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Herbicidal Activity of Isolated Fractions and Identified Compounds from the Ethyl Acetate Extract of *Parthenium hysterophorus* L. Leaves on *Echinochloa colona* (L.) Link and *Hedyotis verticillata* (L.) Lam.

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ABSTRACT

Ethyl acetate extract of *Parthenium hysterophorus* leaves was fractionated through vacuum liquid chromatography resulting in nine main fractions which were tested against germination, plumule, and radicle lengths of *Echinochloa colona* and *Hedyotis verticillata*. Fractions 5 and 6 completely inhibited the germination of the bioassay weeds. The presence of five major bioactive compounds [stigmasterol (ST), ursodeoxycholic acid (UDCA), naphthalene (NAP), bis (2-ethylhexyl) adipate (DEHA) and bis (2-ethylhexyl) phthalate (DEHP)] was confirmed through GC-MS in these fractions. Dose-response studies were carried out at 0.5, 1.0, 1.5 and 2.0 mg/ml and 0.5, 1.0, 1.5 and 2.0 kg a.i./ha in the laboratory and greenhouse, respectively. In the laboratory experiments, DEHA and DEHP exhibited potent inhibition on germination ($P<0.05$) of *E. colona* and *H. verticillata* by 82-86 and 83-91%, respectively, at the highest concentration of 2.0 mg/ml. All the five compounds inhibited seed germination ranging from 61-73% for *E. colona* and 35-40% for *H. verticillata* under greenhouse. Plumule length of *H. verticillata* was strongly suppressed at 2.0 mg/ml of UDCA with 80% inhibition under laboratory, while 57% inhibition was obtained under greenhouse at 2.0 kg a.i./ha. Similarly, radicle length was suppressed with >90% on both the species under laboratory by UDCA. However, in the greenhouse UDCA was more phytotoxic to *E. colona* with a 72% reduction in radicle length as compared to *H. verticillata* with 42% radicle retardation. The present study revealed that DEHP, DEHA and UDCA could be potential sources of natural herbicide.

Key words : Bioactive compounds, bioassay species, germination inhibition, herbicidal activity, plumule, radicle length, *P. hysterophorus*

INTRODUCTION

Agriculture is essential in securing food security, human capital development about enhancing the standard of living and more than 60% of the global populace rely on it as a source of livelihood (Lopez-Ridaura *et al.,* 2019). It has been estimated that the global human population is about 7.7 billion and may hit 10.2 billion in 2050. To this end, food production has to step up to meet the food and energy requirements of the ever-increasing population. To achieve this, the use of farm inputs to boost food production becomes necessary and this warrants the use of synthetic agrochemicals (Pati and Chowdhury, 2015a). These agrochemicals have been steadily applied in the control and management of weeds in the production of crops because of their exceptional efficacy and low price (Monteiro and Santos, 2022). However, the excessive utilization of these synthesized agrochemicals has created a negative impact on the natural environment as well as the sustainable management of weeds. Therefore, investigation on the application of natural compounds from plants and other life forms for the management and control of weeds on farms has been extensively carried out (Bajwa *et al.*, 2020).

Lately, researchers have been preoccupied with securing a substitute for synthetic herbicides for the control and management of weeds in many crops. Allelopathy has become the center of focus in the quest for the search alternatives to synthetic herbicides lately

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(Sathishkumar *et al.*, 2020). Allelopathic weeds were proposed to be used against other weeds as weeds species were regarded as abundant sources of allelochemicals. These secondary metabolites can inhibit seed germination and the growth of many more weeds, of which many are herbicide-resistant (Cheng and Cheng, 2015).

Parthenium hysterophorus L. (Asteraceae), often referred to as Rumpai Miang Mexico in Malaysia, is an annual aggressive and pernicious weed native to north-east Mexico and it also possessed the ability to successfully invaded many ecosystems due to its allelopathic properties (Maszura *et al.*, 2018; Bristone *et al.*, 2020). Research on the phytochemistry of parthenium has detected many secondary metabolites leaves, stems, roots and flowers (Bajwa *et al.*, 2020). The phytotoxicity of parthenium has been attributed to several sesquiterpene lactones and phenolics identified from its parts (Pati and Chowdhury, 2015b). In addition to phenolics and sesquiterpenes in parthenium, alkaloids and flavonoids have also been reported to be amongst the secondary metabolites produced by the plant (Bajwa *et al.,* 2020).

The phytotoxic activity of this noxious weed in the control of other weeds has been established by earlier researchers. For instance, Bristone *et al.* (2021) reported that the ethyl acetate and methanol leaf extracts of *P. hysterophorus* reduced seed germination, plumule, and radicle length of *E. colona* and *Ludwigia decurrens* by 100% inhibition at 2.5% concentration. Pati and Chowdhury (2015a) reported that ethyl acetate and methanol extracts of aerial parts of *P. hysterophorus* inhibited the germination of *Vigna radiana* L. seeds at concentration levels as low as 10% and inhibition increased with an increase in concentration from 10-100%. However, there is limited information on the activities of isolated fractions and identified compounds from the crude ethyl acetate extract of *P. hysterophorus* leaves in Malaysia. As a result, the current study was carried out to identify the functional groups of the identified compounds from *P. hysterophorus* and to assess their herbicidal activity on jungle rice [*Echinochloa colona* (L.) Link] and woody borreria (*Hedyotis verticillata* (L.) Lam in both the laboratory and greenhouse conditions.

MATERIALS AND METHODS

P. hysterophorus samples were collected from invaded fields in Kampung Kongsi Nam (5°63'21'' N Latitude and 100°49'23'' E Longitude) in Kedah state of Malaysia. Seeds of the two bioassay species jungle rice [*Echinochloa colona* (L.) Link] were obtained from rice fields in Pasir Mas Kelantan, Malaysia (20°4'39" N Latitude and 102°14'37"E Longitude) while woody borreria [*Hedyotis verticillata* (L.) Lam] was collected from oil palm plantation at Sungai Rual (5°39'39" N and 101°47'40" E) Jeli, Kelantan, Malaysia.

Analytical grade solvents : ethyl acetate, hexane, methanol and Dimethyl sulfoxide (DMSO) were purchased from HmbG Chemicals (Hamburg, Germany), while Dibutyl phthalate (DBP) PESTANAL, Stigmasterol, Naphthalene, Ursodeoxycholic acid and Dibutyl adipate were procured as high purity standards from Sigma Chemical Company (Selangor, Malaysia). Silica gel for column chromatography (70-230 mesh) was bought from Merck (Darmstadt, Germany).

The ethyl acetate extracts of *P. hysterophorus* leaves were prepared according to the procedure of Pati and Chowdhury (2015a) and Bristone *et al.* (2021). Five kg plant leaves were collected from the field and washed with distilled water to remove contaminants. The plant samples were air-dried under the shade at a temperature of 25-32°C and relative humidity of 22-40% for 10 days. The dried samples were then ground into a 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 ethyl acetate solvent by vigorously shaking at 200 rpm for 72 h on an orbital shaker at 25°C (resulting in a 20% stock solution). The crude extract was then filtered through filter paper Whatman No.1. A 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. The original crude of ethyl acetate was bioassay using *E. colona* and *H. verticillata* as test plants.

The isolation of phytotoxic compounds was carried out using the vertical liquid chromatography as described by Yinusa *et al.* (2015). The ethyl acetate extract was subsequently directed to a vacuum liquid

chromatography in regular Merck silica gel with 70-230 mesh (63-200 µm) by using a Buchner funnel (10 x 10 cm). Silica gel (170 g) was weight out and poured into the column and the sides of the funnel were tapped gently with rubber tubing for even distribution and proper packing of the column. The pre-absorbed sample to be loaded on the column was prepared by dissolving 10 g of the ethyl acetate extract in 150 ml of ethyl acetate. To the solution, 70 g of silica was added and mixed by stirring with a glass rod. The mixture was allowed to dry at room temperature in a fume hood. Ten grams of the pre-absorbed sample mixture was evenly layered on the top of the column layer bed. Then sea sand (extra pure) was evenly layered on top of the pre-absorbed sample and finally covered with filter paper. The column was consecutively eluted with different ratios of petroleum ether (PE) and ethyl acetate (EA) at 100 : 0, 80 : 20, 60 : 40, 40 : 60, 20 : 80 and 0 : 100 followed by ethyl acetate (EA) and methanol (MeOH) at 100 : 0, 80 : 20, 60 : 40, 40 : 60 and 20 : 80. Nine major fractions were collected, and tested for their phytotoxic effect on germination of *E. colona* and *H. verticillata* as outlined below.

The procedure of Chuah and Lim (2021) was adopted for the evaluation of the phytotoxic activity of the isolated fractions. Twenty seeds of each of *E. colona* and *H. verticillata* were placed in 9 cm diameter petri dishes lined with two layers of filter paper (Whatman No. 1) and moistened with 5 ml of each fraction at 5 mg/ml. Petri plates that were merely moistened with distilled water were the control group. For 14 days, the petri plates were maintained at 30/20°C day and night with a 12 h photoperiod in the laboratory. When the radicle length reached 1 mm seeds were considered emerged. The emerged seeds were calculated as a percentage of the total number of viable seeds in each replication at the end of the incubation period. Also measured and recorded were the radicle and plumule lengths of germinated seeds. The percentage of the respective control was used to express the data.

The compounds of the fraction that exhibited the most phytotoxicity were identified using a gas chromatography-mass spectrometry (GC-MS) Agilent 7890B GC/MS System with a 7693 autosampler and an Agilent 5977A Series GC/MSD-mass selective detector (from Agilent Technologies, Beijing, China) with analytical column (HP-5ms Ultra Inert 30 m × 250 µm, 0.25 µm) (p/n 19091S-433UI). The conditions employed for the column included GC grade helium at a flow rate of 1 ml/min, injection mode with split less. The inlet temperature was set at 280°C, the oven was set at 60°C for 1 min, then 40°C/min to 170°C, then 10°C/min to 310°C, then held for 2 min. The settings of the transfer line, source and quadrupole temperatures were 280°C, 250°C, and 150°C, respectively, while the solvent delay was 3.5 min. SIM and electron ionization (EI) were the acquisition modes (Liu *et al.,* 2019). Identification of secondary metabolites was done using an automatic system of processing data (Agilent MassHunter Workstation Software qualitative analysis) supplied by the National Institute of Standards and Technology (NIST version 2.0) database and also by comparison of retention time and mass spectra with those of authentic and standard samples.

A total of 25 seeds of both *E. colona* and *H. verticillata* were placed in 9 cm diameter petri plates that were inlaid with double sheets of filter paper. The filter paper previously was moistened with 5 ml (0.5, 1.0, 1.5 and 2.0 mg/ml) of stigmasterol, ursodeoxycholic acid, naphthalene, bis (2-ethylhexyl) adipate, and bis (2-ethylhexyl) phthalate, respectively, which were dissolved in 2% dimethyl sulfoxide (DMSO). Distilled water was used as a control. For 14 days, the petri plates were maintained at 30/20°C day and night with a 12 h photoperiod in the laboratory. When the radicle length reached 1 mm, seeds were considered to emerge. The emerged seeds were calculated as a percentage of the total number of viable seeds in each replication at the end of the incubation period. Also measured and recorded the radicle and plumule lengths of germinated seeds. The percentage of the respective control was used to express the data.

The top soil collected from Agro-Techno Park, University Malaysia Kelantan, Jeli Campus was brought to the greenhouse (temperature 27-32°C, humidity 65-80%) and spread in the plastic tray until dried. The dried soil was then mixed and sieved carefully to remove the trash. Five hundred g of the soil was put in plastic pots (20 cm diameter x 10 cm height), and saturated with tap water. Twenty five seeds of *E. colona* and thirty seeds of *H. verticillata* were sown on the soil surface. After one day, the weed species were treated with the identified

compound [stigmasterol, ursodeoxycholic acid, naphthalene, bis (2-ethylhexyl) adipate, and bis (2-ethylhexyl) phthalate] that previously was dissolved in 2% DMSO at 0.5, 1.0, 1.5 and 2.0 kg a.i./ha. The treatments were applied by using a micropipette to provide 10 ml of liquid per pot. Non-treated seeds were used as control treatments. The number of emerged *E. colona* and *H. verticillata* seedlings were counted and recorded after 14 days. The radicle length and plumule length were then measured, and the data were expressed as percentages of the respective controls (Chuah and Lim, 2021).

The germination and dose-response tests were arranged in a completely randomized design (CRD) with three replications. The data were subjected to a one-way analysis of variance (ANOVA). Tukey HSD 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

Vacuum liquid chromatography (VLC) conducted resulted in 19 fractions, however, upon carrying the TLC profiling it yielded nine fractions. The nine fractions displayed significant (P<0.05) phytotoxic activity at varying levels, however, some demonstrated promotion activities (Fig. 1). Fractions 5 and 6 completely inhibited the germination, plumule, and radicle length of both *E. colona* and *H. veticillata* seeds by 100%. The other fractions gave germination inhibition that ranged from 13-73 and 53-80% for *E. colona* and *H. verticillata,* respectively (Fig. 1A). The remaining fractions also demonstrated phytotoxic activity on the plumule length of the two bioassay species. A decrease from 8-26 and 6-39% in plumule length was observed in *E. colona* and *H. verticillata,* respectively (Fig. 1B). Similarly, radicle length was significantly reduced by the other fractions as there was a

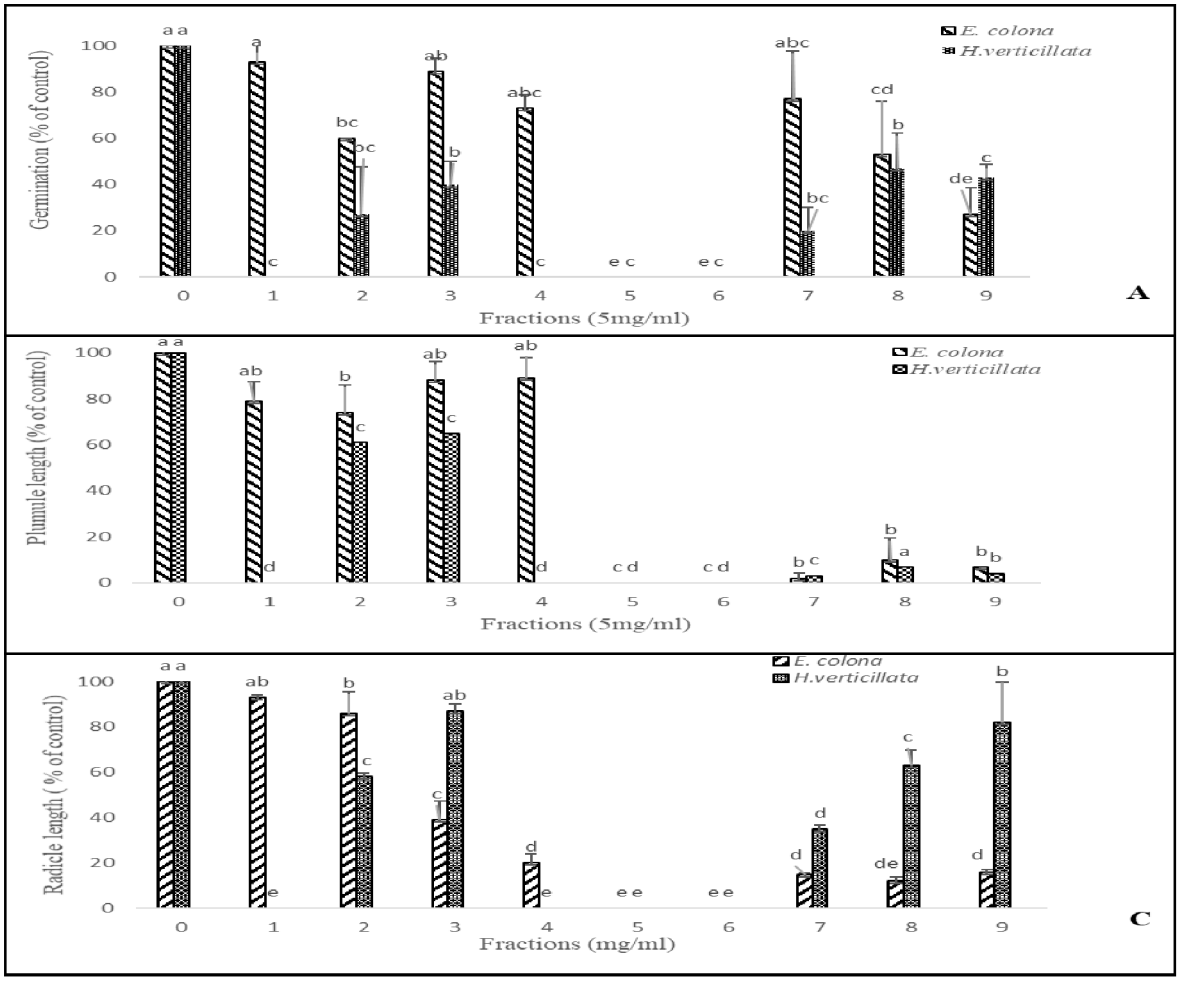

Fig. 1. The effect of ethyl acetate leaf fractions of *P. hysterophorus* on germination (A), plumule length (B) and root length (C) of *E. colona* and *H. verticillata.* Vertical bars are the standard deviation of the mean.

reduction of 7-85 and 13-65%, respectively, for *E. colona* and *H. verticillata* (Fig. 1C). This study revealed that fractions 5 and 6 significantly reduced the germination of the two bioassay species. This suggestd that the two fractions possessed allelochemicals that demonstrated inhibitory effects on *E. colona* and *H. verticillata* giving a 100% inhibition. The results of this study concur with those of Pati and Chowdhury (2015b) who reported that ethyl acetate and methanol fractions of *P. hysterophorus* demonstrated maximum inhibition on the germination and growth of *Vigna radiata*. Similarly, Boonmee *et al.* (2020) reported that the ethyl acetate fraction of *Nephrolepis cordifolia* aerial parts demonstrated greater inhibitory effects on the shoots and roots of *Lepidium sativum* (garden cress) compared with the aqueous fraction. Furthermore, Krumsri *et al.* (2022) revealed that the aqueous and ethyl acetate fractions of *Senna garrettiana* leave extract had significant inhibitory effects on the seedlings of *Lepidium sativum* L. and *Echinochloa crus-galli* compared with the control ($P<0.05$). The IC_{50} values of the bioassay species revealed that the suppression by the ethyl acetate fraction was higher than that by the aqueous fraction and *L. sativum* was the most susceptible to the two fractions.

The GC-MS analysis of the most phytotoxic fractions (fractions 5 and 6) revealed the presence of 5 major compounds viz., Stigmasterol (ST) (MW 414 g/mol, per cent area volume 4.62), Ursodeoxycholic acid (UDCA) (MW 392.56 g/mol, per cent area volume 25.51), Naphthalene (NAP) (MW 128.170 5 g/mol, per cent area volume 5.35), Bis(2-ethylhexyl) phthalate (DEHP) (MW390.56, per cent area volume 14.2), Bis (2-ethylhexyl) adipate (DEHA) (MW 390.6, per cent area volume 12.07) with their respective standards and structural formulae [(Fig. 2 (A-E)]. These compounds were also reported by many workers as components of plants identified via GC-MS. For instance, stigmasterol was isolated from various medicinal plants such as *Odontonema strictum, Argemone mexicana* and *Fritillaria roylei* (Luhata and Moses, 2015; Praveen *et al.,* 2015; Kaur *et al.*, 2020). Ursodeoxycholic acid was reported to be produced by some soil isolates of bacterial species and fungi as well as from the gut microbiota (Guzior and Quinn, 2021; Yang *et al.,* 2021). Naphthalene derivatives were reported to be isolated from plants such as *Rheum australe, Cassia fistula, Kniphofia foliosa,* etc. (Ibrahim and Mohamed, 2016). Bis (2-ethylhexyl) phthalate was reported to be isolated from different natural sources such as from soil and wheat grains (Mei *et al.,* 2019), in vegetable and fruit samples (Cao *et al.,* 2015), in the stem of *Datura stramonium* (Durak and Aysu, 2016). Bis (2-ethylhexyl) adipate migrated when packaging material was in direct contact with high-fat content foods. Bis (2-ethylhexyl) adipate can be released into the environment during its synthesis and distribution and in consumer use of finished plastic products (Cao *et al.,* 2015). Furthermore, Bis (2-ethylhexyl) adipate was isolated from the Antarctic krill (*Euphausia superb;* Xiangning and Daicheng, 2018).

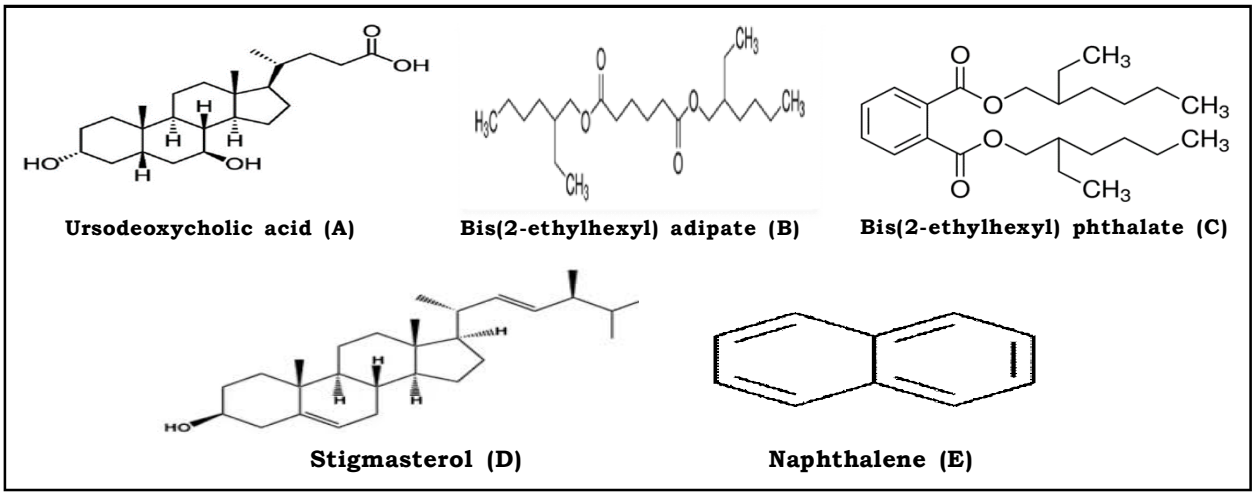

Fig. 2. Structural formula of identified compounds : (A) Ursodeoxycholic acid, (B) Bis (2-ethylhexyl) adipate, (C) Bis (2-ethylhexyl) phthalate, Stigmasterol, (D) and Naphthalene (E).

Laboratory Condition

The effect of the five compounds : Stigmasterol (ST), Ursodeoxycholic acid (UDCA), Naphthalene (NAP), Bis (2-ethylhexyl) adipate (DEHA) and Bis (2-ethylhexyl) phthalate (DEHP) on the germination and seedling growth of *E. colona* and *H. verticillata* in the laboratory is presented in Table 1. The germination percentage of *E. colona* decreased with increasing concentration of the compounds, highest inhibition was at 2 mg/ml, while 0.5 mg/ml gave the lowest inhibition. DEHP gave the highest germination inhibition percentage of 14%, followed by DEHA at 18%, NAP gave 19%, while ST and UDCA both gave 21% inhibition. The effect of the five compounds on the germination of *H. verticillata* seeds revealed that there was differential response across the concentrations. Three compounds UDCA, DEHP and DEHA inhibited seed germination with an increase in the concentration of the compounds giving 7, 9 and 12%, respectively, at 2 mg/ml, while ST and NAP gave the highest inhibition of 9 and 15%, respectively at 0.5 mg/ml, while lowest inhibition was at 2 mg/ml. The result showed that the compounds significantly ($P<0.05$) inhibited seed germination of the bioassay species when compared to the control.

The effect of the compounds on plumule length showed varying degrees of suppression on the two bioassay species when compared to the control. The highest plumule length inhibition of 65% on *E. colona* was given by DEHP at 2.0 mg/ml concentration, while the highest reduction in the plumule length of *H. verticillata* was 20% through UDCA at 2.0 mg/ml (Table 2). Radicle elongation of the two bioassay species was significantly suppressed by the five compounds. UDCA gave the highest radicle growth suppression percentage of 7 and 4 for *E. colona* and *H. verticillata,* respectively, at 2 mg/ml concentration when compared to the control (Table 2).

The effect of the isolated and identified compounds on the germination, plumule and radicle length *of E. colona* and *H. verticillata* in the nursery is presented in Table 2. Two compounds DEHP and UDCA gave the highest germination inhibition on the two bioassay species when compared to the control experiment. DEHP inhibited the germination of *E. colona* by 17% at 1.0 kg a.i/ha, while UDCA suppressed the germination of *H. verticillata* by 41% at 1.5 kg a. i./ha (Table 2). The response of the plumule length of *E. colona* and *H. verticillata* to the five compounds showed that the highest plumule length suppression was obtained at 2 kg a. i./ha by UDCA with

Table 1. Effect of identified compounds on germination, plumule length and radicle length of *E. colona* and *H. verticillata* in the laboratory condition. Means carrying different alphabets denote significant differences in germination percentage, plumule and radicle lengths ($P<0.05$)

Bioassay species	*E. colona*					*H. verticillata*				
Identified compound/ Concentration (mg/ml)	ST	UDCA	NAP	DEHA	DEHP	ST	UDCA	NAP	DEHA	DEHP
	Germination (% of control±S. D.)					Germination (% of control±S. D.)				
0.0	100±2a	100±2a	100±2a	100±2a	100±2a	100±0a	100±0a	100±0a	100±0a	100±0a
0.5	27±6b	36±4b	27±12b	35±6b	33±9b	9±5b	21±5b	15±2b	23±2b	21±14b
1.0	35±9c	33±4b	24±12b	27±2bc	25±5c	12±4b	12±4bc	24±7b	12±0c	14±6b
1.5	33±8c	30±9b	24±4b	20±5cd	19±4d	17±2b	9±6c	75±2c	14±2c	11±5b
2.0	21±2b	21±6c	19±6b	18±4d	14±7d	47±16c	7±2c	77±2c	12±2c	9±4c
	Plumule length (% of control±S. D.)					**Plumule length (% of control±S. D.)**				
0.0	100±2a	100±2a	100±2a	100±2a	100±2a	100±2a	100±2a	100±2a	100±2a	100±2a
0.5	83±3b	89±6b	89±4b	82±8b	83±2b	20±0b	50±8b	88±6ab	91±4ab	91±4ab
1.0	80±4b	93±6ab	85±5b	75±10bc	85±5b	35±5c	37±9b	82±2b	83±4b	91±2ab
1.5	81±2b	94±2ab	85±6b	81±6b	80±0b	49±3d	21±2c	87±4ab	80±2b	92±4ab
2.0	75±2c	81±2c	80±0b	69±4c	65±5c	46±4d	20±0c	79±8b	75±2b	70±5c
	Radicle length (% of control±S. D.)					**Radicle length (% of control±S. D.)**				
0.0	100±3a	100±3a	100±3a	100±3a	100±3a	100±3a	100±3a	100±3a	100±3a	100±3a
0.5	61±4b	22±2b	83±2b	74±7b	78±5b	32±0b	6±6b	81±6b	77±3b	57±13b
1.0	70±10bc	11±5c	83±8b	56±22bc	77±4b	69±3c	6±2b	85±2b	65±3c	69±3c
1.5	73±7bc	8±2c	78±5bc	67±14b	73±2b	74±2c	5±5b	74±5c	76±4b	73±2d
2.0	57±2c	7±0c	70±5c	50±8c	55±4c	61±4d	4±2b	65±2d	58±9d	55±7c

ST (Stigmasterol), UDCA (Ursodeoxycholic acid), NAP (Naphthalene), DEHA (Bis (2-ethylhexyl) adipate) and DEHP (Bis (2-ethylhexyl) phthalate).

Table 2. Effect of identified compounds on germination, plumule length and radicle length of *E. colona* and *H. verticillata* in the greenhouse condition. Means carrying different alphabets denote significant differences in germination percentage, plumule and radicle lengths (P<0.05)

Bioassay species	*E. colona*					*H. verticillata*				
Identified compound/ Concentration (kg a. i./ ha)	ST	UDCA	NAP	DEHA	DEHP	ST	UDCA	NAP	DEHA	DEHP
	Germination (% of control±S. D.)					Germination (% of control±S. D.)				
0.0	100±0 a	100±0a	100±0a	100±0a	100±0a	100±0a	100±0a	10±0a	100±0a	100±0a
0.5	29±9b	25±11b	26±2b	31±13b	29±9b	60±7b	76±2bc	62±12b	71±7b	69±7b
1.0	26±6b	24±5b	23±8b	20±8b	17±6c	65±14b	68±4bc	69±5b	64±6b	73±12b
1.5	39±13b	39±10b	27±5b	29±8b	39±13b	59±9b	41±27c	60±9b	68±2b	72±7b
2.0	39±4b	39±4b	27±4b	39±7b	32±4b	61±12b	61±3bc	59±8b	65±8b	60±2b
	Plumule length (% of control±S. D.)					**Plumule length (% of control±S. D.)**				
0.0	100±0a	100±0a	100±0a	100±0a	100±0a	100±2a	100±2a	100±2a	100±2a	100±2a
0.5	68±5b	72±8b	75±3b	72±6b	68±5b	80±0b	86±5b	80±0b	83±3b	92±3ab
1.0	66±7b	66±7bc	88±4c	50±5c	66±7b	77±6bc	75±5b	77±3b	86±2b	89±5b
1.5	61±4b	57±3c	75±5b	62±6b	61±4b	59±9cd	75±5b	63±3c	83±3b	86±2b
2.0	53±5c	43±3d	66±5b	65±2b	47±5c	61±9d	58±3c	59±3c	61±3c	64±3c
	Radicle length (% of control±S. D.)					**Radicle length (% of control±S. D.)**				
0.0	10±2a	100±2a	100±2a	100±2a	100±2a	100±2a	100±2a	100±2a	100±2a	100±2a
0.5	72±6bc	67±5b	81±2bc	91±6ab	72±6b	80±6b	91±3b	80±3b	88±0b	90±3ab
1.0	67±6c	52±4c	88±2bc	82±4b	67±6b	71±3c	90±3b	76±6b	85±3b	86±6b
1.5	53±2c	56±6c	77±6c	86±4b	53±2c	81±3b	83±5b	71±3b	85±3b	86±3b
2.0	43±2d	28±4d	65±2d	81±5b	43±2d	67±3d	64±3c	66±3c	69±6c	65±6c

ST (Stigmasterol), UDCA (Ursodeoxycholic acid), NAP (Naphthalene), DEHA (Bis (2-ethylhexyl) adipate), and DEHP (Bis (2-ethylhexyl) phthalate).

suppression percentages of 43 and 58%, respectively, when compared with the control experiment (Table 2). The five compounds similarly displayed significant effects on the growth of the radicle of the two test plants at the different concentration levels when compared to the control. The highest radicle growth suppression of *E. colona* and *H. verticillata* was obtained at 2 kg a. i./ha by UDCA giving suppression percentages of 28 and 64%, respectively (Table 2).

The present study on the evaluation of the herbicidal activity of isolated fractions and identified compounds from the ethyl acetate extract of *P. hysterophorus* leaves on *E. colona* and *H. verticillata* revealed that the five compounds exhibited different levels of phytotoxicity on the germination and growth of bioassay plants in both the laboratory and nursery experiments. The germination of *E. colona* was strongly inhibited by DEHP in both the laboratory and nursery experiments. This was in agreement with the findings of previous reports of Deng *et al.* (2017), who reported that high concentrations (>0.5 mmol/l) of identified phthalates caused significant (P<0.05) inhibition of tobacco seed germination and seedling growth. Similarly, Khaliz *et al.* (2016) disclosed that the germination process was affected adversely by the application of phthalates in *Cucumis sativus* (cucumber) and *Brassica chinensis* (rape). UDCA was more phytotoxic on the germination of *H. verticillata* seeds in both the laboratory and nursery experiments. Although, no literature has quoted the use of this compound in agriculture either as a pesticide or a plant growth regulator, however, research on UDCA acid indicated that it adversely suppressed the spore germination and toxin activity of various *Clostridium difficile* strains *in vitro* (Winston *et al.*, 2020). DEHA also demonstrated some level of phytotoxicity on the germination of the two bioassay species after DEHP and UDCA in both the laboratory and nursery experiments, however, no specific study was conducted on the herbicidal activity of DEHA. Though other studies carried out suggested that this compound seriously inhibited the survival and growth of algae *Heterosigma akashiwo* and *Gymnodinium breve* at 5.82 and 2.74 mg/l concentrations, respectively. Similarly, the growth of planktonic crustacean *Daphnia magna* was inhibited at very low concentrations of 0.087-0.180 mg/ml (Xiangning and Daicheng, 2018). In terms of germination inhibition of *E. colona* and *H. verticillata* seeds in both laboratory and nursery, NAP came fourth among the five compounds under study although information on its

herbicidal activity in literature was lacking, however, Pajaro-Castro *et al.* (2017) reported that naphthalene and benzene inhalation were able to induce alterations in reproduction, development, metamorphosis, oxidative stress, metabolism, neurotransmission and death of the red flour beetle (*Tribolium castaneum*). Stigmasterol was least phytotoxic on the germination of the seeds of the bioassay species in both the laboratory and nursery conditions. The information on the herbicidal activity of this compound is lacking, however, it has been evaluated for its pharmaceutical potentials such as antiosteoarthritic, anti-hypercholesterolemic, cytotoxicity, antitumor, hypoglycemic, antimutagenic, antioxidant, antiinflammatory and insecticidal (Aaron *et al.,* 2018). The study found that the five compounds were more phytotoxic in the laboratory (filter paper) experiment than the nursery experiment (soil). The same scenario was observed by Kumari and Kaur (2020) that in the early stages of germination, seeds soaked water from surrounding soils but organic compounds with moderately low water solubility were easily imbibed to soil organic matter rather than dissolved in the soil pore. This made them less likely to enter the seed and yield lethal effect by either suppression or triggering of seed germination.

On the plumule and radicle growth of the two bioassay species, the five compounds also demonstrated certain degrees of phytotoxicity. UDCA gave the highest suppression of plumule and radicle length of *E. colona* and *H. verticillata* in both the laboratory and nursery experiments. Even though there are no documented herbicidal activities of this compound, however, research on UDCA indicated that it adversely suppressed the growth of various *Clostridium difficile* strains *in vitro* (Winston *et al.*, 2020). The compound that followed UDCA in terms of shoots and root length suppression of the two bioassay species in both the laboratory and nursery experiments was DEHP. DEHP was reported to significantly inhibit the growth of the stem and root of *Lactuca sativa* at 100 µg/ml (Ren *et al.*, 2015). Furthermore, Gao *et al.* (2017) reported that DBP and DEHP at 5, 10, 20, 30, 40 and 125. 50 µg/ml concentrations demonstrated severe toxic effects on root and shoot elongation of wheat (Jinnong 7) seedlings. DEHA came third in terms of plumule and radicle length suppression followed by ST and NAP. However, there was no documented evidence to suggest that these three compounds possessed herbicidal properties.

CONCLUSION

The five compounds possessed varying degrees of phytotoxicity on the germination and seedling growth of *E. colona* and *H. verticillata* in the following order from the most phytotoxic to the least : Bis (2-ethylhexyl) phthalate > Ursodeoxycholic acid > Bis (2-ethylhexyl) adipate >Stigmasterol >Naphthalene. However, Bis (2-ethylhexyl) phthalate demonstrated higher phytotoxicity on the germination of the *E. colona* than *H. verticillata,* therefore, can be valuable in the development of natural herbicide. Notwithstanding, there is the need for further studies to ascertain the exact sequence of physiological activities that are responsible for the inhibition of seed germination and growth of the bioassay species.

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