Pak. J. Weed Sci. Res., 19(3): 239-256, 2013

QUALITATIVE DETERMINATION OF ALLELOCHEMICALS IN *Psidium guajava* L. LEAVES FOR THEIR EFFECT AGAINST WHEAT AND CANARY GRASS

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ABSTRACT

Excessive use of herbicides in fields threatens the environment by destroying flora and fauna. Allelopathic substances might be proved as a substitute of chemical herbicides to suppress the target weeds. In this study, the Psidium guajava L. leaves were extracted with 70% (v/v) methanol for the constitutional composition. The allelopathic effects were investigated against pre- and post-germinated wheat and canary grass seeds. Among the evaluated 16 allelochemicals, some eight components (Ellagic acid, 3,4-dihydroxybenzoic acid, Feruic acid, Galluic acid, Vivillic acid, 3-hydroxy-benzoic acid, Gallic acid and p-coumaric acid) were identified by comparing the chromatograms of the unknown with standard ones. The pre- and post-germinated bioassays were taken into consideration for wheat and canary grass separately. The results showed that over all 70% methanol extract of P. guajava L. leaves (2.5-10%) increased the shoot length and root linearly for both pre-germinated wheat bioassays (14.0 – 16.5cm and 12.9 - 15.0cm and post germinated wheat bioassays 13.85 - 16.25 cm and 11.15 - 14.50 cm, respectively) as compared with control ones. Similarly, allelochemicals of P. guajava L. suppressed both the pre-germinated and post-germination bioassays of canary grass as compared with control ones (7.35 - 0.45cm and 6.50 - 2.75cm for shoot and 1.65 - 0.05cm and 2.15 - 0.75cm for root, respectively). These results suggest that P. guajava L. may contain growth inhibitory substances and may possess allelopathic potential. Therefore, leaves of P. guajava L. may be a possible candidate for the isolation and identification of allelopathic substances and for the development of natural herbicides for sustainable agricultural production.

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Key words: P*. guajava* L., identification, allelopathy, wheat, canary grass.

INTRODUCTION

The term allelopathy refers to any process involving secondary metabolites (allelochemicals) produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems including positive and negative effects. Allelochemicals from plants are released into the environment by exudation from roots, leaching from stems and leaves or decomposition of plant material (Rice, 1984; Lovett and Ryuntyu, 1992). Plants or organisms that release these compounds are called "donor species", while those that are influenced in their growth and development are called "target or recipient species". Allelopathy includes plant-plant, plant-microorganisms, plant-virus, plant-insects and plant-soil-plant chemical interactions. Allelopathic effects can be stimulatory or inhibitory, depending on the identity of the active compound on the static and dynamic availability, persistence and fate of organics in the environment and on the particular target species (Inderjit and Keating, 1999). Also, allelopathy is generally accepted as a significant ecological factor in determining the structure and composition of plant communities (Scrivanti *et al*., 2003). Literature justifies that the research in allelopathy includes isolating, identifying and quantifying specific active allelochemicals from plant kingdom. Once these substances are identified and characterized they can be used either as natural herbicides. Medicinal plant had inhibitory effects on selected weeds and its allelochemicals inhibiting weed growth was identified (Lin *et al*., 2003; 2004). Literature also explains that one of the main reasons of product reduction in crops is weed infestation. Interference of weeds in the crops which are in the form of compound competitive and allopathic effects caused to million dollars damages in crops in all over the world. Most of weeds species have deterrent effects on crops, but some of them stimulated seed germination and also the production of crops (Provide references).

In modern countries, with use of different methods, weed damages have been reduced to 5%, and a large number of weed remnant after harvesting entered the soil and formed the most important of soil sources (Jaskuish, 1997). On the other hand in developing countries where weed infestation has become a menace, which deteriorated plant products as a result of the weed competition and allelopathy. The higher amount of herbicide application led to an increase in production cost as well as severe environmental problems, like ground water contamination, decline of the number of beneficial flora and fauna and also human health hazards directly or indirectly.

To avoid the detrimental effects of herbicides, researchers of the different corners of the world are searching for novel natural plant products to develop bio-herbicides. So due to allelochemical potential of wild plants and to search for the bio-herbicides, presently analysis and allelochemical effects of P*. guajava* L. leaves against wheat and canary grass were taken into consideration.

MATERIALS AND METHODS

Plant Material

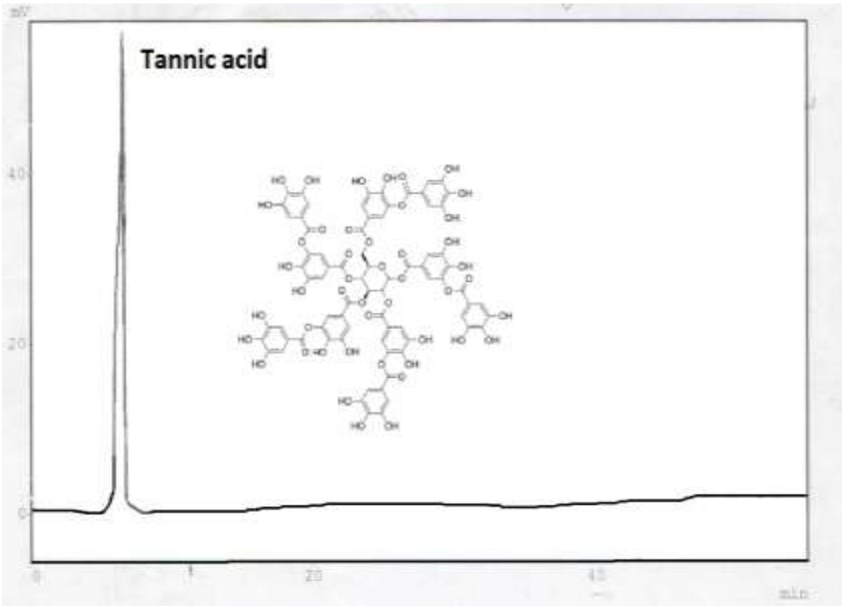
The leaves of the *P. guajava* L. were collected in triplicate from three different trees from GC University, Faisalabad new campus situated on Jhang road Faisalabad, Pakistan. The leaves were washed with distilled water, shade dried, grinded into powder form in order to increase the surface area and then were stored in plastic bottle at low temperature before further use**.**

Seeds of the test plants

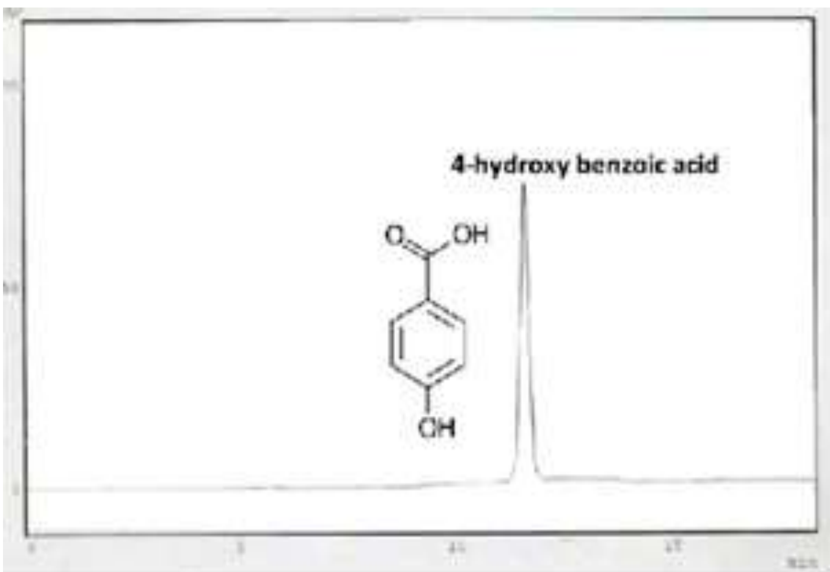
Healthy seeds of the wheat were purchased from the Faisalabad grain market. Similarly healthy seeds of the canary grass were obtained from Ayub Agriculture Research Institute, Faisalabad, Pakistan. The canary grass was chosen as a test plant for bioassay along with wheat due to its existence as weed in the crop field through out the country. Pre- and post- germinated bioassays of both of these test plants were studied in order to find out the allelopathic effects of the *P. guajava* L. leaves. During pre-and post-germinated bioassays study of these test plants, shoot, root and fresh biomass were taken into consideration.

Extraction

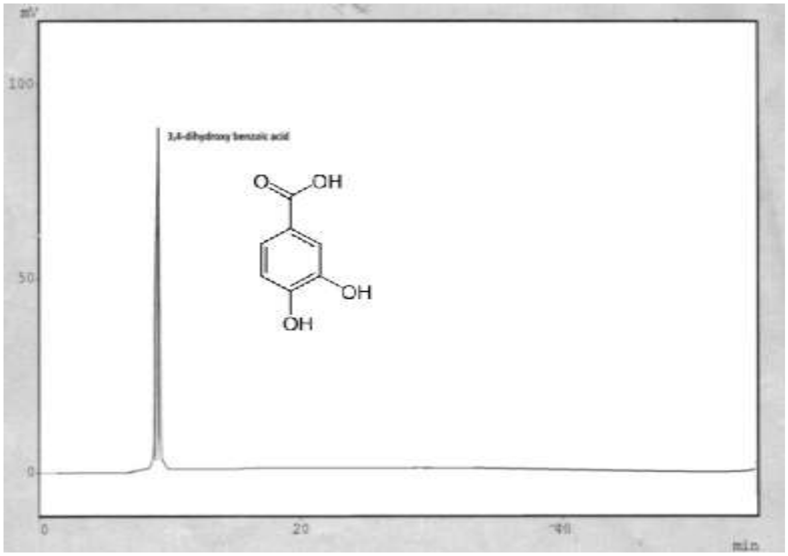
Dried and grinded leaves (50g) of *P. guajava* L. were extracted with 500CC of 70% (V/V) aqueous methanol for two days. The extract was filtered under vacuum. The residue was re-extracted with 500CC of same aqueous methanol for one day and was filtered again. The two filtrates were then combined and extracted with n-hexane to remove the less polar fraction. The original extract was then evaporated by rotary evaporator up to dryness at 40^0C. The residue was re-dissolved into 2CC of HPLC grade methanol for analysis with normal stationary phase column and UV-visible detector at 278nm. The components of the *P. guajava* L. leaves were then identified qualitatively by comparing the retention time of the standard chromatograms with that of unknown one (Fig 1).



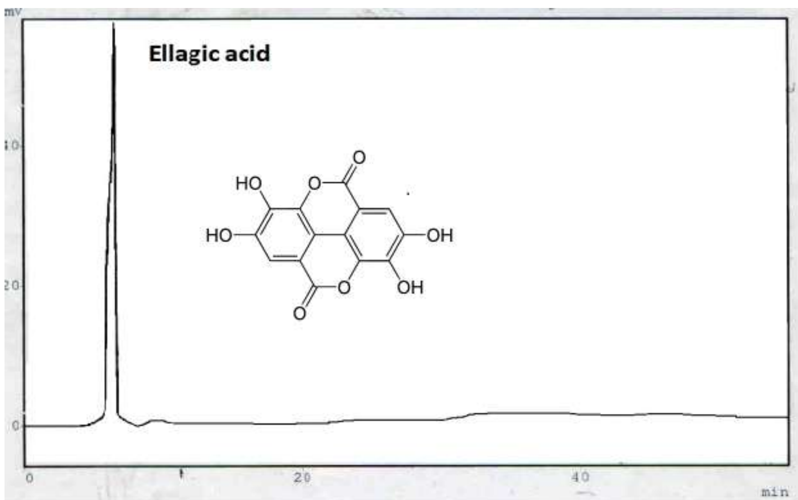
Tannic Acid



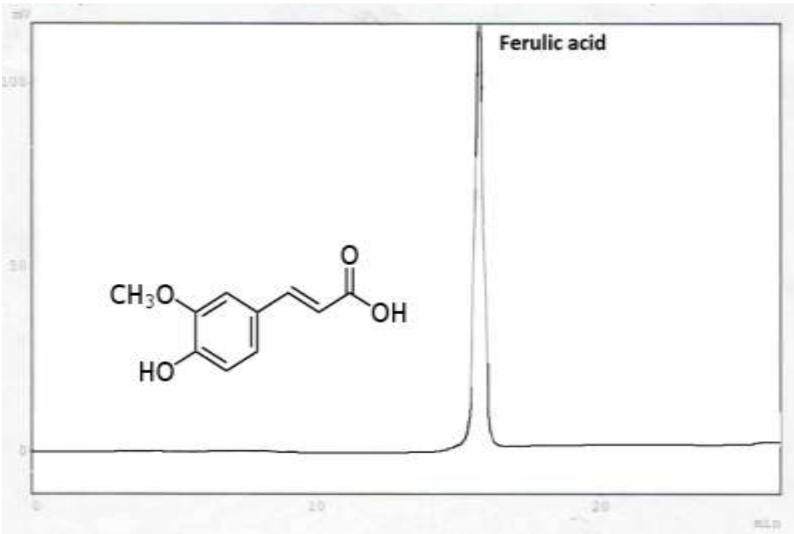
4-hydroxy benzoic acid



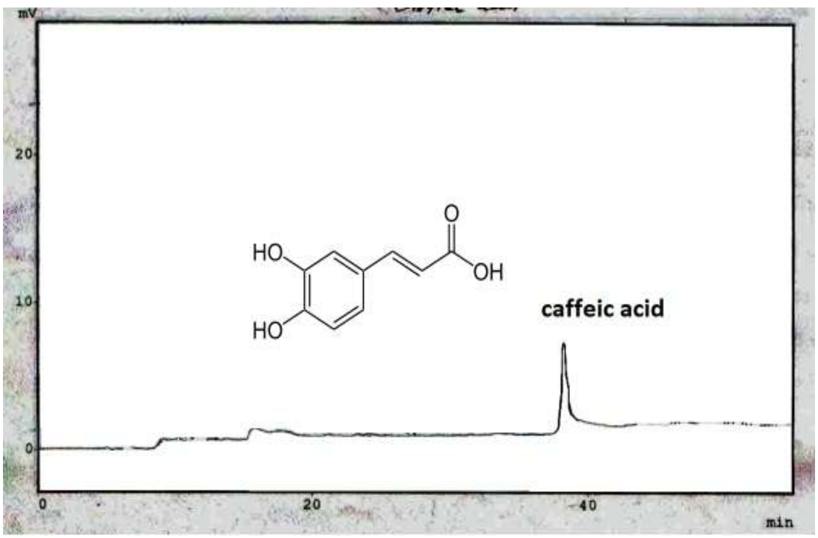
3,4-dihydroxy benzoic acid



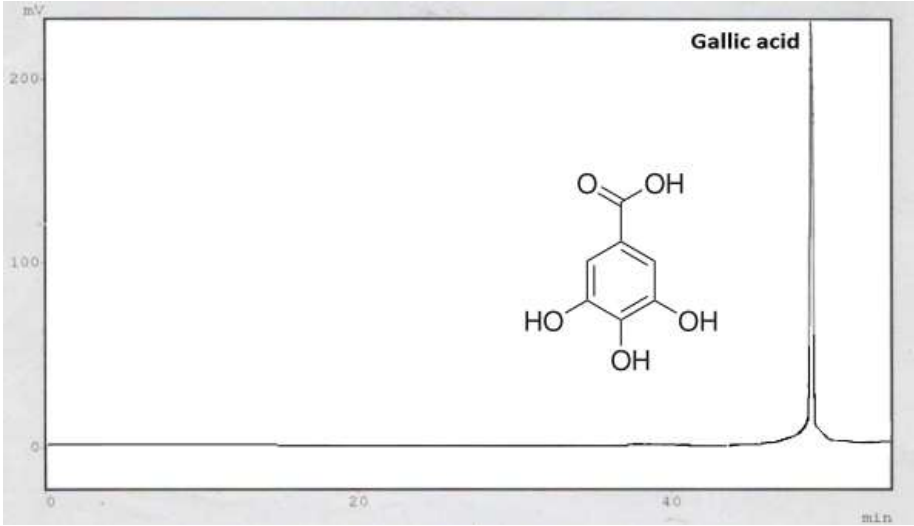
Ellagic acid



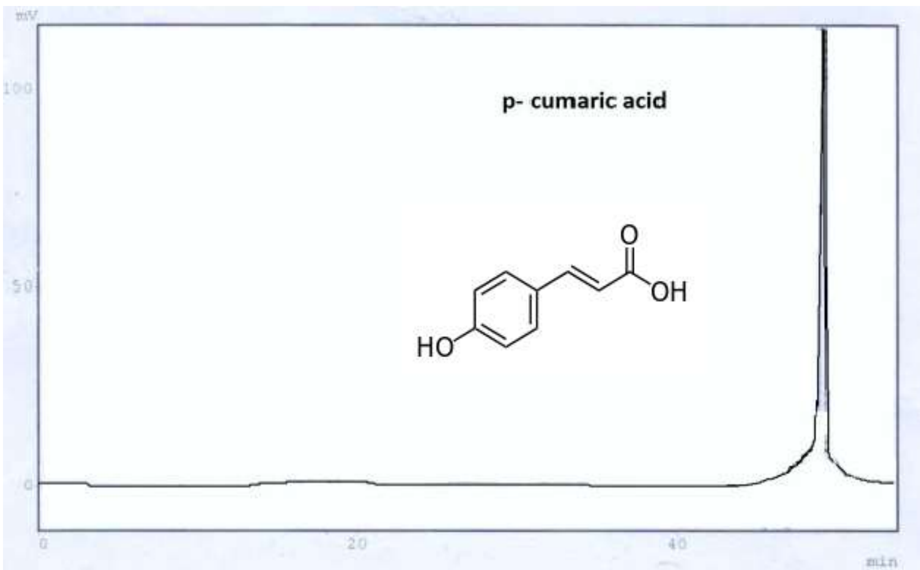
Ferulic acid



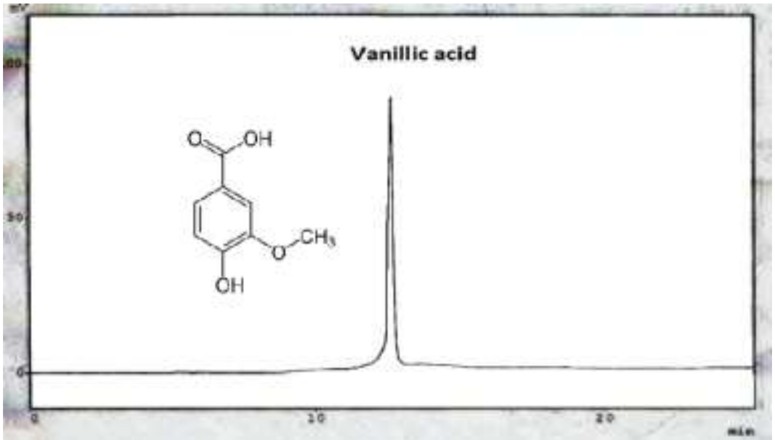
Caffeic acid



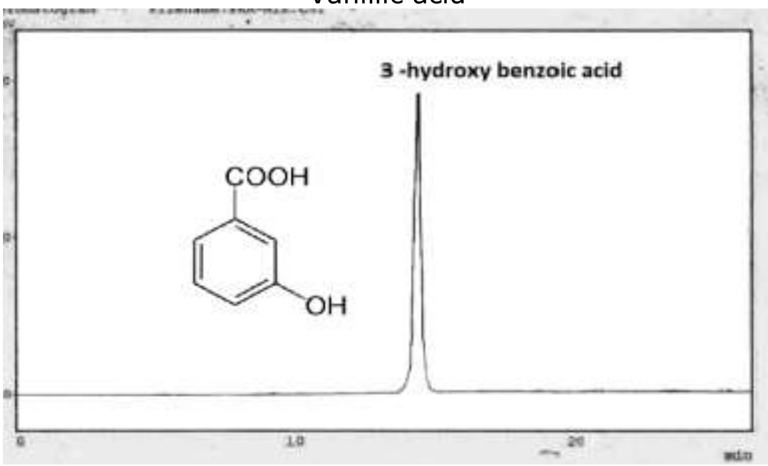
Gallic acid



p - cumaric acid



Vanillic acid



3-hydroxy benzoic acid

Figure 1. Chromatograms of the standard allelochemicals

Pre-Germinated Bioassays

Two hundred (200) wheat seeds were placed in six petri-dishes (10 cm diameter) between the two layers of filter paper Whatman No. 1. When almost all seeds were germinated, 10 germinated seeds were transferred into each petri-dish, put them at equal distances and covered the petri-dishes. Four ml of each extract was applied in each Petri dish except control and petri dishes were placed in an incubator at 24 ± 2 ºC. Equal volume of distilled water was served as control. Later distilled water was applied in all the treatments to provide the required moisture, if needed. Three replicates were arranged in a randomized complete block design under the experimental conditions mentioned above. Shoot, root lengths and fresh biomass of each plant in each petri-dish were noted 15 days after sowing. Then dry weight of all plants in each petri-dish was noted after oven drying at 40°C till constant biomass (Nouri *et al*., 2012). Same procedure was adopted for canary grass. All the fractions (concentrations) were tabulated in ascending order (2.5%, 5.0%, 7.5% and 10.0%). Similarly for post germinated bioassays ten seeds of wheat and ten seeds of canary grass were sown separately in the sterilized petri dishes between the two layers of filter paper Whatman No.1. The remaining procedure was same as for the pre-germinated bioassay study.

Pre-germinated wheat bioassay

Pre-germinated canary grass bioassay

Post-germinated wheat bioassay

Post –germinated canary grass bioassay

Figure 2. Pre- and post-germinated bioassays of the wheat and canary grass

RESULTS AND DISCUSSION

After extracting the *Psidium guajava* L. leaves with 70% aqueous methanol, less polar fraction was separated with n-hexane. The polar methanol fraction was then evaporated upto dryness under vacuum. The residue was re-dissolved in 2CC of methanol and then was filtered through silica cartridge prior to HPLC analysis. 20ul was then injected for analysis by using methanol/water (3:1) mobile phase, normal stationary phase column and uv-visible detector

(278nm). The allelochemicals of the extract were identified qualitatively by comparing the retention time of the standard chromatograms (Fig. 1) with unknown chromatogram of the *P. guajava* L. leaves extract. A total of 16 allelochemicals were eluted from the *P. guajava* leaves (a-p) (Fig. 3) but, among these, 8 were identified (a, b, f, h, i, j, n and o) while allelochemicals that were eluted were at retention time 11.00, 15.50, 16.00, 17.95, 30.00, 37.00, 44.00, and 51.55 minutes (c, d, e, g, k, l, m and p) were not identified due to unavailability of respective standards. Among the identified components, the first allelochemical to be eluted was ellagic acid (6.55 minutes) while the last one to be eluted was p-cumaric acid (49.55 minutes). All others to be identified were eluted with in this time limit (Table-2). After identification the same extract was used in different concentrations (2.5%, 5%, 7.5% and 10%) to study the allelopathic effect of the experimental plant against pre-germinated and post-germinated wheat and canary grass seeds.

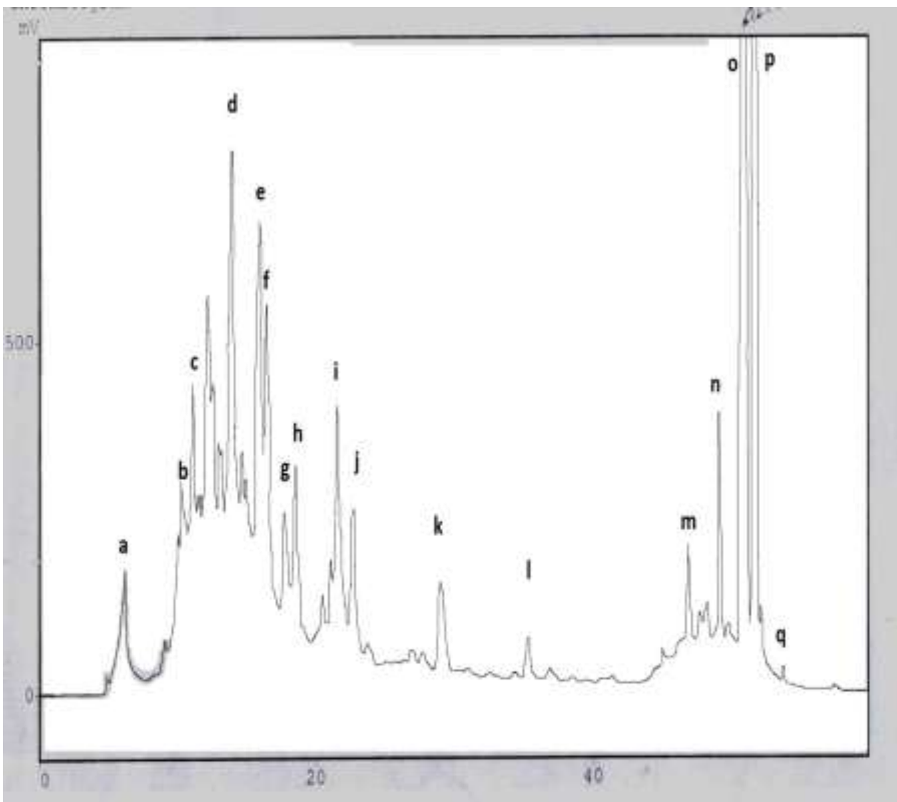


Figure 3. Chromatogram of the various allelochemicals in *P. guajava* L. leaves

Table-1. Allelochemicals evaluated/identified with their retention time

Sr. no.	Phenolic compounds	Retention Time
A	Ellagic acid	6.55
B	3,4- dihydroxy benzoic acid	9.5
C	Unknown	11
D	Unknown	12.55
E	Unknown	15.55
F	Feruic acid	17
G	Unknown	17.95
H	Galuic acid	19
I	Vanillic acid	22
J	3-hydroxy / 4-hydroxybenzoic acid	23.5
K	Unknown	30
L	Unknown	40.45
M	Unknown	46.9
N	Gallic acid	46.5
O	p- coumaric acid	49.55
P	Unknown	51.5

The bioassays study of the pre-germinated wheat and canary grass Table-2 and 3, explain that in case of wheat allelochemicals / phenolic compounds from *P. guajava* L. enhanced the length of shoot as well as root linearly as the concentrations of these phenolic compounds was increased (14.00 and 12.95cm at 2.5% while 16.50cm and 15.00 at 10% concentration, respectively as compared with the control ones (12.45cm and 10.00cm) while the effect of these allelochemicals/phenolic compounds was observed inhibitory for both the development of shoot (7.35cm/2.5%, 7.00cm/5%, 4.15cm/7.75% and 0.45cm/10%) and root of canary grass (5.75cm/2.5%, 4.00cm/5%, 2.75cm/7.75% and 0.45cm/10%, respectively) as compared with control (7.50cm and 5.75cm) ones. The fresh weight of the wheat was increased (2.65mg—3.25mg) while canary grass was decreased (0.10—0.05mg) linearly as the concentration was increased. Whole discussion is illustrated in Fig. 4 and 5.

Table-2. Effect of allelochemicals on pre-germinated Wheat seeds

Concentration %	Shoot length (cm)	Root length (cm)	Fresh weight (mg)
Control	12.45	10	2.45
2.50%	14	12.95	2.65
5%	14.75	13.55	2.95
7.50%	15.25	14.95	3
10%	16.5	15	3.25

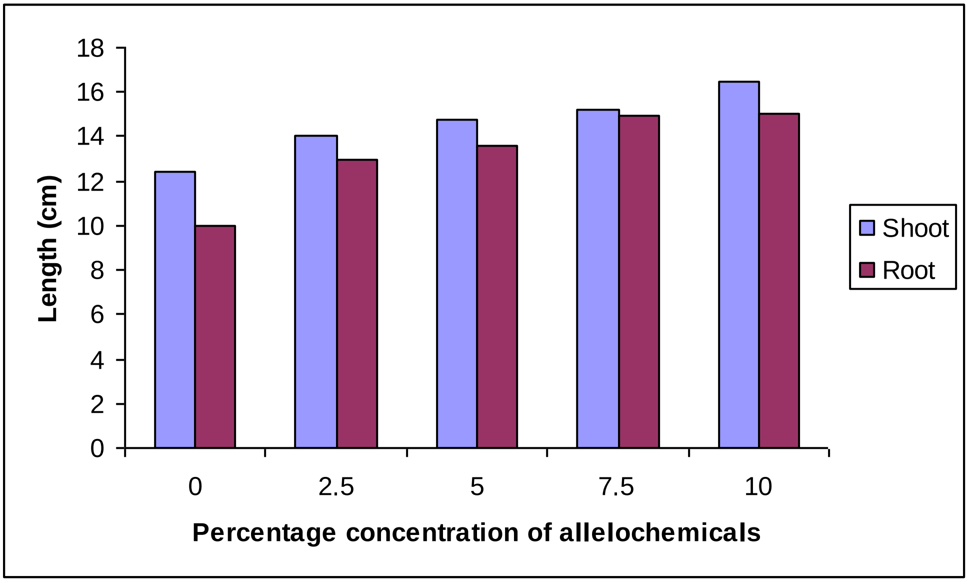


Figure 4. Effect of allelochemicals on pre-germinated wheat seeds

Table-3. Effect of allelochemicals on pre-germinated Canary grass seeds

Concentration %	Shoot length (cm)	Root length (cm)	Fresh weight (mg)
Control	8.45	4.55	0.1
2.50%	7.35	1.65	0.1
5%	7	1.15	0.1
7.50%	4.15	0.35	0.06
10%	0.45	0.05	0.05

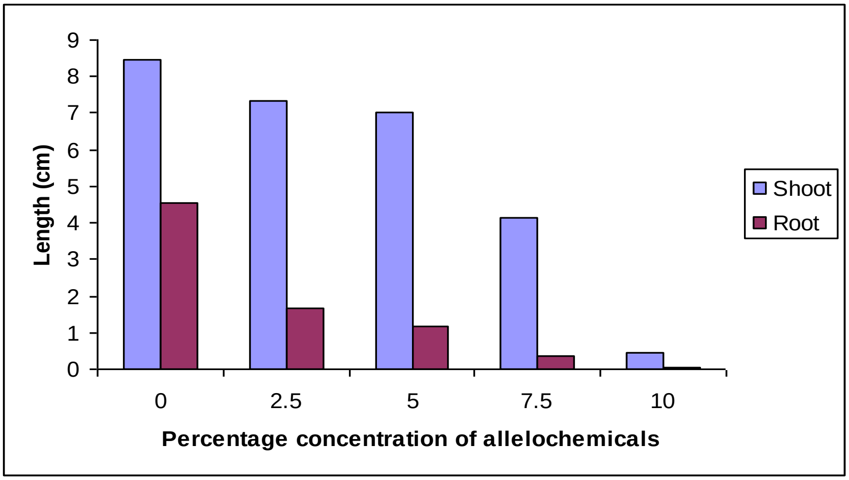


Figure 5. Effect of allelochemicals on the pre-germinated canary grass seeds

Similarly the results of the post-germinated wheat and canary grass seeds explain (Table-4 and 5) that the effect of the allelochemicals / phenolic compounds on the development of shoot as well as root at all the 4 concentrations of the allelochemicals / phenolic compounds (2.5% , 5%, 7.5% and 10%) was about similar to that pre-germinated one. The shoot length of the wheat was enhanced at all the concentrations as compared with the control (13.15cm) being maximum(16.25cm) at 10% concentration and minimum (13.85cm) at 2.5% while root development was also increased as compared with control (10.25cm) being maximum(14.50cm) at 10% and minimum(11.15cm) at 2,5%, respectively. Both the shoot and root of the canary grass showed inhibitory response at all the concentrations of the allelochemicals / phenolic compounds being minimum at 10% concentration (2.75cm and 0.75cm) as compared with control (7.45cm and 2.75cm) ones. In case of wheat the fresh weight was increased (1.85—2.25mg) while in case of canary grass it was decreased (0.10mg—0.05mg) linearly as compared with control ones. The whole results are further illustrated in Fig. 6 and 7. Earlier Allelopathic effect medicinal plants such as *Euphorbia guyoniana* and *Retama retam,* (donor species) was studied to control the weeds (*Bromus tectorum* and *Melilotus indica*) growth. The results verified that the germination efficiency, plumule and radicle length of *Bromus* was completely inhibited at the highest concentration of aqueous extracts of the donor species level (10%). Further it was observed that the inhibition was markedly in obvious *Bromus tectorum* than in *Melilotus indica* indicating that *Bromus tectorum* is more sensitive to all of tested donors, while the *Melilotus indica* is more adapted to the aqueous extract than the *Bromus tectorum* (Nasrine *et al*., 2011). In another study, it was observed that allelopathy offers the potential for biorational weed control through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots of living or decomposing plant materials (Weston, 1996). Similarly the effect of weed allelopathic of sorghum (*Sorghum halepense*) on germination and seedling growth of wheat (Alvand cultivar) was observed. Aqueous extract of leaves, stems, seeds and roots of sorghum had a significant deterrent effect on wheat seeds germination. Allelopathic effect of leaves and stems of this plant had a significant deterrent effect on the growth of wheat seedling length although the roots extract increased partial longitudinal growth of wheat seedling. Extracts of different tissues of sorghum reduced also the wheat seedling fresh weight (Nouri *et al*., 2012). It was found that allelopathic effect of Neem (*Azadirachta indica* A. Juss) against the germination of certain weeds was found inhibitory. Germination, root and shoot growth of all the tested plant species were inhibited at concentration greater than 0.001g dry weight equivalent extract / ml. It was further observed that the allelopathic effect was much higher on the root development than on the shoot growth of the test plants (Abdus-Salam and Hisashi, 2010).

Table-4. Effect of allelochemicals on post-germinated Wheat seeds

Concentration %	Shoot length (cm)	Root length (cm)	Fresh weight (mg)
Control	13.15	10.25	1.75
2.50%	13.85	11.15	1.85
5%	13.95	12.55	1.9
7.50%	14.5	13	2
10%	16.25	14.5	2.25

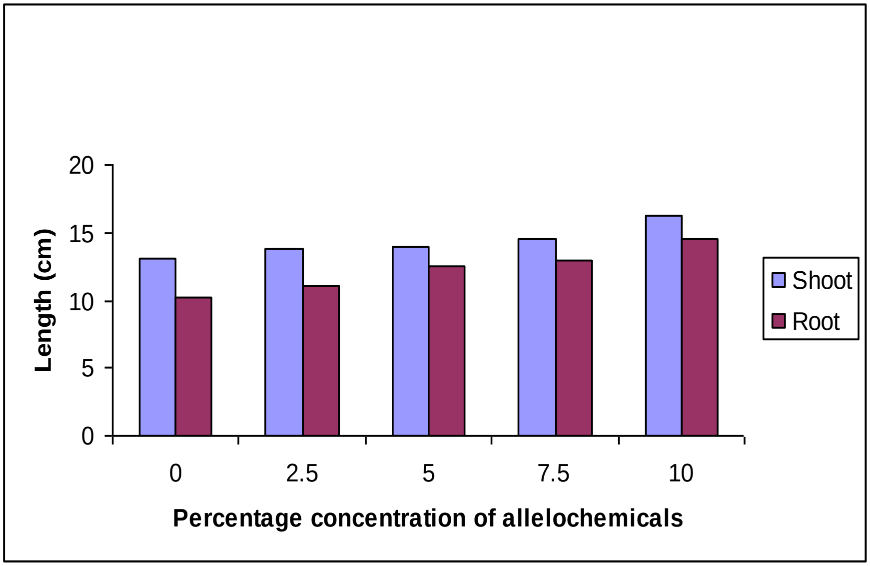


Figure 6. Effect of allelochemicals on the post-germinated wheat seeds

Table-5. Effect of allelochemicals on the post-germinated Canary grass seeds

Concentration %	Shoot length (cm)	Root length (cm)	Fresh weight (mg)
Control	7.45	2.75	0.15
2.50%	6.5	2.15	0.1
5%	5.55	1.75	0.1
7.50%	3	1.25	0.05
10%	2.75	0.75	0.05

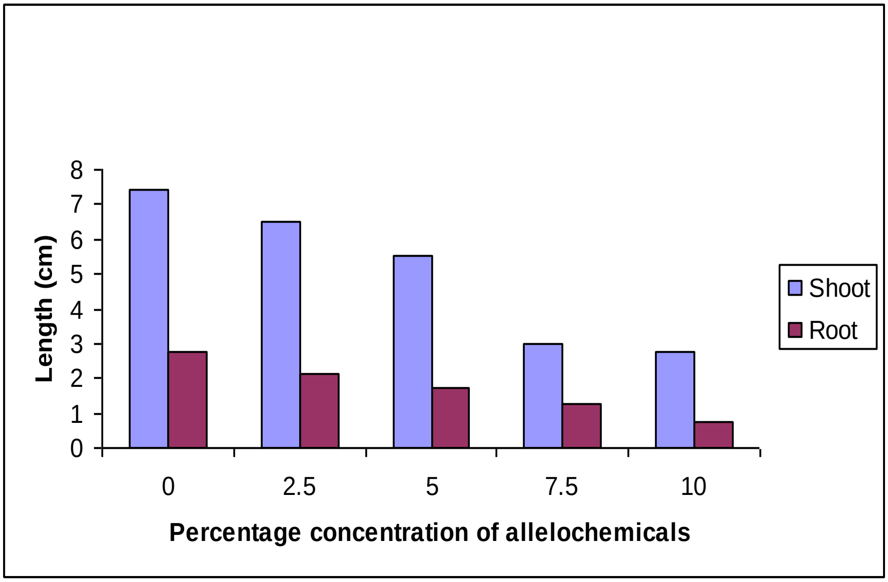


Figure 7. Effect of allelochemicals on the post-germinated canary grass seeds

CONCLUSION

In light of the results, it is concluded that *Psidium guajava* possesses some growth inhibitory substances and also has somewhat allelopathic potential. Consequently, the leaves of *P. guajava* might be a potential contender for the isolation and identification of allelopathic substances. Moreover, for a sustainable agricultural production it is also helpful in the development of natural herbicides.

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