

Preliminary Phytochemical Analysis and Assessment of Herbicidal Activity of *Parthenium hysterophorus* L. Extract on Germination and Initial Development of Two Selected Rice Weeds in Malaysia

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ABSTRACT

The excessive use of synthetic herbicides for weed control has resulted in adverse environmental problems such as pollution, herbicide resistance and diseases. Therefore, the search for alternative sources of herbicide by using natural sources that are ecofriendly, effective and safe, becomes imperative. The present study was undertaken to investigate the presence of phytochemicals in *Parthenium hysterophorus* L. leaf, stem and root extracts using the different polarity of solvents (methanol, hexane, ethyl acetate and water) and to evaluate the phytotoxic effect of the extracts on germination and seedling growth of two rice weed species : *Echinochloa colona* (L.) Link. and *Ludwigia decurrens* Walt. under laboratory conditions. The standard procedure for qualitative phytochemical analysis was adopted for the analysis. For the germination test, the extracts were reconstituted at 2.5, 5.0, 7.5 and 10% concentration levels with distilled water as a control. Phytochemical analysis showed both the presence as well as the absence of alkaloids, flavonoids, anthraquinones, steroids, phenols, saponins, tannins, resins and terpenoids from the different solvents and plant parts. The study revealed that ethyl acetate leaf extract had the highest number of phytochemicals followed by the methanol leaf extract. Extracts in different solvents and plant parts demonstrated distinctly varying herbicidal actions towards the bioassay weed species. It was also found that both ethyl acetate and methanol leaf extracts significantly reduced ($P < 0.05$) seed germination, plumule, and radicle lengths by 100% inhibition at a concentration as low as 2.5%. Conversely, less inhibition was provided by hexane and water extracts across all plant tissue concentrations. The results of this study suggested that ethyl acetate and methanol leaf extracts of *P. hysterophorus* L. can be utilized as an organic herbicide for the management of rice weeds.

Key words : Phytochemical analysis, bioassay species, crude extracts, germination inhibition, plumule, radicle length

INTRODUCTION

Synthetic herbicides have been used for weed control in farms to boost crop yields to meet the food needs of the increasing human population globally, however, these chemical components of the herbicides cause serious negative impacts on human and animal health, environmental pollution and herbicide resistance by the weeds (Carvalho, 2017; Farooq *et al.*, 2018). These problems, therefore, necessitated the need to search for alternatives to synthetic chemicals from natural sources in plants and other life forms

for the sustenance of the environment (Shahzad *et al.*, 2016).

Plants produce secondary metabolites, also referred to as allelochemicals are found in almost all plant parts such as leaflets, stalks, florets, berries, nuts and root stocks (Zamdahl, 2018). It has been reported to inhibit germination in neighbouring plants, nutrient uptake and suppress crop plant growth. Some common plant secondary metabolites are phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates and amino acids with admixtures of several compounds sometimes possessing a higher allelopathic

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effect than individual compounds alone (Cecchin *et al.*, 2017). These allelochemicals are environmentally-friendly as these are easily degraded in nature, void of halogenated compounds, and possess new target sites that are different from synthetic chemicals. Furthermore, these also display a high level of novelty and structural diversity and are highly sought after for the discovery of new bioherbicide with new modes of action for weed control (Pati and Chowdhury, 2015).

Phytotoxicity assays were reported to be a fundamental strategy for recognizing crop plants which probably could be the origin of herbicidal compounds of delight (Norhafizah *et al.*, 2013). Furthermore, Hanan (2016) reported that the phytotoxicity of individual parts of plants such as leaves, stems, bark, florets, roots, leaf mulch and litter, soil leachates and their derivative compounds may differ in their allelopathic action. It is established that plant secondary metabolites that suppress the growth of certain plant species at a particular concentration might trigger the growth of similar or distinct species at varying concentrations (Norhafizah *et al.*, 2013). A lot of research works have been conducted globally on the phytotoxic effects of allelochemicals on seed germination inhibition, plumule, and radicle length, seedling growth retardation and poor seedling survival (Norhafizah *et al.*, 2013; Chuah and Lim, 2015; Pati and Chowdhury, 2015).

There are lot of documented reports on weeds that are phytotoxic. Among the weeds, *P. hysterophorus* L. is an aggressive and pernicious weed native to North-east Mexico (Saini *et al.*, 2014). It was classified among the 10 worst weeds of the world and is listed in the global invasive species database (Bhateria *et al.*, 2015). In Malaysia, parthenium is called Rumpai Miang Mexico (Karim, 2013). The ability of parthenium to successfully invade many countries of the world including Malaysia is due largely to its allelopathic properties which enable it to compete favourably with crops and other weed species (Karim, 2013; Kaur *et al.*, 2014). Allelochemicals released by parthenium were reported to significantly reduce the germination of seeds and the growth of several crop plants (Pati and Chowdhury, 2015). The aim of this paper, therefore, was to conduct preliminary phytochemical analysis and to

assess the herbicidal potential of *P. hysterophorus* (leaf, stem and root part) extracts using four different solvents on selected two rice weeds : *Echinochloa colona* (L.) Link (junglerice) and *Ludwigia decurrens* Walt. (willow rimrose) under laboratory condition.

MATERIALS AND METHODS

Parthenium weed samples were collected from invaded fields in KampungKongsi Nam (5°63'21" N Latitude and 100°49'23" E Longitude) in Kedah state of Malaysia. Seeds of bioassay species *E. colona* and *L. decurrens* were obtained from rice fields in Pasir Mas Kelantan, Malaysia (20°4'39" N Latitude and 102°14'37" E Longitude). Analytical or laboratory grade solvents (methanol, hexane and ethyl acetate) used in this experiment were purchased from HmbG Chemicals (Hamburg, Germany). The laboratory-based experiment was conducted in the post-graduate laboratory of the University of Malaysia Kelantan, Jeli Campus during May and July 2018.

The extracts of parthenium plant parts were prepared according to the procedure of Norhafizah *et al.* (2013) and Pati and Chowdhury (2015). Plant parts collected were separated into leaves, stems and roots and washed with distilled water to remove contaminants. The plant samples were air-dried under the shade for 10 days. The dried samples were then ground into fine powder using a laboratory blender and kept at 4°C until use. For the preparation of the crude extracts, 50 g of *P. hysterophorus* powdered raw material was macerated in 250 ml of respective solvents i. e. hexane, ethyl-acetate, methanol and water with exhaustive sequential extraction by vigorously shaking at 200 rpm for 72 h on an orbital shaker at 25°C (resulting in a 20% stock solution). The crude extracts were then filtered through filter paper Whatman No.1. Digital rotatory evaporator (Yamato, RE 801100-240V) at 40°C was used to evaporate the excess solvent from the filtrate to obtain the crude extract. To obtain the different concentration levels for the germination trial, the crude extract was then diluted with 1% of each of three solvents and remaining with distilled water and reconstituted to get 2.5, 5.0, 7.5 and 10.0% (g/v) test extracts for the phytotoxicity studies.

Qualitative phytochemical analysis was carried out for the methanol, ethyl acetate, hexane and water extract of leaf, stem and root of *P. hysterophorus* using standard procedures to identify the constituents as described by Gul *et al.* (2017) and Chintalapani *et al.* (2018).

0.5 g of the extracts was measured into a watch glass and little amount of dilute hydrochloric acid and 1 ml of Mayer's reagent was added to the solution; the formation of a white precipitate indicated the presence of alkaloids. 0.5 g of extracts was treated with sodium hydroxide (NaOH) solution, followed by the addition of 2 ml diluted hydrochloric acid (HCl). The presence of flavonoids was detected when a yellow colour solution formed in sodium hydroxide and turned colourless with diluted HCl.

0.5 g of extract in a test tube was added with 2 ml of chloroform and 3 ml of concentrated sulphuric acid (H_2SO_4). Reddish brown colour of interface was formed, indicating the presence of steroids. 0.5 g of extracts in a test tube was added with 1 ml of 10% ferric chloride solution, followed by 1 ml of HCl. The solution was immersed in a boiling water bath for 5 min and allowed to cool. Then the mixture was shaken with 1 ml of diethyl ether and the resultant solution was treated with ammonia solution. Formation of a pink or deep red colouration of aqueous layer indicated the presence of anthraquinones.

0.5 g of extract was dissolved in distilled water. Then, 5-6 drops of 10% ferric chloride and 1 ml of HCl were added. The formation of dark green colour indicated the presence of phenols. The 0.5 g of extract was mixed with few drops of distilled water and the mixture was shaken vigorously. A copious lather formation was noticed which indicated the presence of saponins, and the absence of the copious lather meant the absence of saponins.

10% of alcoholic ferric chloride was added to 0.5 g of extracts in a 1:1 ratio. Formation of a dark blue or greenish grey colouration of the solution indicated the presence of tannins. To 0.5 g of the extract, 1 ml of chloroform was added followed by 2-3 ml of acetic anhydride. Then 1 to 2 drops of concentrated sulphuric acid (H_2SO_4) were added into the solution. The formation of pink or red colouration indicated the presence of terpenoids. 0.5 g of extract was treated with 3-4 ml of copper sulphate solution and mixed properly for 1-2 min. Appearance of

green precipitation indicated the presence of resins. 0.5 g of extract was dissolved in water followed by the addition of 2 ml aqueous solution of sodium hydroxide (NaOH). Formation of yellow colour showed the presence of glycosides.

Twenty-five seeds of *E. colona* and 30 seeds of *L. decurrens* were placed in the respective 9 cm diameter Petri plates having double sheets of filter paper soaked with 5 ml of the extract. Distilled water was used for the negative or control experiments. The Petri plates were kept in a growth chamber at 30/20°C day/night with a 12-h photoperiod for 14 days. Seeds were deemed emerged when the radicle attained a length of 1 mm. At the end of the incubation period, the emerged seeds were recorded as a percentage of the total number of viable seeds used in each replication. The radicle length and plumule length of germinated seeds were also measured and recorded. The data for the radicle and plumule lengths were also presented as a percentage of their individual control (Chuah *et al.*, 2014). The laboratory bioassays were arranged in a completely randomized design (CRD) with three replications. The data were subjected to analysis of variance (ANOVA). Tukey test was used to compare the mean among the treatments. Differences were regarded as significant when the p-values were less than 0.05 ($P < 0.05$).

RESULTS AND DISCUSSION

The preliminary qualitative phytochemical analysis of crude extracts of methanol, ethyl acetate, and hexane and aqueous of *P. hysterophorus* leaf stem and root are shown in Table 1. The following secondary metabolites were seen in the extracts viz., alkaloids, flavonoids, anthraquinones, steroids, phenols, saponins, tannins, resins, terpenoids and glycosides. The ethyl acetate leaf extract tested positive for all the 10 secondary metabolites tested for, seconded by the methanol and the aqueous leaf extracts both with eight phytochemicals, while the hexane leaf extract tested was positive for six secondary metabolites. Similarly, the ethyl acetate stem extracts also showed the presence of eight secondary metabolites, followed by the methanol stem extracts with seven, then the aqueous with six and lastly the hexane stem

Table 1. Preliminary phytochemical screening of ethyl acetate, methanol, hexane and water crude extracts of *Parthenium hysterophorus* L. leaf, stem and root

S. No.	Phyto constituents	Methanol			Ethyl acetate			Hexane			Water		
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
1.	Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
2.	Flavonoids	+	-	-	+	+	-	-	-	-	+	+	+
3.	Steroids	-	-	-	+	-	+	-	-	-	-	-	-
4.	Anthraquinones	+	+	+	+	+	+	-	-	-	+	-	-
5.	Phenol	+	+	+	+	+	+	+	+	+	+	+	+
6.	Saponins	+	+	+	+	+	+	+	+	+	+	+	+
7.	Tannins	+	+	+	+	+	+	+	+	+	+	+	+
8.	Terpenoids	-	-	-	+	-	-	-	-	-	-	-	-
9.	Resins	+	+	-	+	+	-	+	-	-	+	-	-
10.	Glycosides	+	+	+	+	+	+	+	+	+	+	+	+

+=Positive and -=Negative.

extract with five. Analysis of the ethyl acetate root indicated the presence of seven phytochemicals, seconded by the methanol and aqueous root extracts that tested positive for six phytochemicals, while the hexane root extract tested positive for five.

The phytotoxic effects of leaf, stem and root of *P. hysterophorus* methanolic extracts on seed germination, plumule and radicle length of *E. colona* and *L. decurrens* are presented in Fig. 1. The results indicated that methanolic leaf extracts completely inhibited the emergence

of *E. colona* and *L. decurrens* at a low concentration of 2.5 and 7.5%, respectively (Fig. 1A). Similarly, methanolic stem and root extracts exhibited strong inhibition on the two weed species germination (90-97% inhibition) at the highest concentration of 10% (Fig. 1D and G). It was found that the plumule and radicle length of *E. colona* and *L. decurrens* were significantly reduced ($P < 0.05$) by 30 to 45% and 50 to 60%, respectively, at a similar concentration of these extracts (Fig. 1B, E, H and 1C, F, I).

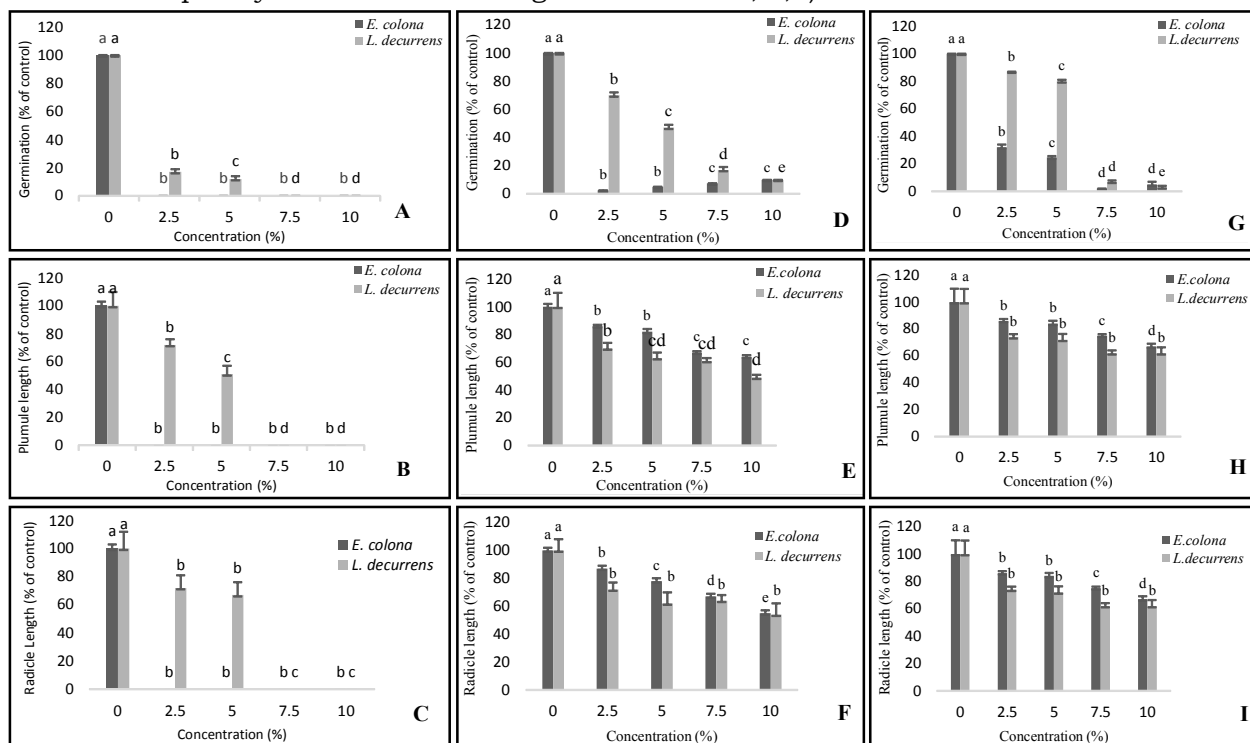


Fig. 1. Inhibitory effect of parthenium leaves (A-C), stem (D-F) and root (G-I) of methanolic extract on germination, plumule and radicle length of *Echinochloa colona* and *Ludwigia decurrens*. Vertical bars are standard deviation of the mean. Means carrying different alphabets denote significant differences in germination percentage, plumule and radicle lengths ($P < 0.05$).

Fig. 2 shows the phytotoxic effects of hexane extracts of parthenium leaf, stem, and root on the germination and growth of the two bioassay weed species. The results showed that the hexane leaf extract inhibited seed germination of *E. colona* and *L. decurrens* at 2.5% with inhibition of 71 and 47%, respectively (Fig. 2A). The stem and the root extracts inhibited germination by 80 and 76% in *E. colona* at 10 and 2.5%, respectively (Fig. 2D and G). On the other hand, it was discovered that plumule and radicle length of *E. colona* and *L. decurrens* were reduced by 46-69 and 33-36% at 2.5% concentration (Fig. 2B, E, H and 2C, F, I). The results showed that hexane extracts did not significantly ($P>0.05$) affect seed germination, plumule and radicle lengths.

The results of the phytotoxic effect of ethyl acetate extract of parthenium parts on seed germination, radicle and plumule length of *E. colona* and *L. decurrens* are presented in Fig. 3. The results showed a significant effect ($P<0.05$) on seed germination, plumule and radicle lengths of the two weed species. The leaf extracts completely inhibited seed germination of the two weed species at all the concentration levels. Likewise the stem and root extracts at 7.5-10.0%

also completely inhibited the emergence of *E. colona* by 100% and inhibited emergence in *L. decurrens* by 20-47% at 2.5% (Fig. 3A, D and G). The results also indicated that at all the concentration levels, the ethyl acetate extracts inhibited completely plumule and radicle development. Plumule length was reduced by 53-72% and radicle length by 31-36%, at 2.5% concentration was observed on *E. colona* and *L. decurrens*, respectively (Fig. 3B, E, H and 3C, F, I). The phytotoxic effect of aqueous extracts of *P. hysterophorus* parts on *E. colona* and *L. decurrens* on seed germination, plumule and radicle length is shown in Fig. 4. The results indicated that the leaf, stem and root extract significantly inhibited seed germination of *E. colona* seeds at 2.5% concentration with an inhibition rate of 88%, while at 10% concentration the germination of *L. decurrens* seeds was inhibited by 67, 22 and 63% for the leaf, stem and root extracts, respectively (Fig. 4A, D and G). It was also found that the plumule and radicle length of *E. colona* and *L. decurrens* were significantly reduced ($P<0.05$) by 30 to 49% and 35 to 64%, respectively, at 2.5 and 10.0% concentration of the leaf, stem and root extract (Fig. 4B, E, H and 4C, H, I).

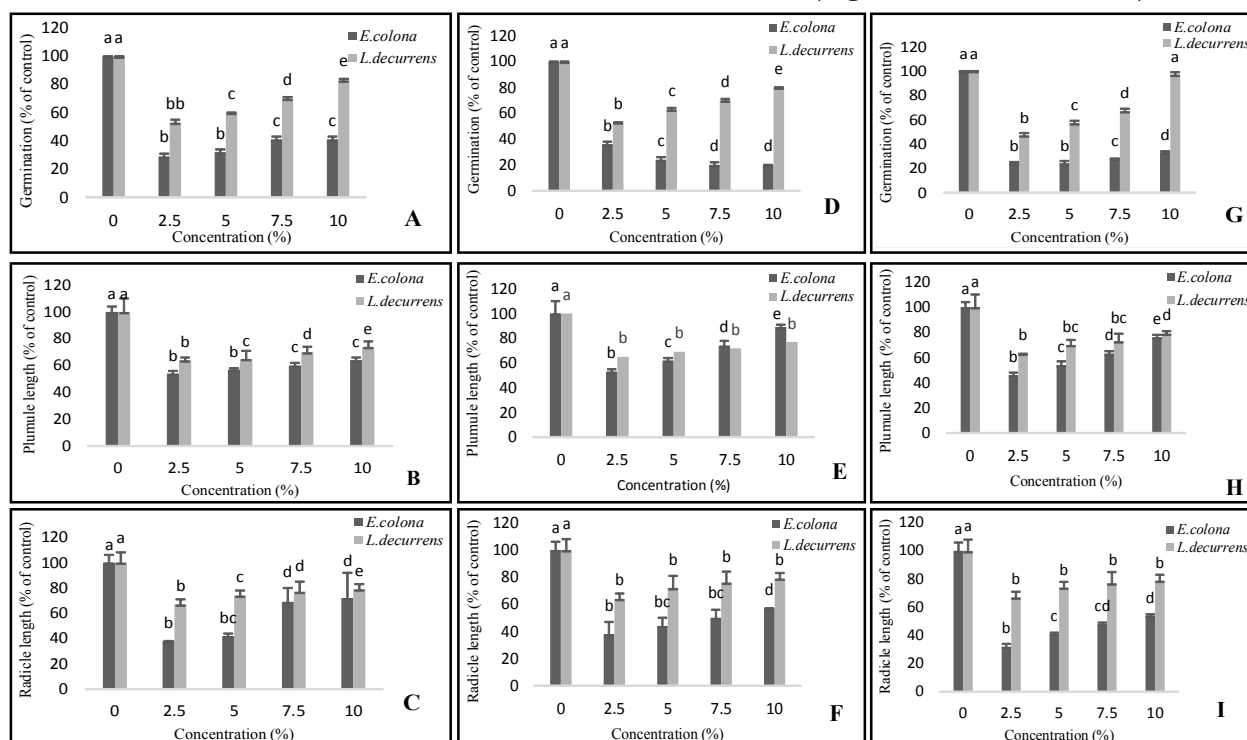


Fig. 2. Inhibitory effect of parthenium leaves (A-C), stem (D-F) and root (G-I) of hexane extract on germination, plumule and radicle length of *Echinochloa colona* and *Ludwigia decurrens*. Vertical bars are standard deviation of the mean. Means carrying different alphabets denote significant differences in germination percentage, plumule and radicle lengths ($P<0.05$).

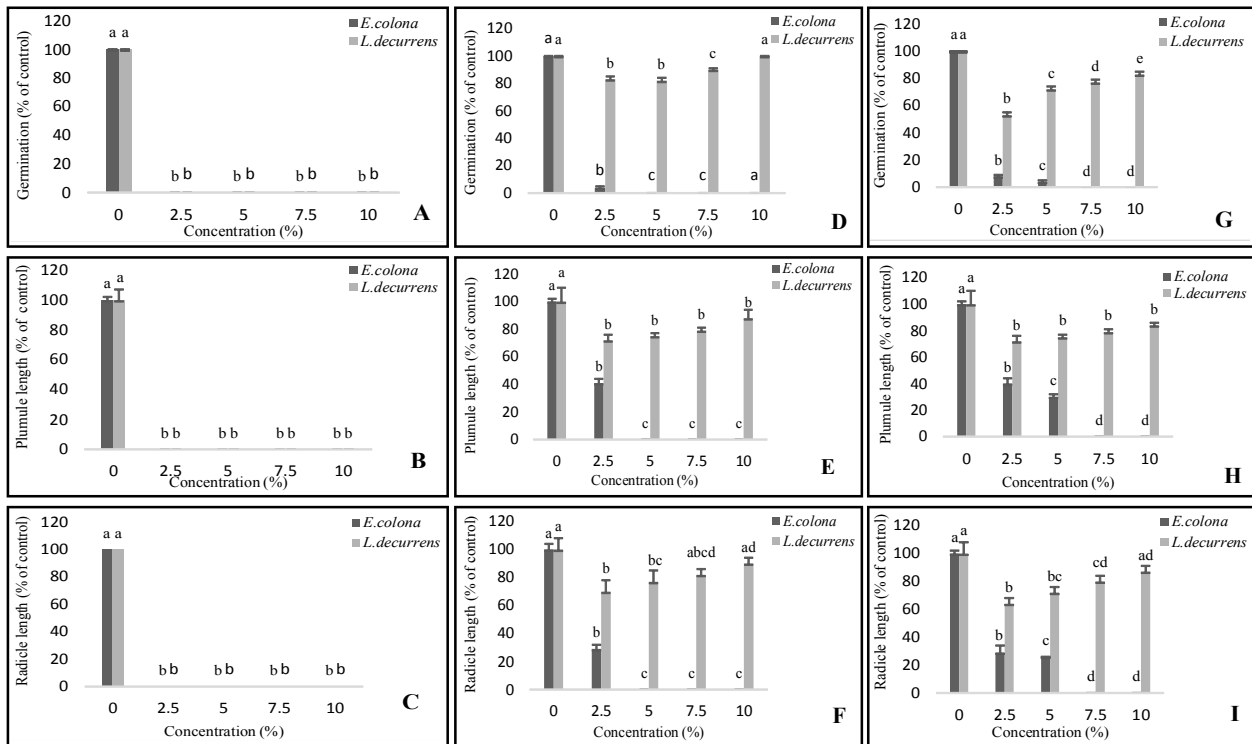


Fig. 3. Inhibitory effect of parthenium leaves (A-C), stem (D-F) and root (G-I) of ethyl acetate extract on germination, plumule and radicle length of *Echinochloa colona* and *Ludwigia decurrens*. Vertical bars are standard deviation of the mean. Means carrying different alphabets denote significant differences in germination percentage, plumule and radicle lengths ($P < 0.05$).

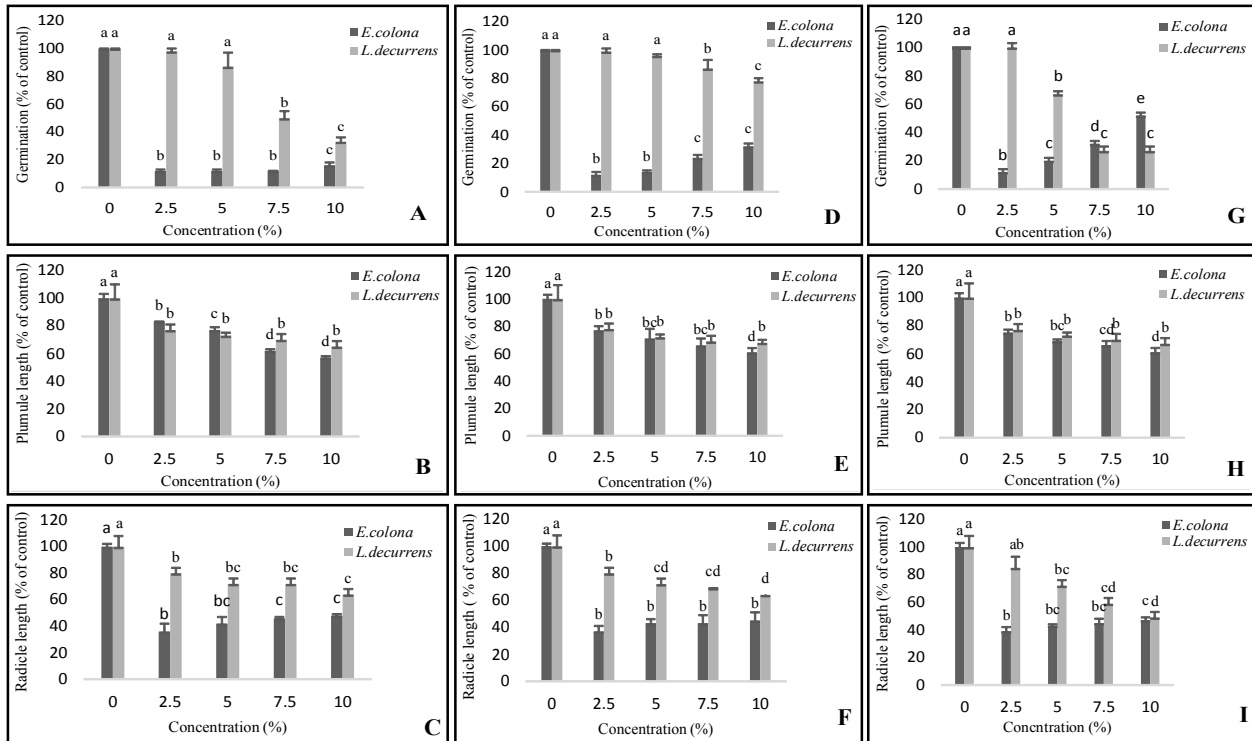


Fig. 4. Inhibitory effect of parthenium leaves (A-C), stem (D-F) and root (G-I) of water extract on germination, plumule and radicle length of *Echinochloa colona* and *Ludwigia decurrens*. Vertical bars are standard deviation of the mean. Means carrying different alphabets denote significant differences in germination percentage, plumule and radicle lengths ($P < 0.05$).

Plant secondary metabolites are reported to be an important source of allelochemicals that may play a very important role in the evolution of bio-herbicides that will be eco-friendly against synthetic herbicides (Cheng and Cheng, 2015). The phytochemical analysis of the extracts of leaf, stem and root of *P. hysterophorus* using different solvents in the present study indicated that the plant contained compounds that have herbicidal potentials. Other researchers also reported similar findings using different solvents for extraction, for example, Devi *et al.* (2014) reported the qualitative phytochemical analysis of methanol and aqueous extract of parthenium leaves. The methanol extracts revealed the presence of alkaloids, flavonoids, phenols, glycosides, cardiac glycosides, terpenoids, saponins, steroids, and tannins, whereas in the aqueous extract glycosides, terpenoids, saponin and tannin were absent. Many botanical extracts most especially those from weeds possess the ability to suppress the germination of other weed species (Abdel-Gawad *et al.*, 2015; El-Mergawi and Al-Humaid, 2019). The phytotoxic actions of different parts of the same species of weed also vary for their impacts on seed germination and initial seedling growth and development (Abdel-Gawad *et al.*, 2015; Qasem, 2017). Plant secondary metabolites had also been stated to suppress the germination of seed and radicle development in many monocotyledonous and dicotyledonous plants that were released into the soil via leaf litter decomposition (Khaliq *et al.*, 2016, Qasem, 2017). It had also been reported that each plant part/organs accumulate different kinds of secondary metabolites and solvents of different polarity with different degrees of extracting compounds from plant parts (Pati and Chowdhury, 2015; Roopashree and Naik, 2019). In the current study, the phytotoxicity of *P. hysterophorus* leaf, stem and root using different solvents was evaluated on seed germination, plumule and radicle lengths of two weeds of rice in Malaysia *E. colona* and *L. decurrens*. The ethyl acetate leaf extracts (Fig. 3) were noted as best inhibitors on seed germination, plumule and radicle lengths at all the concentrations (2.5, 5.0, 7.5 and 10.0%), followed by the methanol (Fig. 1) and water extracts (Fig. 4). Meanwhile, the hexane extract (Fig. 2) gave the lowest inhibition. This finding was consistent with the results of a previous study by Pati and

Chowdhury (2015) who reported that ethyl acetate and methanol extracts of *P. hysterophorus* inhibited the germination of *Vigna radiata* L. seeds at concentration level as low as 10% and inhibition increased with increase in concentration from 10-100%. Tessema and Tura (2018) also reported that aqueous leaf, stem and root extracts of parthenium caused a complete failure in *Triticum aestivum* and *Hordeum vulgare* under high concentration of *P. hysterophorus* extracts at 10%. Similarly, Sorecha and Bayissa (2017) further reported that the extracts of *P. hysterophorus* parts inhibited seed germination, shoot heights, and root lengths of peanut and soybean at 1%. Furthermore, Pélagie-Michelin *et al.* (2016) reported that solvent polarity impact was strongly influenced and the decrease in germination percentage was amplified with rising extract polarity, implying a rise in active ingredients with polarity. The ethyl acetate extract, which was less polar than the aqueous and methanol fractions showed the strongest stimulatory actions resulting in a larger germination percentage than the negative control. However, Wang *et al.* (2017) hypothesized that it might contain secondary metabolites with suppressive effects on the germination of seed. The same scenario may apply to the effect ethyl acetate extract in the present study on the bioassay plant as it had been reported that secondary metabolites (allelochemicals) present in leaf extracts of certain plants suppressed embryo growth or results in its death. Furthermore, the discernable decrease in seed germination, as well as obstruction of water imbibition by seeds, may be explained by other different mechanisms such as changes in gibberellic acid synthesis and actions (Cheng and Cheng, 2015; Amb and Ahluwalia, 2016).

In this current study, the hexane extracts at all concentrations stimulated seed germination, plumule and radicle lengths of the bioassay species (Fig. 2). This result was also in concordance with the findings of Céspedes *et al.* (2015) who reported that some compounds of secondary metabolism in plants acted as inhibitors, while others as stimulators as a portion of plant chemical defense system. Pélagie-Michelin *et al.* (2016) also further confirmed that hexane extracts of *Callistemon viminalis*, *Tephrosia vogelii*, *Senna spectabilis*, *Cupressus lusitanica* and *Polyscias fulva*

contained non-polar substances which promoted increase in shoot and root lengths and diameters. Therefore, these extracts had appreciable quantities of allelochemicals that can induce visible growth in shoot and root length. Root and shoot growth was also significantly reduced in the ethyl acetate, methanol and aqueous extracts at the different concentration levels particularly in the leaf extract because of the presence of phytotoxic allelochemicals. Allelochemicals were reported to meddle straight away with physiological and biochemical reactions implicated in the growth and development of plant tissues. Céspedes *et al.* (2015) also reported that many allelochemicals and extracts from botanical sources possessed plant growth suppressing effects in the course of seed germination and other plant physiological activities such as plumule and radicle of seedlings, respiration of seeds during the process of germination, uptake of oxygen, hydrogen, and chloroplast and mitochondrial redox inhibition. Kong *et al.* (2019) reported that allelochemicals or plant secondary metabolites inhibited the germination of seeds as well as the emergence of seedlings because they contained some compounds to be utilized directly as herbicides or as precursor templates for the development of herbicides. The inhibition of root length showed that parthenium could successfully thrive and could replace native vegetation because of its phytotoxic allelopathic potential, which is a serious threat to biodiversity (Hassan *et al.*, 2018).

On the whole, the two bioassay species were more susceptible to ethyl acetate extract as the leaf extract completely inhibited seed germination, plumule and radicle growth at all concentration levels, while the stem and the root extracts completely inhibited seed germination, plumule and radicle length at 7.5 and 10%. This showed that the ethyl acetate leaf extracts had more growth-inhibiting compounds compared to the solvent extracts.

CONCLUSION

In conclusion, the ethyl acetate, methanol, and aqueous parthenium leaf crude extracts had demonstrated strong inhibitory effects on seed germination, plumule and radicle lengths of the two bioassay plants. However, the strongest inhibition effect was observed in the ethyl

acetate leaf extracts at as low as 2.5% concentration than the stem and roots, followed by methanol and aqueous extracts. This showed that the extract containing phytotoxic secondary metabolites (alkaloids, flavonoids, anthraquinones, steroids, phenols, saponins, tannins and resins) could be both suppressive and stimulatory in action as shown on the parameters studied. The secondary metabolites in the ethyl acetate extracts may possess excellent biotechnological utilization prospects in crop production.

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