 h aged onion root meristem cells were exposed to two different concentrations (0.5 and 2 mg/ml) of AAEAL for 2, 4, 6, 12 and 24 h. The untreated roots were maintained simultaneously in distilled water. Root tips were processed and squashed for light microscopic analysis as described earlier [11]. The frequency of condensed interphase nucleus and mitotic index depressions in relation to the untreated controls were calculated.

2.4 Scoring and Statistical analysis
Green gram seedlings growth was recorded and the growth retardation percentages were calculated. The difference between the untreated and treated groups for the seedling lengths and cytometric variations were analysed with the Student's t test. Differences between corresponding controls and exposure treatments were considered statistically significant at
$p < 0.001$.

RESULTS

3.1 Green gram root growth retardation
Data clearly indicate that AAEAL could induce dose dependent growth retardation on green-gram seedlings. The growth inhibition was calculated as 30, 31, 38, 42, 54, 62% respectively for the concentrations 0.25, 0.5, 1, 2, 4, 5 mg/ml of AAEAL at 96 h. (Table. 1).

3.2 AAEAL induced cyto-metric variation in onion root tip cells
Light microscopic study on AAEAL treated onion root tip cells revealed increased percentage of condensed interphase cells (0.91, 3.24, 4.36, 4.87% after treatment with 0.5 mg/ml of AAEAL and 8.67, 12.39, 15.24, 17.87%

after treatment with 2 mg/ml of AAEAL for 2, 4, 6, 24 h respectively as compared to control groups which were 0.60, 1.16, 0.95, 1.42% for the aforesaid hours respectively (Figure. 1, 2). The extract also induced dose and time dependent reduction in cellular length and nuclear diameter (Table. 2).

Table. 1 Pooled data showing AAEAL induced green gram seedlings growth retardation effect

AAEAL Concentration (mg/ml)	Seedling Length (cm)		
	Range	**(Mean ± SEM)**	**Inhibition %**
0.00	3.7-23.5	13.30±1.58	00
0.25	1.1-20.9	09.22±2.06***	30
0.50	0.7-18.5	09.12±1.74***	31
1.00	1.0-18.5	08.19±1.71***	38
2.00	0.6-15.3	07.64±1.06***	42
4.00	0.2-15.0	06.10±1.36***	54
5.00	0.4-12.8	05.21±1.15***	62

****Significant at $p < 0.001$ with Student's t- test.*

3.3 AAEAL induced c-metaphase formation in onion root apical meristems:

AAEAL (0.5-2 mg/ml; at 2-6 h) induced high frequency of c-metaphase (haphazardly arranged shorter and condensed chromosomes) in onion root tip cells, where the frequency was maximum (84.44% c-metaphase of total metaphase cells) at a concentration of 2 mg/ml at 2 h (Figure.1, 3)

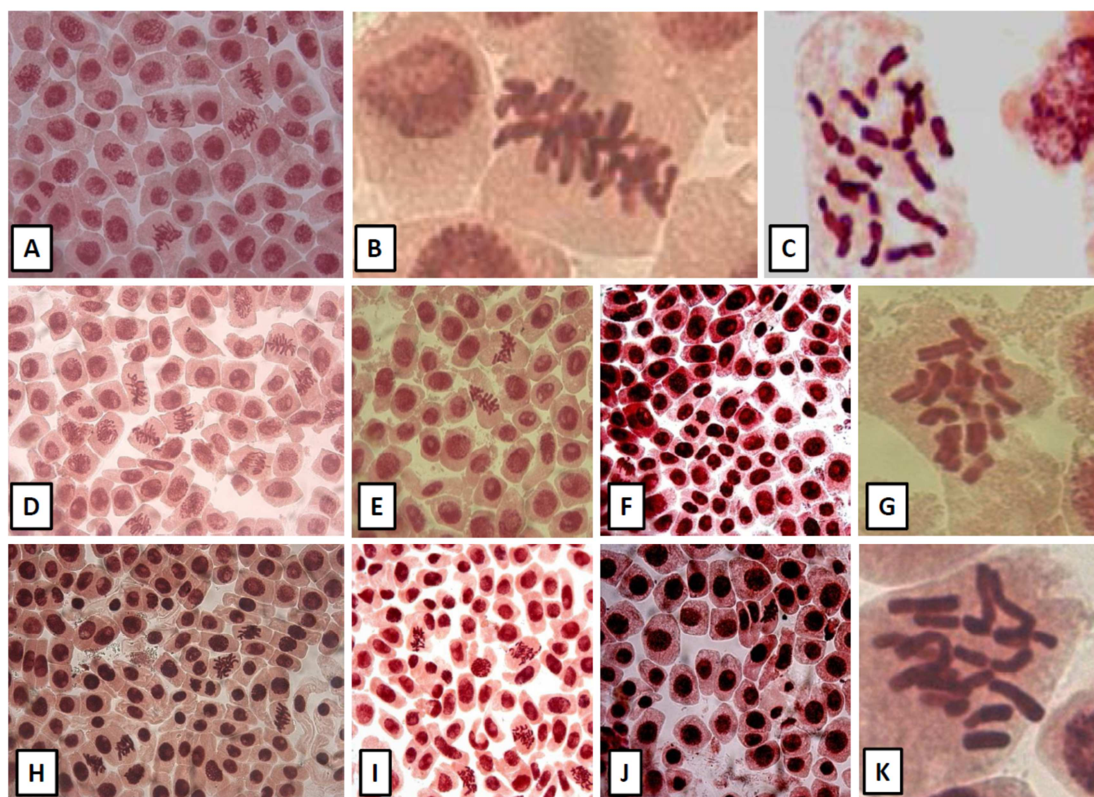

Figure 1. Photomicrographs showing AAEAL induced interphase chromatin condensation and c-metaphse in onion root tip cells. A-B (untreated control) and C-K (AAEAL treated) squashed and aceto-orcein stained onion root tip cells. A; squashed root apical meristem cells showing normal characteristics of cell cycle phases, B; ideal metaphase chromosome arrangement, C, G and K; AAEAL (2.0 mg/ml) induced c-metaphase, D, E and F; respectively for 2, 6 and 12 h at 0.5 mg/ml AAEAL treated and H, I and J; respectively for 2, 6 and 12 h at 2.0 mg/ml AAEAL treated

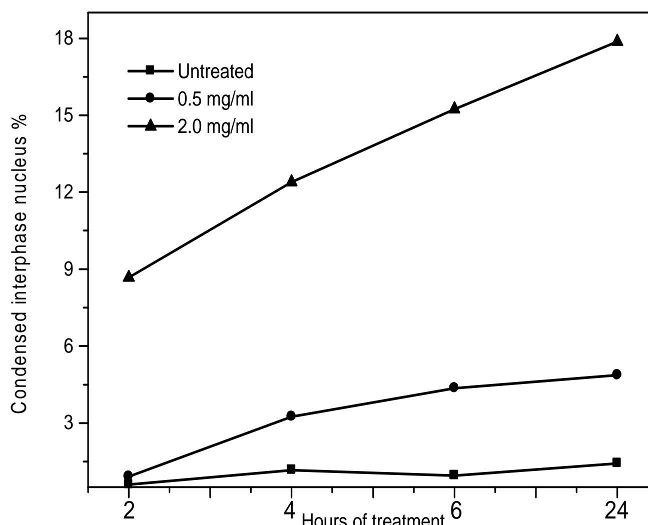

Figure 2. Showing AAEAL induced condensed interphase nucleus % in onion root tip cells

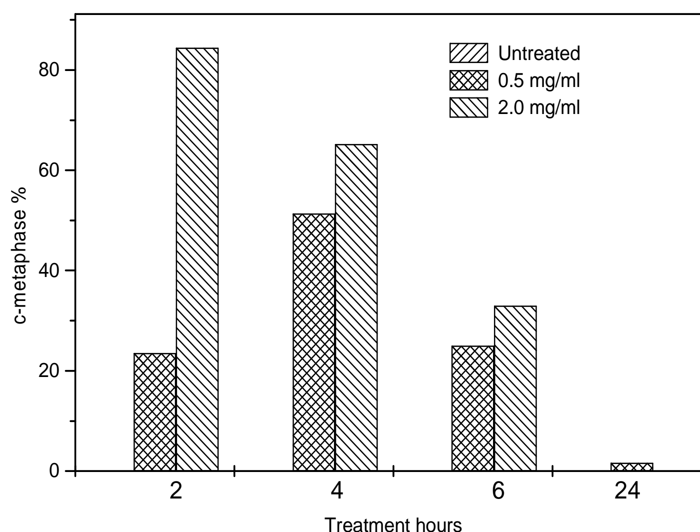

Figure 3. Showing AAEAL induced c-metaphase % in onion root tip cells

Table. 2 Pooled data showing the influence of AAEAL on nuclear diameter and cell length of onion root tip cells

Dose (mg/ml)	H	CS	ND(µm)		CL(µm)		ND/CL	
			Range	Mean±SEM	Range	Mean±SEM	Range	Mean±SEM
0	4	63	12-30	20.92±0.41	28-70	39.47±0.94	0.30-0.73	0.54±0.01
0.5		90	6-27	14.77±0.38***	18-63	31.65±0.78 ***	0.26-0.74	0.48±0.01***
2.0		83	10-18	13.20±0.21***	20-37	28.69±0.44 ***	0.31-0.60	0.47±0.01***
0.5	6	80	10-21	15.22±0.30***	20-49	35.28±0.80 ***	0.21-0.67	0.44±0.01***
2.0		48	11-20	14.15±0.28***	24-57	34.83±0.94 ***	0.26-0.58	0.42±0.01***
0.5	24	66	9-21	13.98±0.35***	22-56	34.03±0.83 ***	0.23-0.65	0.42±0.01***
2.0		80	5-18	13.21±0.28***	19-53	33.15±0.80 ***	0.17-0.61	0.40±0.01***

****Significant at $p < 0.001$ as compared to their respective control with Student's t- test. H; Hours, CS; Cells scored, ND; Nuclear diameter, CL; Cell length.*

DISCUSSION

Allelochemicals are secondary metabolites or natural products that help to regulate the structure of plant communities [1]. It may be present in all plant organs but their quantities vary from one organ to another [15, 16]. The chemical exudates from allelopathic plants play a vital role in the allelopathic action. In the present study

allelopathic activity of aerial parts aqueous extracts of *A. latifolia* (AAEAL) was evaluated to explore the underlying allelopathic mode of actions.

AAEAL showed allelopathic potential in terms of seedling growth inhibition, delayed cell cycle kinetics, chromosomal and nuclear abnormalities. In the initial experiments, a wide range (0.25–5 mg/ml) of AAEAL concentrations were used for morphometric bioassays on green gram seedlings and onion roots, and finally two concentrations 0.5 and 2 mg/ml were selected for cytological analysis on onion root tip cells. The AAEAL treatment could significantly reduce the lengths of green gram seedlings in a dose dependent manner. Growth retardation percentage increased from 30 to 60% for increased concentration from 0.25 to 5 mg/ml ($p<0.001$) (Table. 1). The phytotoxicity and alleopathic effects of plant extracts in terms of seedling growth inhibition are well documented in the literature [16, 17]. This can be attributed to the fact that seedling growth is characterized by high metabolic rates and are therefore highly susceptible to allelochemicals [18].

In our previous study we have shown AAEAL induced root morphological and cytological alterations like rottening, swelling, atrophication of root hairs in the treated wheat seedlings as compared to untreated controls maintained in distilled water [11]. Our study also indicated phytotoxic, cytogenotoxic and antiproliferative potentials of leaf aqueous extracts of *A. latifolia* where mitotic index depression bioassay on onion root apical meristem cells revealed that the AAEAL treatment could reduce the mitotic index. Such a dose dependent reduction in mitotic index percentage suggested that the exposure of AAEAL to root apical meristem cells led to cytotoxic stress, reduction in cell numbers entering into mitotic cycle and all together increased interphase cell frequency [11, 12]. Moreover, increased cumulative frequency of prophase-metaphase and decreased cumulative frequency of anaphase-telophase indicate AAEAL induced delayed cell cycle kinetics in onion root apical meristem cells [12]. Levan first introduced onion root tip assay and later it was proposed as a standard method to study toxicity of the toxicants [19-23]. A number of earlier studies have also suggested that the level of growth inhibition increases with increasing extract concentrations [11, 24, 25]. Our present study indicates, dose-and time dependent increased condensed interphase cell frequency, where the percentage (17.87%) was highest at 2mg/ml at 24 h (Figure. 1, 2). Light microscopic study on AAEAL treated onion root tip cells also revealed the presence of interphase cells with reduced nuclear diameter that supports the phenomenon of AAEAL induced allelopathic antiproliferation in meristem cells (Figure. 1, Table. 2). Furthermore, the light microscopic study also revealed that the AAEAL treatment could induce formation of higher frequency of c-metaphase (84.44%) than normal metaphase (15.56) and which might have occurred due to the microtubule disruption (Figure. 1, 3). Microtubule disrupting agents arrest the cells in mitosis by triggering activation of a mitotic check point, which ensures accurate attachment of chromosomes to the mitotic spindle, before entering into anaphase. When drug treatment causes microtubules to fail to attach to the kinetochores, mitotic checkpoint continues to generate signals that inhibit metaphase to anaphase transition leading to metaphase arrest and induction of c-metaphase [26, 27].

In our previous study detailed phytochemical profiling of AAEAL was done and revealed the presence of various phytoconstituents like phenolics, tannins, flavonoids, anthraquinones, saponins, alkaloids, carbohydrates, glycosides and terpenoids and also indicated that AAEAL contains higher percentage of phenolics [12], which may contribute in these allelopathic activities. Among various phytochemicals, phenolics are the most abundant substances that affect seedling growth and cell division [28, 29]. Total phenolics in AAEAL was also determined (21.03±0.9 %) as tannic acid equivalents on dried extract matter basis [12].

Therefore, the novel findings of the present study are the exploration of allelopathic potentials of AAEAL, wherein the antiproliferative, cytogenotoxic and phytotoxic activities are the underlying principle of this activity and which may occur due to the presence of polyphenolics in AAEAL.

CONCLUSION

In conclusion, the AAEAL possess significant allelopathic potentials where the interphase chromatin condensation, chromosomal aberrations, microtubule disruptions, phytotoxic and cytotoxic activities were possibly the underlying mechanisms of allelopathic interactions. Thus *A. latifolia* may hold future prospect as a biological herbicidal and weedicidal agent.

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