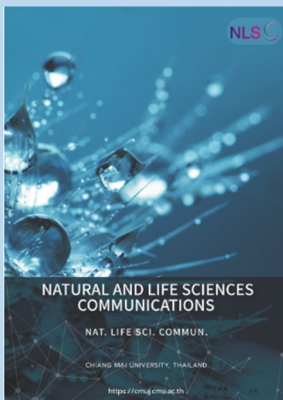


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Sida cordifolia L. Methanol Leaf Extract has Bioherbicidal Potential on Selected Noxious Weed Species in Malaysia

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ABSTRACT

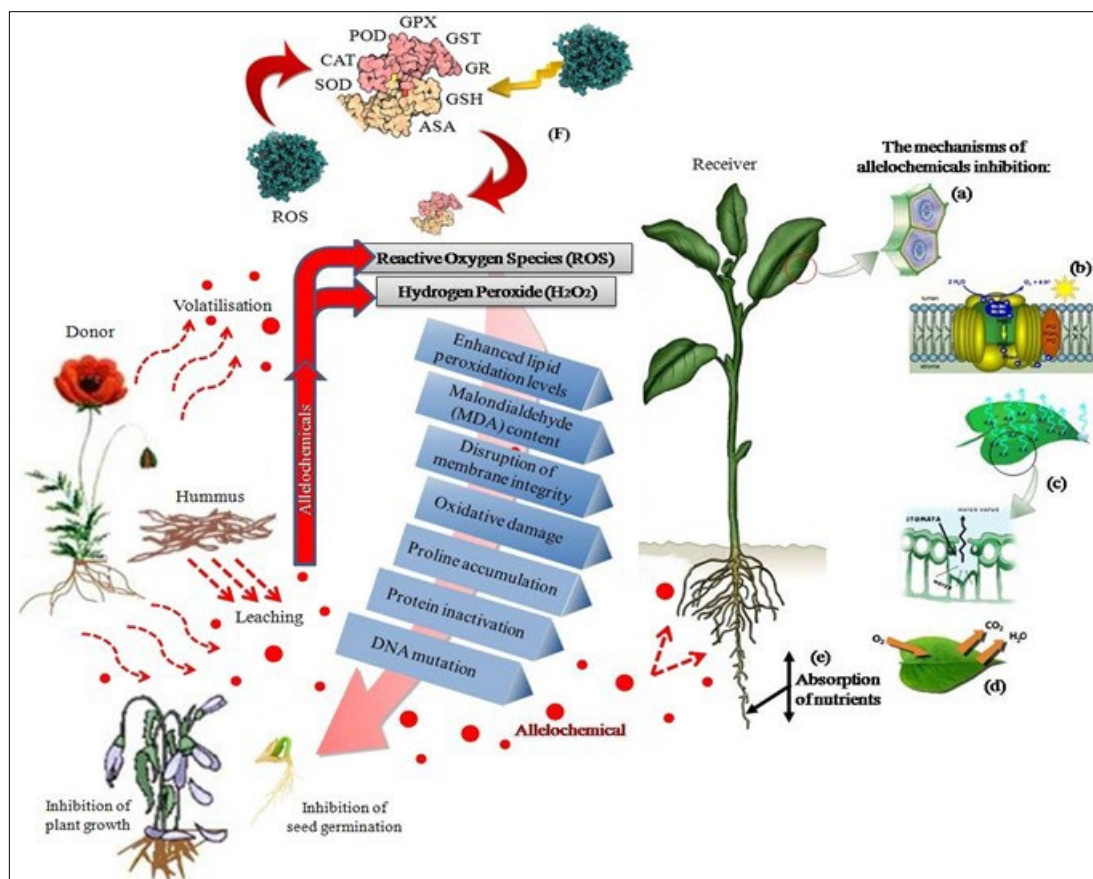
Allelopathy is implicated for a decline in agricultural output. *Sida cordifolia* is an invasive herb that is found in all the tropical and sub-tropical regions. The study assessed effect of *S. cordifolia* methanol leaf extract on germination and growth of noxious weed species (*Ageratum conyzoides*, *Asystasia gangetica*, *Parthenium hysterophorus*, *Echinochloa crus-galli*, *E. colona* and *Oryza sativa*). Fifty grams of *S. cordifolia* leaf powder was extracted with 1,000 ml methanol and tested against the weeds in the laboratory and 3.0, 2.5 and 1.5 gL⁻¹ crude extract were tested in the glasshouse. Seed germination bioassay was conducted in a growth chamber. Data on shoot and root dry weight, leaf area, chlorophyll pigments, photosynthesis, stomatal conductance, and transpiration were determined at 3 weeks after foliar spray of *S. cordifolia* leaf extract in a glasshouse. CRD and RCBD were adopted in the laboratory and glasshouse experiments. *Parthenium hysterophorus* showed the greatest overall sensitivity with lowest EC₅₀ for germination (0.34) and lowest root EC₅₀ (0.04) while *A. conyzoides* (2.66), *O. sativa* (2.81), *E. crus-galli* (2.10) and *P. hysterophorus* (2.78) on seedling elongation. Result in glasshouse revealed that *A. conyzoides*, at highest concentration, had severely decreased in chlorophyll pigments and net photosynthesis (29.7% and 15.79%). Liquid Chromatography Mass Spectrophotometry analysis revealed high rutin and DL-phenylalanine. Crude methanolic extract of *S. cordifolia* exhibits dose-dependent responses in both lab and glasshouse assays. Isolation of active constituents in the *Sida* extract, selectivity tests on crops, mode of action, formulation and field trials is recommended.

Keywords: Allelopathy germination, Inhibition, Phytochemical, *Sida cordifolia*

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Graphical Abstract:



INTRODUCTION

Finding alternatives to synthetic herbicides for weed control is receiving attention due to rising demand for sustainable agriculture, and concerns about the excessive use of synthetic agrochemicals such as herbicides, pesticide, fumigants and repellents, as well as environmental pollution and rising costs of synthetic herbicide. Allelopathy refers to plant interference to dominate other neighbouring species in a competitive environment. It is a natural control approach with prospect to manage and enhance agricultural productivity. The role of allelochemicals as natural products is well documented (Baratelli et al., 2012; Inderjit and Keating, 2012; Fabro et al., 2014; Yulianto and Xuan 2018). *Sida cordifolia* contains multiple bioactive compounds, particularly alkaloids, flavonoids, and saponins that exhibits phytotoxicity and allelopathic effect (Ghani and Hussain, 2002; Singh and Kumar, 2013). Phytotoxicity studies reveal that extracts of *S. cordifolia* inhibited seed germination and seedling growth in *Ageratum conyzoides* and *Oryza sativa* (Ahmed et al., 2025). Allelochemicals generated from plants have the potential to be employed directly as natural herbicides or as a model for future herbicide development. For instance, syringic acid and quercetin inhibit seed germination, root elongation and seedling growth in various plant species (Li et al., 2010; Hussain and Reigosa, 2011). DL-phenylalanine inhibits seedling growths by disrupting protein synthesis and enzyme function (Einhelling, 1995) while p-anisic acid disturbs cell membrane integrity and enzyme activities leading to impaired water uptake and growth suppression (Gniazdowska and Bogatek, 2005). These allelochemicals may be structurally altered to produce a more active product.

Sida cordifolia is a noxious weed that colonizes farmland, field and sides of roads in the south and western regions of Sokoto, Nigeria. It is extensively used in ethnomedicine (Ahmed et al., 2018). However, there is little knowledge about its

herbicidal ability. Visual inspection of the *S. cordifolia* invaded area showed a decline in the number of native plant species. The amount and type of secondary metabolites in medicinal plants can change greatly depending on where the plants are grown, and this is due to environmental and edaphic factors (Yakubu and Afolayan, 2010). As a result, plants from different regions may show difference in their biological activity and efficacy. This study tested the herbicidal ability of *S. cordifolia* leaf extract on germination and growth of *E. crus-galli*, *E. colona*, *O. sativa* (weedy rice), *A. gangetica*, *A. conyzoides* and *P. hystrophorus*. The aim is to quantify inhibition of germination and seedling growth across concentrations; evaluate foliar effects on biomass and physiology in glasshouse; and identify major phenolics in *S. cordifolia* leaf extract via LC-MS.

MATERIALS AND METHODS

Germination bioassay was conducted in a growth chamber (Climas model 361/HR, Spain) at Seed Technology Laboratory, Department of Crop Science, Universiti Putra Malaysia while glasshouse experiment was carried out in Ladang 15 Faculty of Agriculture, Universiti Putra Malaysia.

Seeds collection and reagents

Seeds of monocots (*Echinochloa crus-galli*, *E. colona*, *Oryza sativa*) and dicots (*Asystasia gangetica*, *Ageratum conyzoides* and *P. hystrophorus*) weed species were obtained from Weed Science Laboratory, Universiti Putra Malaysia and Ladang Infoternak, Sungai Siput, Perak. Methanol solvent (HPLC- grade) was purchased from Fisher Scientific, UK while Rutin, Syringic acid, Quercetin, DL-phenylalanine, and p-anisic acids (standard chemicals) were obtained from International Laboratory, USA and supplied by Chemtron Biotechnology, Sdn Bhd, Malaysia.

Preparation of *S. cordifolia* methanol extract

Fresh leaves of *S. cordifolia* at vegetative stage were collected from Wammako area, Sokoto, Nigeria and washed thoroughly with running tap water, dried under shade, pulverized and preserved. Fifty grams dried leaf powder of *S. cordifolia* leaf was extracted twice each with 1,000 ml methanol (80%) for 12 hours at 30°C. The resultant extraction was combined and sieved through one layer of Whatman filter paper. The filtrate was evaporated to dryness at 40°C using a rotary evaporator (RE-2L, Labfreez, China) and lyophilized with Labconco Freezone 2.5 (USA) to remove solvent (methanol) residuals. Sterile distilled water was added to the lyophilized crude extract to give a volume of 1,000 ml as stock (100%), and the stock was serially diluted to give a concentration of 0% (distilled water, (T1)), 10%(T2), 20%(T3), 30%(T4), 40%(T5), 50%(T6), 75%(T7) and 100%(T8). These concentrations were adopted for the germination study.

Laboratory bioassay and seed treatment

Healthy and uniform seeds of the target weed species each were surface sterilized with 5% sodium hypochloride for five minutes and then rinsed three times with distilled water prior to planting. Ten seeds of the weed species each were placed onto double layers of Whatman (No. 1) filter paper in 9 cm petri dish (VanVolkenburg et al., 2020) and then moistened with 10 mL of *S. cordifolia* leaf extract, 5 mL of distilled water was used to maintain moisture. Germination test was conducted in a growth chamber at 30°C/25°C day/night ($\pm 2^\circ\text{C}$), 70 % relative humidity and 12 hours photoperiod. Germination was monitored daily. Radicle protrusion was considered as germination. Data on radicle and shoot lengths (cm) were measured using Leica Microscope (Leica EZ4 HD, Germany). The experiment was terminated 5 days after planting.

$$\% \text{ of inhibition} = \frac{\text{Mean germination in control} - \text{mean germination in treatment}}{\text{Mean germination in control}} \times 100$$

Glasshouse experiment

Four weed species (*A. conyzoides*, *P. hysterophorus*, *E. crus-galli* and *O. sativa*) with greater susceptibility to *S. cordifolia* extract from germination study were selected and further subjected to glasshouse experiment. Seedling of the weed species at 2 and 4 leaf growth stages (monocot and dicot) were raised and transferred into 30 cm × 30 cm pot (1 plant per pot) containing 4 kg of soil mixture at 3:1 ratio (soil and peat moss). The lyophilized crude powder of leaf extract were prepared at 3.0, 2.25, 1.5 and 0 g/L (distilled water) and sprayed twice 10 ml per pot at 7 days interval using a hand atomizer in the glasshouse under 12 hour photoperiod and temperatures at 34°C/22°C day/night. Data was collected 21 days after the last spray and the experiment was terminated at 6 weeks after planting.

Growth measurement

Four leaves per plant were measured using leaf area meter (LI-3,000 Li-COR, USA) settings (CO₂ concentration ~400 µmol·mol⁻¹, light intensity light (PAR) = 1,000), leaf cuvette area (6 cm²), flow rate (200 – 400 µmol), and expressed as cm² plant⁻¹. Samples were oven dried at 50°C for 72 hours, and dry weights were determined using digital balance scale.

Physiological parameters

Four healthy leaves two each from young fully expanded matured leave on the same node of weed species were randomly selected and analyzed for total chlorophyll content using a chlorophyll meter (Soil Plant Analysis Development-SPAD- 502, Minolta, Japan), while net photosynthetic rate (A), stomatal conductance (gs) and transpiration rate (E) were evaluated using a portable photosynthetic system (LI COR-6400, Lincoln, NE, USA) between 10:00 am to 11:00 am (Mahdavi et al., 2017).

Phytochemical analysis

Twenty milligrams crude powder of *S. cordifolia* was diluted with 20 ml methanol (HPLC grade) and filtered with nylon membrane 0.2 µm to 15 mm syringe filter. The analysis was performed using Liquid chromatography equipped with Mass Spectrometry (MS) and Single Quadrapule Detector (SQD) at 150°C, capillary voltage 2.0 (kv) and 3v extractor using gradient eluent system. Identification of the compounds was achieved by using ACQUITY UPLC BEH C18 column 100 mm x 2.1 mm x 1.7 µm at 35°C eluted with a 10 min 5-95% acetonitrile and Isopropanol at a flow rate of 0.2 ml min⁻¹. The extract sample was analyzed with mobile phase [water (A) and methanol (B) LC-MS grades], and formic acid (0.1%) was added to both A and B. Linear gradients starting with 75% A and 25% B (25:75) followed by 20% A and 80% B (20:80) in 5 min and 75% A and 25% B (25:75) in 7 min at a flow rate of 0.2 ml min⁻¹ in positive and negative ionization mode.

Experimental design and statistical analysis

Germination bioassay was laid in a completely randomized design CRD with three replications according to Aslani et al. (2013) and least significant difference (LSD) at *P* < 0.05 using Statistical Analysis Software (SAS version 9.4) while glasshouse treatments were arranged in a randomize complete block design RCBD with four replications and means comparison was conducted using Duncan multiple range test at *P* ≤ 0.05. Effective doses capable of inhibiting 50% (EC₅₀) of tested parameters were calculated. EC₅₀ values were calculated using Probit analysis based on inhibition percent of the parameters.

$R_e = EC_{g50}, EC_{sl50}, EC_{rl50}, EC_{sdw50}$ (shoot dry weight) + EC_{S50} (shoot) + EC_{rdw50} (root dry weight) + EC_{la50} (leaf area) + EC_{cc50} (chlorophyll content) + EC_{sc50} (stomata conductance) + EC_{p50} (photosynthetic rate) + EC_{tr50} (transpiration rate).

RESULTS

Effect of *S. cordifolia* leaf extract on germination and seedling vigor of weeds species

Sida cordifolia methanol leaf extract showed significant ($P < 0.05$) reduction on seed germination of all the target species except *O. sativa* (weedy rice). At the highest concentration (T8), no seed germination was recorded in *P. hystrophorus* (Table 1). Seed germination appeared to decrease with the increase in the concentrations of the *Sida* extract. The results showed significant ($P < 0.05$) reduction in shoot and radicle elongation of all the weed species in a concentration dependent pattern. A significant decrease in the root elongation was recorded in all the weed species except *O. sativa* and the specie was less affected with an inhibition index of 88.24 % at T8 concentration (Table 3). In general, decline in shoot and root lengths appeared to follow inhibition order of *P. hystrophorus* > *A. conyzoides* > *A. gangetica* > *E. crus-galli* > *E. colona* > *O. sativa* and *P. hystrophorus* > *A. gangetica* > *A. conyzoides* > *O. sativa* < *E. crus-galli* > *E. colona* > (Table 2-3). Relatively, two representatives each from broad leaf and grasses were selected and considered for further study in the glasshouse.

The EC_{g50} for germination was lowest in *P. hystrophorus* (0.34), indicating that *P. hystrophorus* seed germination was most sensitive to the *Sida* leaf extract. EC_{g50} values for seedling length were similar across species, with *A. conyzoides* having 2.66, *O. sativa* with 2.81, *E. crus galli* at 2.10, and *P. hystrophorus* 2.78, suggesting comparable effects of the treatment on seedling elongation among the tested plants. Root length EC_{rl50} was lowest for *P. hystrophorus* (0.04) and highest for *A. conyzoides* (2.75), with *O. sativa* and *E. crus galli* showing intermediate sensitivities (1.12 and 1.68, respectively) indicating that *P. hystrophorus* root growth is extremely susceptible compared to the other species. *Parthenium hystrophorus* exhibited the greatest overall sensitivity while *O. sativa* was the most tolerant in terms of germination but showed moderate sensitivity in root and seedling lengths (Table 4).

Table 1. Methanol leaf extract of *S. cordifolia* on the germination (%) of target species.

Treatment	<i>E. crus-galli</i>	<i>E. colona</i>	<i>O. sativa</i>	<i>A. conyzoides</i>	<i>A. gangetica</i>	<i>P. hystrophorus</i>
T1	80.0a	76.7a	100.0a	100.0a	96.6a	96.7a
T2	70.0ab	66.9a	96.7a	96.6a	76.7ab	43.3b
T3	63.3abc	62.0ab	95.3a	93.3ab	73.3ab	33.3bc
T4	60.0bc	60.0ab	91.6a	86.7ab	70.0ab	20.0cd
T5	53.3bcd	43.3bc	86.7a	83.3ab	60.0bc	16.7de
T6	50.0cd	33.3cd	83.3a	81.3ab	53.3cd	16.1de
T7	36.7d	16.7d	76.6a	73.3b	36.7cd	3.3ef
T8	16.7e	6.70e	74.2a	40.0c	26.7d	0.0f

Note: Data represent means of three replicates. Same superscript letters within column are not significantly different at $P < 0.05$ using LSD.

Table 2. Methanol leaf extract of *S. cordifolia* on the shoot length (cm) of target species.

Treatment	<i>E. crus-galli</i>	<i>E. colona</i>	<i>O. sativa</i>	<i>A. conyzoides</i>	<i>A. gangetica</i>	<i>P. hystrophorus</i>
T1	3.72ab	3.42a	5.18a	5.00a	6.32a	1.64a
T2	3.62ab	3.41a	4.63bc	4.62ab	5.95a	1.57a
T3	3.47b	3.29ab	3.71ab	4.19ab	5.72a	1.19ab
T4	3.43c	3.22b	3.48cd	3.28cd	3.87b	0.91abc
T5	3.41a	3.13ab	2.9cde	2.05ab	3.76b	0.36bc
T6	2.27bc	2.84ab	2.47def	1.53bc	3.52b	0.15abc
T7	1.45d	1.46c	2.09ef	1.19cd	1.40c	0.11bc
T8	0.86e	0.81d	1.67f	0.08d	0.54c	0.00c

Note: Data represent means of three replicates. Same superscript letters within the column are not significantly different at $P < 0.05$ using LSD.

Table 3. Methanol leaf extract of *S. cordifolia* on the root length (cm) of target species.

Treatment	<i>E. crus-galli</i>	<i>E. colona</i>	<i>O. sativa</i>	<i>A. conyzoides</i>	<i>A. gangetica</i>	<i>P. hystrophorus</i>
T1	2.36a	2.96a	6.63a	2.61a	1.72a	2.30a
T2	1.37b	1.46a	3.54b	1.98ab	1.30ab	1.07b
T3	1.15bc	1.25a	2.03bc	1.78abc	1.17b	0.97b
T4	1.04bc	0.97a	1.94bc	1.31bcd	0.84bc	0.33bc
T5	0.50bc	0.91a	1.82c	0.84cde	0.58cd	0.28bc
T6	0.42c	0.75a	1.58c	0.48de	0.48cd	0.17c
T7	0.37c	0.68a	1.22c	0.42de	0.40dc	0.03c
T8	0.32c	0.42a	0.78c	0.29e	0.17d	0.00c

Note: Data represent means of three replicates. Same superscript letters within column are not significantly different at $P < 0.05$ using LSD.

Effects of foliar spray of *S. cordifolia* leaf extract on biomass of weed species

The result of foliar spray of methanol leaf extract of *S. cordifolia* on biomass showed that all the species (*A. conyzoides*, *P. hystrophorus*, *E. crus-galli* and *O. sativa*) tested in the glasshouse have different response to the treatment. The inherent capacity of each species to produce biomass was a determinant factor to overall dry weights (Figure 1), at the highest concentration (3.0 g L^{-1}) of the Sida leaf extract, *P. hystrophorus* shoot and root dry weights were severely reduced by 51.36% and 71.90% while *E. crus-galli* and *O. sativa* recorded minimum shoot dry mass reduction (14.04% and 20.70%) as compared with the control (Figure 1A and 1B). Shoot dry weight was most sensitive in *A. conyzoides* ($\text{EC}_{\text{sdw}50} = 1.30$), while *P. hysterosphorus* (2.50) and *O. sativa* (2.76) had intermediate $\text{EC}_{\text{sdw}50}$ values. *Echinochloa crus-galli* demonstrated the least effect with an $\text{EC}_{\text{sdw}50}$ of 2.95. However, *A. conyzoides* and *E. crus-galli* displayed similar efficacy regarding root dry weight, with low $\text{EC}_{\text{rdw}50}$ values of 1.34 and 1.40 respectively. In contrast, *O. sativa* (2.70) and *P. hysterosphorus* (2.21) had higher $\text{EC}_{\text{rdw}50}$ values, indicating a reduced capacity to inhibit root biomass accumulation.

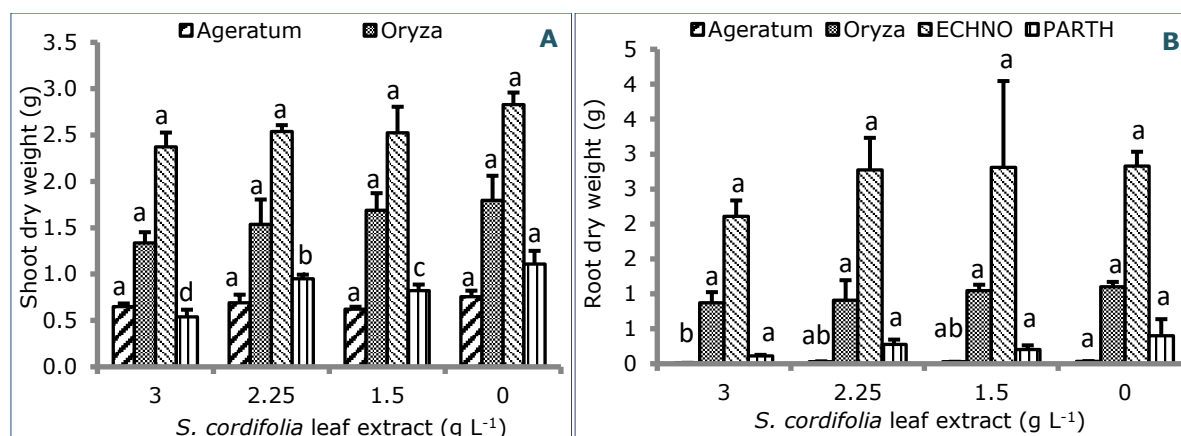


Figure 1. Shoot (A) and root (B) dry weight per plant treated with different concentrations of *S. cordifolia* methanol leaf extract. Each bar represents the mean $\pm$ SEM. All species had 4 four replicates. Statistics performed by two-way ANOVA with DMRT. Different letters at each bar within the concentration indicate significant difference at $P < 0.05$ as compared to the control. ECHNO = Echinochola, PARTH = Parthenium.

Effects of foliar spray of *S. cordifolia* leaf extract on physiological parameters of weed species

The leaf area of the target species is shown in Figure 2A. *Parthenium hysterophorus* leaf area declined by 23.00% (107.18 ± 5.34) at higher concentrations. All the species showed slight decrease in leaf area after folia spray of the Sida extract. In terms of leaf area reduction, *A. conyzoides* had an EC_{50} of 1.66. The EC_{50} values for *O. sativa* (2.86), *E. crus-galli* (2.46), and *P. hysterophorus* (2.71) were comparatively higher, pointing to a less potent effect of the treatments on leaf size. Chlorophyll content decreased significant ($P < 0.05$) in all the species, Figure 2B revealed that the chlorophyll pigment of *A. conyzoides* declined by 29.70% (26.83 ± 1.18) at higher concentration (3.0 g L^{-1}) as compared with the control, similar reduction pattern was observed with *E. crus-galli*, *P. hysterophorus* and *O. sativa* respectively. The treatment effects on chlorophyll content varied with *P. hysterophorus* achieved the lowest EC_{50} (1.26), followed by *E. crus-galli* (2.15) and *A. conyzoides* (2.91), indicating strong potency in reducing chlorophyll concentration. *Oryza sativa*, however, had a higher EC_{50} (2.87) which suggests a minimal impact on chlorophyll levels.

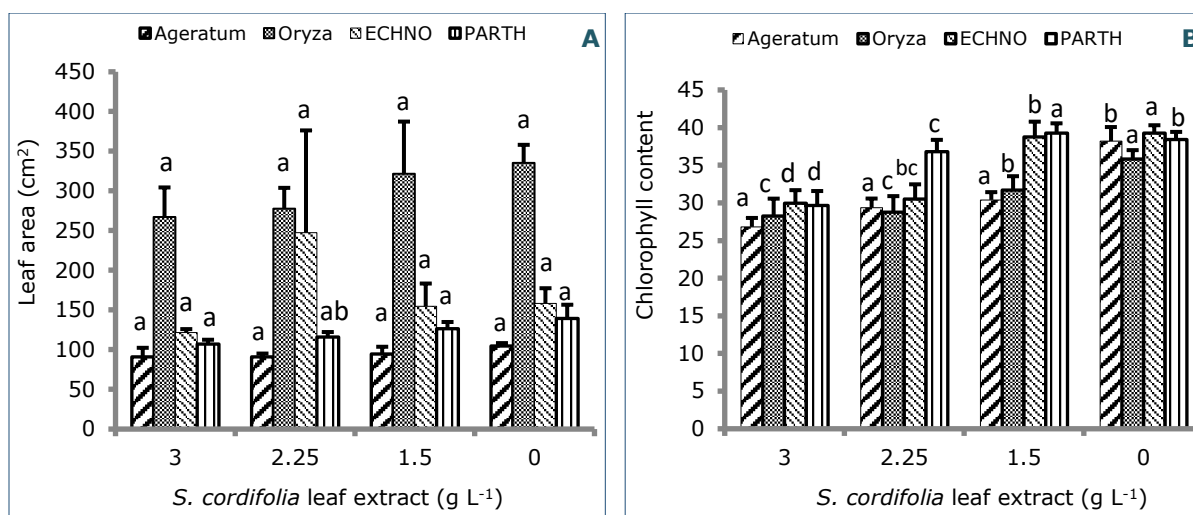


Figure 2. Leaf area (A) and Chlorophyll content (B) per plant treated with different concentration levels of *S. cordifolia* methanol leaf extract. Each bar represents the mean $\pm$ SEM. All species had 4 four replicates. Statistics performed by two-way ANOVA with DMRT. Different letters at each bar within concentrations indicate significant difference at $P < 0.05$ as compared to the control. ECHNO = Echinochola, PARTH = Parthenium.

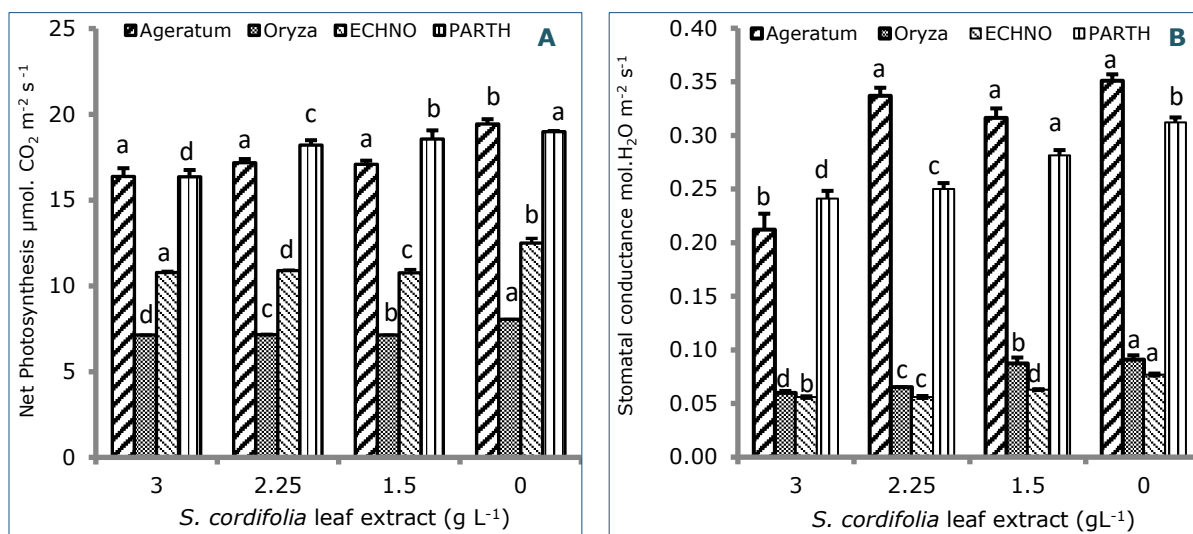


Figure 3. Net photosynthesis (A) and stomatal conductance (B) per plant treated with different concentration levels of *S. cordifolia* methanol leaf extract. Each bar represents the mean $\pm$ SEM. All species had 4 four replicates. Statistics performed by two-way ANOVA with DMRT. Different letters at each bar among concentrations indicate significant difference at $P < 0.05$ as compared to the control. ECHNO = Echinochola, PARTH = Parthenium.

The rate of photosynthesis in all the weed species showed significant ($P < 0.01$) declined. Photosynthetic rate in *A. conyzoides* showed the lowest EC_{50} (1.83), suggesting that it is the most susceptible while the EC_{50} values for *O. sativa* (2.68), *E. crus-galli* (2.73), and *P. hysterosphorus* (2.49) were higher indicating a comparatively reduced efficacy in suppressing photosynthetic activity (Table 4). High inhibition 15.79% and 13.85% (16.37 ± 0.49 and 16.36 ± 0.39) were observed at the maximum concentration (3 g L^{-1}) of the Sida extract in *A. conyzoides* and *P. hysterosphorus* as shown in Figure 3A. As a limitation to regulation of gaseous

exchange in the cellular tissue, the foliar spray of *S. cordifolia* leaf extract significantly inhibited stomatal conductance and rate of transpiration in *A. conyzoides* and *O. sativa* 39.56% and 25.70% (0.21 ± 0.01 and 1.97 ± 1.0) and 34.1% and 58.8% (0.06 ± 0.001 and 0.57 ± 0.017) at higher concentrations (Figure 3B and 4).

Table 4. Effective concentration (EC_{50}) for germination (EC_{g50}), shoot length (EC_{sl50}), root length (EC_{rl50}), shoot dry weight (EC_{s50}), root dry weight (EC_{r50}), leaf area (EC_{la50}), chlorophyll content (EC_{cc50}), stomata conductance (EC_{sc50}), photosynthesis (EC_{p50}) and transpiration rate (EC_{tr50}) of selected weed species exposed to leaf methanol extract of *S. cordifolia* in glasshouse condition.

Target plant	<i>A. conyzoides</i>	<i>O. sativa</i>	<i>E. crus-galli</i>	<i>P. hysterosphorus</i>
EC_{g50}	5.21	11.75	4.30	0.34
EC_{sl50}	2.66	2.81	2.10	2.78
EC_{rl50}	2.75	1.12	1.68	0.04
EC_{sdr50}	1.30	2.76	2.95	2.50
EC_{rdr50}	1.34	2.7	1.4	2.21
EC_{la50}	1.66	2.86	2.46	2.71
EC_{cc50}	2.91	7.30	2.15	1.26
EC_{sc50}	1.17	1.35	1.67	2.39
EC_{p50}	1.83	2.68	2.73	2.49
EC_{tr50}	2.05	1.07	2.53	0.94
Rank	22.88	36.4	23.97	17.66

Note: EC_{g50} , EC_{sl50} , EC_{rl50} , EC_{s50} , EC_{r50} , EC_{la50} , EC_{cc50} , EC_{sc50} , EC_{p50} and EC_{tr50} are the concentrations of extracts that inhibits 50% of the parameter.

The EC_{tr50} values for transpiration differ considerably among treatments. *Parthenium hysterosphorus* exhibited the lowest EC_{tr50} (0.94), followed by *O. sativa* (1.07), indicating substantially higher potency in reducing transpiration. In contrast, *A. conyzoides* (2.05) and *E. crus-galli* (2.53) demonstrated less pronounced inhibitory effects. *Ageratum conyzoides* performed prominently with the lowest EC_{sc50} (1.17) for stomatal conductance followed by *O. sativa* (1.35) and *E. crus-galli* (1.67) whereas *P. hysterosphorus* (2.39) exhibited the least effect on stomatal movement among the treatments. The results indicate that the inhibitory potency varies across treatments. *Ageratum conyzoides* and *P. hysterosphorus* generally exhibit superior efficacy in suppressing several physiological parameters compared to *O. sativa* and *E. crus-galli*. These differential effects suggest that the active compounds in the leaf extract of *S. cordifolia* could have significant implications for application in weed management. Physiological parameters indicated positive correlation matrix (Table 5).

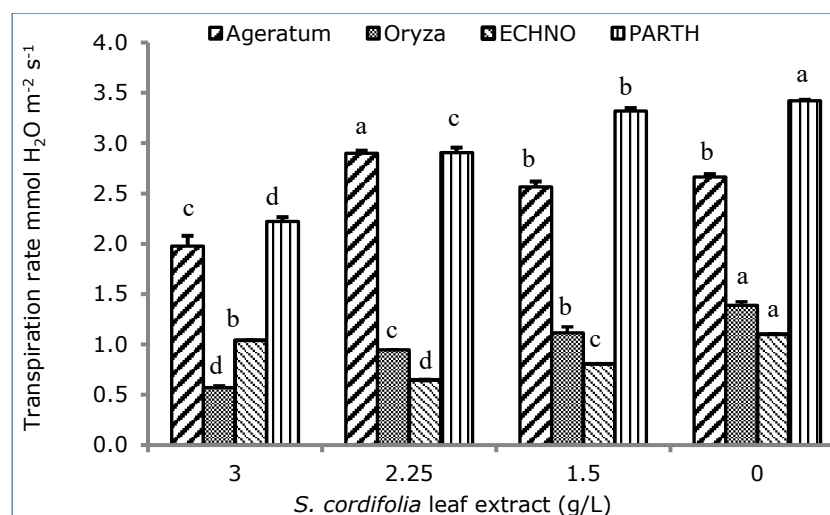


Figure 4. Transpiration rate per plant treated with different concentration levels of *S. cordifolia* methanol leaf extract. Each bar represents the mean $\pm$ SEM. All species had 4 four replicates. Statistics performed by two-way ANOVA with DMRT. Different letters at each bar within concentrations indicate significant difference at $P < 0.05$ compared to control. ECHNO = Echnochola, PARTH = Parthenium.

Table 5. Pearson correlation matrix of physiological parameters.

Factor	Photosynthesis	Stomatal conductance	Transpiration
Chlorophyll	0.598**	0.530*	0.586**
Photosynthesis		0.911**	0.965**
Stomatal conductance			0.966**

Note: Significant at $P < 0.001$ (**), $P < 0.05$ (*).

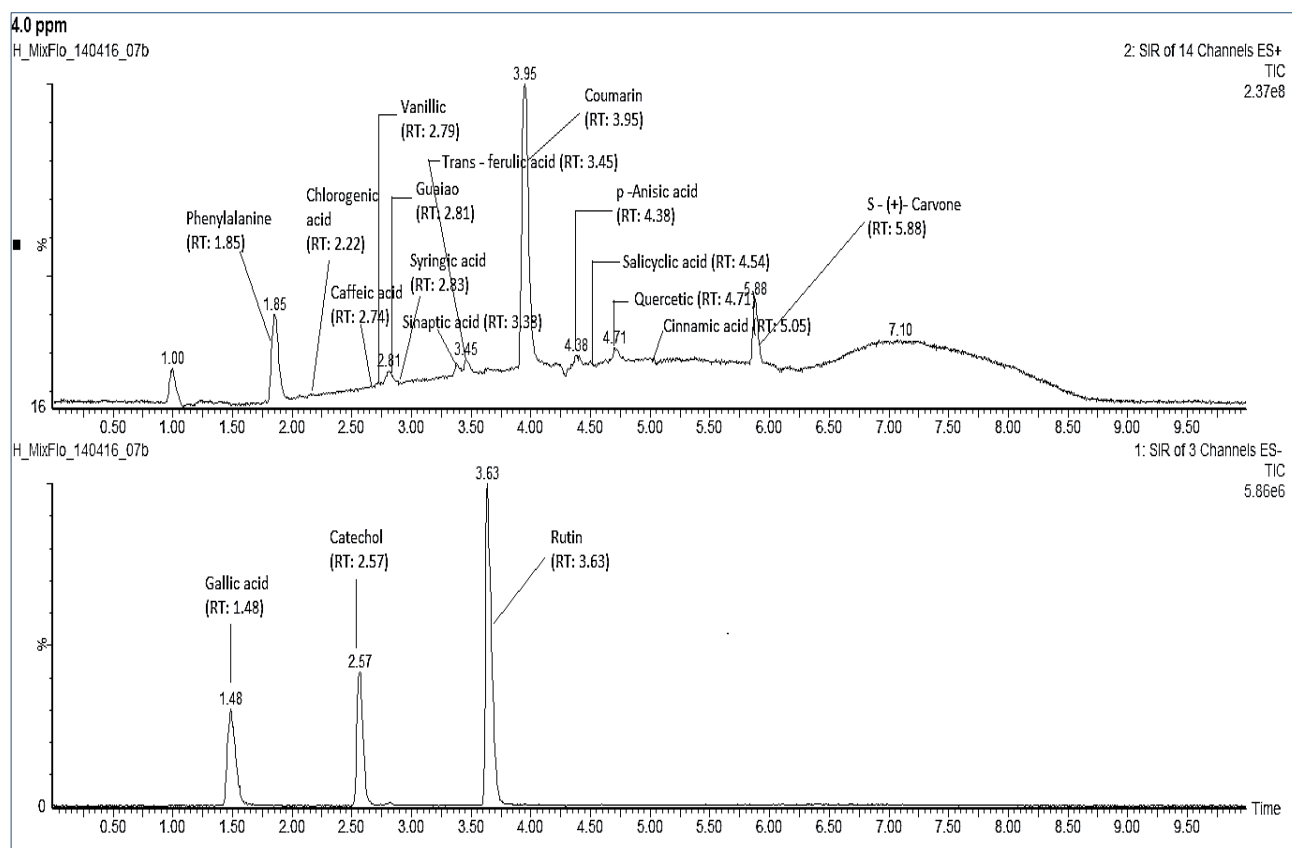


Figure 5. LC-MS chromatograms of standard compounds showing retention time.

Phenolics and flavonoid compounds present in the *S. cordifolia* methanol leaf extract were identified using standard chemicals of 17 compounds (Figure 5) and quantified using LC-MS. The phytochemical profile revealed presence of rutin, DL-phenylalanine (Figure 6), P-anisic acid, quercetin and syringic acid (Table 6).

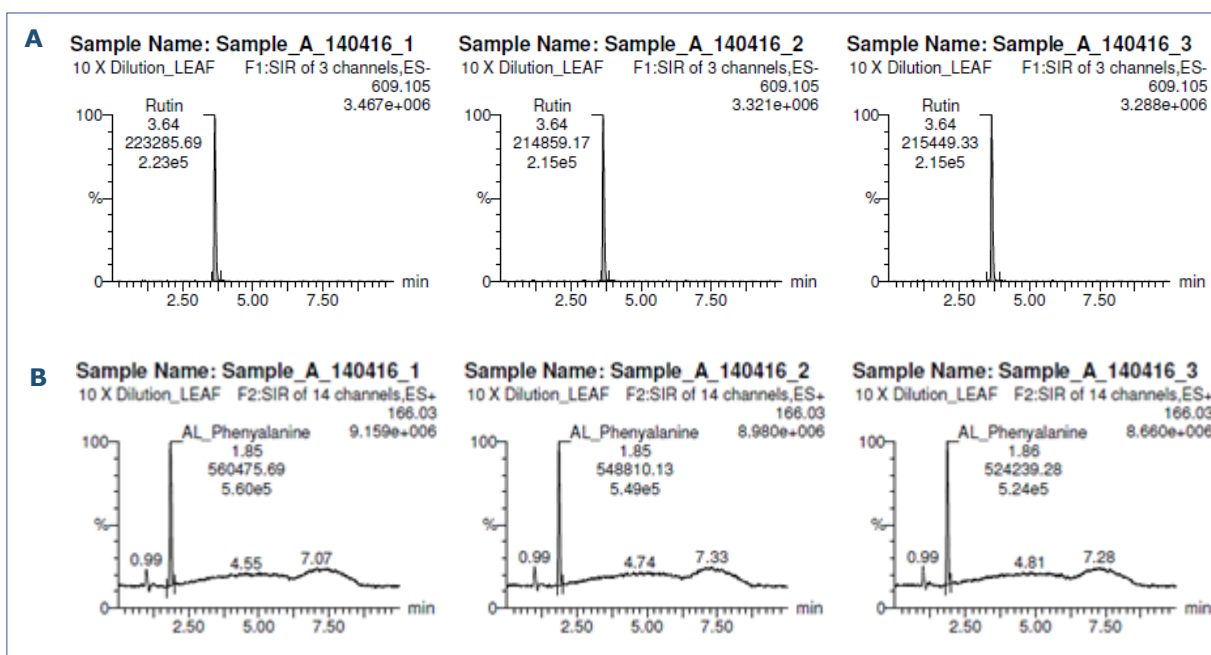


Figure 6. A chromatogram showing peak area and retention time of rutin(a) and phenylalanine(b) analyzed using LC-MS.

Table 6. Phytochemical compounds identified in *S. cordifolia* leaf.

No.	Compounds	RT (min)	Area	Formula	Molecular weight	Conc. µg/g
1	Rutin	3.64	217,864.729	C ₂₇ H ₃₀ O ₁₅	664.57	26.01 ± 0.50
2	DL- Phenylalanine	1.85	544,508.365	C ₉ H ₁₁ NO ₂	165.19	5.67 ± 0.05
3	Quercetin	4.71	5,615.432	C ₁₅ H ₁₀ O ₇	302.24	0.84 ± 0.07
4	Syringic acid	2.91	13,171.729	C ₉ H ₁₀ O ₅	198.17	0.64 ± 0.77
5	<i>P</i> – anisic	4.29	22,497.832	C ₈ H ₈ O ₃	152.15	1.24 ± 1.62

Note: Data are means ± standard deviation of four replicates. RT (Retention time).

Phytotoxicity of *Sida* extract is more severe in broadleaf species (*P. hysterophorus* and *A. conyzoides*) and this appeared to caused darken, swollen, and rotten of radicle with substantial decreased in germination and seedling growth in a concentration dependent pattern (Figure 7). This may be considered a potent plant growth and seed germination inhibitor.



Figure 7. An image (×8) of *P. hysterophorus* seedlings after 5 days treatment with methanol leaf extract of *S. cordifolia* at different concentrations. PT = Parthenium extract; PT6 = 50% concentration; PT5 = 40% concentration; PT4=30% concentration; PT3 = 20% concentration; PT2= 10% concentration; Control-a = Showing fuzzy root hair development.

DISCUSSION

The result of our experiment showed inhibition impact on germination, shoot and radicle elongation of the tested weed species in a concentration dependent pattern. Several studies have reported the inhibitory effect of leaf extract in some weed species and their potential in sustainable weed management (Jabran et al., 2010; Bali et al., 2017; Araniti et al., 2018; Chaib et al., 2021). Extract from *Melia azedarach*, *Rhus corivaria*, *Artemisia aborescon* and *Lantana camara* species inhibited and suppressed seed germination and growth of *Eruca sativa*, *Brassica napus*, *Araujia sericifera* and *Plantago psyllium* (Labruzzo et al., 2017). Several studies report pronounced phytotoxic and allelopathic effects of plant extracts on germination and seedling growth. For instance, extracts of *Calotropis procera*, *Peganum harmala* and *Tamarix aphylla* significantly inhibited root and shoot growth in wheat and mustard (Aslam et al., 2016). Mugwort and Mulberry-leaf extracts inhibited seed germination, suppressed root and shoot lengths, and reduced dry mass in bermudagrass and

Sinapis alba (Haq et al., 2010; Panniaci et al., 2015). The strong inhibition of *P. hystrophorus* seed germination observed in the present study is particularly important from a weed management perspective, as this species is recognized as one of the world's most invasive and difficult-to-control weeds (Bajwa et al., 2023). The high EC_{50} for *O. sativa* suggests that rice can tolerate higher concentrations of Sida extract, making it a promising candidate for integrated weed management strategies. These findings underscore that diverse plant secondary metabolites can impair early plant development across species.

The EC_{50} values for seedling length were relatively uniform among the tested species. This convergence in EC_{50} values suggests that, at the seedling stage, the allelochemicals present in the extract act through mechanisms that are not highly specific to the genetic or physiological differences among these species. Wang et al. (2023) reported that aqueous extracts from *Lantana camara* inhibited seedling growth of both weed and crop species at comparable concentrations, highlighting a lack of strong selectivity at the seedling elongation stage. Phenolic acids and flavonoids commonly found in Sida and other medicinal plants can interfere with microtubule organization and mitochondrial function, leading to reduced cell expansion in multiple species (Li et al., 2022). The EC_{150} values for root length revealed pronounced species-specific differences in sensitivity to Sida leaf extract. *Parthenium hystrophorus* displayed an extremely low EC_{150} , indicating a high susceptibility of root growth. The heightened sensitivity of *P. hystrophorus* roots may be attributable to differences in root surface area, permeability, or lower antioxidant defense mechanisms, making them more vulnerable to oxidative damage, and cellular disruption caused by phenolic and flavonoid compounds commonly found in Sida extracts (Li et al., 2022). While the intermediate responses in *O. sativa* and *E. crus-galli* are consistent with earlier studies indicating that monocot crops and weeds may share similar moderate levels of tolerance to certain allelopathic agents (Wang et al., 2023).

Methanol leaf extract of *S. cordifolia* caused changes in biomass in the target species, and this may be due to chemical interference of different allelochemicals. All the species exhibited sharp and gradual decrease in leaf area after foliar spray of Sida extract. Several studies revealed decrease in leaf area of plant species treated with different concentrations of plant extracts (Jabran et al., 2015; Algandaby and El-Darier, 2018). The EC_{150} results reveal concentration-dependent differences in the potency of the four weed species (*Ageratum*, *Oryza*, *Echinochloe*, and *Parthenium*) on physiological functions and biomass accumulation. *Echinochloe crus-galli* exhibited moderate weight, particularly on root dry weight, possibly reflecting differential root uptake, in line with findings that allelochemicals can have distinct above- and below-ground effects (Inderjit and Duke, 2003).

The use of physiological data to evaluate allelochemicals stress and mode of activity of plant extract is common in allelopathic study. Aqueous root extract of cucumber contain derivatives of benzoic and cinnamic acids caused decreased in stomatal conductance, intercellular CO_2 concentration and transpiration of cucumber seedlings (Li et al., 2010). Photosynthetic activity mainly depend on mesophyll tissues metabolism and stomatal movement which facilitate assimilation of CO_2 , therefore enhancement or decreased in photosynthetic activity is primarily associated with diffusion rate of CO_2 within the leaf tissue via stomatal conductance (Ahmed et al., 2010).

Chlorophyll content is an important factor in photosynthetic activity and was observed to be decreased as the Sida extract concentrations increases. *Sida cordifolia* leaf extract contain phenolic compounds (Singh et al., 2009), and this class of phytochemicals are well known for phytotoxicity in reducing chlorophyll content, photosynthesis, inhibit cell division and alter cell ultra-structure (Bercnaccia and Furini, 2004; Mahdavi et al., 2017). Reduction in chlorophyll pigment could be attributed to inhibition of chlorophyll biosynthesis and photosynthesis, destruction of ultra cellular and root activity (Bercnaccia and Furini, 2004). Reduction in net photosynthesis has been ascribed to inhibition of photosynthesis mechanism and ROS production (Lawlor, 2002), decrease in photosynthetic metabolite, chloroplast activity,

carboxylation and increase in enzymes activity (Jose and Gillespie, 1998). The variation in chlorophyll EC_{50} values, with *Parthenium* and *Echinochloe* produced much stronger reductions than *Oryza*, may be attributed to differences in active compound composition in Sida leaf extract. Wang et al. (2024) recently demonstrated that allelopathic root extracts from *Phytolacca americana* inhibited crop seedling biomass and chlorophyll content while simultaneously inducing stress-related enzymes.

There is significant decreased in stomatal conductance and transpiration in the target species after 21 days of foliar spray of Sida extract. Similar assertions were reported (An et al., 2017), where allelopathin (Juglone) released from *Juglan nigra* inhibit net photosynthesis and transpiration in *Glycine max* and *Zea mays*. Solutions from benzoic and cinnamic acids reduced transpiration and stomatal conductance in cucumber seedling (Hong et al., 2005). Ageratum consistently produced the lowest EC_{50} for shoot dry weight, photosynthesis, and stomatal conductance, indicating strong susceptibility to the Sida leaf extract. *Parthenium* meanwhile, showed high potency in reducing both transpiration and chlorophyll content, pointing to a strong influence on water regulation and pigment stability. Allelochemicals from plant extracts can disrupt gas exchange and photosynthetic function, often through effects on stomatal closure, chlorophyll biosynthesis, or direct damage to the photosynthetic machinery (Taiz and Zeiger, 2010; Cheng and Cheng, 2015). Furthermore, under stress, photosynthesis, transpiration, and stomatal conductance can become decoupled, and their responses are not always proportional (Wang et al., 2026).

Liquid chromatography analysis revealed presence of rutin (26.01 $\mu\text{g/g}$) in the Sida leaf extract, and this may be a strong indicator of invasive phenomenon of the *S. cordifolia*. Rutin at 80 $\mu\text{g/mL}$ concentration inhibited growth of mung bean (*Vigna radiata*) but showed slight growth stimulation at concentration lower than 40 $\mu\text{g mL}$ (Fan et al., 2010). Many studies reported significant inhibition effect of flavonoids and phenolics (allelochemicals) on germination and seedling growth (Singh et al., 2009; Aslani et al., 2016; Ahmed et al., 2017). Phenolic compounds can reduce oxidative stress by scavenging excess free radical (Musa et al., 2025), and modulate physiological responses that reduce ROS production. Phenolic accumulation is associated with the stress signaling that down regulates photosynthetic activity (Noctor et al., 2014). Rutin, DL-phenylalanine, quercetin and p-anisic acid mitigate oxidative stress and act as antioxidant or a signaling metabolite that trigger protective response. As a result, their presence is commonly associated with reduced chlorophyll degradation, down regulate net photosynthesis, lower stomatal conductance and transpiration. These effect limits may reduce ROS production and cellular integrity under stress condition.

Rutin, Quercetin and P-hydroxybenzoic acid detected in *Acacia melanoxylon* leaf extract decreased shoot growth of *Dactylis glomerata*, *Lolium prene*, *Rumex acetosa*, *Lactuca sativa*, *Acanthopanax seiboldianum* and *A. gracillstylus* (Hussain and Reigosa, 2011). Phenylalanine and syringic acid have phytotoxic effect on seedling growth of *Alternaria alternate* and *Triticum aestivum* (Bobylev et al., 1999; Wu et al., 2001). Quercetin, rutin, iso-quercetin inhibited seed germination and seedling growth of *Brassica pekinensis* and *Ficus carica* (Ladhari et al., 2020; Ming et al., 2020). In contrast, (Rial et al., 2018) revealed that the allelochemical (α -tomatine) is allelopathic against weed species by stimulating seed germination (77%) at 100 μM of *Phelipanche ramosa* while *Orobancha cumana* and *O. crenata* were not stimulated, hence concluded that the *P. ramosa* could use α -tomatine as a signal to confirm present of its host.

CONCLUSION

In general, methanol leaf extract of *S. cordifolia* at 50 g/L inhibited seed germination (100%) of *P. hystrophorus*, 70%, 69.3%, 63.3%, 60, and 25% in *E. colona*, *A. gangetica*, *E. cruss-galli*, *A. conyzoides* and *O. sativa*, while other growth, physiological parameters and biochemical (stress) indices decreased and increased in a concentration dependent pattern. Sida leaf extract at 3 gL⁻¹ may contain allelochemicals with bioherbicidal potential; further validation (mode-of-action, crop safety, formulation, field trials) is required. Rutin and DL-phenylalanine were previously not reported in *Sida cordifolia*. Detected compounds (rutin, quercetin, phenylalanine, etc.) are plausible contributors; activity-guided fractionation and bioassays of purified compounds are needed to attribute activity in the Sida leaf extract. While EC₅₀ values provide comparative potency, further research is needed to elucidate mechanisms of action, confirm selectivity, and evaluate long-term and field effects. Assessment of crop safety and non-target impacts are recommended. Exploration on formulation techniques through nanotechnology and potency assessment in-situ for sustainable weed management in agriculture is worthy.

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AUTHOR CONTRIBUTIONS

Hassan Ahmed: Conceptualization (Lead), Methodology (Lead), Formal Analysis (Lead), Writing – Original Draft (Lead); **Abdul Shukor Juraimi:** Data Curation (Equal), Writing – Review & Editing (Equal), Investigation (Lead); **Dzolkhifli Omar:** Data Curation (Equal), Formal Analysis (Equal), Investigation (Lead); **Muhammad Saiful Ahmad-Hamdani:** Writing – Review & Editing (Equal), Investigation (Lead); **Yusof Muhammad Rafii:** Methodology (Supporting), Formal Analysis (Supporting), Validation (Equal), Data Curation (Lead), Investigation (Supporting), Supervision (Equal).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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Quantify sample summary report

Quantify Sample Summary Report MassLynx 4.1 SCN805
Identification of Phytochemical compounds

Dataset: Untitled

Last Altered: Friday, April 15, 2016 17:09:45 Malay Peninsula Standard Time
Printed: Friday, April 15, 2016 17:13:10 Malay Peninsula Standard TimeMethod: C:\Metabolomics services\2014.PRO\MethDB\H_MixFlo_01.mdb 15 Apr 2016 16:43:14
Calibration: C:\Metabolomics services\2014.PRO\CurveDB\H_MixFlo_01.cdb 15 Apr 2016 16:46:28

Name: Sample_A_140416_1, Date: 14-Apr-2016, Time: 17:09:59, ID: , Description: 10 X Dilution_LEAF

	# Name	Trace	RT	Area	IS Area	Response Detecti...	Conc.	%Dev
1	1 Catechol	108.887	2.56	36.454		36.454 bbl		
2	2 Gallic acid	168.896	1.64	146.397		146.397 bbl		
3	3 Rutin	609.105	3.64	223285.688		223285.688 bb	26.2	
4	4 Syringic acid	TIC	2.91	13171.729		13171.729 bb	0.1	
5	5 p_anisic acid	152.93	4.41	18246.936		18246.936 bbl		
6	6 S_+ Carvone	150.997	5.82	3477.651		3477.651 bbl		
7	7 Coumarin	146.92	4.05	1780.296		1780.296 bbl		
8	8 AL_Phenylalanine	166.03	1.85	560475.688		560475.688 bb	5.7	
9	9 Vanillic acid	168.92	4.05	1423.464		1423.464 bbl		
10	10 Trans_ferulic acid	194.89	3.57	1722.083		1722.083 bbl		
11	11 Sinaptic acid	224.977	3.46	3259.324		3259.324 bbl		
12	12 Quercetic	303.017						
13	13 Chlorogenic	355.02	2.24	1807.033		1807.033 bbl		

Name: Sample_A_140416_2, Date: 14-Apr-2016, Time: 17:21:13, ID: , Description: 10 X Dilution_LEAF

	# Name	Trace	RT	Area	IS Area	Response Detecti...	Conc.	%Dev
1	1 Catechol	108.887	2.60	41.593		41.593 bbl		
2	2 Gallic acid	168.896	1.54	176.146		176.146 bbl		
3	3 Rutin	609.105	3.64	214859.172		214859.172 bb	25.2	
4	4 Syringic acid	TIC	3.11	23894.295		23894.295 bb	1.2	
5	5 p_anisic acid	152.93	4.29	22497.832		22497.832 bb	0.1	
6	6 S_+ Carvone	150.997	5.90	633.450		633.450 bbl		
7	7 Coumarin	146.92						
8	8 AL_Phenylalanine	166.03	1.85	548810.125		548810.125 bb	5.6	
9	9 Vanillic acid	168.92	3.93	11979.832		11979.832 bbl		
10	10 Trans_ferulic acid	194.89						
11	11 Sinaptic acid	224.977	3.44	5246.528		5246.528 bbl		
12	12 Quercetic	303.017	4.74	5615.432		5615.432 bb	0.9	
13	13 Chlorogenic	355.02	2.23	2196.205		2196.205 bbl		

Name: Sample_A_140416_3, Date: 14-Apr-2016, Time: 17:32:26, ID: , Description: 10 X Dilution_LEAF

	# Name	Trace	RT	Area	IS Area	Response Detecti...	Conc.	%Dev
1	1 Catechol	108.887	2.66	273.704		273.704 bbl		
2	2 Gallic acid	168.896						
3	3 Rutin	609.105	3.64	215449.328		215449.328 bb	25.3	
4	4 Syringic acid	TIC	3.01	3002.741		3002.741 bbl		
5	5 p_anisic acid	152.93	4.29	63991.582		63991.582 bb	2.4	
6	6 S_+ Carvone	150.997	5.91	1018.655		1018.655 bbl		
7	7 Coumarin	146.92	4.12	1127.133		1127.133 bbl		
8	8 AL_Phenylalanine	166.03	1.86	524239.281		524239.281 bb	5.3	
9	9 Vanillic acid	168.92	3.75	11887.139		11887.139 bbl		
10	10 Trans_ferulic acid	194.89	3.37	3745.582		3745.582 bbl		

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Name: Sample_A_140416_3, Date: 14-Apr-2016, Time: 17:32:26, ID: , Description: 10 X Dilution_LEAF

	# Name	Trace	RT	Area	IS Area	Response Detecti...	Conc.	%Dev
11	11 Sinaptic acid	224.977	3.25	1177.788		1177.788 bbl		
12	12 Quercetic	303.017	4.73	3741.319		3741.319 bb	0.8	
13	13 Chlorogenic	355.02	2.16	385.415		385.415 bbl		

LCMS Analysis (Quantitative analysis)																	
Liquid Chromatography (LC)																	
Model/ Brand	Waters ACQUITY UPLC																
Mobile phase	Group 1: Solution A: Water (LCMS Grade) + 0.1% Formic acid Solution B: Methanol (LCMS Grade) + 0.1% Formic acid Group 2: Solution A: Acetonitrile (LCMS Grade) Solution B: Isopropanol (HPLC Grade)																
Column type / Temperature	ACQUITY UPLC BEH C18 column (100 mm x 2.1 mm x 1.7µm) Temperature: 35 °C (Group 1), 30 °C (Group 2)																
LC Method	Group 1: Mixture phenolics																
	<table><tr><th>Flow rate (ml/min)</th><th>Time (min)</th><th>% A</th><th>% B</th></tr><tr><td rowspan="3">0.2</td><td>Initial</td><td>75</td><td>25</td></tr><tr><td>5.0</td><td>20</td><td>80</td></tr><tr><td>7.0</td><td>75</td><td>25</td></tr></table>	Flow rate (ml/min)	Time (min)	% A	% B	0.2	Initial	75	25	5.0	20	80	7.0	75	25		
	Flow rate (ml/min)	Time (min)	% A	% B													
	0.2	Initial	75	25													
		5.0	20	80													
7.0		75	25														
Run time: 10 min																	
Group 2: Mixture Fatty Acids																	
<table><tr><th>Flow rate (ml/min)</th><th>Time (min)</th><th>% A</th><th>% B</th></tr><tr><td>0.3</td><td>Isocratic</td><td>95</td><td>5</td></tr></table>	Flow rate (ml/min)	Time (min)	% A	% B	0.3	Isocratic	95	5									
Flow rate (ml/min)	Time (min)	% A	% B														
0.3	Isocratic	95	5														
Run time: 5 min																	
Ionization mode	Positive and Negative																
Injection volume	1 µL																
Mass Spectrometry (MS)																	
Model/ Brand	Waters Single Quadrupole Detector (SQD)																
Capillary voltage (kV)	2.0 (Group 1), 3.9 (Group 2)	Cone gas flow (L/Hr)	50														
Source temperature (°C)	150	Desolvation temperature (°C)	250														
Extractor (V)	3.0	Desolvation gas flow (L/Hr)	500														
Cone voltage (V)	Group 1: Mixture phenolics Positive mode: - Vanillic Acid: 22.0 - trans-Ferulic Acid: 18.0 - Syringic Acid: 22.0 - Guaiaao: 50.0 - Salicyclic acid: 18.0 - Coumarin: 36.0 - Trans_cinnamic acid: 20.0 - S_+_Carvone : 16.0 - p_anisic acid: 22.0 - DL - Phenylalanine:20 - Caffeic acid: 12.0 - Sinaptic acid: 16.0 - Quercetic: 52.0 - Chlorogenic acid: 16.0 Negative mode: Gallic Acid: 30.0 Catechol: 44.0 Rutin: 56.0																