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Allelopathic potential of tannic acid and its equivalent phenolics extracted from aerial parts of *Ampelocissus latifolia* (Roxb.) Planch.

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Abstract: *This study was aimed to compare allelopathic effect of tannic acid with the successive solvent extract fractions of aerial parts of Ampelocissus latifolia and to identify the qualitatively superior allelopathic (inhibitory) phenolic fraction in terms of growth retardation effects in wheat root apical meristems in laboratory conditions. Five extract fractions were prepared using soxhlet apparatus by sequentially passing organic solvents with increasing polarity index through the fixed amount of dried leaf powder and the allelopathic inhibitory action of the extract fractions was studied using wheat seedlings and that was compared with the allelopathic action of tannic acid. All the test fractions and tannic acid were found to be effective regarding wheat root growth retardation in a dose dependent manner. IC_{50} values were calculated as 2.8, 1.6, 0.8, 1.1, 1.6 and 0.17 mg/ml respectively for petroleum ether (PEEF), chloroform (CEF), ethyl acetate (EAEF), methanolic (MEF) and aqueous extract fraction (AEF) and tannic acid. Tannic acid caused 93.67% growth inhibition at 1 mg/ml after 48 h. Comparative data indicated that MEF caused maximum growth inhibition (94.76% growth inhibition at 4 mg/ml after 48 h) as it contains highest amount of plant phenolics, followed by EAEF, CEF, AEF and PEEF, but while considering % root growth inhibition in terms of per phenolic mg %, EAEF showed the highest potency. Here, not only the quantity of phenolics but also the quality of phenolics is an important factor for allelopathic actions. Thus tannic acid and EAEF phenolic component may be considered as substitute of chemical herbicides.*

Key Words: *Allelochemicals, allelopathy, phytotoxicity, polyphenolics.*

I. Introduction

Allelopathy is considered as a vital ecological process that influences the primary and secondary plant succession and the structure, composition and dynamics of plant communities [1]. It is defined as stimulatory or inhibitory influence of plants on other plants due to the release of allelochemicals into the environment [2]. The phenomenon of allelopathy among plant species are responsible for natural selection in plant communities e.g. protecting the donor plant against microorganisms, insects and other pathogens or even inhibiting the growth of neighbouring plant species or stimulating the growth of the seeds [3]. In recent years, increase in the use of synthetic herbicides for weed management has raised environmental and health concerns. The researchers are now seeking alternate ways of weed management [4]. Application of allelochemicals has shown tremendous scope in agricultural pest management [5].

Allelochemicals are secondary metabolites present in all parts of the plant in varying quantities. Various phytochemicals act as potential allelopathic agents like steroids, terpenoids, carbohydrates, glycosides, alkaloids, flavonoids, anthraquinones, saponins, tannins etc. which are present in various plant parts [6]. Amongst the various phytochemicals, phenolics are the most abundant substances to affect seedling growth, cell division and cell morphology [7, 8]. They play vital roles in defense against predators and pathogens and contribute to physiological functions such as dormancy and seed maturation [9]. Phenolics are also regarded as bioactive compounds to have allelopathic potentials [10, 11].

Tannic acid is chemically called penta-m-digalloyl glucose and it occurs widely in leaves, stems, barks, fruits, root exudates, decaying plant residues and soil and is distributed throughout plant kingdom. Tannic acid, a plant polyphenol exerts anticarcinogenic activity in chemically induced cancers [12]. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidant property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds [13, 14]. There are previous study reports showing tannic acid induced fungal growth reduction and conidial germination inhibition [15]. Tannins also inhibited the germination of rice and groundnut and affected radicle development and elongation. Moreover, tannic acid exposure reduced the number of microorganisms present in the soil [16]. They are also reported to influence growth, development and reproduction of higher plants by interacting with auxin and gibberelic acid [17]. In

fact, there are various study reports emphasizing the role of tannic acid in agriculture, ecology, human health and welfare and many other aspects [12, 13, 16].

Besides phenolics, alkaloids are also associated with medicinal values and one of their common biological properties is their cytotoxic activity [18]. Alkaloids also exhibit allelopathic potential. Different alkaloids like cocain, physostigmine, caffeine, quinine, berberine, atropine, piperine are effective in inhibiting seed germination to some extent. Triterpene glycosides isolated from Caribbean sponges *Erylus formosus* and *Ectyoplasia ferox* possess defensive and allelopathic roles [19]. Anthraquinones isolated from *Polygonum sachalinense* are also reported to significantly inhibit lettuce seedling growth [20]. Thus allelochemicals play significant role in agro-ecosystems. Allelochemicals also affect seed germination, growth, quality and quantity of crop products [2]. Synthetic chemicals are widely used to control unnecessary herbs or weeds but their indiscriminate use is continuously being stopped out because of their adverse effects on the environment. As a result, the use of plant secondary metabolites as herbicides or weedicides is gaining renewed interest. Some of the allelochemicals exhibit biological activity and have been used in the pharmaceutical and agrochemical industries [21, 22]. Allelopathic effects of plant extracts in terms of seedling growth inhibition are well documented in the literature [23]. Seedling growth is characterised by high metabolic rate and therefore is highly susceptible to allelochemicals [24]. Sensitivity of seedlings to allelochemicals is one of the characteristics that best indicate the phytotoxicity of plant extracts [25]. Wheat seedlings are widely used in allelopathy tests for their sensitivity to plant extracts.

Ampelocissus latifolia (Roxb.) Planch. (Family: Vitaceae) is herbaceous climber and is native to Indian subcontinent. This plant is used extensively for its medicinal properties. Recently antibacterial, antioxidant [26, 27] cytotoxic and phytotoxic [28], antiproliferative [29] and allelopathic activities of *A. latifolia* have been reported [30, 31]. This plant exhibits anti-inflammatory activity by inhibiting histamine kinin and prostaglandin pathway [32]. Epicalmistrin, uvaribonin and chalcone obtained from the root of *Ampelocissus* showed important cell growth inhibitory activity against human cancer cell lines [33]. In our previous studies we have shown AAEAL induced root morphological and cytological alterations like rottening, bulging, atrophication of root hairs in the treated onion, wheat and moong bean seedlings with significant reduction in mitotic index and induced chromosomal abnormalities in the root tip cells [28, 29]. Our study also indicated allelopathic activity of different polar and non polar extract fractions of this plant [30, 31].

Although various studies have been done so far regarding allelopathy, phenolic quality based allelopathic potential of successive soxhlet extract fractions of *A. latifolia* along with the determination of most effective allelopathic (inhibitory) extract fractions and use of tannic acid for allelopathy are not well studied using wheat root apical meristem cells. Therefore, the novel aspect of the present study was to determine tannic acid and *A. latifolia* leaf extracts induced root growth retardation of wheat seedlings and the root morphology alteration in relation to quality of phenolics and the polarity of solvents used for phenolics extraction.

II. Materials and Methods

2.1 Chemicals

Tannic acid powder, petroleum ether, chloroform, ethyl acetate and methanol 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 leaves of *A. latifolia* were collected from The Burdwan University Golapbag campus, West Bengal and the plant species was taxonomically identified by Prof. A. Mukherjee (Taxonomist), Department of Botany, The University of Burdwan. Collected leaves were washed thoroughly with normal tap water. Then the leaves were shade dried, powdered using grinding machine and the leaf powder was stored in air tight container for future use.

2.3 Extract preparation

Powdered leaf material (30g) 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 then condensed using rotary vacuum evaporator and kept for evaporation to remove solvents in hot air oven at 50^{o}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 the study of allelopathic action.

2.5 Culture and treatment of wheat seedlings

Wheat seedlings were cultured following the method as described earlier in detail [28]. 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^{o}C and 65% humidity in dark in Environmental test chamber. 48 h aged, equal sized germinated wheat seedlings were treated continuously with five different extract fractions (PEEF, CEF, EAEF, MEF, AEF) at 0.5, 2 and 4 mg/ml concentrations prepared in 1% DMSO solution for 24 and 48 h. Growth retardation pattern was compared to tannic acid, a standard phenol, used as positive control (using concentrations of 0.05, 0.2, 0.4, 0.6, 1 mg/ml). Seedling root lengths (in cm) were recorded at 24 and 48 h.

In another set of experiment 48 h germinated wheat seedlings were treated with the extract fractions keeping their phenolic content in equal amount (i.e.0.26 mg), and thus varying their concentrations (PEEF 30 mg/ml, CEF 5.2 mg/ml, EAEF 2 mg/ml, MEF 0.6 mg/ml and AEF 0.7 mg/ml) to find out the most effective extract fraction in terms of % wheat root growth retardation based on per mg % phenolics

2.6 Total phenolics content

Total phenol content was estimated following the procedure of Makkar *et. al*.,[34], with slight modification [31]. 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.

2.7 Statistical analysis

All the assays were performed in triplicate and all the data points were expressed as Mean± SEM. Wheat root growth was recorded and the growth retardation percentage was calculated. Differences between negative control and treated groups were compared by Students' t test. Level of significance was considered at $p<0.001$. Correlation of coefficient (r) and coefficient of determination (r^2) were calculated using Microsoft Excel. IC_{50} values of the extract fractions and that of tannic acid standard were calculated using probit analysis.

III. Results

3.1 Allelopathic activity in terms of wheat root growth retardation

Data indicated that the leaf extract fractions of *A. latifolia* have differential capacity to induce dose dependent wheat root growth retardation as compared to untreated control (Figure 1, 2). Here, the maximum root growth was recorded in untreated control groups that were maintained in 1% DMSO, while the minimum root length was recorded after treatment with MEF at our highest concentration of 4 mg/ml at 48 h (0.22±0.02 cm), compared to tannic acid control (1mg/ml) which was 0.28±0.04 cm (Figure 3, 4). The growth inhibition after 24 h of extract, at concentrations of 4 mg/ml, exposure was calculated as 35.63, 81.61, 86.21, 87.36 and 68.97% respectively for PEEF, CEF, EAEF, MEF and AEF. The growth inhibition after 48 h of extract exposure, at 4mg/ml concentration, was also calculated as 68.57, 91.43, 93.81, 94.76 and 75.24% respectively for PEEF, CEF, EAEF, MEF and AEF. Tannic acid was used as positive control that also could induce 55.6, 81, 85.07 and 93.67% growth retardation respectively at 0.2, 0.4, 0.6 and 1 mg/ml after 48 h (Figure 3). IC_{50} values were calculated for all the fractions and compared to tannic acid standard as PEEF-2.8 mg/ml, CEF-1.6 mg/ml, EAEF-0.8 mg/ml, MEF-1.1 mg/ml, AEF-1.6 mg/ml, Tannic acid-0.17 mg/ml after 48 h of treatment.

While considering % root growth inhibition in terms of per phenolic mg %, EAEF (2 mg/ml) showed highest potency (91.35% growth inhibition at 48 h) followed by PEEF, CEF, MEF and AEF (Figure 5, 6) signifying the fact that not only the quantity but also the nature of plant phenolics determine the potency of plant extracts to act as allelopathic agents, which is evidenced by the highest activity of EAEF, showing the most efficacy compared to the other fractions in spite of containing equal amount of phenolics. EAEF (phenolic content 0.26 mg/2 mg EAEF) was found to be the most effective causing 91.35% wheat root growth retardation at 48 h, compared to PEEF (phenolic content 0.26 mg/30 mg PEEF), which caused 89.19% growth retardation, CEF (phenolic content 0.26 mg/5.2 mg CEF), which caused 85.95% growth retardation, MEF (phenolic content 0.26 mg/0.6 mg MEF) which caused 32.43% growth retardation and AEF (phenolic content 0.26 mg/0.7mg AEF), which caused 7.57% root growth retardation (Figure 5).

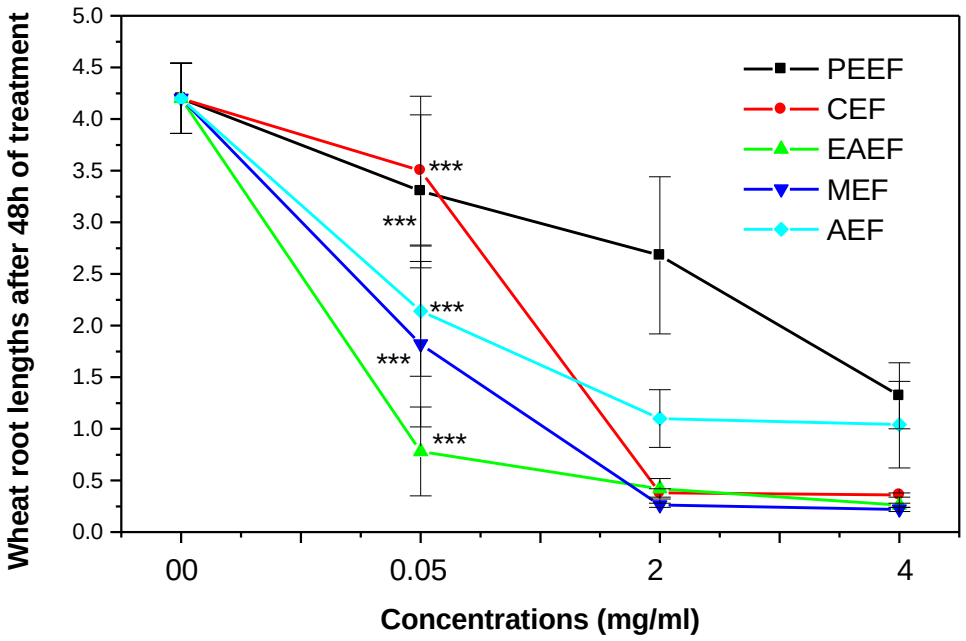


Figure 1: Showing influence of five successive extract fractions of *A. latifolia* (PEEF, CEF, EAEF, MEF, AEF) leaves on wheat root lengths after 48 h of treatment and compared to negative control. Each data point is expressed as Mean ± SEM for triplicate set of experiments. ***Significant at $p<0.001$ with Student's t- test.

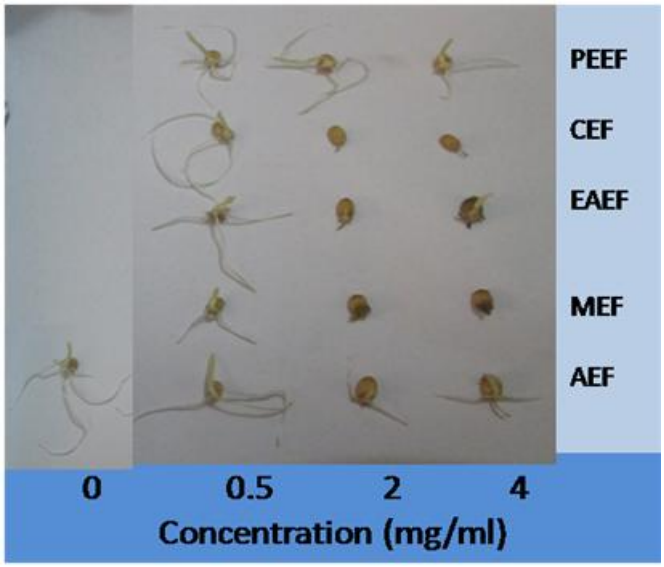


Figure 2: Showing influence of five successive extract fractions (PEEF, CEF, EAEF, MEF, AEF) of *A. latifolia* leaves (0.5, 2, 4 mg/ml concentrations each) on wheat root lengths after 48 h of treatment and compared to negative control (1% DMSO).

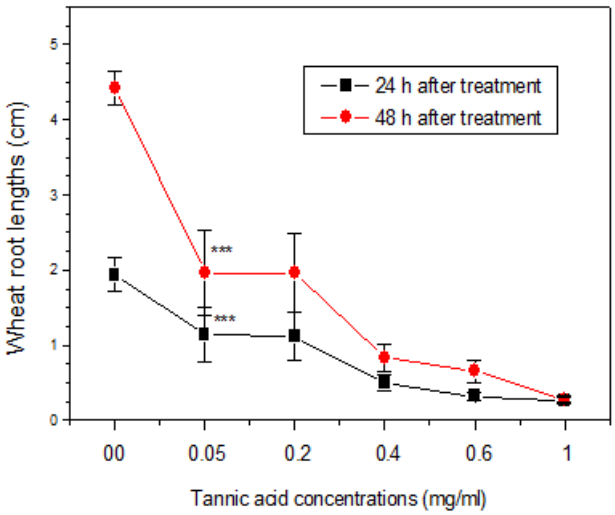


Figure 3: Showing wheat root lengths after 24 h and 48 h of treatment with Tannic acid (0.05, 0.2, 0.4, 0.6 and 1 mg/ml concentrations) compared to negative control. Each data point is expressed as Mean±SEM for triplicate set of experiments. ***Significant at $p<0.001$ with Student's t- test.

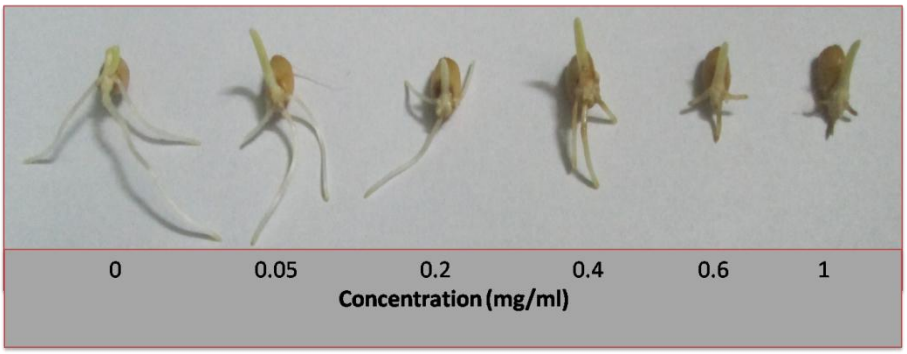


Figure 4: Showing effect of Tannic acid (0.05, 0.2, 0.4, 0.6 and 1 mg/ml concentrations) on wheat root growth after 48 h of treatment.

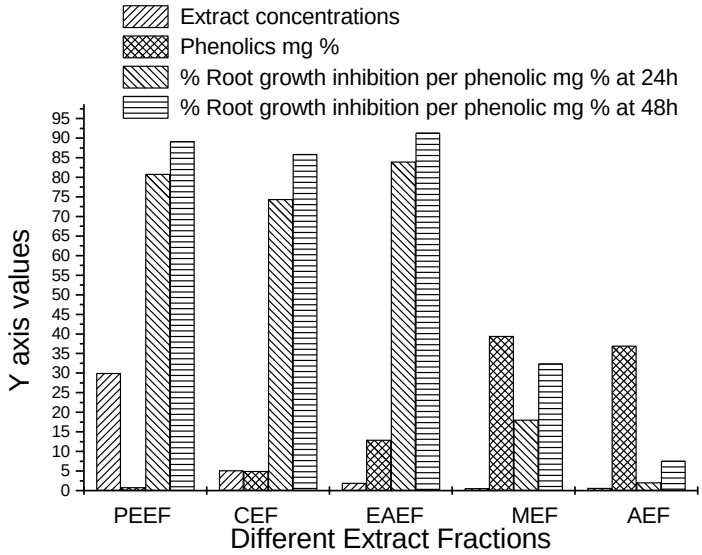


Figure 5: Showing extract concentrations, phenolics mg % and % root growth retardation per mg % phenolics at 24 and 48 h of treatment (Y axis values) in relation with five extract fractions: PEEF, CEF, EAEF, MEF and AEF (X axis parameters).

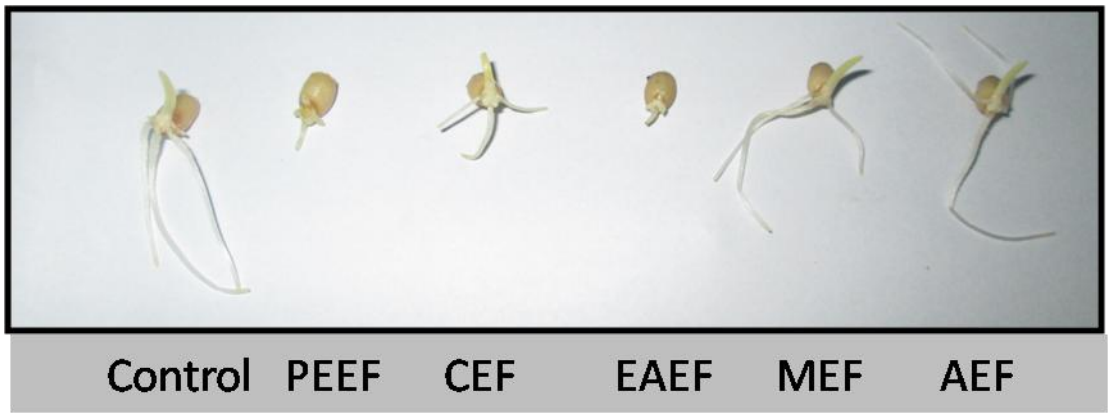


Figure 6: Showing influence of the five extract fractions (PEEF, CEF, EAEF, MEF and AEF) containing equal amount of phenolics (0.26 mg) on wheat root lengths after 48 h of treatment.

3.2 Correlation between increasing concentrations of extract fractions, standard tannic acid and wheat root growth inhibition

Allelopathic effect of plant extracts in terms of seedling growth inhibition is well documented in the literature [31, 35]. There are various study reports showing that plant growth inhibition increases with increasing extract concentrations. Here, in this study correlation of coefficient (r) and coefficient of determination (r^2) between increasing concentrations of five extract fractions as well as tannic acid and root growth retardation percentage were determined (Table 1, Figure 7-12). Our results indicate a linear positive correlation between increasing extract concentrations and percentage of root growth retardation.

Table 1. Correlation of coefficient (r) and coefficient of determination (r^2) between increasing concentrations of five extract fractions of *A. latifolia* as well as tannic acid and wheat root growth retardation percentage.

Extract Fractions and Positive Control used	r (r^2)
PEEF	0.991(0.983)
CEF	0.825(0.680)
EAEF	0.955(0.912)
MEF	0.834(0.696)
AEF	0.849(0.720)
Tannic acid	0.868(0.753)

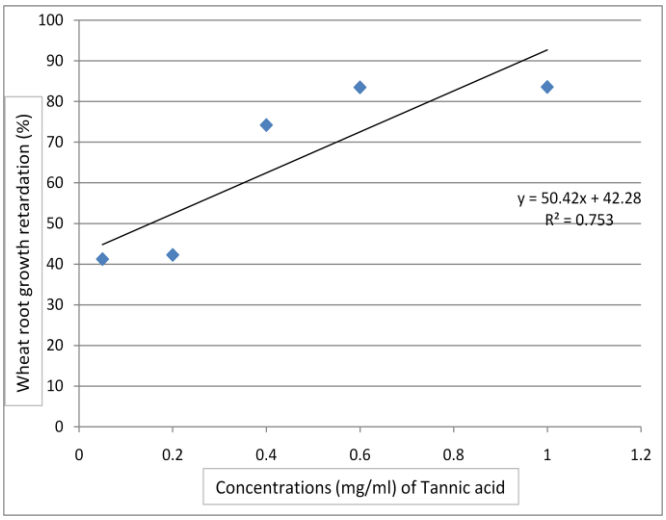


Figure 7: Showing a positive linear correlation between different concentrations of Tannic acid standard (X) and wheat root growth inhibition (Y).

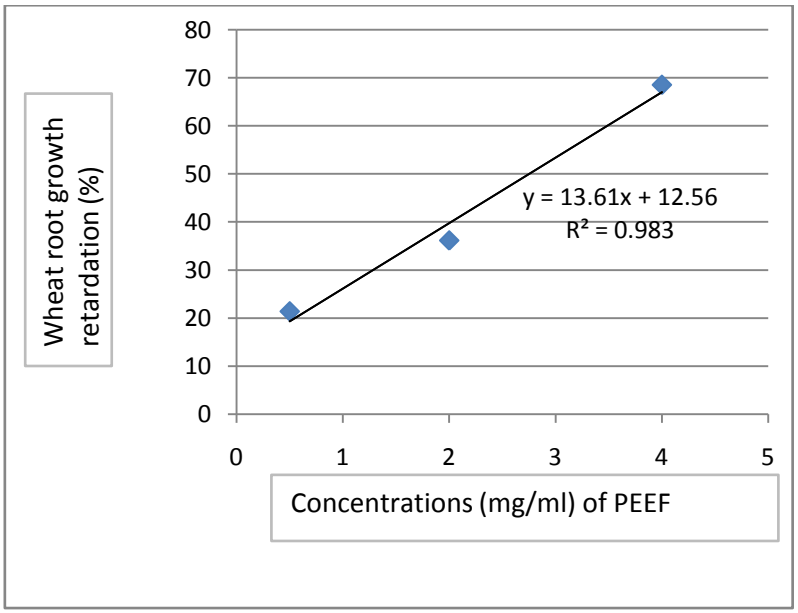


Figure 8: Showing a positive linear correlation between different concentrations of PEEF (X) and wheat root growth inhibition (Y).

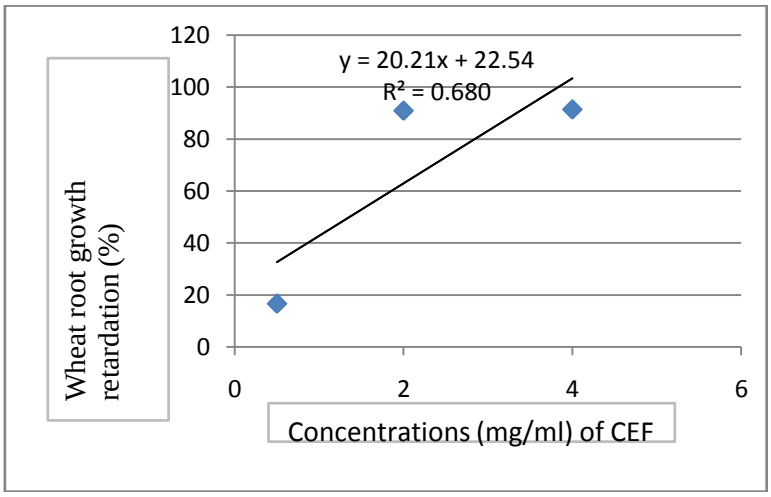


Figure 9: Showing a positive linear correlation between different concentrations of CEF (X) and wheat root growth inhibition (Y).

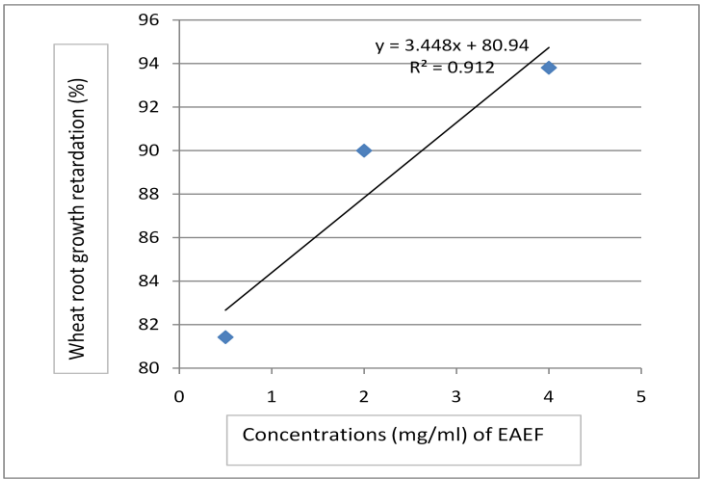


Figure 10: Showing a positive linear correlation between different concentrations of EAEF (X) and wheat root growth inhibition (Y).

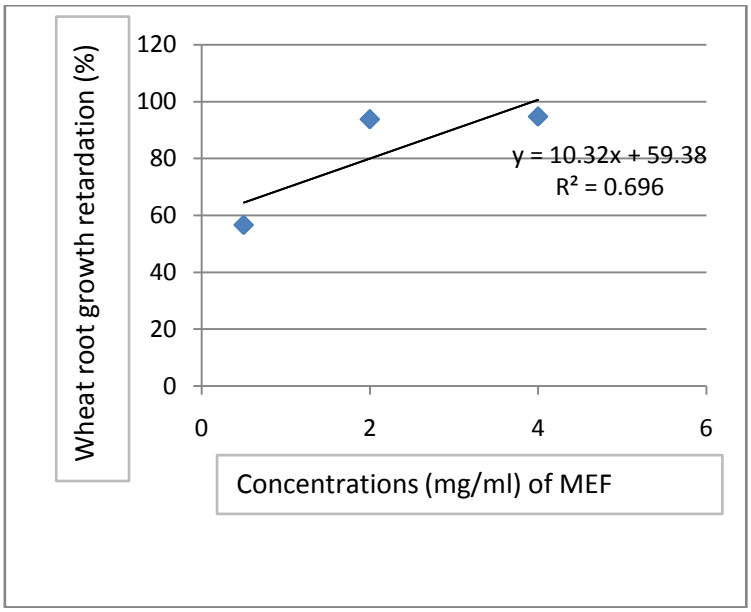
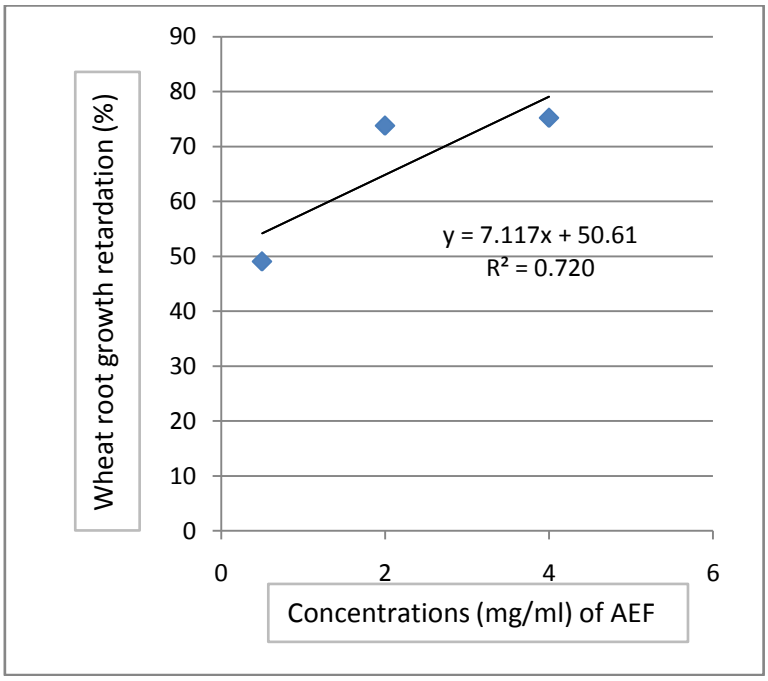


Figure 11: Showing a positive linear correlation between different concentrations of MEF (X) and wheat root growth inhibition (Y).



(E)

Figure 12: Showing a positive linear correlation between different concentrations of AEF (X) and wheat root growth inhibition (Y).

3.3 Total phenolic 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 AEF, 37.01±0.67. PEEF contains the least phenolics, 0.85±0.28, while EAEF and CEF contain moderate amounts respectively as 12.75±0.26 and 5.14±0.23 mg per 100 mg of dry extract matter (Figure 5) [31].

IV. Discussion

Here, in the present study allelopathic potential of tannic acid and the most effective extract fraction of *A. latifolia* was determined in terms of % wheat root growth retardation based on per mg % phenolics where treatment to wheat seedlings were given keeping the amount of phenolics in equal amount for all the extract

fractions to test whether the mode of inhibitory allelopathy also depends on the quality of phenolics in addition to the phenolics quantity.

In the present study we have analyzed tannic acid (concentrations of 0.05, 0.2, 0.4, 0.6, 1 mg/ml) induced root growth inhibition in wheat seedlings. Tannic acid induced dose-dependent growth retardation (Figure 3, 4) and the IC_{50} value was calculated as 0.17 mg/ml after 48 h of treatment indicating a good future prospect to be used as natural herbicidal agent. In our previous study, we have shown tannic acid induced phytotoxicity in wheat root apical meristem cells with fluorescence microscopic analysis [28]. There are study reports showing anticarcinogenic [12], antifungal [15], antimicrobial [16] and antioxidant [13] activities of tannins.

In the present study the ground leaf powder of *A. latifolia* was successively extracted with the different solvents, having increasing polarity index, to get various extract fractions like PEEF, CEF, EAEF, MEF and AEF. All the extract fractions, from non polar to polar, were found to have some extent of effective allelochemicals having wheat root growth inhibitory actions (Figure 1, 2). Here, different concentrations (0.5, 2, and 4 mg/ml) of all the extract fractions were used and treatment was given to the germinated wheat seedlings for 48 h, data of root length was recorded after 24 and 48 h of extract exposure. Thus, the allelochemicals were distributed over several fractions, indicating that more than one compound is responsible for this allelopathic inhibitory action. Dose dependent study with all the extract fractions as compared with that of tannic acid equivalent denotes that MEF is the richest in plant phenolics (39.53±0.75 mg tannic acid equivalent phenolics per 100 mg of dry extract matter) and the most potent regarding growth retardation (Figure 1, 2) and root morphology alterations like rottening and change in root colouration followed by EAEF. The growth retardation patterns due to exposure of our extract fractions seemed to be comparable with the tannic acid induced growth arrest in wheat root apical meristem cells (Figure 3, 4, 7). Here, the concentrations of MEF and EAEF were considerably higher than that of tannic acid that may be due to the fact that the extract fractions were in crude form.

The coefficient of determination (r^2) and correlation of coefficient (r) of the different concentrations of the five extract fractions and their root growth inhibitory actions were determined (Table 1, Figure 8-12). Data indicate the growth inhibitory effects of the various concentrations of all the solvent fractions showed increased inhibition that positively correlates with the increasing concentrations of all extract fractions (Table 1, Figure 8-12).

Qualitative phytochemical analysis revealed the presence of various secondary metabolites like steroids, terpenoids, carbohydrates, glycosides, flavonoids, alkaloids, saponins, anthraquinones and tannins in varied quantities in the extract fractions while phlobatannins were found to be totally absent in all the extract fractions [31]. Anomalies like rottening, 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* [28, 29]. The crude aqueous extracts of aerial parts of *A. latifolia* showed allelopathic (inhibitory) action and it may be due to phenolic acids and other soluble allelopathic compound [30]. Quantitative measurement of total phenolics was done following standard protocols. Plant phenolics play essential roles in defense against pathogens and predators and contribute to physiological functions such as seed maturation and seed dormancy [9]. Our previous study also indicated cytogenotoxic, phytotoxic and antiproliferative potentials of leaf aqueous extracts of *A. latifolia* (LAEAL) wherein the mitotic index depression bioassay on onion root apical meristem cells revealed that LAEAL treatment could reduce the mitotic index. This dose dependent decreased mitotic index percentage suggested that the application of LAEAL to root apical meristem cells caused cytotoxic stress with reduced number of cells entering into mitotic cycle and all together increase in interphase cell frequency [28, 29] which is also correlated to our present findings. Allelopathic bioassays indicate that the methanol and ethyl-acetate fractions of *A. latifolia* leaves were effective fractions. They caused more than 50% growth inhibition at only 0.5 mg/ml of extract concentrations. The allelopathic activity of *A. latifolia* may occur due to the interaction of different classes of phytochemicals such as fatty acids, phenolics, alkaloids etc. present in this plant. Allelopathic potential can be attributed to the fact of disruption of mitochondrial respiration, disruption of the activity of metabolic enzymes involved in glycolysis and in pentose phosphate pathway [35]. In our earlier studies, seedling root growth retardation effect of crude aqueous extract and the successive solvent extract fractions of *A. latifolia* leaves were shown in onion, wheat and moong bean root apical meristems. Our results indicated that root apical meristems are sensitive to plant extracts; these results are also in agreement with the previous study reports [36, 37] which discloses that root growth retardation is a result of suppression of cell division and chromosomal aberrations [28, 31]. When the cell cycle becomes altered or deregulated by some indigenous or exogeneous agents, different check points become activated to halt cell cycle machinery to allow for recovery or to proceed to cell death. Internucleosomal degradation, chromatin condensation, metaphase arrest due to microtubular disruption, delayed entry into anaphase stage etc. are the underlying causes behind delayed cell cycle kinetics resulting in significant growth retardation and altered cellular morphology. A number of earlier studies have suggested that levels of root growth inhibition increase with increasing extract

concentration [28, 29, 31, 37] and there is linear positive correlation between the two variables (Figure 7-12). In our previous report we have hypothesized that allelochemicals' extract value, quality and quantity of phytochemicals and corresponding phytotoxic/allelopathic (inhibitory) potential may vary in relation to the change in polarity index of solvents [31].

Here, 94.76% growth inhibition was observed at 4 mg/ml of MEF after 48 h and a comparable growth retardation effect was seen with tannic acid treatment, 93.67% at 1 mg/ml after 48 h (Figure 1-4), but when % root growth inhibition per phenolic mg % was considered (Figure 5, 6), EAEF (phenolic content 0.26 mg/2mg EAEF) was found to be the most effective extract fraction, 84.04% wheat root growth inhibiting capability at 24 h and 91.35% at 48 h and subsequently in decreasing order as found with PEEF (89.19% root growth retardation at 48 h, with phenolic content 0.26 mg/30 mg PEEF), CEF (85.95% root growth retardation at 48 h, with phenolic content 0.26 mg/5.2 mg CEF), MEF (32.43% root growth retardation at 48 h, with phenolic content 0.26 mg/0.6 mg MEF) and finally AEF with 7.57% root growth retardation at 48 h, with phenolic content 0.26 mg/0.7 mg of AEF. Thus it can be inferred that although the amount of plant phenolics play a vital role in plant defense, the nature of phenolics extracted with ethyl acetate solvent and their quality based functional role cannot be ignored. The MEF at 4 mg/ml shows the highest potency in terms of root growth retardation with 1.73 mg phenolics /4 mg extract, while reduction in the phenolic content to 0.26 mg and thus reduction in the amount of MEF used (0.6 mg) is not so much efficient like EAEF, having far greater effectiveness with 0.26 mg of phenolics only (91.35% growth retardation by EAEF compared to 32.43% by MEF, both of them containing 0.26 mg phenolics equivalent). Thus we can see that although polar solvents are more effective in extracting phenolics (Figure 5) than non polar solvents, the two sorts of solvents i.e. one polar and the other non polar also differ regarding the nature of phenolics they extract. Here, although the quantity of phenolics were found to be higher in both MEF and AEF compared to other fractions, the allelopathic inhibitory activity was greater for non polar extract fractions (EAEF, PEEF and CEF) when these fractions were compared for their allelopathic potential keeping their phenolics in equal amount (Figure 5, 6). Thus we have seen that in general the phenolics extracted by non polar solvents are much more efficient as allelochemicals as compared to those extracted by polar solvents when applied in equivalent amounts. Though we have hypothesized that not only the quantity but also the quality of plant phenolics play important role in plant defense mechanism, the allelopathic function of various other phytochemicals present in different extract fractions cannot be overlooked. Moreover, though the quality of phenolics is considered to be the major player in this context, the other secondary metabolites may also work together to contribute in plant defense, acting as inhibitory or stimulatory allelochemicals.

V. Conclusion

Tannic acid and *Ampelocissus latifolia* extract fractions could inhibit wheat root growth indicating that *A. latifolia* leaf extracts contain bio-active allelopathic compounds wherein ethyl acetate extract fraction contained the superior qualities of phenolics with allelopathic inhibitory actions as compared with the other extract fractions. Thus the effectiveness of allelopathic action depends on both the quantity and quality of phenolics and *A. latifolia* may hold future prospect as a source of biological herbicidal as well as weedicidal agents, though, further field studies are needed to evaluate the potential allelopathic actions on the neighborhood species co-occurring in nature with this species.

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References

[1]. A.E. Smith, and L.D. Martin, Allelopathic characteristic of three cool-season grass species in forage ecosystem, *Agron. J., 86, 1994, 243-246.*
[2]. E.L. Rice, *Allelopathy*, (2nd Ed. Acadamic Publishers, New York, USA, 1984), pp 424.
[3]. F.A. Einhelling, Interactions involving allelopathy in cropping systems, *Agronomy Journal, 88, 1996, 886-893.*
[4]. M. Jamil, Z.A. Cheema, M.N. Mushtaq, M. Farooq and M.A. Cheema, Alternative control of wild oat and canary grass in wheat fields by allelopathic plant water extracts, *Agron. Sustain. Dev., 29, 2009, 475–482.*
[5]. M. Farooq, K.H.M. Siddique, H. Rehman, T. Aziz, A. Wahid and D. Lee, Rice direct seeding experiences and challenges, *Soil Till. Res., 111, 2011, 87–89.*
[6]. J.P. Barnes and A.R. Putnam, Role of benzoxazinones in allelopathy by rye (*Secale cereale* L.), *J. Chem. Ecol., 13, 1987, 889.*
[7]. J. Angayarkanni, K.M. Ramkumar, T. Poornima and U. Priyadarshini, Cytotoxic activity of *Amorphophallus paeoniifolius* tuber extracts *in vitro*. *Am. Eurasian. J. Agric. Environ. Sci., 2, 2007, 395–398.*
[8]. J.M. Fachinetto, M.D. Bagatini, J. Durigon, A.C.F. Silva and S.B. Tedesco, Anti-proliferative effect of infusions of *Achyrocline satureioides* on the *Allium cepa* cell cycle, *Rev. Bras. Farmacogn., 17, 2007, 49–54.*
[9]. B. Winkel-Shirley, Biosynthesis of flavonoids and effects of stress, *Curr. Opin. Plant Biol., 5, 2002, 218-223.*
[10]. A. Baghestani, C. Lemieux and G.D. Leroux, Determination of allelochemicals in spring cereal cultivars of different competitiveness, *Weed Sci., 47, 1999, 498–504.*

[11]. S.U. Chon and Y.M. Kim, Herbicidal potential and quantification of suspected allelochemicals from four grass crop extracts, *J. Agron. Crop. Sci., 190, 2004, 145–150.*

[12]. S. Nam, D.M. Smith and Q.P. Dou, Tannic acid potently inhibits tumor cell proteasome activity, increases p27 and Bax expression, and induces G1 arrest and apoptosis, *Cancer Epidemiol. Biomarkers Prev., 10(10), 2001, 1083-1088.*

[13]. Z.Y. Wang, J.Y. Hong, M.T. Huang, K.R. Reuhl, A.H. Conney and C.S. Yang, Inhibition of nitrosamine-induced tumorigenesis by green tea and black tea. In: Phenolic Compounds in Food and Their Effects on Health. II. Antioxidants and Cancer Prevention, *Cancer Res., 52, 1992, 1943–1947.*

[14]. H.F. Stich and M.P. Rosin, Naturally occurring phenolics as antimutagenic and anticarcinogenic agent. In: Nutritional and Toxicological Aspects of Food Safety, (Friedman, W., Ed., Adv. Exp. Med. Biol., Plenum Press, New York and London, 1984), volume. 177 p 1-29.

[15]. S.H. Abdollahzadeh, R.Y. Mashouf, H. Mortazavi, M.H. Moghaddam, N. Roozbahani, and M. Vahedi, Antibacterial and antifungal activities of *Punica granatum* peel extracts against oral pathogens, *Journal of Dentistry, 8(1), 2011, 1–6.*

[16]. A. Scalbert, Antimicrobial properties of tannins, *Phytochemistry, 30, 1991, 3875.*

[17]. M.R. Corcoran, T.A. Geissman and B.O. Phinney, Tannins as Gibberellin Antagonists, *Plant Physiol., 49, 1972, 323-330.*

[18]. A.I. Waechter, A. Cave, R. Hocquemiller, C. Bories, V. Munoz and A. Fournet, Antiprotozoal activity of aporphine alkaloids isolated from Unonopsis buchtienii (Annonaceae), *Phytother. Res., 13, 1999, 175-177.*

[19]. J. Kubanek, K.E. Whalen, S. Engel, S.R. Kelly, T.P. Henkel, W. Fenical and J.R. Pawlik, Multiple defensive roles for triterpene glycosides from two Caribbean sponges, *Oecologia, 131, 2002, 125-136.*

[20]. M. Inove, H. Nishimura, H.H. Li and J. Mizutani, Allelochemicals from Polygonum sachalinense Fr. Schm. (Polygonaceae), *Journal of Chemical ecology, 18(10), 1992, 1833-1840.*

[21]. M. Hamburger and K. Hostettmann, Bioactivity in plants: The link between phytochemistry and medicine, *Phytochemistry, 30(12), 1991, 3864-3874.*

[22]. J.R. Vyvyan, Allelochemicals as leads for new herbicides and agrochemicals, *Tetrahedron, 58(9), 2002, 1631-1646.*

[23]. P.U. Grisi, M.A. Ranal, S.C.J. Gualtieri and D.G. Santana, Allelopathic potential of *Sapindus saponaria* L. Leaves in the control of weeds, *Acta Scientiarum Agronomy, 34, 2012, 1-9.*

[24]. R. Cruz-Ortega, A.L. Anaya, B.E. Hernandez-Bautista and G. Laguna-Hernandez, Effects of allelochemical stress produced by *Sicyos deppei* on seedling root ultrastructure of *Phaseolus vulgaris* and *Cucurbita ficifolia*, *Journal of Chemical Ecology, 24(12), 1998, 2039-2057.*

[25]. I. Chung, M. Ahn and S.J. Yun, Identification of allelopathic compounds from rice (Oryza sativa L.) straw and their biological activity, *Can. J. Plant Sci., 81, 2001, 815–819.*

[26]. S. Choudhury, H.R. Chowdhury and S. Mandal, Pharmacognostic studies of *Ampelocissus latifolia* (Roxb.) Planch. an important ethnomedicinal plant, *Intern. J. Curr. Res., 5(3), 2013, 643-648.*

[27]. P.A. Pednekar and B. Raman, Antimicrobial and antioxidant potential with FTIR analysis of *Ampelocissus latifolia* (Roxb.) Planch. leaves, *Asi. J. Pharmac. and Clin. Res., 6(1), 2013, 157-162.*

[28]. A. Chaudhuri and S. Ray, Evaluation of phytotoxic and cytogenotoxic potentials of leaf aqueous extract of *Ampelocissus latifolia* (Roxb.) Planch. in relation to its total polyphenol content, *Int. J. Pharm. Bio. Sci., 5(4), 2014, 225 – 235.*

[29]. A. Chaudhuri and S. Ray, Antiproliferative activity of phytochemicals present in aerial parts aqueous extract of *Ampelocissus latifolia* (Roxb.) Planch. on apical meristem cells, *Int. J. Pharm. Bio. Sci., 6(2), 2015a, 99 – 108.*

[30]. A. Chaudhuri, L.M. Kundu, S. Dutta, S. Chatterjee, S. Goswami, G.C. Roy and S. Ray, Allelopathic effects of aerial parts aqueous extract of *Ampelocissus latifolia* (Roxb.) Planch. in apical meristem cells, *Asian Journal of Plant Science and Research, 5(3), 2015, 11-16.*

[31]. A. Chaudhuri and S. Ray, Determination of effective allelopathic (inhibitory) extract fractions of *Ampelocissus latifolia* (Roxb.) Planch. Leaf**.** *European Journal of Experimental Biology, 5(8), 2015b, 1-7.*

[32]. C.T. Tamilarashi, U. Subasini, S. Kavimani and B. Jaykar, Phytochemical and pharmacological evaluation of *Ampelocissus latifolia, Anc. Sci. of Life, 20(1), 2000, 14-18.*

[33]. G.R. Pettit, V.J. Mukku, G. Craqq, D.L. Herald, J.C. Knight and C.L. Herald, Antineoplastic agents.558. *Ampelocissus* sp. cancer cell growth inhibitory constituents, *J. Nat. Prod., 71(1), 2008, 130-133.*

[34]. H.P.S. Makkar, M. Blummel, N.K. Borowy, and K. Becker, Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods, *J. Sci. Food Agric., 61,1993, 161-165.*

[35]. A. Muscolo, M.R. Pauccio and M. Sidari, The effects of phenolic acids extracted from two different soils in Aspromonte on germination of *Pinus laricio* seeds, *Fresenius Environmental Bulletin, 10, 2001, 659–663.*

[36]. S. Ray, S. Chatterjee and C.S. Chakrabarti, Antiproliferative activity of allelochemicals present in aqueous extract of *Synedrella nodiflora* (L.) Gaertn. in apical meristems and Wistar rat bone marrow cells, *Iosr. J. Pharm., 3(2), 2013, 1-10.*

[37]. A.K. Choudhary, D. Singh and J.A. Kumar, Comparative study of screening methods for tolerance to aluminum toxicity in pigeonpea [Cajanus cajan (L.) Millspaugh], *Australian Journal of Crop Science, 5(11), 2011, 1419-1426.*