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Identification of Phytochemicals and Mineral Nutrients of Selected Malaysian Plant Extracts and Its Effects on Seed Priming of Maize

Mingzhao Han, Susilawati Kasim*, Md Kamal Uddin, Halimatul Sa'adiah Abdullah, Shah Ahmed Reza and Effyanti Mohd Shuib

Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Plants contain a variety of phytochemicals, which act as natural bioactive compounds to help plants enhance abiotic tolerance and promote growth. Therefore, plant extracts are considered to have great potential as environmentally friendly biostimulants in sustainable agriculture. This study aimed to identify the phytochemical compounds and quantify nutrients present in three plant extracts, namely *Euphorbia hirta*, *Polygonum minus*, and *Eleusine indica*, as well as to explore the effect on the growth of maize seedlings (*Zea mays* L.). The plant powder was extracted using methanol, followed by a solid-liquid extraction procedure. The phytocompounds were analyzed by liquid chromatography-mass spectrometry, while mineral nutrients were quantified using inductively coupled plasma. Five concentrations of plant extracts, i.e., 5, 15, 25, 50, and 100%, were designed to evaluate seed germination and priming. The result showed that 53, 45, and 39 phytocompounds were identified from *E. hirta*, *P. minus*, and *E. indica*, respectively, and classified into different chemical groups (such as flavonoids and amino acids) and rich nutrients (for example, N, P, and K). Besides, *P. minus* and *E. hirta* extracts with lower concentrations (5 and 15%) showed a positive effect on germination, shoot length and fresh weight, and the Highest concentration (100%) of *E. hirta* and *E. indica* extracts exhibited strong toxicity. This study emphasizes that weed extracts containing abundant secondary metabolites and nutrients can be used as natural biostimulants for maize seed priming.

Keywords: Maize, mineral nutrient, plant extract, phytochemical, seed priming

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E-mail addresses:
hmzh95@outlook.com (Mingzhao Han)
susilawati@upm.edu.my (Susilawati Kasim)
mkuddin07@gmail.com (Md Kamal Uddin)
halimatul81@gmail.com (Halimatul Sa'adiah Abdulla)
rezashahahmed@gmail.com (Shah Ahmed Reza)
effyantims@gmail.com (Effyanti Mohd Shuib)
* Corresponding author

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INTRODUCTION

Plants can produce a variety of bioactive compounds through metabolism, among which numerous antioxidant compounds have been identified in vegetables and fruits, such as phenolics, flavonoids, tocopherols, and anthocyanins (Ali et al., 2021). Certain medicinal plants have gained widespread use in the pharmaceutical industry for treating related diseases (Hao et al., 2020). Meanwhile, phytochemicals, which accumulate in high concentrations within plants, may protect against damage caused by abiotic stress (Hussein et al., 2015). Additionally, some beneficial phytochemicals serve as natural antioxidants and can supplement the human body's requirements (Boots et al., 2008), thereby increasing immunity and mitigating age-related health issues (Forni et al., 2019). As a result, there has been an increase in the global consumption of medicines and health products derived from plants.

In the world's tropical regions, a wide spread of weeds in agricultural lands affects crop production, and Malaysia is no exception (Dilipkumar et al, 2020). Karim et al. (2004) reported that weed invasion can cause rice yield losses ranging from 5 to 85% in Malaysia. Besides, weeds compete intensively for nutrients, resulting in a yield decrease of 5 to 20% (Sahid et al., 1992). However, farmers and administrators usually ignore the disadvantage of weeds. On the other hand, studies have demonstrated the potential benefits of certain weeds. For instance, *P. minus* is believed to be associated with antioxidant activities, such as flavonoids and phenolic acids (Baharum et al., 2010). *Euphorbia hirta* exhibited high radical scavenging and antioxidant activity, while *E. indica*, native to the tropics and subtropics, also possesses antioxidant properties (Iqbal & Gnanaraj, 2012; Rattanata et al., 2014). Considering that weeds are abundant and inexpensive sources of materials, developing biostimulants from weeds to supplement traditional fertilizers may not only mitigate the negative effects of weeds on crops but also enhance plant growth, development, and the quality of agricultural products.

Several plant extracts are being studied as biostimulants to evaluate their impact on plant growth. Islam et al. (2022) investigated the effects of banana pseudostem sap on sweet corn seedling growth and confirmed the stimulating role of identified phytochemicals and mineral nutrients in banana pseudostem sap. Yasmeen et al. (2013) illustrated that *Moringa oleifera* leaf extract can act as a seed priming agent to effectively enhance seed emergence, seedling vigor, leaf area and yield-related attributes in wheat. Talukder et al. (2015) also examined herbal plant extract and its ability to promote the germination of different vegetable seeds. The foliar application of licorice extract was found to mitigate drought stress in sesame (Pourghasemian et al, 2020), while seaweed extract enhanced stress growth traits and antioxidant contents in *Spiraea* and *Pittosporum* (Elansary et al., 2016). However, there has been limited research on the application of weed extracts for plant growth. Besides, most studies on phytochemicals in weeds have only focused

on specific phytocompounds, with a few observations that are difficult to quantify. Liquid chromatography-mass spectrometry (LC-MS), a methodology-based metabolic profiling technique, has been improved and enables the investigation of the diversity of non-target phytochemicals via a specialized database. Moreover, it provides structural information without extra tandem mass spectrometry (MS/MS) analysis (Matsuda et al., 2009). Thus, the hypothesis of this study is that weed extracts contain abundant phytochemicals and mineral elements, and it may have a beneficial effect on seed priming. Therefore, the objectives of our research were to (1) identify and quantify the phytochemicals in weed extracts, (2) quantify their phytocompounds and mineral nutrients, and (3) evaluate the effects of weed extracts on seedling growth. The findings of this study will contribute to the exploration of weed extracts as natural and cost-effective biostimulants for sustainable agriculture.

MATERIALS AND METHODS

Collection of Selected Plants

Three plants, namely *E. hirta*, *E. indica*, and *P. minus*, were selected for the study (Figure 1). *Polygonum minus* was purchased from a local supplier. In contrast, the others were collected from a local field in the Faculty of Agriculture (2°98' N, 101°73' E) at the Universiti Putra Malaysia in Selangor, Malaysia. The mature and whole plants were washed with fresh tap water to remove mud from the roots and then rinsed twice with distilled water. The washed plants were air-dried for 7 days at room temperature, then pulverized using a blender and stored in sealed plastic bags.

Extraction of Plant Extracts with Methanol Solvent

Extraction of selected weeds was done in Erlenmeyer flasks: 10 g plant materials were mixed with methyl alcohol 99.95% (HPLC grade, Sigma-Aldrich, USA) using

(a) (b) (c)

Figure 1. Selected plants: (a) *Euphorbia hirta*; (b) *Polygonum minus*; and (c) *Elusine indica*

an optimum ratio of 1/8 (w/v) sample weight to solvent volume (Alupului et al., 2012). After that, the mixtures were continuously shaken at 150 rpm for 24 hr via an orbital shaker. The extraction samples were filtered with Whatman No.1 filter paper (diameter 150 mm, Cytiva, USA), and the alcohol and excess water were evaporated under vacuum at 30℃ using a rotary evaporator (CCA-111, EYELA, Japan). Finally, a part of the concentrated sample solutions was collected and preserved in a refrigerator at -20℃ for phytocompounds analysis using liquid chromatography-mass spectrometry (LC-MS), while the other part of the concentrated sample solution was preserved at 4℃ to determine the chemical properties and used for seedling growth.

LC-MS Studies

Separation was performed using a Thermo Scientific™ (USA) C18 column (Acclaim™ Polar Advantage II, 3 mm × 150 mm, 3 µm particle size) on an UltiMate™ 3000 UHPLC systems (Dionex). Gradient elution was performed at a flow rate of 0.4 ml/min and 40°C column temperature using distilled water + 0.1% formic acid (A) and 100% acetonitrile (B) with 22 min total run time. The injection volume of the sample was 1 µl. The gradient started at 5% B (0–3 min), 80% B (3–10 min), 80% B (10–15 min), and 5% B (15–22 min). The sample was diluted in 1:10 methanol. All the chemicals were purchased from Chemiz (Malaysia).

Determination of Chemical Properties in Selected Plant Extracts

The pH and electrical conductivity (EC) were measured from plant extract solution (100 g/L), which concentrated extract solution from 10 g plant materials was dissolved in 100 ml distilled water utilizing a digital pH meter (HI 2211 pH meter,

Table 1
Chemical properties of selected plant extracts

Chemical characters	*Euphorbia hirta* extract	*Polygonum minus* extract	*Elusine indica* extract
pH	4.25	4.79	4.66
Electrical conductivity (µS/cm)	10.83	16.39	16.06
Total C (%)	51.46	49.94	46.10
Total N (%)	0.66	7.10	3.69
Total P (g/kg)	0.67	2.26	0.32
Total K (g/kg)	22.07	38.54	62.08
Total Ca (g/kg)	0.26	0.25	0.39
Total Mg (g/kg)	0.37	0.26	1.31
Fe (mg/kg)	45.79	67.13	52.00
Cu (mg/kg)	5.14	7.17	4.29
Zn (mg/kg)	7.52	20.72	61.82
Mn (mg/kg)	2.36	3.14	6.72
B (mg/kg)	7.24	4.37	3.20

Hanna Instruments, USA) and digital EC meter (Hanna 2300, Hanna Instruments, USA). The plant extract (on a dry weight basis) was for measure the total C and N using TruMac® CNS analyzer (LECO, USA) and characteristics of total mineral nutrients, namely P, K, Ca, Mg, Fe, Cu, Zn, Mn, and B using inductively coupled plasma (ICP)—optical emission spectroscopy (Optima 8300, PerkinElmer, USA), respectively. The chemical properties of weed extract samples are described in Table 1.

Preparation of Selected Plant Extracts for Seedling Growth

The plant extract solution (100 g/L) was diluted with distilled water to prepare 5, 15, 25, and 50% solutions. Meanwhile, plant extracts (100 g/L) and distilled water were considered a 100% concentration solution and controlled to evaluate seedling growth. Maize seeds (*Zea mays* L., Hybrid F1 316, Malaysia) were first soaked for 12 hr in water, and then 10 sprouting seeds were placed in the Petri dish with equal distance between them. According to the treatments, 8 ml of plant extract was poured into a Petri dish with pieces of tissue (material-solution ratio is 1:4) and followed by 5 ml of a similar solution in each Petri dish was added every day to keep humidity, which was continued for all the experimental period (10 days). Each treatment was performed in three repetitions. The experiment was carried out in the Faculty of Agriculture, Universiti Putra Malaysia Lab, where the temperature was 26°C and the humidity 70% conditions. The date of germination (%), shoot length (cm), fresh and dry weight (g), and soil plant analysis development (SPAD) value (SPAD-502Plus, Konica Minolta, Japan) were recorded.

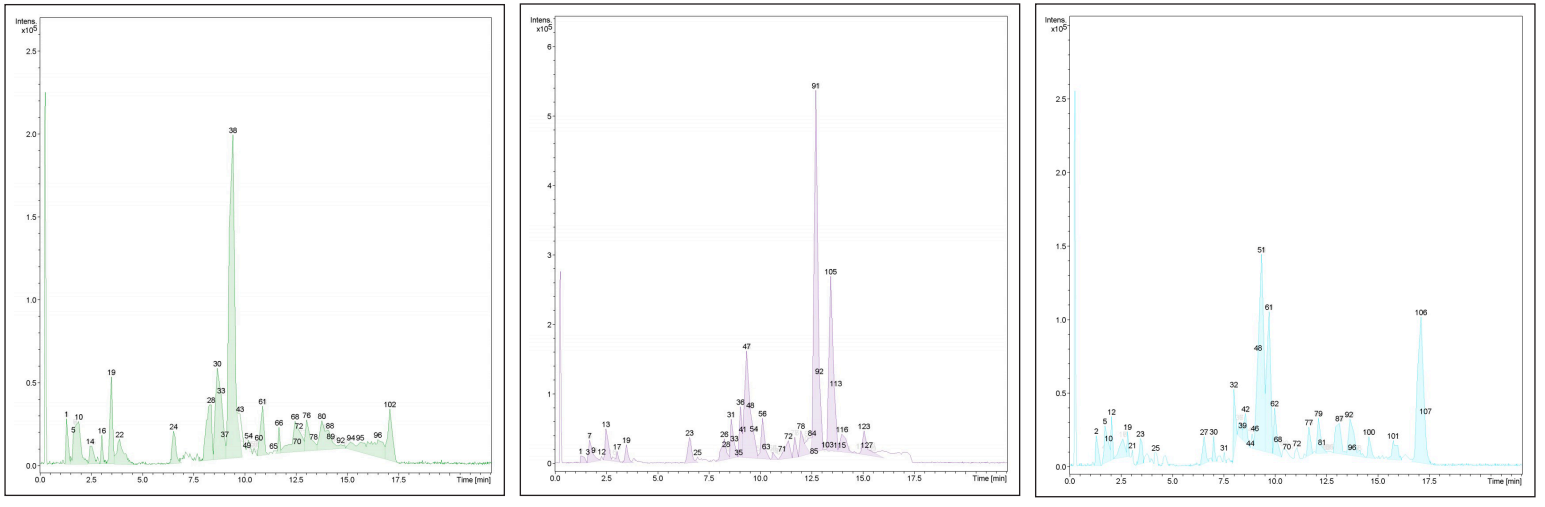
Statistical Analysis

Data were analyzed using the SPSS 25.0 (IBM, USA). Significant differences between treatments were calculated by one-way analysis of variance (ANOVA) with the least significant difference (LSD) test at $p < 0.05$. The shoot lengths of maize seedlings were measured using Image J software (version 1.8).

RESULTS

Characterization of Phytocompounds in Selected Plant Extracts

Phytochemical compounds identified with methanolic extraction of *E. hirta*, *P. minus*, and *E. indica* using LC-MS were shown in Table 2. A total of 45, 53, and 39 phytocompounds were identified by given chromatographic peaks under the retention time of 1.30 to 17.3 min (Figure 2). A total of 112 phytocompounds were classified into different chemical groups, including 19 flavonoids, 14 amino acids, 24 alkaloids, 11 polyketides, 24 terpenoids, 5 phenylpropanoids, 2 carbohydrates, 2 fatty acids, 2 vitamins, as well as 9 others (Table 2). Among the three plant extracts, *P. minus* contains the most phytocompounds, containing 53, followed by *E. hirta*, containing 45; the least is *E. indica*, containing 39. Besides, the three weed extracts contained five identical phytocompounds: lotaustralin, carolinianine,



(a) (b) (c)

Figure 2. Total positive charged ions chromatogram of (a) *Euphorbia hirta*, (b) *Polygonum minus*, and (c) *Elusine indica* obtained by liquid chromatography-mass spectrometry through methanolic extraction

Table 2
Identification of phytochemical compounds in selected plants by liquid chromatography-mass spectrometry

Serial Nr	Compounds name	Chemical group	Chemical formula	Mass	Plant beneficial Yes (Y)/Not (N)	E. H. Y/N	P. M. Y/N	E. I. Y/N
1	Cupressuflavone	Flavonoids	$C_{30}H_{18}O_{10}$	538.09	Y	Y	N	N
2	Quercetin 3, 4', 7-trissulfate		$C_{15}H_{10}O_{16}S_3$	541.91	Y	Y	N	N
3	Azaleatin		$C_{16}H_{12}O_7$	316.05	Y	Y	Y	N
4	Epigallocatechin gallate		$C_{22}H_{18}O_{11}$	458.08	Y	Y	N	Y
5	Hesperidin		$C_{28}H_{34}O_{15}$	610.18	Y	N	Y	N
6	Pachypodol		$C_{18}H_{16}O_7$	344.08	N	N	Y	N
7	Procyanidin B4		$C_{30}H_{26}O_{12}$	578.14	Y	N	Y	N

Table 2 *(continue)*

Serial Nr	Compounds name	Chemical group	Chemical formula	Mass	Plant beneficial Yes (Y)/Not (N)	E. H. Y/N	P. M. Y/N	E. I. Y/N
8	Piperaduncin B		$C_{29}H_{30}O_8$	506.54	N	Y	N	N
9	Myricitrin		$C_{21}H_{20}O_{12}$	464.09	Y	N	Y	Y
10	Myricetin		$C_{15}H_{10}O_8$	318.03	Y	N	N	Y
11	Morin		$C_{15}H_{10}O_7$	302.04	Y	N	N	Y
12	Isovitexin		$C_{21}H_{20}O_{10}$	432.10	Y	Y	N	Y
13	Isoterchebin		$C_{41}H_{30}O_{27}$	954.09	Y	N	Y	N
14	Tricin		$C_{17}H_{14}O_7$	330.07	Y	Y	N	N
15	Vicenin-2		$C_{27}H_{30}O_{15}$	594.15	Y	N	Y	N
16	Ptaerochromenol		$C_{15}H_{14}O_5$	274.08	Y	Y	N	Y
17	Pelargonidin 3-O-3, 6-O-dimalonylglucoside		$C_{27}H_{25}O_{16}$	605.11	Y	N	Y	N
18	Silychristin		$C_{25}H_{22}O_{10}$	482.12	Y	Y	Y	N
19	Isoscutellarein		$C_{15}H_{10}O_6$	286.04	Y	N	Y	N
20	Pidolic acid	Amino acid	$C_5H_7NO_3$	129.04	Y	N	Y	N
21	Amaranthin		$C_{30}H_{34}N_2O_{19}$	726.17	Y	Y	N	N
22	N-Succinyl-LL-2,6-diaminoheptanedioate		$C_{11}H_{18}N_2O_7$	290.11	N	N	Y	N
23	Homocapsaicin		$C_{19}H_{29}NO_3$	319.21	Y	N	Y	N
24	Indicaxanthin		$C_{14}H_{16}N_2O_6$	308.10	Y	N	Y	N
25	Lotaustralin		$C_{11}H_{19}NO_6$	261.12	Y	Y	Y	Y
26	L-Tyrosine		$C_9H_{11}NO_3$	181.07	Y	N	N	Y
27	L-Valine		$C_5H_{11}NO_2$	117.07	Y	Y	N	N
28	L-Phenylalanine		$C_9H_{11}NO_2$	165.07	Y	Y	N	N
29	Glucobrassicin		$C_{16}H_{20}N_2O_9S_2$	448.06	Y	N	Y	N
30	N-Carbamoylputrescine		$C_6H_{13}N_3O$	131.10	N	N	N	Y
31	Neoglucobrassicin		$C_{17}H_{22}N_2O_{10}S_2$	478.07	Y	N	Y	N
32	L-Homoserine		$C_4H_9NO_3$	119.05	Y	Y	N	N
33	Methylthio-2-oxobutanoic acid		$C_5H_8O_3S$	148.01	N	Y	N	N
34	Alangiside	Alkaloids	$C_{25}H_{31}NO_{10}$	505.19	Y	N	N	Y

Table 2 *(continue)*

Serial Nr	Compounds name	Chemical group	Chemical formula	Mass	Plant beneficial Yes (Y)/Not (N)	E. H. Y/N	P. M. Y/N	E. I. Y/N
35	Casimiroin		$C_{12}H_{11}NO_4$	233.06	N	N	N	Y
36	Cinnamoylcocaine		$C_{19}H_{23}NO_4$	329.16	Y	Y	N	N
37	Carolinianine		$C_{16}H_{24}N_2O_2$	276.37	Y	Y	Y	Y
38	Echitovenine		$C_{23}H_{28}N_2O_4$	396.20	N	N	Y	N
39	Deacetylisoipecoside		$C_{25}H_{33}NO_{11}$	523.20	Y	Y	N	N
40	Finaconitine		$C_{33}H_{46}N_2O_{10}$	630.31	N	N	Y	N
41	Heliotrine		$C_{16}H_{27}NO_5$	313.18	N	N	Y	N
42	Leiokinine A		$C_{14}H_{17}NO_2$	231.12	N	N	Y	N
43	Lophocerine		$C_{15}H_{23}NO_2$	249.17	N	N	Y	N
44	Lunamarine		$C_{18}H_{15}NO_4$	309.10	N	Y	N	N
45	Norhyoscyamine		$C_{16}H_{21}NO_3$	275.15	Y	Y	N	N
46	N-Methyltyramine		$C_9H_{13}NO$	151.09	Y	N	Y	N
47	Melicopine		$C_{17}H_{15}NO_5$	313.09	N	N	N	Y
48	Mesembrinol		$C_{17}H_{25}NO_3$	291.18	N	N	Y	N
49	Clivoline		$C_{21}H_{27}NO_7$	405.17	N	N	N	Y
50	Lobelanidine		$C_{22}H_{29}NO_2$	339.21	N	Y	Y	Y
51	Terpendole K		$C_{32}H_{39}NO_5$	517.28	N	Y	N	N
52	Tyramine		$C_8H_{11}NO$	137.08	Y	N	Y	N
53	Reserpine		$C_{33}H_{40}N_2O_9$	608.27	N	N	N	Y
54	Salutaridine		$C_{19}H_{21}NO_4$	327.14	Y	N	Y	N
55	Vasicinol		$C_{11}H_{12}N_2O_2$	204.08	Y	N	Y	N
56	21, 22-Diprenylpaxilline		$C_{36}H_{49}NO_4$	571.36	N	N	Y	N
57	(6s)-Hydroxyhyoscyamine		$C_{17}H_{23}NO_4$	305.16	Y	N	Y	N
58	Hamaudol	Polyketides	$C_{15}H_{16}O_5$	276.09	N	Y	N	N
59	Aclacinomycin S		$C_{36}H_{45}NO_{13}$	699.28	Y	N	N	Y
60	Ansamitocinoside P-3		$C_{37}H_{51}ClN_2O_{14}$	783.06	Y	N	N	Y
61	dTDP-D-glucuronate		$C_{18}H_{16}O_7$	344.08	N	N	Y	N

Table 2 *(continue)*

Serial Nr	Compounds name	Chemical group	Chemical formula	Mass	Plant beneficial Yes (Y)/Not (N)	E. H. Y/N	P. M. Y/N	E. I. Y/N
62	5-O-Methylalloptaeroxylin		$C_{16}H_{16}O_4$	272.10	Y	N	Y	N
63	Glucofrangulin B		$C_{26}H_{28}O_{14}$	564.14	Y	Y	N	Y
64	Leucomycin A7		$C_{38}H_{63}NO_{14}$	757.42	N	N	N	Y
65	Leucomycin A8		$C_{39}H_{63}NO_{15}$	785.41	N	Y	Y	N
66	Niddamycin		$C_{39}H_{63}NO_{14}$	769.42	N	Y	N	N
67	Troleandomycin		$C_{41}H_{67}NO_{15}$	813.45	N	Y	N	Y
68	Rifamycin		$C_{37}H_{47}NO_{12}$	697.30	N	N	Y	N
69	Frullanolide	Terpenoids	$C_{15}H_{20}O_2$	232.14	Y	N	Y	N
70	Tutin		$C_{15}H_{18}O_6$	294.11	N	Y	N	N
71	Thevetin B		$C_{42}H_{66}O_{18}$	858.42	N	Y	N	N
72	Taxine B		$C_{33}H_{45}NO_8$	583.31	N	N	N	Y
73	Terpenoid EA-T		$C_{30}H_{40}O_8$	528.27	N	Y	N	N
74	Petasin		$C_{20}H_{28}O_3$	316.20	Y	Y	N	N
75	Phorbol 12-tiglate 13-decanoate		$C_{35}H_{52}O_8$	600.36	Y	Y	N	N
76	Picrasin C		$C_{23}H_{34}O_7$	422.23	N	N	N	Y
77	Baliospermin		$C_{32}H_{50}O_8$	562.35	N	N	Y	Y
78	Diterpenoid EF-D		$C_{27}H_{38}O_7$	474.26	Y	Y	N	N
79	Dehydron gaione		$C_{15}H_{20}O_3$	248.14	Y	N	Y	N
80	Deltonin		$C_{45}H_{72}O_{17}$	884.47	Y	Y	N	N
81	Diterpenoid SP-II		$C_{20}H_{32}O_4$	336.23	Y	Y	N	N
82	Erioflorin acetate		$C_{21}H_{26}O_7$	390.16	N	N	Y	N
83	Germacrene		$C_{15}H_{22}O_2$	234.16	Y	N	Y	N
84	Lathyrol		$C_{20}H_{30}O_4$	334.21	Y	N	N	Y
85	Lycoxanthin		$C_{40}H_5O_6$	552.43	Y	Y	N	Y
86	Alpha-Irone		$C_{14}H_{22}O$	206.16	Y	N	N	Y
87	Alpha-Zeacarotene		$C_{40}H_{58}$	538.45	Y	N	Y	N
88	Alpha-Vetivone		$C_{15}H_{22}O$	218.16	Y	N	Y	N

Table 2 *(continue)*

Serial Nr	Compounds name	Chemical group	Chemical formula	Mass	Plant beneficial Yes (Y)/Not (N)	E. H. Y/N	P. M. Y/N	E. I. Y/N
89	Arbusculin A		$C_{15}H_{22}O_3$	250.15	Y	N	Y	N
90	Agavoside A		$C_{33}H_{52}O_9$	592.36	Y	N	Y	N
91	7-Deoxyloganate		$C_{16}H_{24}O_9$	360.14	N	N	N	Y
92	12-O-Tetradecanoylphorbol 13-acetate		$C_{36}H_{56}O_8$	616.39	N	N	Y	N
93	Coniferyl alcohol	Phenylpropanoids	$C_{10}H_{12}O_3$	180.07	Y	N	Y	N
94	Cleistanthin A		$C_{28}H_{28}O_{11}$	540.16	Y	N	Y	N
95	Eudesobovatol		$C_{33}H_{44}O_4$	504.32	Y	Y	N	N
96	Peucedanin		$C_{15}H_{14}O_4$	258.08	Y	Y	N	N
97	Umbelliferone		$C_9H_6O_3$	162.03	Y	N	N	Y
98	D-Glucose	Carbohydrate	$C_6H_{12}O_6$	180.06	Y	N	N	Y
99	Neuraminic		$C_9H_{17}NO_8$	267.09	N	Y	N	Y
100	Gamolenic acid	Fatty acid	$C_{18}H_{30}O_2$	278.22	Y	Y	N	Y
101	3-Indoleacrylate		$C_{11}H_9NO_2$	187.06	Y	Y	Y	Y
102	Phylloquinol	Vitamin	$C_{31}H_{48}O_2$	452.36	Y	Y	N	Y
103	5, 6, 7, 8-Tetrahydromonapterin		$C_9H_{15}N_5O_4$	257.11	N	N	N	Y
104	Actinorhodin	Others	$C_{32}H_{26}O_{14}$	634.13	N	N	Y	N
105	Butirosin A		$C_{21}H_{41}N_5O_{12}$	555.27	N	N	Y	N
106	4-Deoxy-4-thio-alpha-D-digitoxosyl-calicheamicin T0		$C_{30}H_{38}N_2O_{11}S_4$	730.13	N	N	Y	N
107	Pedunculagin		$C_{34}H_{24}O_{22}$	784.07	Y	N	N	Y
108	Pyrogallic acid		$C_6H_6O_3$	126.03	N	Y	N	N
109	Prostaglandin E3		$C_{20}H_{30}O_5$	350.20	N	Y	N	N
110	Uroporphyrin I		$C_{40}H_{38}N_4O_{16}$	830.22	Y	Y	Y	Y
111	7,8-Dihydroneopterin3"-triphosphate		$C_{17}H_{22}N_2O_{10}S_2$	494.99	N	N	Y	Y
112	Glucosyl-4, 4'-diaponeurosporenoate		$C_{36}H_{50}O_7$	594.35	N	N	N	Y

Note. *E. H.*, *P. M.*, and *E. I.* represent *Euphorbia hirta*, *Polygonum minus*, and *Elusine indica* extracts, respectively

lobelanidine, 3-indoleacrylate, and uroporphyin I. The overall study found that 68 molecules were beneficial to the plant, of which *E. hirta*, *P. minus*, and *E. indica* contained 29, 33, and 23, respectively.

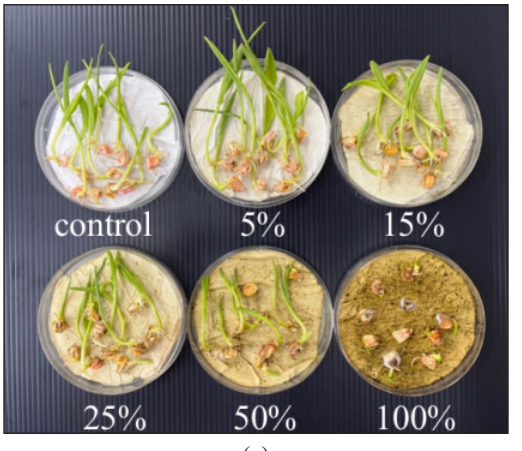
Effects of Selected Plant Extracts on Seedling Growth of Maize

The germination of maize seed was strongly affected by plant extract application, and the effects differed with different concentrations (Table 3, Figure 3). Overall, low concentrations (5 and 15%) of plant extracts can promote seed germination, while seed germination was significantly inhibited by high concentrations (p<0.05). Correspondingly, 100% *E. hirta* and *E. indica* extract treatments significantly decreased germination compared with control (p<0.05). Results also showed that the germination of *E. indica* extract treatments was higher than that of *E. hirta* and *P. minus* extract treatments at the same concentration except for 100% concentration.

The change in shoot length after applying weed extract was the same as germination (Table 3, Figure 3); the data on root length were not shown because that was not evident. Especially compared to the control, 100% *E. hirta* and *E. indica* extract treatments significantly decreased by 78.1 and 80.8%, respectively (p<0.05). In addition, 5% *E. hirta* and *E. indica* extract treatments were significantly higher than other concentrations (p<0.05). A similar trend was found in fresh weight, in which treatments with the highest concentration

(a)

(b)



(c)

Figure 3. Effect of different concentrations: (a) *Euphorbia hirta*; (b) *Polygonum minus*; and (c) *Elusine indica* extracts on seedling growth, respectively

Table 3
Effect of different concentrations of selected plant extracts on seedling growth of maize

Treatments	Germination (%)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Soil plant analysis development value
Control	86.67 ± 5.77	7.55 ± 1.78	0.38 ± 0.04	0.10 ± 0.03	21.20 ± 2.01
5% *Euphorbia hirta*	90.00 ± 10.00a	6.80 ± 0.47a	0.35 ± 0.08a	0.11 ± 0.01a	19.23 ± 0.57a
15% *Euphorbia hirta*	80.00 ± 0.00ab	4.99 ± 0.75b	0.26 ± 0.04ab	0.07 ± 0.01b	15.00 ± 1.30b*
25% *Euphorbia hirta*	76.67 ± 11.55ab	4.76 ± 1.22b	0.26 ± 0.07ab	0.09 ± 0.02ab	14.60 ± 1.92b*
50% *Euphorbia hirta*	70.00 ± 10.00b	4.42 ± 0.82b	0.31 ± 0.04ab	0.10 ± 0.02a	14.80 ± 0.60b*
100% *Euphorbia hirta*	50.00 ± 10.00c*	1.65 ± 0.22c*	0.21 ± 0.05b*	0.09 ± 0.02ab	9.47 ± 0.85c*
5% *Polygonum minus*	93.33 ± 5.77a	7.62 ± 0.77a	0.41 ± 0.02a	0.10 ± 0.01ab	19.27 ± 0.65a
15% *Polygonum minus*	96.67 ± 5.77ab	7.76 ± 0.95a	0.35 ± 0.06ab	0.08 ± 0.03b	20.47 ± 1.78a
25% *Polygonum minus*	73.33 ± 15.28bc	3.54 ± 0.54c	0.29 ± 0.06b	0.12 ± 0.03a	18.87 ± 0.86a
50% *Polygonum minus*	76.67 ± 5.77bc	7.56 ± 0.51a	0.37 ± 0.06ab	0.08 ± 0.01b	20.67 ± 2.15a
100% *Polygonum minus*	76.67 ± 12.58c	5.33 ± 0.26b	0.28 ± 0.06b*	0.07 ± 0.03b	18.37 ± 2.05a
5% *Elusine indica*	96.67 ± 5.77a	9.47 ± 0.93a	0.44 ± 0.10a	0.11 ± 0.02a	19.40 ± 1.76b
15% *Elusine indica*	100.00 ± 0.00a	6.77 ± 0.81b	0.37 ± 0.05ab	0.10 ± 0.03a	23.03 ± 1.56a
25% *Elusine indica*	80.00 ± 20.00a	5.93 ± 0.35b	0.35 ± 0.03ab	0.11 ± 0.01a	13.67 ± 2.32c*
50% *Elusine indica*	90.00 ± 10.00a	4.03 ± 0.79c	0.25 ± 0.05bc*	0.08 ± 0.03a	14.47 ± 1.29c*
100% *Elusine indica*	50.00 ±17.32b*	1.45 ± 0.32d*	0.22 ± 0.08c*	0.08 ± 0.01a	-

Note. Values mean ± standard error of three replicates. Different letters represent significant differences between the same plant extract with different concentrations; "*" represents a significant difference between the treatment with control (p<0.05)

(100%) significantly reduced fresh weight (*E. hirta*: 44.7%; *P. minus*: 26.3%; *E. indica*: 42.1%), whereas it was not significant effect on dry weight ($p<0.05$).

The SPAD value in the seedling varied due to different weed extracts and concentrations. All *E. hirta* extract treatments, except for 5% concentration, were significantly lower than that of the control. Besides, 25 and 50% *E. indica* extract treatments significantly decreased by 35.5 and 31.7% compared to control, respectively, while the effect of *P. minus* extract treatments was not significant ($p<0.05$). Notably, the visual fungal colonies were found in the Petri dish with 100% *E. indica* extract treatment (Figure 2), while the other treatments had no external growth.

DISCUSSION

Phytochemicals and Chemical Properties in Selected Plant Extracts

Phytochemicals play crucial roles in the plant's secondary metabolism, including pest repellence and growth regulation (da Silva et al., 2016). Plants that contain a large number of bioactive compounds have the potential to improve human health (Forbes-Hernández et al., 2014). In our study, 45, 53, and 39 secondary metabolites were identified in *E. hirta*, *P. minus*, and *E. indica*, respectively, which can be categorized into a variety of groups (Table 2). Additionally, diverse important flavonoid compounds were identified and quantified in plant extracts compared with previous studies, such as tricin, myricitrin, and isovitexin. Flavonoids are considered to have antioxidant and anti-stress effects, which can usually help plants cope with drought, salinization and other environmental stresses (Stolarzewicz et al., 2013), and have beneficial functions, including regulation of plant respiration and photosynthesis (Cushnie et al., 2005), drivers of symbiosis between rhizobacteria and plants (Weston & Mathesius, 2013), furthermore promoting the growth and development of plants, which may help to increase yield and improve quality.

Results also revealed the presence of various amino acids, such as methylthio-2-oxobutanoic acid, pidolic acid, and lotaustralin, in plant extracts (Table 2). Amino acids are known to act as chelators of metal ions in agricultural products and interact with trace elements to form small, electrically neutral molecules that facilitate their absorption and transport in plants (Paleckiene et al., 2007) and promote growth and development (Qiu et al., 2020). In addition, alkaloids are another group of vital secondary metabolites related to plants and humans (War et al., 2012). In our investigation, a total of 24 alkaloids were discovered. It is worth noting that only 10 are beneficial to the plant because alkaloids usually play a role in plants' defense against pests and diseases, so they may be toxic and even have a negative effect on plants and the human body. Moreover, in our study, 4 polyketides, 15 terpenoids, 5 phenylpropanoids, 2 carbohydrates, 2 fatty acids, and one vitamin benefit plants. Accumulation of these bioactive compounds in plants can inhibit reactive oxygen species (ROS) inside cells through ROS scavenging

and decreasing ROS-related enzyme activity. Thus, the balance of redox reactions in the cell is maintained. Additionally, our study showed that all three weed extracts were rich in nutrient elements, such as N, P, and K. Among them, *P. minus* extracts contained the highest content of N and P (Table 1). Based on the above information, the identified phytochemical compounds in weed extracts have the potential to serve as natural biostimulants in terms of plant protection, growth, and development in sustainable agriculture.

Application of Selected Plant Extracts on Maize Seeding Growth

The study showed that three different plant extracts greatly influence the growth and development of sweet corn (Table 3, Figure 3). Results illustrated that high concentration (100%) plant extracts severely restricted seed germination and decreased the shoot height and fresh weight. These findings are in accordance with Ghodake et al. (2012), who found that the allelopathic effect of *Euphorbia* species caused inhibition in germination percentage and shoot-root length on wheat. Agarwal et al. (2002) and Gella et al. (2013) have also shown that weed extracts reduce the seed germination, plumule length, radicle, and weight of wheat. Besides, a high concentration of banana pseudostem sap inhibited any expansion in germination on sweet corn (Islam et al., 2022). It might be because some unknown compounds and pathogenic microorganisms cause negative effects on the germination rate of seeds (Talukder et al., 2015). On the other hand, weed extracts of *P. minus* and *E. indica* with low concentrations (5 and 15%) exhibited a slight promoting effect on germination and shoot length; this finding corroborates the results illustrated by Aslam et al. (2016), who reported that plant extract with low concentrations promoted seed germination. However, all *E. hirta* extract treatments in this study reduced shoot length (9.9~78.1%) compared with control. Therefore, different plant species and extract concentrations have diverse effects on seeding growth.

In this study, there was a negative trend of SPAD value as the concentrations of *E. hirta* and *E. indica* extracts increased, which is similar to the result of Joshi and Joshi (2016), who revealed the total chlorophyll accumulation in seedlings of wheat after being treated with six different weed extracts. Besides, Oyerinde et al. (2009) have also shown that allelochemicals in weed extract may affect chlorophyll content and photosynthesis in plants. It may be because weed extracts contain some toxic metabolites, such as alkaloids, that cause adverse effects on crop growth (Qasem, 2002). On the other hand, easier development of fungal colonies at higher concentrations also inhibits seedling growth. However, there was no significant effect on the SPAD value under all concentrations of *P. minus* extract treatments compared with the control (Table 3). It may be because *P. minus* extract contains fewer toxic metabolites and more beneficial phytochemicals and nutrients such as N, P, and K (Tables 1 and 2); thus, *P. minus*

extract has great potential for improving crop production.

CONCLUSION

The study identified phytochemical compounds in extracts of *E. hirta*, *P. minus*, and *E. indica* extracts, classifying them into 11 categories, including flavonoids, amino acids, alkaloids, polyketides, terpenoids, phenylpropanoids, carbohydrate, fatty acid, vitamin and others. Besides, *P. minus* extracts have the highest content of nitrogen and phosphorus. These phytocompounds and soluble nutrients are related to the growth and development of plants. The application of weed extracts has a significant impact on maize seed priming. In particular, *E. hirta* and *E. indica* extracts exhibited inhibitory effects at higher concentrations, while *P. minu*s extract maintained a higher germination rate, indicating lower toxicity. This finding emphasizes the importance of phytochemicals in seed germination and plant development. However, there is still limited knowledge regarding the specific effects of molecules on plants and human health. Future quantitative studies of beneficial phytocompounds will help to better understand how the application of weed extracts in agriculture can be economical and environmentally friendly.

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