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# Identification of Phytochemicals in *Cleome rutidosperma* DC. Methanol Extract and Evaluate its Efficacy on Some Common Rice Field Weeds

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## ABSTRACT

Screening different plant species for herbicidal activity and identifying new allelochemicals with novel structures and phytochemical activity could be promising candidates for reducing the negative consequences of chemical herbicides. Our study aims to investigate the allelopathic substance(s) and herbicidal efficacy of *Cleome rutidosperma* DC. on rice filed weeds in the lab and glasshouse. The phytochemical constituents of the methanol extract of *Cleome rutidosperma* were analyzed by high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (HPLC-ESI-QTOF-MS). The allelopathic effect of *C. rutidosperma* has been further studied on the germination and early development of five common rice field weeds: *Echinochloa crus-galli* (L.) P. Beauv., *Fimbristylis miliacea* (L.) Vahl, *Oryza sativa* f. *spontanea* Roshev., *Leptochloa chinensis* (L.) Nees, and *Cyperus iria* L. The seed germination and growth of tested weeds under lab and glasshouse conditions were compared to three concentrations of *C. rutidosperma* methanol extract at 2.5, 5, and 10% with the control (only distilled water). The results indicated the presence of 64 and 10 known chemicals using positive and negative ionization techniques, the majority of which were toxic. The inhibitory effect of *C. rutidosperma* was stronger in the lab than

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in the glasshouse. No seed germination of the tested species was observed when 10% *C. rutidosperma* extract was applied. The photosynthesis rate of *C. iria* exhibited a higher reduction (70.56%) compared to other species at higher doses (10%) of *C. rutidosperma*. These findings demonstrated that *C. rutidosperma* is a significant source of phytotoxic components and can be used to develop future bio-herbicides. The outcome of this study can be employed in the organic management of weeds and reduce our heavy reliance on synthetic herbicides.

*Keywords*: Allelopathy, *Cleome rutidosperma*, germination, growth, physiology, phytochemicals

## INTRODUCTION

Rice (*Oryza sativa* L.), a primary food for more than half of the world's population, is cultivated in >100 countries, with Asia accounting for 90% of global production (Fukagawa & Ziska, 2019). Ray et al. (2013) predicted that world rice demand would more than double by 2050. In lowland and highland environments, weed infestations are a significant biological restriction to rice production at all seasons (Reynolds et al., 2015).

The most threatened weed is *Echinochloa crus-galli* (L.) P. Beauv. (barnyard grass), which can decrease rice production by up to 64%, depending on the rice variety (Yang et al., 2021). *Echinochloa crus-galli* possesses the adaptive characteristics and competitive qualities required for effective competition and survival in various geographical and environmental situations (Clements & Ditommaso, 2011). In rice fields, *Fimbristylis miliacea* (L.) Vahl, sometimes known as hoorahgrass, is an invasive sedge with an emergence density ranging from 54 to 3,074 plants per square meter in Southeast Asia (Siddique & Ismail, *2013).* According to Siddique and Ismail (2013), *F. miliacea* is ranked third and fifth among the most troublesome weeds in Malaysia. *Oryza sativa* f. *spontanea* Roshev. (weedy rice), popularly known as "red rice", is now one of the major weeds in many places of the world that produce rice (Juliano et al., 2020; Mispan et al., 2019; Ziska et al., 2015). Countries switching to direct seeding rice instead of transplanting are facing a serious problem (Nadir et al., 2017). *Leptochloa chinensis* (L.) Nees (Chinese sprangletop) is a major global agricultural grass weed spread in rice fields and has developed resistance to cyhalofop-butyl herbicide (Yu et al., 2017). *Leptochloa chinensis* can grow in flooded and upland environments, making it a common weed in rice and other crops (Wang et al., 2022). According to Jiang et al. (2018), *Cyperus iria* L. has successfully adapted to habitats and is a problematic weed in rice cultivation. Approximately 5,000 seeds can be produced by one *C. iria* plant, and the first rice seedlings often emerge within a few weeks after planting (Awan et al., 2022).

Weeds are a growing issue than diseases and pests when it comes to reducing rice yields in tropical Asian countries (Motmainna et al., 2021a; Juraimi et al., 2013). In rice production, the use of herbicides and other types of chemical control is the most common practice (Hasan, Ahmad-Hamdani, et al., 2021; Motmainna, Juraimi, Uddin, Asib, Islam, Ahmad-Hamdani, & Hasan, 2021; Sherwani et al., 2015). However, by 2020, global pesticide usage has been estimated to increase to 3.5 million tons (Sharma et al., 2019). Herbicide-resistant weeds are developed when similar herbicides are used repeatedly at the same field site. Eight of the 16 weed species detected in Malaysia that have been documented to be resistant to various herbicides were found in rice fields (Ruzmi et al., 2017). The global interest in inorganic farming supports alternative methods that prevent herbicide-resistant weed development (Motmainna et al., 2021b). Such circumstances have promoted using other alternatives, such as bioherbicide, to control the population of weeds.

Screening different plant species for herbicidal activity and identifying new allelochemicals with novel structures and phytochemical activity could be promising candidates for reducing the negative consequences of chemical herbicides. Taking an example, WeedLock is a commercial bioherbicide obtained from *Solanum habrochaites* S. Knapp & D. M. Spooner (wild tomato) extract and showed promising weed control efficacy in both glasshouse and field conditions (Hasan, Mokhtar, et al., 2021). Verdeguer et al. (2020) reported that, by 2020, six commercial bioherbicides, i.e., Matratec, GreenMatch, GreenMatchEX, WeedZap, Weed Slayer, and Avenger Weed Killer, derived from essential oils and/or their compounds were registered and available in the USA. Bioherbicides such as BioWeed, Avenger Weed Killer, and Weed Slayer successfully controlled *Ochna serrulata* Walp., *Digitaria sanguinalis* (L.) Scop., and *Echinochloa crus-galli* (L.) P. Beauv., respectively (Travlos et al., 2020).

Invasive weeds may release chemicals into the environment that suppress nearby plants trying to compete with them (Kato-Noguchi et al., 2014; Lorenzo et al., 2012). Phytochemicals are plant-based substances and non-nutritional secondary metabolites that are omnipresent in plants and can be beneficial to human health and reduce the risk of major chronic diseases (Mendoza & Silva, 2018). Phytochemicals can be found in many parts of plants, such as leaves, roots, and seeds, and have the potential as bioherbicides (Mushtaq et al., 2020). Seeds, leaves, and roots of *Cleome* plants have been used medicinally for several purposes, including as an antiscorbutic, stimulant, anthelmintic, vesicant, carminative, and rubefacient (Prabha et al., 2017; Singh et al., 2016). Some species of *Cleome* have the potential to serve as an alternative pesticide due to the presence of chemical pesticide components responsible for poisonous and insecticidal activities (Upadhyay, 2015). *Cleome*'s crude extracts were extremely poisonous to egg-masses of *Meloidogyne javanica* root-knot nematode (Krishnappa & Elumalai, 2013; Stephan et al., 2001). However, antioxidant activity was observed in methanolic (MeOH) extracts of leaves from five different *Cleome* species. *Cleome viscosa* L. had strong insecticidal action, suggesting it could replace pesticides against *Spodoptera litura* (Lakshmanan et al., 2018; Mali, 2010). It has been previously reported that *C. rutidosperma* possesses antiplasmodial action (Bose et al., 2007). Many scientists have reported the healing effects of *C. rutidosperma*, but nowadays, the phytochemicals of *C. rutidosperma* can be used in environmentally friendly weed control. The objective of the present study is to identify the phytochemicals profiling using HPLC-ESI-QTOF-MS and evaluate the phytotoxic effect on *E. crus-galli*, *F. miliacea, O. sativa*, *L. chinensis*, and *C. iria*.

## MATERIALS AND METHODS

### Test Plants

This experiment used five different weed species as the control group: *E. crus-galli*, *F. miliacea*, *O. sativa*, *L. chinensis*, and *C. iria*. The weed seeds were taken from Farm 15, Faculty of Agriculture, Universiti Putra Malaysia.

### Extraction

Previously, many scientists revealed the insecticidal and medicinal effects of *C. rutidosperma* (Prabha et al., 2017; Singh et al., 2016; Upadhyay, 2015), but little information is available regarding its herbicidal effect. Therefore, it was selected for use in this study to determine the herbicidal effect. *Cleome rutidosperma* was harvested at its most vegetative and matured stage in a natural environment of weed infestation from Universiti Putra Malaysia. The whole plant was harvested, hand-cleaned running water was used to eliminate dirt or debris, and ten were air-dried for three weeks. Then, the grinder was used to grind the gathered plant material to a powder. In a paraffin-wrapped conical flask, 100 g of *C. rutidosperma* were soaked in 1,000 ml of methanol (80%, Merck, Germany). The flask was agitated with an orbital shaker for 48 hr at room temperature (24–26°C). The solution was centrifuged for 1 hr at 1,107 ×*g* after being filtered using four layers of cheesecloth and then re-filtered using a 0.2-μm, 15-mm syringe filter (Phenex, non-sterile, luer/slip, LT Resources, Malaysia). The collected supernatant was evaporated using a rotary evaporator set to 40°C. The extraction percentage is calculated as follows:

$$\text{Extraction percentage} = \frac{\text{Extract weight (g)}}{\text{Powder weight (g)}} \times 100\%$$

For bioassay purposes, various concentrations of extracts were prepared by diluting the stock extracts with sterile distilled water. Before being used, all extracts were stored in the fridge at 4°C in the dark. The methanol extracts were obtained following the procedure described by Aslani et al. (2016).

### HPLC-ESI-QTOF-MS Analysis

For HPLC-ESI-QTOF-MS analysis, the crude sample (20 mg) was diluted in 100% high-performance liquid chromatography (HPLC) Grade methanol (20 ml, Merck, Germany) and filtered through 0.2-μm, 15-mm syringe filters (Phenex, non-sterile, luer/slip, LT Resources, Malaysia). The chemical contents of the *C. rutidosperma* sample obtained from the methanol extract were examined following the approach described in Tamsir et al. (2020), with a few minor changes. A dual electrospray ionization (ESI) source Agilent 6520 Accurate-Mass Q-TOF mass spectrometer (Germany) and an Agilent 1290 Infinity LC system (Germany) were used to analyze the chemical substances. To perform a more accurate analysis of the chemical profile, the settings of the mass spectrometry (MS), as well as the type of column used, were all subjected to optimization.

With the goal of achieving rapid and efficient separations at lower column pressures (Guiochon & Beaver, 2011), an ACQUITY UPLC BEH C18 column (150 mm × 2.1 mm × 3.5 m, Germany) was used and maintained at 50℃ with a constant flow rate of 0.4 ml/min during the entire liquid chromatography (LC) run time of 26 min. In this study, a mobile phase was used for sample elution. It consisted of water liquid chromatography–mass spectrometry (LC-MS Grade) containing 0.1% formic acid (solvent A, Merck, Germany) and acetonitrile (LC-MS Grade, Merck, Germany) containing 0.1% formic acid (solvent B, Merck, Germany). The MS/MS investigations were conducted at 325℃ with a drying gas flow of 10 L/min and a nebulizer pressure of 40 psi. Analysis of positive and negative ion modes at varying collision energy (CE) was performed to optimize signals and extract maximum structural information from ions in the mass range of 100 to 3,200 m/z, achieving the most sensitive ionization effect for analytes. MassHunter Qualitative Analysis software (version B.07.00) was used to process the data, and peaks were identified by comparing them with values from the literature and an online database (Abu Bakar et al., 2020).

**Laboratory Bioassay**

A bioassay was performed in a growth chamber at the Seed Technology Laboratory, Department of Crop Science, Universiti Putra Malaysia. The most consistent and healthy seeds were selected and soaked in potassium nitrate ($KNO_3$, Merck, Germany) at a concentration of 0.2%. They were soaked in water for 24 hr, then cleaned, and placed in an incubator (between 24 and 26°C) until a radicle measuring 1 mm in length appeared. Twenty-five seeds of *E. crus-galli*, *F. miliacea*, weedy rice, *L. chinensis*, and *C. iria* that had already sprouted were put in Petri dishes with two sheets of Whatman No. 1 filter paper. After that, 10 ml of methanol extracts from *C. rutidosperma* were applied to the filter paper in concentrations of 0 (distilled water), 2.5, 5, and 10%, respectively. The experiment was carried out using a completely random design with four replicates. The Petri dishes were placed in a growth chamber with fluorescent light (8,500 lux) at 30℃ (day) and 20℃ (night) on a 12-hr day, 12-hr night schedule. The relative humidity ranged between 30 and 50%. Due to the need to prevent anaerobic conditions and allow for gas exchange, the covers of the Petri dishes were not attached. At 7 days following treatment, the survival rate, hypocotyl, and radicle length were measured.

### Glasshouse Experiment

**Experimental Site, Treatments, and Design.** The efficacy experiment was carried out between June and July of 2021 at the Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia. Before being placed in germination trays, seeds were soaked in a solution of 0.2% $KNO_3$ (Merck, Germany) for 24 hr. One healthy, pre-germinated seedling was successfully transplanted into each soil-filled plastic pot (9 cm diameter). River sand, peat growth, and topsoil at a 3:2:1 ratio were used to prepare the soil of each pot. The weeds were treated with methanol extract of *C. rutidosperma* at three rates (2.5, 5, and 10%) and left untreated (control) when they reached the 2-3 leaf stage. A 1 L multipurpose sprayer (Deluxe pressure sprayer, Malaysia) was used to spray where the spray volume is 100 ml/m$^2$. The experiment was set up with a randomized complete block design (RCBD) and four replications.

**Data Collection.**

***Plant Injury.*** Injury to plants was visually evaluated 21 days after spraying using a scale established by Burrill et al. (1976), where 0 indicates no effect (all foliage is still green and healthy), >70% indicates acceptable control, and 100% indicates complete kill (dead).

***Photosynthetic Rate, Transpiration, and Stomatal Conductance***. From 9 a.m. to 11 a.m., the LI-COR-6400XT Portable Photosynthetic System (USA) was used to measure photosynthetic rate, transpiration, and stomatal conductance. The observations were performed at a carbon dioxide ($CO_2$) flow rate of 400 µmol/m$^2$/s on the abaxial surface, with the saturating photosynthetic photon flux density (PPFD) set at 1,000 mmol/m$^2$/s.

***Plant Height, Fresh and Dry Weight.*** At 21 days after spray (DAS), the height of all plant species was measured using a measuring tape from the top of the soil. At 21 DAS, weeds were picked 1 cm above the ground. The samples' fresh weight was measured using a digital balance, and dry weight was measured after drying them in an oven at 65°C for 72 hr. Weed control efficiency was determined using the following equation:

$$\text{Weed control efficiency (\%)} = \frac{\text{Dry weight of untreated pot} - \text{Dry weight of treated pot}}{\text{Dry weight of untreated pot}} \times 100\%$$

### Statistical Analysis

A two-way analysis of variance (ANOVA) was carried out to determine any significant differences between each treatment and the control; the differences among the treatment's means were grouped using Tukey's test with a 0.05 probability level. Statistical analysis system (SAS, version 9.4, USA) software was used to conduct the analysis.

## RESULTS

### Identification of Phytotoxic Components in *C. rutidosperma*

The methanol extract of *C. rutidosperma* was analyzed and profiled by LC-MS analysis in positive and negative ionization modes to characterize chemical constituents qualitatively. To our knowledge, this is the first validated method for detecting active compounds in the whole plant of *C. rutidosperma* using LC-MS analysis. The results obtained from the LC-MS analysis allowed 64 and 10 proposed known compounds to use positive and negative ionization modes between 1 and 20 min, respectively (Table 1). There are six different phenolic compounds (anthranilic acid, quercitrin, irisolidone 7-O-glucuronide, 1,6-hexanediol dimethacrylate, auraptene, and ferujol), three alkaloids (indoline, quinoline, and indole-3-acrylic acid), four amino acids (thyroliberin N-ethylamide, hexadecasphinganine, 15-methylhexadecasphinganine, and eicosasphinganine) and some amines, benzofurans, terpenoid, and fatty acids were detected. Trichothecine ($C_{19}H_{24}O_5$), a terpene, was identified and exhibited the $[M+H]^+$ ion at 12.183 min with 332.1618 m/z. The $[M-H]^-$ ion at 448.0614 m/z was proposed at 2.834 retention time for glucobrassicin ($C_{16}H_{20}N_2O_9 S_2$). Quercitrin ($C_{21}H_{20}O_{11}$), a well-known flavonoid, was exhibited at 7.24 min at 449.108 m/z. Our study found that in positive-ion mode, two indole-type alkaloids were tentatively named indoline (RT 2.626 with 119.0739 m/z) and indole-3-acrylic acid (RT 3.674 with 187.0632 m/z). Five amines (hercynine, phytosphingosine, dioctylnitrosamine, laurixamine, and sphinganine) were identified and all exhibited a $[M+H]^+$ ion. Hercynine was identified at 1.321 min, and its fragment ion is 197.1167 m/z.

Table 1
*Chemical composition of a* Cleome rutidosperma *methanol extract as determined by liquid chromatography–mass spectrometry*

| Sl no | RT (min) | Determined compound | Molecular formula | Mass fragment (m/z) | Polarity | Error (ppm) |
|---|---|---|---|---|---|---|
| 1 | 1.321 | Hercynine | $C_9H_{15}N_3 O_2$ | 197.1167 | Positive | -1.3 |
| 2 | 1.44 | Anthranilic acid | $C_7H_7NO_2$ | 137.0473 | Positive | 2.8 |
| 3 | 1.441 | W-5 hydrochloride | $C_{16}H_{23}ClN_2O_2S$ | 342.1168 | Negative | 0.25 |
| 4 | 1.442 | Pyroglutamic acid | $C_5H_7NO_3$ | 129.0422 | Positive | 2.92 |
| 5 | 1.445 | 3-Diazo-1-[(4-methylphenyl) sulfonylamino]-1-methylsulfonylurea | $C_9H_{11}N_5O_5S_2$ | 333.0202 | Negative | -0.16 |
| 6 | 1.448 | Diethadione | $C_8H_{13}NO_3$ | 171.0892 | Positive | 2 |
| 7 | 1.764 | 2-Coumaranone | $C_8H_6O$ | 118.0408 | Positive | 9.01 |
| 8 | 2.626 | Indoline | $C_8H_9N$ | 119.0739 | Positive | -3.65 |
| 9 | 2.834 | Glucobrassicin | $C_{16}H_{20}N_2O_9 S_2$ | 448.0614 | Negative | -0.85 |
| 10 | 2.86 | Quinoline | $C_9H_7N$ | 129.0578 | Positive | 0.1 |
| 11 | 3.674 | Indole-3-acrylic acid | $C_{11}H_9NO_2$ | 187.0632 | Positive | 0.89 |

Table 1 (*continue*)

| Sl no | RT (min) | Determined compound | Molecular formula | Mass fragment (m/z) | Polarity | Error (ppm) |
|---|---|---|---|---|---|---|
| 12 | 3.675 | Benzoylacetonitrile | $C_9H_7NO$ | 145.0528 | Positive | -0.3 |
| 13 | 8.81 | [4-[[N-(4-Acetyloxybutanoyl)-C-[4-(azidomethyl)piperidin-1-yl]carbonimidoyl]amino]-4-oxobutyl] acetate | $C_{19}H_{30}$ $N_6O_6$ | 438.2232 | Positive | -1.08 |
| 14 | 9.005 | 4-[4-(4-Azidophenyl)-6-morpholin-4-yl-1,3,5-triazin-2-yl]-N,N-dimethylpiperazine-1-carboxamide | $C_{20}H_{26}N_{10}O_2$ | 438.2234 | Positive | 1.49 |
| 15 | 9.286 | Quercetin | $C_{15}H_{10}O_7$ | 302.0431 | Positive | -1.38 |
| 16 | 9.288 | Irisolidone 7-O-glucuronide | $C_{23}H_{22}O_{12}$ | 490.1115 | Positive | -0.8 |
| 17 | 9.971 | Biochanin A 7-(6-malonylglucoside) (Isoflavonoids) | $C_{25}H_{24}O_{13}$ | 532.122 | Positive | 0.71 |
| 18 | 10.51 | 2-Amino-7-methyl-3,7-dihydropyrrolo[3,2-d]pyrimidin-4-one;ethane;9-methylpurine-2,6-diamine | $C_{17}H_{28}N_{10}O$ | 388.2442 | Positive | 1.34 |
| 19 | 11.271 | 1,6-Hexanediol dimethacrylate | $C_{14}H_{22}O_4$ | 254.1524 | Positive | -2.18 |
| 20 | 11.659 | Eudesmin | $C_{22}H_{26}O_6$ | 386.173 | Positive | -0.29 |
| 21 | 11.739 | 2-[2-Amino-4-[2-(methylideneamino) ethyl]pyrimidin-5-yl]-9-(cyclopropylmethyl)-6-morpholin-4-ylpurin-8-amine | $C_{20}H_{26}N_{10}O$ | 422.2285 | Positive | 1.51 |
| 22 | 11.856 | Thyroliberin N-ethylamide | $C_{18}H_{26}N_6O_4$ | 390.2012 | Positive | 0.94 |
| 23 | 11.998 | Hexadecasphinganine | $C_{16}H_{35}NO_2$ | 273.2665 | Positive | 1.14 |
| 24 | 12.037 | Phytosphingosine | $C_{18}H_{39}NO_3$ | 317.2931 | Positive | -0.41 |
| 25 | 12.179 | Dihydroxyethyl lauramine oxide | $C_{16}H_{35}NO_3$ | 289.2618 | Positive | -0.45 |
| 26 | 12.183 | Trichothecine | $C_{19}H_{24}O_5$ | 332.1618 | Positive | 1.66 |
| 27 | 12.195 | Dodecyldimethylamine oxide | $C_{14}H_{31}NO$ | 229.2407 | Positive | -0.46 |
| 28 | 12.29 | Kobusone | $C_{14}H_{22}O_2$ | 222.1611 | Negative | 4.16 |
| 29 | 12.315 | Dioctylnitrosamine | $C_{16}H_{34}N_2O$ | 270.2669 | Positive | 0.61 |
| 30 | 12.321 | 15-Methylhexadecasphinganine | $C_{17}H_{37}NO_2$ | 287.2829 | Positive | -1.75 |
| 31 | 12.328 | N(3)-Benzylthymidine | $C_{17}H_{20}N_2O_5$ | 332.1359 | Positive | 4.05 |
| 32 | 12.344 | Dodecylacrylamide | $C_{15}H_{29}NO$ | 239.2249 | Positive | 0.13 |
| 33 | 12.349 | Tetrabutylurea | $C_{17}H_{36}N_2O$ | 284.2832 | Positive | -1.54 |
| 34 | 12.355 | Lauryl aminopropylglycine | $C_{17}H_{36}N_2O_2$ | 300.2783 | Positive | -2.15 |
| 35 | 12.553 | Laurixamine | $C_{15}H_{33}NO$ | 243.2561 | Positive | 0.39 |
| 36 | 12.701 | Aminopregnane | $C_{21}H_{37}N$ | 303.2937 | Positive | -3.62 |
| 37 | 13.067 | s-Triazine, 2-amino-4-(morpholinomethyl)-6-piperidino- | $C_{13}H_{22}N_6O$ | 278.1863 | Positive | -2.81 |

Table 1 (*continue*)

| Sl no | RT (min) | Determined compound | Molecular formula | Mass fragment (m/z) | Polarity | Error (ppm) |
|---|---|---|---|---|---|---|
| 38 | 13.157 | 4-Methyl-6-{[3-(Piperidin-4-Ylmethoxy)phenoxy]methyl} yridine-2-Amine | $C_{19}H_{25}N_3O_2$ | 327.1948 | Negative | -0.37 |
| 39 | 13.213 | Auraptene | $C_{19}H_{22}O_3$ | 298.1558 | Positive | 3.76 |
| 40 | 13.239 | 6-(Cyclopentylamino)-2-[(3-hydroxypropyl)amino]-9-isopropylpurine | $C_{16}H_{26}N_6O$ | 318.2175 | Positive | -2.03 |
| 41 | 13.273 | Sphinganine | $C_{18}H_{39}NO_2$ | 301.2989 | Positive | -2.81 |
| 42 | 13.313 | Calanone | $C_{27}H_{20}O_5$ | 424.1308 | Positive | 0.56 |
| 43 | 13.315 | Ferujol | $C_{19}H_{24}O_4$ | 316.1663 | Positive | 3.72 |
| 44 | 13.337 | Estriol | $C_{18}H_{24}O_3$ | 288.1741 | Positive | -5.47 |
| 45 | 13.35 | Kinetensin 1-3 | $C_{15}H_{30}N_6O_4$ | 358.2327 | Positive | 0.34 |
| 46 | 13.701 | Olomoucine | $C_{15}H_{18}N_6O$ | 298.1551 | Positive | -3.08 |
| 47 | 13.71 | Stearic acid hydrazide | $C_{18}H_{38}N_2O$ | 298.2982 | Positive | 0.71 |
| 48 | 13.733 | Hexadecyl isocyanate | $C_{17}H_{33}NO$ | 267.2561 | Positive | 0.3 |
| 49 | 13.754 | Myristamidopropylamine oxide | $C_{19}H_{40}N_2O_2$ | 328.3103 | Positive | -4.15 |
| 50 | 13.81 | N’,N’-Bis(carbamoyl) ethylenediamine-N,N-diacetic acid | $C_{10}H_{26}N_6O_6$ | 326.1915 | Negative | -0.27 |
| 51 | 13.95 | 4-Dodecylbenzenesulfonic acid | $C_{18}H_{30}O_3S$ | 326.1914 | Negative | 0.61 |
| 52 | 14.045 | Decylcarnitine | $C_{17}H_{35}NO_3$ | 301.2625 | Positive | -2.73 |
| 53 | 14.179 | Piptamine | $C_{23}H_{41}N$ | 331.3244 | Positive | -1.41 |
| 54 | 14.271 | Angoletin | $C_{18}H_{20}O_4$ | 300.1348 | Positive | 4.48 |
| 55 | 15.044 | Rubrenolide | $C_{17}H_{30}O_4$ | 298.2135 | Positive | 0.2 |
| 56 | 15.195 | Dodecanamide | $C_{12}H_{25}NO$ | 199.194 | Positive | -2.15 |
| 57 | 15.403 | Eicosasphinganine | $C_{20}H_{43}NO_2$ | 329.3301 | Positive | -2.16 |
| 58 | 15.642 | 2-Decoxysulfanyl-7H-purine | $C_{15}H_{24}N_4OS$ | 308.1683 | Positive | -4.05 |
| 59 | 15.644 | [1-(2-Aminoethyl)triazol-4-yl]-(4-cyclopentylpiperazin-1-yl) methanone | $C_{14}H_{24}N_6O$ | 292.2017 | Positive | -1.79 |
| 60 | 15.896 | Nitrosostromelin | $C_{15}H_{32}N_2O_5$ | 320.231 | Positive | 0.49 |
| 61 | 16.528 | 10-Oxo-13-hydroxy-11-octadecenoic acid | $C_{18}H_{32}O_4$ | 312.2305 | Positive | -1.32 |
| 62 | 16.825 | Dodecylsuccinic anhydride | $C_{16}H_{28}O_3$ | 268.2043 | Positive | -1.79 |
| 63 | 16.931 | Lauryl sulfate | $C_{12}H_{26}O_4S$ | 266.1551 | Negative | 0.12 |
| 64 | 17.00 | 1-Azido-2-tridecylpyrrole | $C_{17}H_{30}N_4$ | 290.2475 | Positive | -1.52 |
| 65 | 17.125 | Lagochilin | $C_{20}H_{36}O_5$ | 356.2554 | Positive | 2.56 |
| 66 | 17.262 | 3-[2-(Dimethylamino)propyl]-1-({4-[(1H-1,2,4-triazol-1-yl) methyl]phenyl}methyl)urea | $C_{16}H_{24}N_6O$ | 316.2004 | Positive | 2.56 |
| 67 | 19.04 | Acridorex | $C_{24}H_{24}N_2$ | 340.1936 | Positive | 1.14 |
| 68 | 19.224 | 1,2-Dinaphthalen-1-ylhydrazine | $C_{20}H_{16}N_2$ | 284.1299 | Positive | 5.04 |

Table 1 (*continue*)

| Sl no | RT (min) | Determined compound | Molecular formula | Mass fragment (m/z) | Polarity | Error (ppm) |
|---|---|---|---|---|---|---|
| 69 | 19.224 | Cyclododecanone tritylhydrazone | $C_{31}H_{38}N_2$ | 438.3025 | Positive | 2.34 |
| 70 | 19.589 | Methyl dodecylbenzenesulphonate | $C_{19}H_{32}O_3S$ | 340.2072 | Negative | 0.18 |
| 71 | 19.852 | 2,2-Bis(azidomethyl)-3-decoxypropan-1-ol | $C_{15}H_{30}N_6O_2$ | 326.2435 | Positive | -1.33 |
| 72 | 19.935 | Stearyldiethanolamine | $C_{22}H_{47}NO_2$ | 357.3612 | Positive | -1.55 |
| 73 | 20.292 | Hexadecanamide | $C_{16}H_{33}NO$ | 255.263 | Positive | -0.14 |
| 74 | 20.54 | Benzenesulfonic acid, undecyl- | $C_{17}H_{28}O_3S$ | 312.1758 | Negative | 0.5 |

*Note*. RT = Retention time

### The Survival Rate and Initial Growth of Weed Seeds

*Cleome rutidosperma* extract was found to have a notable effect on the survival rate, hypocotyl, and radicle length of the examined weed species (Table 2). The inhibitory magnitude of all species was enhanced by increasing the extract concentration from 2.5

Table 2
*Effect of* Cleome rutidosperma *on seed survival, hypocotyl length and root length of test weeds*

| Test species | Dose (%) | Survival rate (%) | Hypocotyl length (cm) | Root length (cm) |
|---|---|---|---|---|
| Weedy rice | 0 | 100.00a | 5.01a | 2.29a |
| | 2.5 | 32.00b | 1.02b | 0.44b |
| | 5 | 14.00c | 0.77c | 0.25c |
| | 10 | 0.00d | 0.00d | 0.00d |
| *Cyperus iria* | 0 | 100.00a | 1.70a | 1.57a |
| | 2.5 | 31.00b | 0.72b | 0.44b |
| | 5 | 10.00c | 0.45c | 0.27c |
| | 10 | 0.00d | 0.00c | 0.00d |
| *Fimbristylis miliacea* | 0 | 100.00a | 2.07a | 2.50a |
| | 2.5 | 42.00b | 0.88b | 0.88b |
| | 5 | 18.00c | 0.51c | 0.41c |
| | 10 | 1.00d | 0.00d | 0.00d |
| *Leptochloa chinensis* | 0 | 100.00a | 1.80a | 3.65a |
| | 2.5 | 61.00b | 0.86b | 0.87b |
| | 5 | 26.00c | 0.45c | 0.23c |
| | 10 | 0.00d | 0.00d | 0.00c |
| *Echinochloa crus-galli* | 0 | 100.00a | 3.61a | 6.47a |
| | 2.5 | 60.00b | 2.07b | 3.11b |
| | 5 | 32.00c | 1.38c | 2.09c |
| | 10 | 12.00d | 0.82d | 0.65d |

*Note.* Mean values sharing similar letters for each weed species in the column are considered not significant at $p<0.05$

to 10% in a concentration-response bioassay. Weedy rice, *C. iria*, and *L. chinensis* did not survive at 10%. Meanwhile, weed survival was significantly reduced by different extract concentrations of *C. rutidosperma*. The extracts were more effective against *C. iria* and weedy rice than against *F. miliacea*, *L. chinensis*, and *E. crus-galli*.

The hypocotyls of the selected weeds were considerably reduced ($p$<0.05) by *C. rutidosperma* methanol extract. The hypocotyl growth of weedy rice, *F. miliacea*, *C. iria*, *L. chinensis*, and *E. crus-galli* was reduced by 84.57%, 75.44%, 71.71%, 74.76%, and 61.80% when treated with 5% of *C. rutidosperma* extract. No hypocotyl growth was recorded at the highest concentration (10%) for weedy rice, *F. miliacea*, *C. iria*, and *L. chinensis*. All tested species showed a decrease in root elongation by *C. rutidosperma*. The radicle growth inhibition ranged by 80%–100%, 72%–100%, 64%–100%, 76%–100%, and 51%–90% for weedy rice, *F. miliacea* *C. iria*, *L. chinensis*, and *E. crus-galli*, respectively. As a result, weedy rice showed the greatest degree of inhibition among the species examined.

### The Effect of *C. rutidosperma* on the Growth and Physiology of Weeds

Table 3 represents the effect of *C. rutidosperma* methanol extract on the growth parameter of the tested plants. Additionally, a dose-dependent inhibition effect was identified. The efficacy of *C. rutidosperma* methanol extract on weedy rice, *F. miliacea* *C. iria*, *L. chinensis*, and *E. crus-galli* was assessed visually. At the highest concentration (10%), *C. rutidosperma* efficacy was significantly higher in all tested species. There was a statistically significant ($p$<0.05) decrease in photosynthesis, stomatal conductance, and transpiration rate when compared to the untreated (control) condition across all species. A higher dose of *C. rutidosperma* (10%) showed a 49.76% photosynthesis reduction in weedy rice, 70.56% in *C. iria*, 31.82% in *F. miliacea*, 57.95% in *L. chinensis*, and 64.72% in *E. crus-galli*. The methanol extract of *C. rutidosperma* inhibited the stomatal conductance of more than 50% for all tested weeds except weedy rice (43.59%) and *F. miliacea* (27.57%). All the weeds evaluated showed a dose-dependent response to *C. rutidosperma* extract on their transpiration rate, and this effect was statistically significant ($p$<0.05). However, transpiration rate reduction varied among the tested species. At a lower concentration of *C. rutidosperma* (2.5%), *C. iria* had the highest reduction in transpiration rate at 73.27%, followed by *E. crus-galli* at 69.57%, weedy rice at 60.08%, *L. chinensis* at 58.36%, and *F. miliacea* at 36.09%.

Each weed studied had a unique response to the methanol extract of *C. rutidosperma* on its plant height. However, the highest plant height was observed in untreated (control). *Cleome rutidosperma* extract reduced plant height from 7.72% to 31.04% in weedy rice, 15.50% to 44.56% in *C. iria*, 1.52% to 18.35% in *F. miliacea*, 7.16% to 37.36% in *L. chinensis*, and

Table 3
*Effect of* Cleome rutidosperma *on the growth and physiological parameters of weeds*

| Test plants | Dose (%) | Injury scale | Photosynthesis (µmol/m²/s) | Stomatal conductance (mol/m²/s) | Transpiration (mmol/m²/s) | Plant height (cm) | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|---|---|---|---|---|
| Weedy rice | 0 | 1.00d | 47.40a | 0.59a | 15.29a | 77.50a | 31.93a | 1.44a |
| | 2.5 | 2.00c | 40.61b | 0.51b | 12.63b | 71.50b | 27.79b | 1.17b |
| | 5 | 3.25b | 34.60c | 0.45c | 10.66c | 68.00b | 24.70c | 1.00c |
| | 10 | 4.75a | 23.79d | 0.33d | 6.09d | 53.42c | 18.64d | 0.74d |
| *Cyperus iria* | 0 | 1.00c | 41.21a | 0.42a | 12.30a | 61.00a | 40.20a | 2.14a |
| | 2.5 | 2.50b | 33.71b | 0.35b | 9.15b | 51.50b | 31.03b | 1.68ab |
| | 5 | 3.50b | 24.53c | 0.28c | 6.19c | 43.37c | 25.26c | 1.22bc |
| | 10 | 5.50a | 12.13d | 0.18d | 3.29d | 33.75d | 19.67d | 0.76c |
| *Fimbristylis miliacea* | 0 | 1.00c | 36.48a | 0.36a | 10.66a | 72.45a | 36.67a | 0.83a |
| | 2.5 | 1.50bc | 32.56b | 0.33b | 9.16ab | 71.32a | 35.10b | 0.78a |
| | 5 | 2.50b | 28.47c | 0.30c | 8.02bc | 66.82ab | 32.10c | 0.70b |
| | 10 | 3.75a | 24.87d | 0.26d | 6.76c | 59.20b | 28.04d | 0.57c |
| *Leptochloa chinensis* | 0 | 1.00c | 39.16a | 0.45a | 11.70a | 92.00a | 50.63a | 2.08a |
| | 2.5 | 2.75b | 33.66b | 0.39b | 9.94b | 85.42a | 45.27b | 1.79b |
| | 5 | 3.50b | 24.80c | 0.31c | 7.11c | 74.19b | 39.58c | 1.50c |
| | 10 | 5.50a | 16.46d | 0.21d | 4.85d | 57.58c | 29.83d | 0.98d |
| *Echinochloa crus-galli* | 0 | 1.00d | 43.82a | 0.51a | 13.20a | 39.03a | 30.04a | 0.85a |
| | 2.5 | 3.50c | 33.22b | 0.41b | 9.36b | 33.78ab | 25.46b | 0.67b |
| | 5 | 5.00b | 22.47c | 0.27c | 6.11c | 28.65b | 19.20c | 0.51c |
| | 10 | 6.50a | 15.46d | 0.20d | 4.02d | 22.47c | 14.05d | 0.30d |

*Note.* Mean values sharing similar letters for each weed species in the column are considered not significant at $p<0.05$

13.42% to 42.37% in *E. crus-galli* compared to untreated (control). The dry weight of the examined weeds was also decreased with an increase in *C. rutidosperma* concentration.

The methanol extract of *C. rutidosperma* resulted in a significant ($p<0.05$) reduction in the fresh and dry weights of all the evaluated species. Fresh weight loss was most pronounced in *C. iria* (37.17%) after being treated with a 5% solution of a *C. rutidosperma* extract, followed by *E. crus-galli* (36.01%), weedy rice (22.61%), *L. chinensis* (21.83%), and *F. miliacea* (12.46%). The foliar application of *C. rutidosperma* at the higher dose (10%) reduced the dry weight by 48.40% in weedy rice, 64.13% in *C. iria*, 30.89% in *F. miliacea*, 52.76% in *L. chinensis*, and 64.20% in *E. crus-galli*.

## Weed Control Efficacy of *C. rutidosperma*

The efficiency of weed control was considerably ($p<0.05$) impacted by *C. rutidosperma* methanol extract (Figure 1). However, the control efficacy was

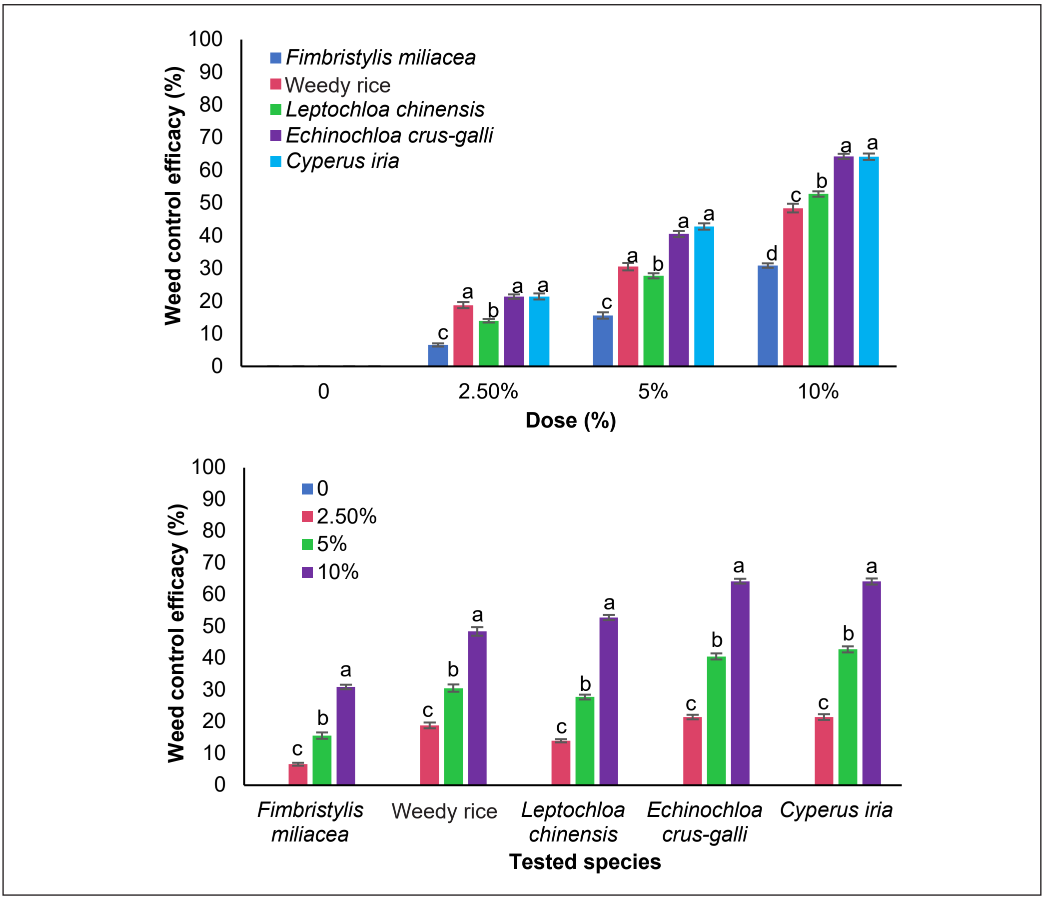


*Figure 1*. Weed control efficacy of *Cleome rutidosperma*. Mean values sharing similar letters are considered not significant at $p<0.05$

measured at 21 DAS and varied among the *C. rutidosperma* application rates compared to the control (untreated). The efficacy of *C. rutidosperma* was highest in *C. iria*, ranging from 21.41% to 64.13%, while *F. miliacea* showed the lowest inhibition, ranging from 6.59% to 30.89% compared to untreated (control). The highest application rate of *C. rutidosperma* (10%) showed the highest weed control efficacy for *E. crus-galli*, 64.20%, followed by 64.13%, 52.76%, 48.40%, and 30.89% for *C. iria*, *L. chinensis*, weedy rice, and *F. miliacea*, respectively. Overall, 5% and 10% application rates exhibited excellent efficacy compared to 2.5% for all tested weed species.

## DISCUSSION

Weed management using agrochemicals in agricultural systems has increased dramatically in recent years. The increased public interest in safe "green" herbicides has resulted in the development of several new bioherbicides for weed management. For example, *C. rutidosperma*, a plant-based bioherbicide, showed a promising weed control efficacy.

Our study detected important fatty acids, indole, amines, amino acids, flavonoids, terpenes, coumarins, carboxylic acids, benzoic acids, benzofuran, and several unknown compounds. Quercetin is a flavonoid of *C. rutidosperma,* which inhibits the shoot growth of *Arabidopsis thaliana* (Weston et al., 2013). Several modes of action of exogenously applied flavonoids on plants have been demonstrated by scientific research. Changes in membrane permeability and inhibition of plant nutrient absorption, suppression of cell division, elongation, and submicroscopic structure, effects on photosynthesis and respiration of the plant, impact on photosynthesis and respiration, enzymatic functions and activities, hormone and protein synthesis, and ATP generation (Shah & Smith, 2020).

Quinoline, indoline, and indole-3-acrylic acid are identified alkaloids of *C. rutidosperma*. Quinoline inhibited the growth of aquatic duckweed and reduced cell division in nion (Shang et al., 2018). Alkaloids caused strong inhibition of coleoptile development and full suppression of protein synthesis, exhibiting antimitotic activity (Hu et al., 2015). Five amines (hercynine, phytosphingosine, dioctylnitrosamine, laurixamine and sphinganine) were detected from *C. rutidosperma*. Phytosphingosine (amines), also found in wheat root exudates, inhibited *Fusarium oxysporum* f. sp. *niveum* (Fusarium wilt of watermelon) (C. Li et al., 2020). Like many other terpenes, trichothecine has allelopathic effects on the seed germination of *A. thaliana* (Malmierca et al., 2015). Terpenes inhibited weed germination and respiratory metabolism. Their strong phytotoxic effects suggest they could be used as a main basis for developing bioherbicides (Z. Li et al., 2019).

Auraptene, a coumarin, was identified in *C. rutidosperma,* which displayed allelopathic effects and stunted seed germination, shoot, and root growth of lettuce (Razavi, 2010). Coumarin decreased gibberellic acid 3 in the hormone system, reducing amylase activity and starch consumption during germination. In addition, coumarin caused oxidative stress by reducing catalase activity, which manifested as an increase in the production of reactive oxygen species such as hydrogen peroxide and malondialdehyde (Yang et al., 2023).

Allelochemicals are not normally released into the environment as a single substance, and the amount of allelochemicals that get released varies depending on the situation. When studying their allelopathic potential, it is important to consider the variety and quantity of allelochemicals produced by plants. Although some allelochemicals may not exhibit allelopathic activity when used alone, they may enhance the allelopathy of other allelochemicals in specific conditions (Cheng & Cheng, 2015). Synergy, antagonism, and additive effects are only some of the interactions between different allelochemicals that need to be explored. The synergistic effects of multiple polyphenol allelochemicals against *Microcystis aeruginosa* were stronger than those of a single polyphenol therapy, as Huang et al. (2020) reported.

*Cleome rutidosperma* methanol extract has modest efficiency against weeds by producing severe damage. Injury symptoms such as chlorosis, stunted development, and burn-down, all of which eventually led to death, were visible. In addition, *C. rutidosperma*, at a higher rate, exhibited mild to moderate damage symptoms. The effectiveness of *C. rutidosperma* increased as the rate of application increased. Similarly, increasing the extract concentration of *Parthenium hysterophorus L.*, *Borreria alata* (Aubl.) DC., and *Cleome rutidosperma* DC. showed excellent efficacy on *Ageratum conyzoides* and *Euphorbia hirta* L. (Motmainna et al., 2021c).

At 10% growth reduction of inhibition of *E. crus-galli*, *C. iria*, *L. chinensis*, weedy rice, and *F. miliacea* was measured at 64.20%, 64.13%, 52.76%, 48.40%, and 30.89%, respectively. Growth reduction occurred due to *C. rutidosperma* methanol extract stress. It is a result of damage to the leaves, specifically necrosis, leaf fire, and wrinkled leaves, all of which inhibit photosynthesis and hinder plant development. The present study agrees with Hasan, Mokhtar, et al. (2021) that foliar application of wild tomato plant extract bioherbicide WeedLock at high concentration hindered the morphological characters of *Euphorbia hirta* L., *A. conyzoides*, *Axonopus compressus* (Sw.) P. Beauv, *F. miliacea* (L.) Vahl, *Eleusine indica* (L.) Gaertn., *C. iria*, *Abelmoschus esculentus* (L.) Moench, *Zea mays* L., *Amaranthus gangeticus* L., and *O. sativa* L.

In our study, the highest reduction in photosynthesis rate was observed when *C. rutidosperma* was applied to *C. iria*. Oxidative stress increased intracellular reactive oxygen species (ROS) production, damaged macromolecules, and reduced plant defense levels, all resulting from a decline in photosynthesis (Hasan et al., 2022; Motmainna, Juraimi, Uddin, Asib, Islam, Ahmad-Hamdani, Berahim, et al., 2021). Photosynthesis is the principal cause of oxidative stress, which considerably impacts plant growth under stressful environmental conditions. Applying *C. rutidosperma* extract reduced the stomatal conductance in the weeds significantly. The stomatal mechanism is a crucial property of plants that minimizes water loss, affecting gas exchanges. Our research demonstrates that methanol extract of *C. rutidosperma* significantly impacted the transpiration rate of tested weeds. Reduced photosynthesis and transpiration rate, regulated by stomatal conductance, are an undeniable result of stress. As the stomata of a plant open, water is lost by evaporation via transpiration, and $CO_2$ is absorbed through photosynthesis (Motmainna, Juraimi, Uddin, Asib, Islam, Ahmad-Hamdani, Berahim, et al., 2021). The result demonstrated in our study was similar to Hasan et al. (2022), who found that WeedLock (a plant-based bioherbicide) inhibited the photosynthetic mechanism in *E. indica* (L.) Gaertn, *A. conyzoides* L., *A. gangeticus* L, and *Z. mays* L.

## CONCLUSION

The current research confirms the herbicidal potential of *C. rutidosperma* extract and shows that it can prevent the germination and growth of test weeds. The *C. rutidosperma* extract was also found to have 74 compounds. Some of these compounds are toxic in different studies. Because of its high potency and selectivity, this weed might be classified as a natural weed control product. This study will promote research toward sustainable weed management programs, especially in the fields of rice and plantation, that could reduce weed infestation and competition over time and less dependence on chemical herbicides.

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