

GC-MS analysis of bioactive compounds in *Wedelia trilobata* L. leaf extract and their herbicidal potential against *Cyperus rotundus* L.

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Abstract: This study investigates the allelopathic and bioherbicidal potential of *Wedelia trilobata* L. leaf extract against *Cyperus rotundus* L., a globally invasive and yield-reducing weed. GC-MS analysis identified 108 bioactive compounds in the extract, predominantly terpenoids (87.6%), including steroids (30.7%), triterpenoids (26.3%), sesquiterpenes (13.8%), diterpenoids (11.5%), and carotenoids (3%). A greenhouse experiment was conducted using three extract concentrations (10%, 20%, 40%) alongside a distilled water control and a gallic acid reference. Purple nutsedge tuber sprout emergence and leaf development were measured at 10 and 30 days after planting. The results showed that the 10% concentration had limited inhibitory effects, while the 20% significantly reduced early-stage sprout emergence. The 40% concentration consistently suppressed both tuber shoot emergence and leaf development across all observation points. These findings confirm that *Wedelia* leaf extract exhibits a concentration-dependent bioherbicidal effect on *Cyperus rotundus*, attributed to its rich terpenoid content. The application of the extract significantly reduces vegetative growth, highlighting its potential as an eco-friendly alternative to synthetic herbicides. This work provides a foundation for further studies on formulation optimization and large-scale application in sustainable agricultural systems.

Keywords: Allelochemicals; allelopathy; bioherbicide; natural herbicides; phytotoxicity; plant extract; plant growth inhibition; sustainable agriculture

Abbreviation	Definition
GC-MS	Gas Chromatography–Mass Spectrometry
CRD	Completely Randomized Design
DAP	Days After Planting
EtOH	Ethanol
UHP	Ultra-High Purity
MS	Mass Spectrometry

Introduction

The world's population projected to reach 9.8 billion in 2050 (K. D. Singh et al., 2021). The significant increase in the global population poses challenges for the agricultural sector in meeting the rising demand for food, necessitating enhanced agricultural inputs. The agricultural technology sector is anticipated to encounter various obstacles in addressing the growing need for food to sustain the rapidly expanding global population (Khursheed et al., 2022). Securing an ample supply of safe and nutritious food is essential for sustaining human life as the global population continues to grow (Kostina-Bednarz et al., 2023). To address these challenges, it is essential to implement innovative and improved agricultural practices while prioritizing sustainability and enhancing productivity (Khursheed et al., 2022).

A decline in plant production can result from both biotic and abiotic factors (Oerke, 2006). Among biotic factors, weeds are identified as the most damaging to agricultural productivity (Gharde et al., 2018). Weeds account for the highest potential yield loss (34%), surpassing losses caused by pests (18%) and diseases (16%). Globally, weed competition poses a threat to over 37% of agricultural productivity, which is significantly higher compared to losses attributed to diseases (11%), viruses (1%), and pests (11%) (Oerke, 2006). *Cyperus rotundus* L., commonly known as purple nutsedge, is considered one of the most problematic weeds globally. Its widespread occurrence in both cultivated and uncultivated lands presents

significant challenges for farmers, as it can reduce crop yields and disrupt native plant communities (Hussain et al., 2017). This weed has been reported to cause yield losses ranging from 20% to 90% in various agronomic and horticultural crops worldwide (Peerzada, 2017).

Losses caused by interference in cultivated land are typically managed through various methods, including cultural, physical, and chemical controls (Peerzada, 2017). Among these, chemical control is the most commonly employed by farmers due to its perceived ease and speed. The application of synthetic herbicides remains the primary approach for weed management and enhancing crop productivity. However, the prolonged use of synthetic herbicides has led to the development of herbicide-resistant weed species, a decline in crop quality, and significant contributions to environmental pollution (El Sawi et al., 2019).

An alternative approach supported by numerous studies involves the allelopathic potential of plant and microbial residues and extracts to inhibit the emergence and growth of sedges (Messiha et al., 2013; Zanuncio, et al., 2013). The toxic secondary metabolites present in these extracts and residues impede the growth and development of sedges. Utilizing allelochemicals in weed management offers a promising strategy to reduce reliance on chemical herbicides, thereby minimizing the risk of herbicide resistance and promoting environmentally friendly weed control methods, particularly in organic vegetable production systems (Peerzada, 2017). Allelopathy plays a crucial role in this process by releasing bioactive chemicals into the environment (Molisch, 2001).

Wedelia (*Wedelia trilobata* L.), a member of the Asteraceae family, is known to contain various bioactive compounds with diverse biological activities (Azizan et al., 2016). GC-MS analysis of its leaf extracts revealed a high abundance of resinous acids, suggesting potential allelopathic properties with herbicidal activity (Azizan et al., 2015). Additionally, LC-QTOF-MS analysis identified diterpenoid and sesterterpenoid compounds in *Wedelia* ethanol and water extracts, while alkaloids, including caffeine and monosaccharides, were exclusively detected in the water extract (Azizan et al., 2016). *Wedelia* has demonstrated allelopathic potential, causing biological suppression in certain plants. For instance, water extracts of *Wedelia* at concentrations of 25%, 50%, 75%, and 100% significantly reduced the growth parameters of *Brassica campestris* L., indicating its phytotoxic effects (Zhang et al., 2013). A previous study also reported that the allelochemicals in *Wedelia* leaf extract inhibited several growth parameters, including plant height, shoot number, leaf number, leaf area, root area, and total root length. Both fresh and dry weights, as well as photosynthetic pigments, decreased with increasing concentrations of *Wedelia* leaf extract. Malondialdehyde levels were highest at the 40% concentration. Furthermore, peroxide activity declined at the highest concentration of *Wedelia* leaf extract, suggesting a failure in the plant's antioxidant defense mechanism. The reduction in growth, photosynthetic pigments, and antioxidant activity suggests that *Wedelia* leaf extract may be effective in controlling the growth of purple nutsedge (Uyun et al., 2024). This research was conducted to determine the compound content in *wedelia* leaf extract and to explore the allelopathic potential of *wedelia* leaf extract as a bioherbicide against sedge weeds as a sustainable weed management strategy.

Results

Identification of *Wedelia* leaf compound content using the GC-MS method

The GC-MS analysis results shown in Supplementary Table S1 and Figure 1. and Figure 2. (a) (b) reveal a total of 108 chromatogram peaks, predominantly from the terpenoid compound group (87.6%), followed by the fatty alcohol group (10.5%) and the fatty acid group (1.9%). Specifically, the GC-MS analysis identified 87.6% of compounds as terpenoids, including 30.7% steroids, 26.3% triterpenoids, 13.8% sesquiterpenes, 11.5% diterpenoids, 3% carotenoids, and 2.1% tetraterpenoids. These findings are consistent with the study by Azizan et al., (2015), which also identified compounds in *Wedelia* extract using GC-MS based on metabolomics and multivariate statistical analysis. Their research reported that *Wedelia* extract contains monosaccharides and diterpene resin acids, including 13-cis-retinoid acid and isopimara acid.

The GC-MS analysis of *Wedelia* leaf extract identified that 26.3% of the compounds belong to the triterpenoid group. Among these, squalene, a compound within the triterpenoid group, was found to have the highest area percentage at 20.76%. Additionally, plant allelochemicals such as betulinic acid, oleanolic acid, and ursolic acid have been reported. Many studies show the cytotoxic effects of triterpenoids including allelopathic effects. These pentacyclic triterpenoids are known to suppress the growth of *Bidens pilosa*, a species commonly found in South and Southeast Asia (Singh et al., 2023).

Triterpenoid derivatives, specifically steroids, are compounds found in animals, plants, bacteria, and fungi ((Noushahi et al., 2022). GC-MS analysis of *Wedelia* leaf extract revealed that 30.7% of the compounds belong to the steroid group, which are derivatives of triterpenoids formed through further biosynthetic pathways, particularly via lanosterol cyclization. Steroids serve various functions in plants, including the inhibition of shoot growth (Singh et al., 1970).

Sesquiterpenes accounted for 13.8% of the total terpenoid compounds identified in the GC-MS analysis of *Wedelia* leaf extract, as shown in Supplementary Table S1 and Figure 1. Among the sesquiterpenes, (-)-Spathulenol exhibited the largest area (4.46%). Spathulenol is a tricyclic sesquiterpene with a 5,10-cycloaromadendrane skeleton, known for its significant bioactivity, including anticholinesterase, antinociceptive, antihyperalgesic, antimycobacterial, antioxidant, anti-proliferative, anti-oedematogenic, and cytotoxic properties (Doorandishan et al., 2021). Additionally, spathulenol has been reported to possess allelopathic activity. For example, spathulenol derived from *O. pulchella* leaf extract inhibited more than 90% of *L. esculentum* coleoptile growth and provided 80% inhibition of *L. esculentum* stem growth (Candido et al., 2016).

Diterpenoids accounted for 11.5% of the total terpenoid compounds identified in the GC-MS analysis of *Wedelia* leaf extract, as shown in Supplementary Table S1 and Figure 1. One of the diterpenoid compounds detected was phytol, which was found to constitute 4.90% of the extract. These results are consistent with previous reports identifying phytol as a diterpenoid

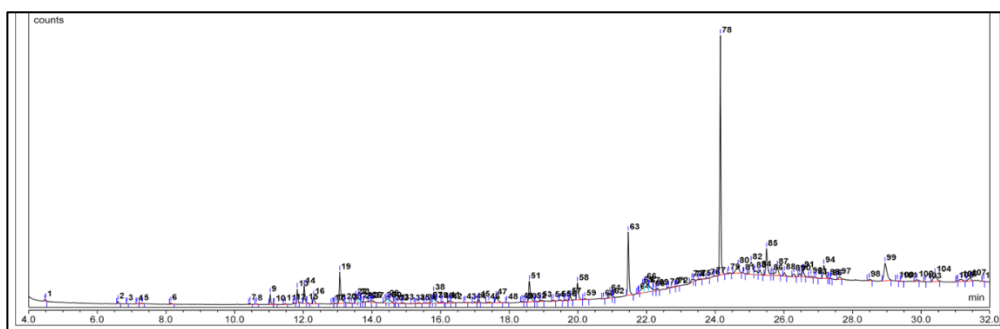


Figure 1. GC-MS chromatogram of the methanol fraction.

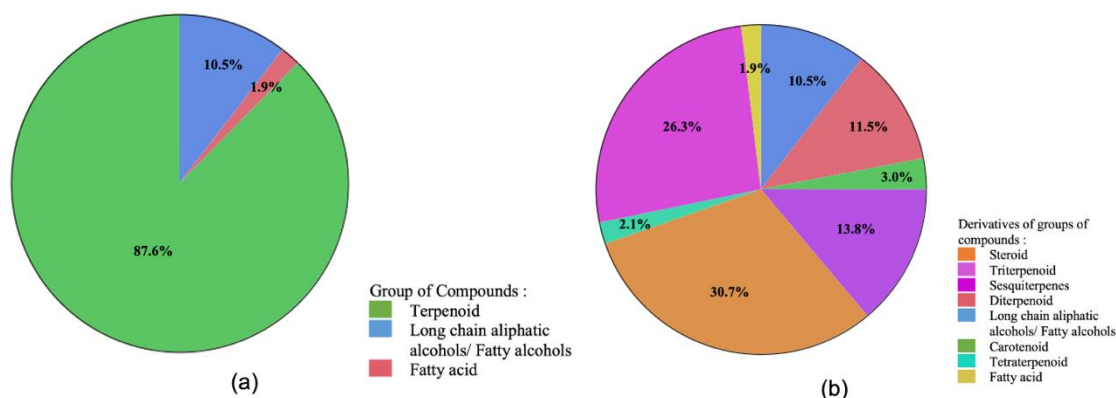


Figure 2 (a) Percentage of wedelia leaf extract compound groups from GC-MS results. (b) Percentage of groups of terpenoid compounds from Wedelia leaf extract from GC-MS results.

compound in Wedelia extract, with a content of 3.70% (Li et al., 2012). Phytol isolated from *A. palmeri* has also been reported to exhibit allelopathic potential, inhibiting the growth of carrots and shallots (Fischer & Quijano, 1985).

Carotenoids, which are derivatives of tetraterpenoids, accounted for 3% of the total terpenoid compounds identified in the Wedelia leaf extract through GC-MS analysis, as shown in Supplementary Table S1 and Figure 1. Recent studies have reported that three carotenoids—neoxanthin, crocin, and β -carotene—exhibit allelopathic activity. These carotenoids were found to inhibit lettuce protoplast cell division by 65-95% at concentrations ranging from 33 to 100 μ M. Additionally, they inhibited the accumulation of yellow pigment in lettuce protoplasts at higher concentrations. Neoxanthin and crocin demonstrated stronger inhibitory effects during the cell division stage than at the cell wall formation stage, with the yellow pigment accumulating in the vacuole. Neoxanthin at 33 μ M and crocin at concentrations greater than 100 μ M were able to inhibit yellow pigment accumulation by more than 100% (Sasamoto et al., 2021).

The Effect of Wedelia Leaf Extract on Inhibiting the Growth of Tuber Sprouts and Leaf Development in Purple Nutsedge (Cyperus rotundus)

The negative control treatment, using distilled water without Wedelia leaf extract, exhibited the highest number of tuber shoots at all observation times (10 and 30 days after planting, DAP). This indicates that in the absence of Wedelia leaf extract, sedge tuber shoots grow uninhibited. The number of emerging tuber shoots serves as an indicator of the weed's vegetative growth, where a reduction in shoot emergence reflects the effectiveness of bioherbicide application in suppressing weed growth (Shahena et al., 2021).

Figure 3 (a) illustrates that the application of Wedelia leaf extract at a 10% concentration did not significantly inhibit tuber shoot growth during the observation periods (10 and 30 DAP) compared to the control group treated with distilled water. However, increasing the concentration of Wedelia extract to 20% resulted in a significant reduction in tuber shoot emergence at 10 DAP, demonstrating its potential to suppress early-stage growth. By 30 DAP, the number of tuber shoots in the 20% concentration group was not significantly different from the control, suggesting a diminished inhibitory effect over time. In contrast, the application of a 40% concentration of Wedelia leaf extract effectively suppressed tuber shoot emergence at both observation times (10 and 30 DAP). These findings reveal a clear trend of decreasing tuber shoot emergence with increasing extract concentration, underscoring the potential of Wedelia leaf extract as a bioherbicide (Hussain et al., 2017).

The application of Wedelia leaf extract demonstrated the ability to reduce the number of secateur leaves. Figure 3 (b) illustrates that Wedelia leaf extract significantly affected the number of secateur leaves. Treatments with extract concentrations of 10%, 20%, and 40% significantly suppressed the growth of secateur leaves during all observation periods (10 and 30 HST) when compared to the control, which received distilled water. However, concentrations of 20% and 40% exhibited more pronounced inhibition at 10 days after planting (DAP). This suggests that increasing the concentration of the extract enhances its allelopathic effect on sedge weeds, particularly at the early stages of sedge growth (10 DAP). The more pronounced inhibitory effect observed in the early stages of growth suggests that allelopathy is most effective during the initial phases of weed development (Rice, 1983). Concentrations of 20% and 40% provided more consistent suppression

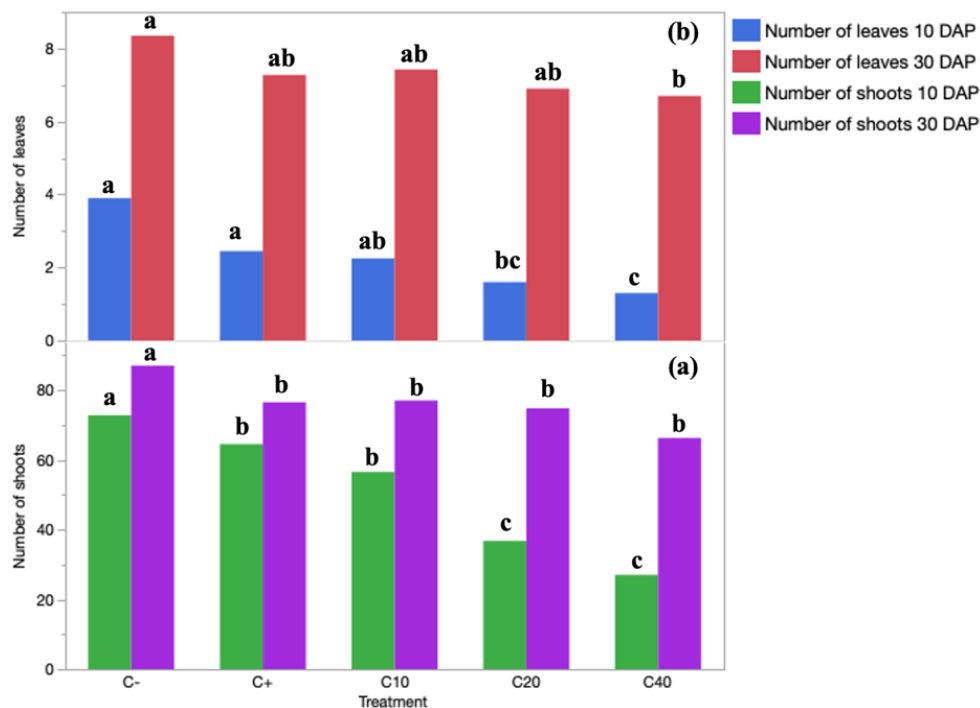


Figure 3 (a) Number of purple nutsedge tubers shoots (b) Number of purple nutsedge leaves.

of secateur leaf numbers at each growth stage, indicating that the allelopathic effect of Wedelia leaf extract is most effective at higher concentrations.

Discussion

The results of this study revealed that the application of Wedelia leaf extract, particularly at higher concentrations (40%), effectively inhibited the growth of *Cyperus rotundus* shoots across all observation periods (10 and 30 DAP). This inhibitory effect is attributed to the presence of terpenoid compounds in Wedelia leaf extract, which are known to influence the activity of plant hormones such as auxin, cytokinin, and gibberellin—hormones critical for cell division and elongation. Disruption in these processes results in stunted plant growth. Specifically, the terpenoid compound 1,8-cineole has been reported to inhibit shoot growth in potato plants by reducing gibberellin production, thereby interfering with the hormone's biosynthesis and suppressing plant growth and development (Suttle et al., 2016). Similarly, diterpenoid compounds such as Podolactone A have been shown to inhibit auxin activity, suppressing shoot formation and growth in *Pisum sativum* (Sasse et al., 1984). Furthermore, Podolactone A, C, and E have been reported to impede chlorophyll biosynthesis and the activity of plant growth hormones, leading to reduced shoot emergence in crops such as lettuce, barley, *Arabidopsis thaliana*, and *Lolium temulentum* (Sasse et al., 1982). Moreover, the diterpenes and sesquiterpenes in Wedelia leaf extract have been reported to significantly inhibit shoot growth in *Cicer arietinum*, *Vigna unguiculata*, and *Vigna radiata* (Shahena et al., 2021). These findings underscore the potential allelopathic effects of Wedelia leaf extract in regulating plant growth through its terpenoid content.

GC-MS analysis revealed that Wedelia leaf extract contains spathulenol, a compound classified within the sesquiterpene group of terpenoids (Supplementary Table S1). Spathulenol, previously extracted from *Eucalyptus camaldulensis*, has been reported to inhibit shoot emergence during the germination of *Amaranthus hybridus* and *Portulaca oleracea* (Verdeguer et al., 2009). Additionally, Wedelia leaf extract contains steroids, which are classified as triterpenoid-type terpenoids. Testosterone, a member of the steroid group, has been documented to exhibit shoot growth inhibitory activity (Singh et al., 1970). Progesterone, also a steroid, was reported to inhibit sunflower shoot growth at a concentration of 0.1 µg/plant. Testosterone application was shown to inhibit sunflower shoot emergence at both high (0.25 µg/plant) and low (0.1 µg/plant) concentrations, while estradiol application at high concentrations (1 µg/plant) similarly suppressed sunflower shoot growth (Bhattacharya & Gupta, 1981). Furthermore, a triterpenoid isolated from *Melilotus messanensis* was reported to inhibit lettuce seed germination (Macias et al., 1997).

Wedelia leaf extract, which contains terpenoid compounds, effectively inhibited the number of leaves across all observation periods. Terpenoids are known to function as allelochemicals (Singh et al., 2023). Among the identified terpenoids, (-)-spathulenol, a sesquiterpene, exhibited the highest abundance (4.46%) as presented in Supplementary Table S1. This compound, previously isolated from *O. pulchella* leaf extract, has been reported to inhibit *L. esculentum* leaf growth by over 90% (Candido et al., 2016). In addition to sesquiterpenes, diterpenoids were also identified in Wedelia leaf extract, as shown in Supplementary Table S1. Diterpenoids have been reported to suppress leaf growth in *Cicer arietinum*, *Vigna unguiculata*, and *Vigna radiata* (Shahena et al., 2021). Furthermore, nanoemulsion formulations derived from *Satureja hortensis*, containing terpenoids such as carvacrol and γ-terpinene, have demonstrated a concentration-dependent reduction in leaf surface area (Hazrati et al., 2017).

Materials and Methods

Experimental site

The field experiment was conducted from April to August 2023 at the experimental field of the Faculty of Agriculture, Universitas Gadjah Mada, located in Banguntapan, Bantul District, Special Region of Yogyakarta, Indonesia (07°48'17" S, 110°24'45" E). The experiments were performed in a greenhouse setting.

Experimental design

The experiment was designed using a completely randomized design (CRD) with a single factor, comprising five different concentrations and four replications. The factor tested was the concentration of *Wedelia* leaf aqueous extract for application. The treatments included five concentrations: three concentrations of *Wedelia* leaf extract (C10%, C20%, and C40%), a reference compound of gallic acid (C+; 92 mg L⁻¹), and a control (C-) using distilled water (aquadest).

Plant materials

The leaves and flowers of *Wedelia* were dried to a constant weight at 40°C for 48 hours. The dried material was then powdered and sifted through a 16-mesh sieve. A portion of the powder (1, 5, and 10 g) was weighed separately, and 100 mL of distilled water was added to each sample. The mixtures were left at room temperature for 24 hours, after which they were filtered to obtain 1%, 5%, and 10% extracts. The filtrates were further processed through muslin cloth (<0.1 mm diameter), followed by filtration using Whatman filter paper No. 1. The final filtrate was stored in a refrigerator (Uyun et al., 2024).

Application of extract

Wedelia leaf extract was applied one day after planting the purple nutsedge tubers, using a soil-to-solution ratio of 1:10 (i.e., 500 g of soil required 50 mL of solution) for each treatment concentration (C10%, C20%, C40%, C+, and C-). To maintain moisture, 20 mL of each solution was added every two days for a duration of 40 day (Uyun et al., 2024).

GC-MS methods

The *Wedelia* leaf extract was prepared by dissolving the sample paste in 1.5 mL of ethanol (EtOH) in a microtube, vortexed to homogenize, and centrifuged at 9500 rpm for 5 minutes if needed. The supernatant was transferred to a GC vial for analysis. GC-MS analysis was performed using an HP-5MS UI column (30 m length, 0.25 mm I.D., 0.25 µm film thickness, maximum temperature 325-350°C). Helium (UHP) was used as the carrier gas. The injector temperature was set to 230°C with a split flow of 50 mL/min and a split ratio of 50:1. The front inlet flow was 1.00 mL/min, and the MS transfer line temperature was 250°C, while the ion source temperature was 200°C. The mass range was set from 40-500 amu. The temperature program included an initial hold at 60°C for 2 minutes, followed by a ramp to 280°C at 10°C/min, held for 8 minutes.

Conclusion

Wedelia leaf extract demonstrates significant bioherbicidal potential against *Cyperus rotundus* L., inhibiting tuber shoot emergence and leaf development through its allelopathic properties particularly at higher concentrations (40%). The bioherbicidal activity of *Wedelia* leaf extract can be attributed to its terpenoid compounds, including sesquiterpenes like (-)-spathulenol and diterpenoids like phytol, which are known to disrupt plant hormone activities such as auxin, cytokinin, and gibberellin, essential for cell division and elongation. These disruptions result in reduced shoot emergence and leaf growth. These results suggest that *Wedelia* leaf extract is a promising, eco-friendly alternative to synthetic herbicides, warranting further research on optimization and field applications.

Competing interests

The authors declare that they have no competing interests.

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