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Determination of effective allelopathic (inhibitory) extract fractions of *Ampelocissus latifolia* (Roxb.) Planch. leaf

Chaudhuri Anwesa and Ray Sanjib*

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

ABSTRACT

The aim of this study was to determine the effective allelopathic (inhibitory) extract fractions of *Ampelocissus latifolia* leaf along with preliminary phytochemical screening and to correlate allelopathy with the quantity and quality of total phenolics and with extraction solvent's polarity index. Five extract fractions were prepared using soxhlet apparatus by sequentially passing organic solvents with increasing polarity through the fixed amount of dried leaf powder and the extract fractions were designated as PEEF (petroleum ether extract fraction), CEF (chloroform extract fraction), EAEF (ethyl acetate extract fraction), MEF (methanolic extract fraction) and finally AEF (aqueous extract fraction) that was collected after boiling in water bath. Comparative allelopathic inhibitory activity of the extract fractions was studied using wheat seedlings and correlated with the qualitative phytochemical analysis and the total phenolics content. Data indicate varied degree of allelopathic potentials of the different extract fractions. Here, MEF showed the highest degree of wheat root growth retardation where PEF showed the least effect. Treatment with the different extract fractions resulted in 73.06, 57.54, 32.33, 10.78 and 4.74% wheat root growth inhibition respectively for MEF, EAEF, CEF, AEF and PEF at a concentration 2 mg / ml for 72 h. Phytochemical analysis revealed that the MEF contains the highest quantity of polyphenolics as compared with the other extract fractions. The present study suggests that *A. latifolia* is very rich in phytochemicals and the allelopathic action increased with the solvent polarity upto methanol in the successive extraction procedure. The MEF also exerts strong allelopathic action which positively correlates with the highest amount of polyphenolic content. Therefore *A. latifolia* leaf polar solvent extracts may be considered as a source of bio-herbicides.

Key words: *A. latifolia*, Allelopathy, Phenolics, Phytochemicals.

1. INTRODUCTION

Allelochemicals are secondary metabolites present in all plant parts but their quantities vary from organ to organ and help to regulate the structure of plant communities [1-3]. The chemical exudates from allelopathic plants play a crucial role in the allelopathic action. Synthetic chemicals are widely used for controlling unwanted herbs or weeds but their indiscriminate use 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.

Ampelocissus latifolia (Roxb.) Planch. (Family: Vitaceae), native to Indian subcontinent is used widely for its therapeutic values [4-7]. This plant exhibits anti-inflammatory activity due to its inhibitory effect on histamine kinin and prostaglandin release [8]. 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 [9]. In our previous study we have shown AAEAL induced root morphological and cytological alterations like rotting, swelling, atrophication of root hairs in the treated wheat seedlings as compared to untreated controls that

were maintained in distilled water [10, 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 aerial parts aqueous extract of *A. latifolia* (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 [10, 11]. Moreover, increased cumulative frequency of prophase-metaphase and decreased cumulative frequency of anaphase-telophase indicated AAEAL induced delayed cell cycle kinetics in onion root apical meristem cells. In our recent study we have also shown the allelopathic activity of aerial parts aqueous extracts of *A. latifolia* where it was positively correlated with its total phenolic content, moreover, the underlying allelopathic mode of actions were explored [12]. However, detailed phytochemical profiling of successive solvent extract fractions of *A. latifolia* leaf along with their allelopathic activities have not been yet studied well. Therefore, the major objectives of the present study were to analyse phytochemicals qualitatively in different extract fractions, to determine the effective allelopathic extract fractions and to correlate allelopathic potentials with their total phenolics content. Here, using soxhlet apparatus and organic solvents with increasing polarity index the different extract fractions were prepared and their allelopathic inhibitory potential was determined by analysing their wheat root growth retardation action in laboratory condition.

2. MATERIALS AND METHODS

2.1 Chemicals

Petroleum ether, chloroform, ethyl acetate, methanol, and tannic acid powder were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. Folin-Ciocalteu's phenol reagent was obtained from Merck Specialities Pvt. Ltd., Mumbai, India. Other chemicals used in this study were of analytical grade from reputed manufacturers.

2.2 Collection and storage of plant leaf material

Fresh plant leaves of *A. latifolia* were collected from The Burdwan University Golapbag campus, West Bengal. This plant species was taxonomically identified by Prof. A. Mukherjee (Taxonomist), Department of Botany, The University of Burdwan. The voucher specimen (No.BUGBAC012) is maintained in the Department of Zoology for future reference. Collected leaves were washed thoroughly with normal tap water. Then the leaves were dried under shaded condition, crushed to powder using grinding machine and the leaf powder was stored in air tight container.

2.3 Extract preparation

Thirty gram powdered leaf material was successively extracted with organic solvents, with increasing polarity index, like petroleum ether, chloroform, ethyl acetate and methanol using soxhlet apparatus continuously for 48 h with 500 ml of the various solvents each and finally remaining leaf powder was boiled in distilled water for 6 h in water bath. The extract fractions (petroleum ether extract fraction-PEEF, chloroform extract fraction-CEF, ethyl acetate extract fraction-EAEF, methanolic extract fraction-MEF, aqueous extract fraction-AEF) were obtained and condensed using rotary vacuum evaporator and kept for evaporation to remove solvents in hot air oven at 50°C till dried completely. These dried extract fractions were then stored in -20°C for future use.

2.4 Experimental plant

Wheat (*Triticum aestivum*) seedlings were used as experimental plant model for allelopathic action where root growth retardation was analysed.

2.4.1 Culture and treatment of wheat seedlings

Wheat seedlings were cultured following the method as described earlier in detail [10]. Briefly, wheat seeds were surface sterilized with 1 % sodium hypochlorite solution for 2 minutes, washed with distilled water vigorously for ten minutes, allowed for seed germination on wet filter paper in glass Petri dishes and left covered with another Petri dish. Petri dishes were maintained at 25±2 °C and 65% humidity in dark in Environmental test chamber. 48 h of germinated seedlings were treated continuously for 72 h with five different extract fractions (PEEF, CEF, EAEF, MEF, AEF) at 2 mg / ml concentration in 1 % DMSO prepared with distilled water. Equal sized germinated wheat seedlings were chosen for each set of experiment and the experiment was set for triplicate of ten seedlings. Seedling root lengths were recorded at 24, 48 and 72 h. 1 % DMSO was used as culture medium for untreated control seedlings.

2.5 Phytochemical screening and estimation

Qualitative phytochemical (carbohydrates, glycosides, saponins, flavonoids, steroids, terpenoids, alkaloids, anthraquinones, tannins, phlobatannins etc.) analysis of the extract fractions was carried out using standard methods [13-16], with minor modifications [17].

2.5.1 Total phenolics content

Total phenol content was estimated following the procedure of [18], with slight modification [10]. Total phenolic content was estimated as tannic acid equivalent and expressed on dried extract mater basis and the data were presented in terms of mg/100 mg of extract.

2.6 Statistical analysis

All the assays were performed in triplicate and all the data points were expressed as Mean \pm SEM. Wheat root growth was recorded and the growth retardation percentage was calculated. Correlation was analysed between extract fractions' phenolic content and wheat seedlings growth inhibition percentage using Microsoft Excel.

3. RESULTS

3.1 Allelopathic activity in terms of wheat root growth retardation

Data clearly indicate that all the extract fractions could induce wheat root growth retardation as compared to untreated control. Here the maximum root growth was recorded from untreated control groups while the minimum root length was recorded after treatment with MEF (Figure. 1, 2). The growth inhibition was also calculated as 4.74, 32.33, 57.54, 73.06 and 10.78% respectively for the extract fractions of PEEF, CEF, EAEF, MEF and AEF at 72 h.

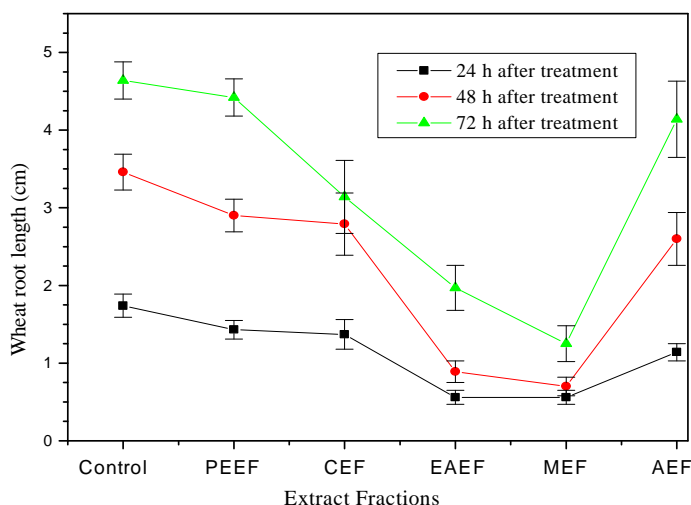


Figure1. Showing growth retardation effect of successively extracted solvent extract fractions of *A. latifolia* leaf on wheat roots with a concentration 2 mg / ml at 24, 48 and 72 h of treatment with PEEF, CEF, EAEF, MEF and AEF
Each data point is expressed as Mean \pm SEM for triplicate set of experiments

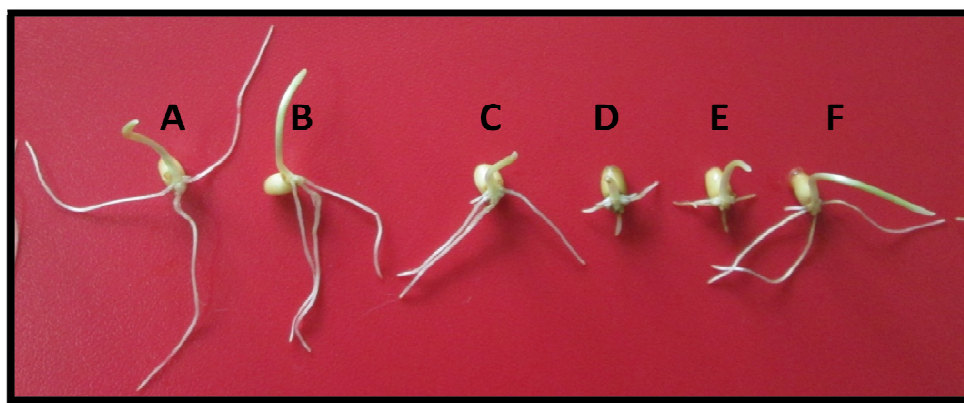


Figure 2. Showing influence of the five solvents extract fractions of *A. latifolia* leaf on wheat root growth at a concentration 2 mg / ml for 72 h where A; control, B; PEEF, C; CEF, D; EAEF, E; MEF and F; AEF

3.2 Phytochemical profile

Preliminary photochemical analysis showed the presence of various phytochemicals in the extract fractions, like steroids, terpenoids, carbohydrates, anthraquinones, glycosides, alkaloids, flavonoids, saponins and tannins, while phlobatannins were absent in all the extract fractions. Among the entire extract fractions methanol extract fraction is the richest containing various phytochemicals followed by ethyl acetate fraction. Abundance of phytochemicals is least in petroleum ether fraction, while rest of the fractions contain moderate amounts (Table. 1).

Table. 1: Showing relative qualitative and quantitative phytochemical abundance in the five successively extracted extract fractions of *A. latifolia* leaf with different solvents of increasing polarity index.

Phytochemicals	Tests performed	PEEF	CEF	EAEF	MEF	AEF
Steroids	Glacial acetic acid and H ₂ SO ₄ test	++	++	+	++	-
	Benedict's test	+	+	++	+++	+
Carbohydrates	Fehling's test	+	+	++	+++	+
	Shinoda test	+	+	+	++	++
Flavonoids	Alkaline solution test	-	-	+	++	++
	Glacial acetic acid and H ₂ SO ₄ test	-	+	+	+++	+
Terpenoids	Mayer's test	-	-	+	+	+
Alkaloids	Wagner's test	-	-	-	+	+
	FeCl ₃ test	-	-	+	++	+++
Tannins	Alkaline reagent test	-	-	+	+++	++
	Aqueous NaOH test	-	-	+	++	-
Glycosides	Fehlings test	-	-	++	++	-
	Borntrager's test	-	-	-	+	++
Anthraquinones	NaHCO ₃ Froth test	-	-	-	+	-
Saponins	HCl test	-	-	-	-	-
Phlobatannins		-	-	-	-	-

Symbols "+" and "-" indicate presence and absence of corresponding phytochemicals respectively and repetition of symbols indicates relative abundance. Here, petroleum ether extract fraction-PEEF, chloroform extract fraction-CEF, ethyl acetate extract fraction-EAEF, methanolic extract fraction-MEF and aqueous extract fraction-AEF.

3.3 Total phenolics content

Data indicate *A. latifolia* leaf MEF contains 39.53±0.75 mg tannic acid equivalent phenolics per 100 mg of dry extract matter which is slightly more than that of the AQF, 37.01±0.67. PEF contains the least phenolics, 0.85±0.28, while EAF and CHF contain moderate amounts respectively as 12.75±0.26 and 5.14±0.23 mg per 100 mg of dry extract matter (Figure. 3).

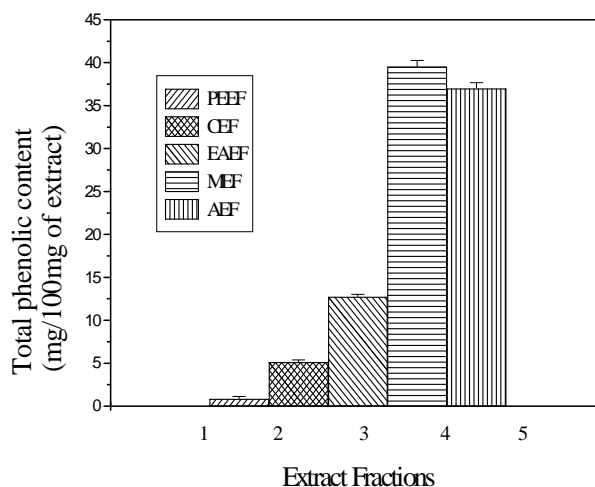


Figure3. Showing total phenolics content (tannic acid equivalent) in the five successive soxhlet extract fractions (PEEF, CEF, EAEF, MEF and AEF) of *A. Latifolia* leaf, extracted with different solvents of increasing polarity index

Data represented as Mean ± SEM of triplicate set of experiments

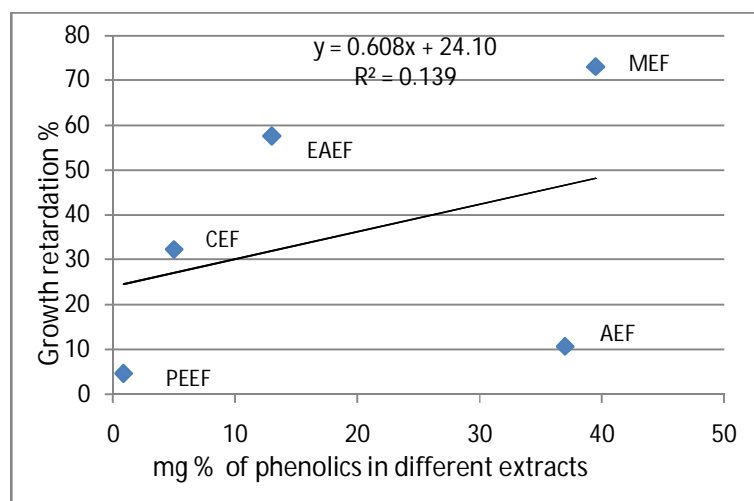


Figure 4. Showing pattern of correlation between growth inhibition capacity (Y) and total phenolic contents (X) of five different extract fractions of *A. latifolia*. Here, petroleum ether extract fraction-PEEF, chloroform extract fraction-CEF, ethyl acetate extract fraction-EAEF, methanolic extract fraction-MEF and aqueous extract fraction-AEF

4. DISCUSSION

Allelopathic effects of plant extracts in terms of seedling growth inhibition are well documented in the literature [3, 19]. Seedling growth is characterised by high metabolic rate, therefore, is highly susceptible to allelochemicals [20]. We hypothesised that allelochemicals' extract value, quality and quantity of phytochemicals and corresponding phytotoxic/allelopathy (inhibitory) potential may vary in relation to the change in polarity index of solvents. The ground leaf powder *A. latifolia* was successively fractionated with different solvents of increasing polarity index. Here, all the extract fractions were found to have some extent of effective allelochemicals having wheat root growth inhibitory actions. Plant based models are regarded as bench top models for rapid evaluation of toxicity of toxicants or other biological parameters [3, 19]. Wheat seedlings are widely used in allelopathy tests and are known for their sensitivity to plant extracts. Anomalies like rotting, necrosis, and complete atrophy of root hairs have been recorded in earlier study with wheat seedlings after treatment with crude aqueous extract of *A. latifolia* [10, 17]. Seedlings sensitivity to allelochemicals is well documented in the literature, as it is one of the characteristics that best indicate the phytotoxicity of plant extracts [12]. The wheat root growth inhibitory effect of the extract fractions of *A. latifolia* leaf showed that the allelopathic phytotoxins can be extracted with the solvents like water, methanol, ethyl acetate, chloroform and petroleum ether. Qualitative phytochemical analysis revealed the presence of various secondary metabolites like steroids, terpenoids, carbohydrates, glycosides, alkaloids, flavonoids, anthraquinones, saponins and tannins in varied quantities in the extract fractions while phlobatannins were found to be totally absent in all the extract fractions. The MEF showed the highest allelopathic inhibitory effects in terms of wheat root growth retardation (73.06% inhibition at concentration 2 mg / ml for 72 h treatment) and also associates with its abundance of allelochemicals. Our previous study with aerial parts aqueous extract of *A. latifolia* and also others studies indicate that, this plant is rich in phytochemicals. The crude aqueous extracts of aerial parts of *A. latifolia* showed the allelopathy (inhibitory) action and it may be due to phenolic acids and other soluble allelopathic compound [10]. The major objective of the present study was to fractionate the allelochemicals using five different solvents and the results indicate that almost all kinds of phytochemicals present in the plant are also present in the MEF (Table. 1). The PEEF contains carbohydrates, steroids and flavonoids, while CEF contains terpenoids in addition to those found in PEEF. Except saponins and anthraquinones, EAEF contains all the allelochemicals that are present in methanolic extract fraction. The aqueous extract fraction contains all sorts of phytochemicals except glycosides, steroids and saponins. As a solvent methanol extracts almost all sorts of phytochemicals and, moreover, the MEF showed the highest phytotoxic effect in terms of wheat root growth retardation and also correlated to its higher phenolic content and abundance of other phytochemicals (Figure. 1, 2, 3, 4). Quantitative measurement of total phenolics was done according to standard protocols (Figure. 3). Saponins are exclusively present in the MEF and also the highest allelopathy growth inhibitory action is exerted by this fraction. Higher concentration of saponins is the most common cause of inhibitory mode of allelopathy though very little is known about its biological activities as there are reports on both the inhibitory (at higher concentration) and stimulatory (at lower concentration) allelopathic activities of saponins [21]. There are reports about purified alfalfa root saponins that could affect germination and seedling growth of some weeds [21] and they concluded that these compounds have potentiality to be used as bio herbicides.

As described earlier all the other extract fractions were also effective to some extent regarding wheat root growth retardation. Amongst the various phytochemicals, phenolics are the most abundant substances that affect seedling growth and cell division [22, 23]. Plant phenolics appear to play vital roles in defense against pathogens and predators and contribute to physiological functions such as seed maturation and dormancy [24]. Phenolics are also regarded as bioactive compounds to have allelopathic potentials [25-27]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [28]. Alkaloids also exhibit allelopathic potential in terms of seed germination inhibition. Cocain, physostigmine, caffeine, quinine and berberine are considered as strong inhibitors whereas atropine, piperine, papaverine are regarded as weak inhibitors of seed germination. Triterpene glycosides isolated from Caribbean sponges *Erylus formosus* and *Ectyoplasia ferox* possess defensive and allelopathic role [21, 29]. Anthraquinones isolated from *Polygonum sachalinense* are also reported to significantly inhibit lettuce seedling growth [30]. *A. latifolia* is rich in various such phytochemicals which have made this plant a good source for agricultural deeds [12]. Thus we can conclude that *A. Latifolia* leaf polar solvent extracts may be an attractive alternative for the use as a natural product for weed control agent avoiding the application of synthetic chemical herbicides. Correlation analysis between phenolic quantity in the different extract fractions and root growth inhibition revealed that up to methanol solvent extract's phenolic quantity and allelopathy (inhibitory) action positively correlated well and with the aqueous extract fraction phenolic quantity and growth inhibition seemed poorly correlated indicating quality of phenolics may also be one of the major factor for determining allelopathic actions (Figure. 4). The best known natural bio-herbicides are phytotoxic water extracts of sorghum (*Sorghum bicolor*) and sunflower (*Helianthus annuus*) [3, 19, 31-35] which can be effectively used in plant protection without yield losses.

CONCLUSION

The induced allelopathic (inhibitory) activities of *A. latifolia* leaf extract fractions could be correlated with both the qualitative and quantitative values of the effective phytochemicals and the used solvent's nature that may be exploited in the future studies focusing on the identification and isolation of the phytotoxic chemicals for integrated weed management.

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