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Allelopathic effects of *Wedelia trilobata (L.) Hitchc* extracts on weed suppression and rice seedling growth

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ABSTRACT

This research evaluates the phytotoxic potential of methanolic extracts derived from Wedelia trilobata (L.) Hitchc. against major rice weeds and rice cultivars under controlled laboratory conditions. Six plant species were tested: three dominant weed species (Leptochloa chinensis, Echinochloa crus-galli, Fimbristylis miliacea), two rice varieties (Oryza sativa L.) OM380, OM5451), and green mustard (Brassica juncea) as an indicator species. The results indicated that root growth of F. miliacea and L. chinensis was completely inhibited (100%) at concentrations between 0.12 - 0.24 g/mL, while shoot elongation was reduced by over 85%. E. crus-galli exhibited only mild inhibition or slight stimulation at lower concentrations. Both rice cultivars showed dose-dependent sensitivity, with OM380 being more responsive to lower concentrations (0.03 g/mL). Phytochemical screening confirmed the presence of high levels of phenolic and flavonoid compounds, particularly in whole plant and leaf extracts (TPC: 158.11 mg GAE/g; TFC: 260.57 mg QE/g). These allelochemicals may interfere with germination and root development of target weeds, suggesting their potential as bioherbicidal agents. The findings highlight the allelopathic potential of W. trilobata for selective weed suppression in rice agroecosystems. Further studies should focus on isolating key bioactive compounds and assessing their efficacy under greenhouse and field conditions to support the development of sustainable weed control strategies in rice production.

1. INTRODUCTION

Weeds are one of the important pests affecting rice plants; they compete for nutrients, water, light, and shelter for insects and diseases. This competition can significantly reduce crop yields and overall plant health. Effective weed management strategies are essential for ensuring a successful rice harvest and maintaining ecosystem balance. Major weed species like red sprangletop - RS (*Leptochloa chinensis* (L.) Nees), barnyard grass - BG (*Echinochloa crus-galli* (L.) Beauv.), and grasslike fimbry - GF (*Fimbristylis miliacea* (L.) Vahl) are major threats to crop productivity and agroecosystem stability in the Mekong Delta's rice-growing regions. Farmers are urged to implement integrated weed management (IWM) strategies, including mechanical, chemical, and cultural control methods, to successfully suppress these hardy weeds. Crop rotation and the use of cover crops are two important cultural techniques that are essential for upsetting weed life cycles and improving soil health. These methods can

significantly reduce weed burden while promoting a more environmentally friendly and sustainable rice production system when paired with timely mechanical interventions and prudent herbicide application (Suk et al., 2022; Chin, 2023). One of the key solutions for effectively managing weeds sustainably, ensuring crop productivity and safety, and maintaining ecosystem balance is the use of plant-based products. This is a promising solution that helps reduce the reliance on chemical herbicides in weed control. In particular, herbicides of natural origin are of interest due to their low toxicity to humans and environmental friendliness, which lead to less resistance and fewer pesticide residues in agricultural products. Many previous studies have demonstrated that plants contain high levels of secondary metabolites such as polyphenols, flavonoids, quinones, and tannins, which are non-cytotoxic and have the ability to biodegrade (Chen et al., 2017; Dehghanian et al., 2022; Rogowska-van der Molen et al., 2023).

Yellow creeping daisy (YCD) has the scientific name *Wedelia trilobata* (L.) Hitchc., belonging to the Asteraceae family, and also has another scientific synonym, *Sphagneticola trilobata* (L.) Pruski. This plant species is widely distributed worldwide (Carolina et al., 2021). In Vietnam, *Wedelia* spp. grows wild everywhere, on all terrains, often referred to as ornamental palm, three - lobed stone flower (Suk et al., 2022). There are many studies on *Wedelia* spp. that demonstrate significant potential as weed inhibitors. According to Nam (2021), a survey of the plant inhibition potential from methanol (MeOH) extracts in *Wedelia chinensis* shows that at a concentration of 1.0 g/mL, *W. chinensis* can inhibit the stems and roots of green mustard (GM) (*B. juncea*) (76.01%; 90.24%), junglerice - JR (*Echinochloa colona*) (81.64%; 100%), and barnyardgrass (BG) (74.43%; 97.15%). Additionally, the ethanol extract from the stems, leaves, and flowers at a concentration of 5.0 mg/mL showed the highest germination inhibition effect on BG seeds, lettuce seeds (*Lactuca sativa* L.), and radish seeds (*Raphanus sativus* L.) (Men et al., 2019). Previous studies on *W. trilobata* have shown strong antioxidant activity and phytotoxicity, as it contains many biologically active compounds, including phenolic, flavonoid, terpenoid, coumarin, saponin, and tannin compounds. (Chethan et al., 2012; Antony & George, 2017; Carolina et al., 2021; Men et al., 2022). However, hardly any studies have evaluated the phytotoxicity of *W. trilobata* against major weed species in rice fields under Vietnamese conditions.

This study initially evaluates the weed suppression ability of *W. trilobata* extract on the studied plant species and determines the content of antagonistic compound groups in the *W. trilobata* extract, laying the groundwork for in-depth research aimed at developing solutions for applying *W. trilobata* in rice field weed management in a biological, safe, and environmentally friendly manner.

2. MATERIALS AND METHOD

Experimental material: Roots, stems, leaves, flowers, and the whole plant sample of YCD were collected at the flowering stage at Can Tho University (10°01'49.4"N 105°46'04.4"E). YCD (The whole plant, roots, stems, leaves, and flowers) was collected, processed, and sorted by each part. The samples were dried at 50°C for 60 hours in a UF260 drying oven (Memmert, Germany) until the weight was constant (Moisture content reached 14-15%) and then ground into powder using a dry sample grinder M8.RT04 (Scilab - Korea). Trang Nong Trading Co., Ltd. (Vinh Loc B, Binh Chanh, Ho Chi Minh City) provided the green mustard (GM) seeds (*Brassica juncea* L.). The rice varieties OM5451 and OM380 were supplied by the Mekong Delta Rice Institute (Tan Thanh, Thoi Lai, Can Tho). Grass seeds (BG, RS, GF) were collected from field plots in Can Tho City during the summer-autumn season (February, 2024). After collecting the grass seeds from the fields, each seed was sorted, with empty seeds removed, and the seeds were naturally dried until the moisture content reached 14-15%. The grass seeds used in the experiment were soaked according to the physiology of each type without the need for dormancy treatment.

Chemicals and laboratory equipment: Methanol (MeOH), distilled water, Folin-Ciocalteu (F-C) (Merck), gallic acid (Sigma), quercetin (Sigma), $FeCl_3$, $Pb(CH_3COO)_2$, $AlCl_3$, Na_2CO_3, $NaNO_2$, NaOH, etc.

2.1. Extraction method

The sample of the whole plant (The roots, stems, leaves, and flowers) is processed and sorted by each part after collection, then dried at a temperature of 50°C for 60 hours using a UF260 drying oven (Memmert, Germany) until the weight is constant and ground into powder using a dry sample grinder M8.RT04 (Scilab, Korea). The extraction technique is performed using the soaking method described by Thi and Kato-Noguchi (2008), with modifications.

Place each sample (100 g) in a 1.0 liter container with MeOH (99%) for 30 minutes, then continue with ultrasonic treatment at 40°C for 45 minutes. The extract is filtered, and the solvent is removed using a rotary evaporator (Yamato, Japan). Add MeOH (50%) to the residue, and repeat the above step until no traces are visible on the glass slide from the extracted droplets.

2.2. Method for extracting allelochemicals present in the methanol extract solution

Fresh samples are cleaned, chopped into small pieces (1 - 1.5 cm), and weighed at 100 g each, then placed into an erlenmeyer containing 1.0 liter of the solvent system MeOH:H_2O (3:2, v/v). After 48 hours (Stirring regularly with a glass rod), the mixture is filtered through a ceramic funnel (Diameter = 100 mm, GenLab), lined with Whatman paper (Filter pore size 20-25 µm, diameter = 90 mm), using vacuum suction to obtain the first extract. The remaining residue is then soaked with 500 mL of MeOH (100%) for another 48 hours, after which the total extract is concentrated using a rotary evaporator (Yamato RE301A-W, Japan) to remove the solvent and obtain the total MeOH extract and its components (Thi & Kato-Noguchi, 2008).

2.3. Preliminary qualitative analysis of the chemical composition in the YCD samples

The antagonistic substances belonging to the two groups of phenolics and flavonoids are qualitatively analyzed in each type of extract: roots, stems, leaves, and flowers, as described by Tiwari et al. (2011) in Table 1.

Table 1. Preliminary qualitative reactions of functional groups in each sample of the YCD

Compound	Reaction process	Positive phenomenon
Test for phenolics	1.0 mL of the extract was added with a few drops of $FeCl_3$ solution.	Dark blue
Test for flavonoids	The extract was added with 1.0 mL $Pb(CH_3COO)_2$ 10%	Yellow precipitate

Phytochemical analysis was conducted to identify the presence of phytochemicals in MeOH extract.

2.4. Determining the total phenolics and flavonoids content

The method for determining the total phenolics content (TPC) and total flavonoids (TFC) in the YCD extract sample was carried out according to the description by Marinova et al. (2005) with modifications. The absorbance (Abs) was measured using a UV-Vis spectrophotometer (Thermo Scientific Multiskan SkyHigh, Singapore). The total phenolics content (TPC) in the YCD extract was determined using the Folin-Ciocalteu (F-C) reagent. Precisely 1.0 mL of the sample solution or standard was placed in a 10 mL volumetric flask, followed by the addition of 5.0 mL of 10% F-C, mixed well, and allowed to stand for 5 minutes. Then, 4.0 mL of 10% Na_2CO_3 solution was added, mixed thoroughly, and incubated in the dark for 40 minutes at 40°C (^{0}LAUDA water bath). The Abs of the standard solution and test sample was measured after the reaction at λ = 765 nm. The TPC was determined based on the standard curve equation of gallic acid at the concentration range of 0, 2, 4, 6, 8, 10, 12, 16, 20 µg/mL: $y = 0.0675x + 0.0005$; ($R^2 = 0.9935$). The experiments were repeated three times. The standard curve equation of gallic acid is used to calculate the total phenolics content using the formula $F = \frac{C \times V}{m}$; the results are expressed as milligrams of gallic acid equivalents per gram of extract sample (mg GAE/g dry weight).

The Folin-Ciocalteu reagent is a reference method for quantifying total phenolics. The oxidizing agent is a mixture of complex compounds, phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). During the reduction of easily oxidizable phenolic hydroxyl groups, this oxidizing agent is reduced to tungsten and molybdenum oxides, producing a blue color with a maximum absorbance at a wavelength of 765 nm, and the intensity of the color will be proportional to the phenolic content in the sample (Rosbash, 1949; Singleton & Rossi, 1965; Ainsworth & Gillespie, 2007; Magalhães et al., 2008).

The total flavonoids content (TFC) is determined using the $AlCl_3$ colorimetric reagent in an alkaline medium. Use a micropipette to draw 1.0 mL of the standard solution or sample into a 10 mL volumetric flask, add 4.0 mL of distilled water, then add 0.3 mL of $NaNO_2$. After 5 minutes, add 0.3 mL of 10% $AlCl_3$, and after 6 minutes, add 2.0 mL of 1.0 M NaOH. Finally, add water to the mark and mix the solution thoroughly. Measure the absorbance of the standard and test samples at 510 nm (Pękal & Pyrzynska, 2014). The TFC is determined based on

the standard curve equation of quercetin at the concentration range of 0, 20, 40, 60, 80, 100 µg/mL: y = 0.0057x - 0.0123; (R^2 = 0.9981). The experiment is repeated 3 times. The standard curve equation of quercetin is used to calculate the total flavonoid content using the formula: $F = \frac{C \times V}{m}$; where F is the total flavonoid content (mg QE/g dry weight), C is the x value from the standard curve with quercetin (µg/mL), V is the volume of the extract (mL), and m is the dry weight contained in volume V (g).

2.5. Phytotoxicity assessment with YCD extract on test plants

The germination inhibition assay was conducted under controlled conditions using a completely randomized design (CRD) with six treatments, each representing a different concentration of YCD extract: 0.03, 0.06, 0.12, 0.24, and 0.48 g/mL. Use a micropipette to draw 1.0 mL of the extract at the corresponding concentrations into Petri dishes (Ø = 50 mm) lined with filter paper. Place the treatments in a fume hood (LFS-180S, Yamato Scientific Co., Ltd., Japan) at 25±1°C until the filter paper is completely dry (approximately 90-120 minutes). Use 1.0 mL of Tween 20 (0.05%) in each Petri dish; Tween 20 is a nonionic surfactant that reduces the surface tension of the solvent system and facilitates the dispersion of insoluble substances (Weiszhár et al., 2012). The experiment is arranged in a completely randomized design, with 3 repetitions and 6 treatments (5 concentrations and a control containing only Tween 20), with each Petri dish containing 10 seeds (GM, BG, RS, GF, rice OM5451, and OM380). The Petri dishes are covered and sealed with paraffin, and placed in a dark cabinet at 25±1°C. After 48 hours, the lengths of the shoots and roots of the test plants are measured (Thi & Kato-Noguchi, 2008). The effect of the MeOH extract on the test plants is calculated using Abbott's formula (1925): I(%) = [(L_0 - L_1)/L_0] x 100, where I is the inhibition rate (%), L_0 is the length of the shoot/root of the control plant, and L_1 is the length of the shoot/root of the treated plant.

2.6. Data analysis

Primary data were calculated using Microsoft Excel (Version 2502). Statistics were processed using SPSS software (Version 25), and data were visualized using R software (Version 4.4.3), employing Duncan's test to compare differences at a significance level of 5%. The data calculating the percentage of inhibition, ranging from 0 to 100%, were transformed to Arcsin$\sqrt{x}$ (Gomez & Gomez, 1984).

3. RESULTS AND DISCUSSION

3.1. The ability of the MeOH extract from YCD to inhibit the seedling stem length of the tested plant species

The results of the survey on the plant inhibition ability of the MeOH extract from YCD on the stem length of GM, as shown in Figure 1, indicate that the extract inhibited 3.81% of the GM stem at the lowest concentration of 0.03 (g/mL), inhibited over 50% at a concentration of 0.24 g/mL (55.13%), and at the highest concentration of 0.48 (g/mL) inhibited 69.79%, with a statistically significant difference at 5% (Figure 4). GM is considered an indicator plant because it shows sensitivity to antagonistic plant effects and antagonistic substances (Kaur & Kaushik, 2005; Asghari & Tewari, 2007).

For GF, the stem showed an inhibition of 14.95%, which is higher than that of the GM stem at the low concentration (0.03 g/mL). As the concentration of the extract increased, the length of GF tended to be inhibited by up to 86.37% (0.48 g/mL) (Figure 1, Figure 4).

The extract of YCD tends to inhibit the stem growth of RS even at a low concentration of 0.03 g/mL (23.77%), and as the concentration increases, the inhibition becomes more pronounced; unlike GM and GF, at a concentration of 0.12 g/mL, the stem length of RS is inhibited by over 50% and reaches 96.33% at the maximum concentration (0.48 g/mL), with a statistically significant difference at the 5% level (Figures 1 and 4).

The extract of YCD does not cause significant inhibition on the stem length of BG; at low concentrations from 0.03 to 0.12 g/mL, it stimulates the growth of the BG stem from (-25.55%) to (-9.49%). However, at a concentration of 0.24 g/mL, the phenomenon of stem inhibition begins to occur, but it is not significant (10.36%), and when increased to the maximum concentration (0.48 g/mL), the stem is only inhibited by 25.69%, showing the least inhibition compared to the other five studied plant species.

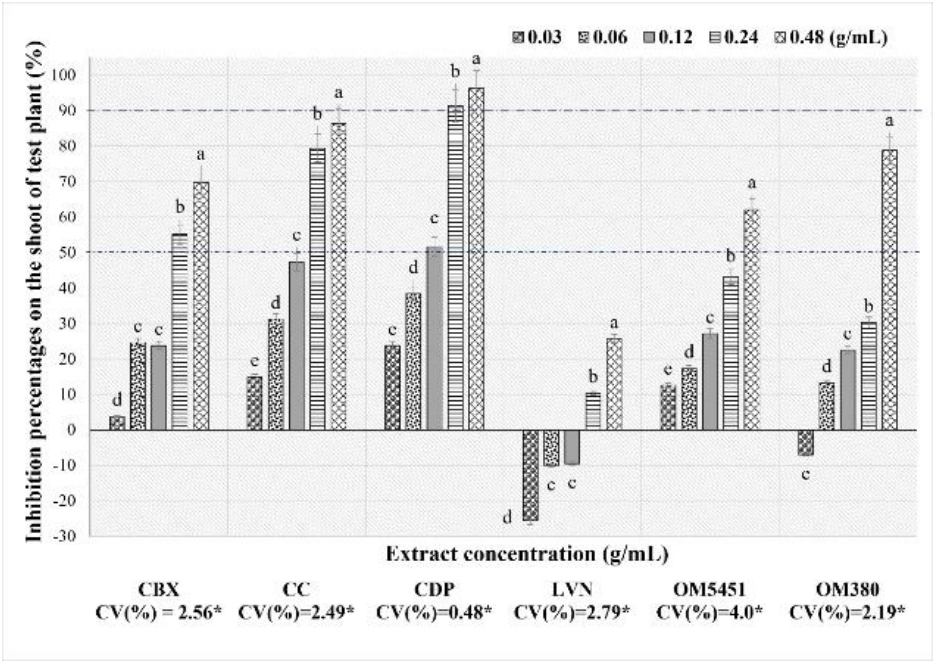

Figure 1. The inhibitory ability (%) of the extract from YCD (*W. trilobata*) at five concentrations on the shoot length of six tested plant species after 48 hours

Note: Bars with the same letter are not significantly different (Duncan test, p<0.05). Negative values in the chart indicate stimulation. CBX: green mustard; CC: grasslike fimbry; CDP: red sprangletop; LVN: barnyard grass.

The results in Figure 1 show that after 48 hours of treatment with the YCD extract, the rice variety OM5451 was almost completely inhibited across all concentrations, with the lowest concentration of 0.03 g/mL achieving an inhibition rate of 12.59%. In contrast, the rice variety OM380 was slightly stimulated (-6.95%) at the same concentration. As the concentration of the extract increased, the stem length of the rice varieties OM5451 and OM380 was progressively inhibited, with the highest concentration (0.48 g/mL) resulting in maximum inhibitions of 78.78% for OM380 and 61.98% for OM5451.

3.2. The inhibitory effect of MeOH extract from YCD on the root length of the seedlings of the tested plant species

The results in Figures 2 and 4 show that the extract from YCD strongly inhibits GF roots, with the lowest concentration inhibiting over 50% of root length, reaching 100% inhibition at just 0.12 g/mL. At a low concentration (0.03 g/mL), the extract slightly stimulates the root length of BG grass and OM380 rice; however, as the concentration increases, the roots of both BG and OM380 rice are also inhibited. For BG roots, the extract does not strongly inhibit at higher concentrations, with a maximum inhibition of only 88.38%, lower than that observed for other plant species at the same concentration. Although at a concentration of 0.12 g/mL, the YCD extract has inhibited over 50% of GM, RS, OM380, and OM5451.

The effect of the MeOH extract from YCD on shoot and root growth of six tested species is presented in Figures 3 and 4. At the lowest concentration (0.03 g/mL), BG grass and OM380 rice showed slight stimulation in shoots and roots (-7% to -25%), whereas other species already exhibited inhibition. GF and RS were the most sensitive: root inhibition exceeded 50% at 0.06 g/mL, reaching 100% at 0.12-0.24 g/mL, while shoot inhibition reached over 90% at 0.48 g/mL. GM and OM5451 also showed clear dose-dependent inhibition, with root suppression above 50% at 0.12 g/mL and maximum shoot inhibition around 62-79% at 0.48 g/mL. By contrast, BG was relatively tolerant, with root inhibition peaking at 88% but shoot inhibition remaining below 26% even at the highest concentration. These results indicate that YCD extract exerts strong inhibitory effects on GF and RS, moderate effects on GM and OM5451, and only limited effects on BG, while low doses may even promote growth in BG and OM380 rice.

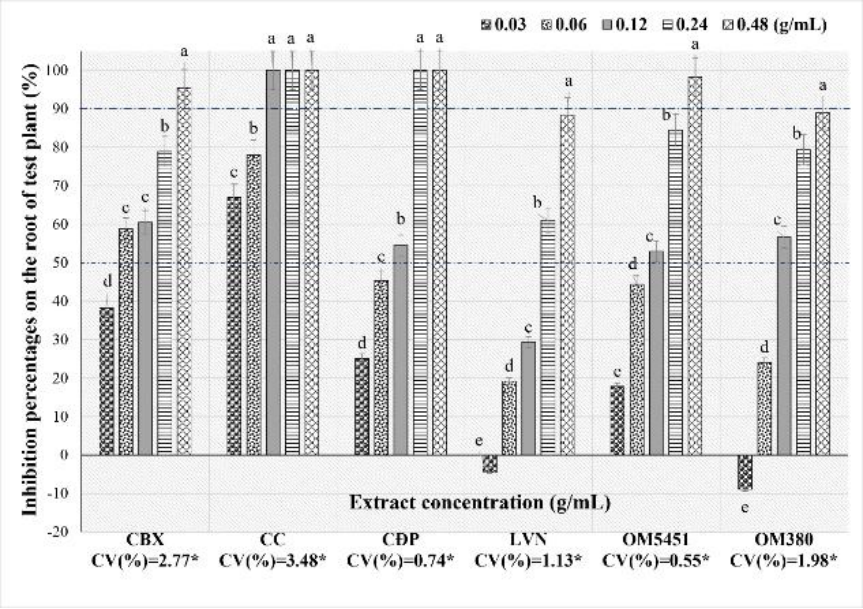

Figure 2. The inhibitory ability (%) of the extract from YCD (*W. trilobata*) at five concentrations on the root length of six tested plant species after 48 hours

Note: Bars with the same letter are not significantly different (Duncan test, p<0.05). Negative values in the chart indicate stimulation. CBX: green mustard; CC: grasslike fimbry; CĐP: red sprangletop; LVN: barnyard grass.

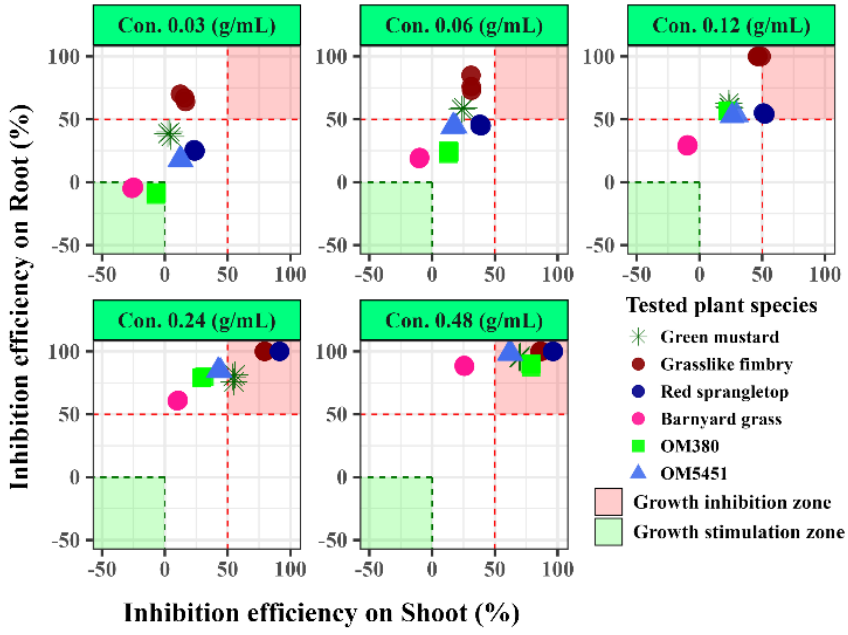

Figure 3. The trend of the effect from YCD extract (*W. trilobata*) on the length of the seedling shoot and root of six tested plant species under laboratory conditions after 48 hours

Note: Each panel represents a different extract concentration (0.03-0.48 g/mL). Values in the upper right quadrant indicate strong inhibition of both shoot and root, whereas values in the lower left quadrant indicate growth stimulation. Species codes: GM - green mustard (Brassica juncea), RS - red sprangletop (Leptochloa chinensis), GF - grasslike fimbry (Fimbristylis miliacea), BG - barnyardgrass (Echinochloa crus-galli), OM5451 and OM380 - rice cultivars

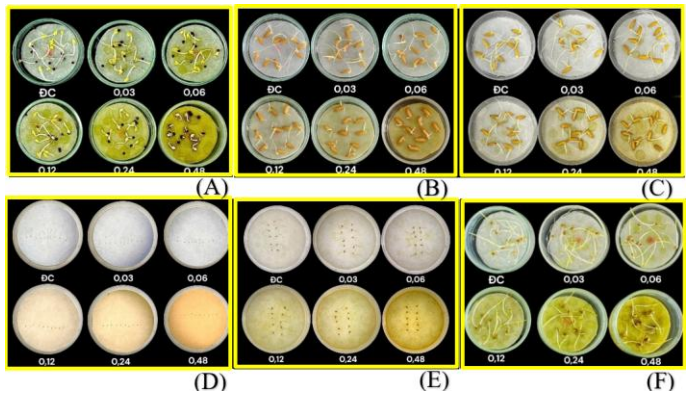

Figure 4. The effect of the extract from YCD (*W. trilobata*) on the shoots and roots of six tested plant species at concentrations (Control; 0.03; 0.06; 0.12; 0.24; 0.48 g/mL)

*Note: (A): **GM**; (B): **OM5451**; (C): **OM380**; (D): **GF**; (E): **RS**; (F): **BG***

3.3. Qualitative results of phenolic and flavonoid compounds in the YCD

The qualitative results of phenolic and flavonoid compounds in the total extract sample of MeOH YCD are presented in Table 2, indicating that the analyzed sample contains phenolic and flavonoid groups (Figure 5). This result is consistent with the study by Mardina (2021), which examined the composition of compound groups in the YCD extract when using MeOH and water as solvents, both of which contained flavonoids and phenolics. According to Nam (2021), *W. chinensis* also contains both of these groups of compounds.

Table 2. The qualitative results of phenolics and flavonoids in the methanol extract from the total sample of YCD

Compound	Phenolics	Flavonoids
YCD	+	+

"+": present; "-": absent

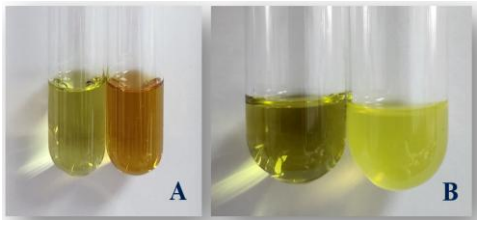

Figure 5. Phytochemical screening (A): Phenolics; (B): Flavonoids

3.4. Quantitative results of total phenolic and flavonoid content in the YCD (Total sample, roots, stems, leaves, flowers)

The TPC and TFC content in the extract samples (Total, roots, stems, leaves, flowers) shown in Table 3 indicates that TPC ranges from 28.85 to 158.11 mg GAE/g of extract, with the roots containing the least (28.85 mg GAE/g) and the total sample having the highest TPC content (158.11 mg GAE/g). The TPC content increases in the order of stem < flower < leaf, corresponding to 61.37 < 104.68 < 111.87 GAE/g of extract, respectively. Phenolics play a key role in pre-emergent herbicides by preventing weed growth from the seed stage, inhibiting germination, altering the permeability of seed cell membranes, and causing ion and water imbalances, which prevent seeds from germinating normally (Soltys et al., 2013). Phenolics can reduce photosynthesis and nutrient synthesis, weakening the weed plants (Kumar et al., 2024). The experimental results suggest that the MeOH extract of the total sample can be used to extract high TPC content from YCD.

The TFC content ranges from 42.63 to 260.57 mg QE/g of extract, with variation among parts. The stem sample contains the least flavonoids (42.63 mg QE/g), while the total sample and leaf sample have very high TFC values of 260.57 mg QE/g and 201.50 mg QE/g, respectively, followed by the root sample (74.41 mg QE/g) and the flower sample (113.59 mg QE/g). Therefore, either the total sample or the leaf sample can be used as a rich source of flavonoids for extraction (Table 3). According to the study by Singhal et al. (2021), the TFC of the MeOH extract from the leaf part was higher than that of the other parts. The differences in secondary metabolite composition may be due to extraction conditions, geographical variations, soil types, extraction methods, solvents, etc., which can lead to variations in TPC and TFC.

The study by Rehman et al. (2017) on the content of polyphenols and total flavonoids in the flowers and

leaves of the Indian crab claw plant, and another study by Liu et al. and Wang (2018), presented contradictory findings. Rehman et al. (2017) indicated that the total polyphenol content in the flowers is higher than in the leaves, but the total flavonoid content in the leaves is higher than in the flowers. In contrast, Liu and Wang (2018) demonstrated that the leaves of the crab claw plant have higher levels of polyphenols and flavonoids than the flowers. This further shows that changes in soil location, geography, etc, can affect the content of various compound groups in the plant (Zhang et al., 2018). Flavonoids and phenolics are two groups of compounds that can inhibit plant growth through various mechanisms. Disruption of cell membranes: Some flavonoids can damage plant cell membranes by altering membrane permeability, leading to ion and water imbalances and causing cell death. Inhibition of auxin transport: Flavonoids can affect the auxin transport system, an important hormone in plant development, causing stunted growth or death in plants (Wu et al., 2023). Inhibition of enzymes: Flavonoids and phenolics can impact key enzymes in the growth process of plants, such as peroxidase and catalase, reducing the antioxidant capacity of plants and causing oxidative stress, affecting enzymes related to protein and DNA synthesis, and disrupting the development of seedlings (Xiong et al., 2024; Patil et al., 2024). Disrupting metabolic processes: These compounds can affect protein and DNA synthesis, thereby interrupting plant development (Wang et al., 2022). Based on these mechanisms, flavonoids and phenolics act as biological herbicides with potential applications in weed control. Additionally, the use of compounds found in YCD opens up prospects for applying these mechanisms to develop effective pre-emergent herbicides to control weeds from the early stages of the growing season.

Comparative studies of other Asteraceae species have demonstrated similar allelopathic potential, supporting the broader relevance of this family to natural weed suppression. For instance, *Parthenium hysterophorus* exhibits strong phytotoxic effects mediated by phenolics, sesquiterpene lactones, and flavonoids, with inhibitory activity comparable to that of synthetic herbicides (Motmainna et al., 2021). Likewise, *Chromolaena odorata* has been reported to release allelochemicals that significantly suppress the germination and early growth of both crops and weeds, indicating its potent competitive influence in agroecosystems (Nirgundikar et al., 2023). Earlier comparative work by Masum et al. (2012) also demonstrated that both *P. hysterophorus* and *C. odorata* strongly interfere with seedling development in multiple crop species. These findings underline that *W. trilobata* shares the allelopathic characteristics of its Asteraceae relatives, yet its selective inhibitory effects on major rice weeds highlight a novel potential for species-specific bioherbicidal development within rice-based systems.

Table 3. The total phenolic and flavonoid content in the MeOH extract from the whole sample and the samples of different parts of YCD

W. trilobata	TPC (mg GAE/g)	TFC (mg QE/g)
Total	158.11 a±1.21	260.57 a±1.22
Root	28.85 e±2.28	74.41 d±1.79
Shoot	61.37 d±2.12	42.63 e±1.01
Leaf	111.87 b±2.03	201.50 b±1.79
Flower	104.68 c±1.59	113.59 c±1.35

Note: Averages in the same column with the same letter are not significantly different (Duncan test, $p<0.05$.

Additionally, the germ roots are inhibited by 100% at 0.24 g/mL, and the stem is highly inhibited by 91.39% to 96.33% at concentrations of 0.24 to 0.48 g/mL. The YCD extract inhibits 50% of LVN roots at a concentration of 0.24 g/mL (60.94%), with the highest inhibition of 88.38% at the maximum concentration; the LVN stem is stimulated at concentrations of 0.03 to 0.12 g/mL, and as the concentration increases, the maximum inhibitory effect only reaches 25.69%.

All extracts contain flavonoids and phenolics, with concentrations varying among parts. The whole plant is always rich in secondary metabolites (Phenolics: 158.11a±1.21 mgGAE/g; flavonoids: 260.57a±1.22 mgQE/g), while the leaf part consistently shows higher levels of TPC (111.87b±2.03 mgGAE/g) and TFC (201.50b±1.79 mgQE/g) compared to the other parts.

The inhibitory effects observed in this study suggest that *W. trilobata* possesses promising bioherbicidal potential for practical weed management. The strong suppression of major rice weeds, particularly *F. miliacea* and *L. chinensis*, indicates that their active compounds can significantly hinder early weed establishment, a critical phase of crop–weed competition. Although the study was conducted under controlled laboratory conditions, the magnitude and consistency of inhibition across species demonstrate that *W. trilobata* extracts could

serve as a viable component of integrated weed management (IWM) programs. However, field-scale application will require further optimization of extract formulation, dosage, and mode of delivery to ensure selectivity toward weeds while minimizing phytotoxicity to rice. Therefore, the results of this study represent an essential preliminary step toward the development of sustainable, plant-based bioherbicides suitable for large-scale rice production systems.

4. CONCLUSION

The methanolic extracts of *Wedelia trilobata* (YCD) demonstrated pronounced allelopathic potential against key rice weeds, with the degree of inhibition increasing proportionally with extract concentration. Among the tested species, *Fimbristylis miliacea* and *Leptochloa chinensis* were the most sensitive, whereas *Echinochloa crus-galli* exhibited a comparatively tolerant response, even showing mild stimulation at lower doses. Rice cultivars (OM380 and OM5451) displayed moderate susceptibility, suggesting that selective application of *W. trilobata* extracts could be optimized for integrated weed management without severely affecting crop growth.

Phytochemical profiling confirmed that all plant parts contained abundant flavonoids and phenolic compounds, particularly in the whole plant and leaves, which are recognized contributors to allelopathic activity. Collectively, these findings underscore the potential of *W. trilobata* as a natural source of bioactive compounds for developing eco-friendly bioherbicides. Future research should focus on the isolation of specific active constituents, elucidation of their mechanisms of action, and evaluation of their greenhouse and field-scale performance and environmental safety to advance sustainable weed management in rice ecosystems.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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