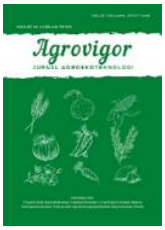
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Article

Profile of Allelochemical Compounds in Ratoon Sorghum Leaf Extract (Sorghum bicolor L.) Based on GC-MS Analysis as a Bioherbicide Candidate

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ABSTRACT

The extensive use of synthetic herbicides has raised environmental concerns and contributed to herbicide-resistant weeds, emphasizing the need for environmentally friendly weed-management alternatives. Sorghum is known for its allelopathic properties, but information on potential allelochemicals in sorghum ratoon leaves remains limited. This study aimed to characterize the chemical constituents of sorghum ratoon leaf extracts and assess their potential relevance to allelopathy based on GC-MS profiling and previously reported biological activities. Ratoon leaves were sequentially macerated using n-hexane, ethyl acetate, and ethanol. Compounds were identified by Gas Chromatography–Mass Spectrometry (GC-MS) and evaluated according to chromatographic peak areas, chemical classes, and reported biological activities. The analysis detected alcohols and phenolic-related compounds, fatty acids, fatty acid esters, amides, sterols, steroids, triterpenoids, and organometallic-related compounds. Several of these groups have previously been associated with allelopathic or phytotoxic activity. Polar compounds may warrant further investigation for aqueous bioherbicide formulations, while fatty acids, esters, and amides may also be relevant to plant growth inhibition. However, their biological effects cannot be confirmed by GC-MS profiling alone. Further bioactivity, dose-response, and target-weed assays are required to validate their phytotoxic potential and suitability for natural bioherbicide development. These findings therefore provide a chemical basis for subsequent experimental validation.

INTRODUCTION

Because competition for nutrients, water, light, and growth space can significantly lower crop output, weed interference remains a major problem in contemporary agricultural systems. Because of their quick and efficient action, synthetic herbicides have been a major part of weed control for decades. However, the frequent and heavy use of herbicides has raised concerns about environmental contamination, hazards to human and ecosystem health, and the emergence of herbicide-resistant weed populations (Indarwati et al., 2023). These difficulties highlight how crucial it is to investigate different weed-management strategies that are more in line with sustainable agriculture principles and the environment.

Plant-derived bioherbicides, especially those based on allelochemicals, are gaining popularity as potential components of long-term weed control. Allelopathy is the term for biological interactions in which an organism releases chemicals that affect other species' germination, growth, development, or physiological functions (Susilo et al., 2024). Since numerous secondary metabolites have been shown to have biological actions that may aid in weed suppression, plant-derived allelochemicals are especially interesting. Although their efficacy, environmental behavior, and potential for resistance development necessitate evaluation for individual compounds and formulations, their natural origin and potential biodegradability offer opportunities to reduce reliance on conventional synthetic herbicides (Amrullah et al., 2024; Mudaningrat et al., 2023).

Therefore, the use of allelopathy in weed control may offer a different approach to lessen reliance on synthetic herbicides while promoting more environmentally friendly farming methods (Indarwati et al., 2023; Najib et al., 2025). Due to their many biological activities and potential uses in crop production systems, plant secondary metabolites have been studied as promising sources of bioherbicidal chemicals (Talahatu & Papilaya, 2015). Sorghum (*Sorghum bicolor* L.) is one of the allelopathic crops that has drawn a lot of interest due to its ability to create and release secondary metabolites that can have an impact on nearby plants. Leaching, volatilization, root exudation, and the breakdown of plant residues are some of the ways that allelochemicals from sorghum tissues might impact weed–crop interactions in agroecosystems (Hussain et al., 2021).

Several classes of bioactive substances, such as phenolic compounds, flavonoids, lipophilic metabolites, and sorgoleone, have been linked in earlier research to the allelopathic characteristics of sorghum (Kostina-Bednarz et al., 2023; Cheng & Cheng, 2015). Under experimental conditions, some of these substances have been linked to phytotoxic reactions and inhibition of plant development. Sorghum is now recognized as a significant plant model and a possible source of natural chemicals for allelopathy-based weed control. However, much of current research has focused on the general allelopathic characteristics of sorghum tissues, specific recognized allelochemicals, or the phytotoxic effects of sorghum extracts or residues. Characterizing the broader chemical profile of leaves formed during the ratoon phase has received relatively little attention, especially when employing sequential extraction to recover compounds with varying polarities.

The regrowth of sorghum after the main crop is harvested is known as the ratoon phase. Newly formed leaves remain physiologically and metabolically active during this stage and provide an additional supply of plant biomass following the primary harvest (Cheema, 2020). Because of this feature, ratoon leaves are both potentially useful for biomass use and scientifically important for allelochemical research. Ratoon leaves may be an understudied source of secondary metabolites that could be further defined for potential agricultural uses, rather than being regarded only as leftover plant material after harvest. However, because there is still little direct comparable data, their chemical makeup should not be taken to be the same or richer than that of leaves from the primary crop.

Even though sorghum allelopathy has been the subject of many investigations, there is still a dearth of precise knowledge regarding the chemical profile of possible allelochemicals in sorghum ratoon leaves. Prior studies have typically focused on phytotoxic reactions, reduced plant growth, or specific bioactive compounds linked to sorghum's allelopathic activity (Anwar et al., 2021). As a result, there is still a disconnect between evidence of sorghum's overall allelopathic potential and our understanding of the variety of chemical components found in ratoon leaf extracts. An essential first step in finding compounds or chemical groupings that might require further biological assessment is characterizing these constituents.

Chemicals within complex plant extracts, especially volatile and semi-volatile compounds susceptible to GC-MS analysis, can be separated and identified using Gas Chromatography–Mass Spectrometry (GC-MS) (Guntoro et

al., 2020). This method has been used in metabolomic and phytochemical research to describe different secondary metabolites linked to biological activities, including substances that may be involved in allelopathic interactions (Anwar et al., 2021). Additionally, compared to extraction using a single solvent, using solvents with distinct polarity, such as n-hexane, ethyl acetate, and ethanol, enables sequential extraction of chemically varied compounds and offers a more comprehensive picture of the metabolite profile.

Instead of assessing sorghum allelopathy largely by plant-growth inhibition or concentrating only on previously identified allelochemicals, the current study is innovative in that it focuses specifically on the chemical profile of sorghum ratoon leaves. By combining sequential extraction with n-hexane, ethyl acetate, and ethanol to recover molecules across various polarity ranges, this study fills the highlighted research gap. The chemical profiles of the compounds are then characterized using GC-MS. As a result, the study offers baseline chemical data on the variety of chemicals found in ratoon leaves and, based on their chemical properties and biological activities already documented in the literature, identifies substances that may be relevant to allelopathy. Crucially, GC-MS profiling does not exhibit phytotoxic or bioherbicidal activity on its own; rather, it is employed here as a first step in chemical characterization.

To identify compounds with potential relevance to allelopathy and future bioherbicide research based on their chemical characteristics and previously reported biological activities, this study used GC-MS to characterize the chemical profile of sequential n-hexane, ethyl acetate, and ethanol extracts of sorghum ratoon leaves. To ascertain the true phytotoxic efficacy and viability of sorghum ratoon leaf-derived chemicals for bioherbicide research, the resulting chemical profile is anticipated to offer a scientific foundation for ensuing bioactivity assays, dose-response studies, and target-weed evaluations.

MATERIALS AND METHODS

Time and Place

This study was conducted from July to September 2023 in the swamp land owned by the Faculty of Agriculture, Bengkulu University. Gas Chromatography–Mass Spectrometry (GC-MS) analysis was performed at the DKI Jakarta Health Laboratory. The main raw material used in this study was sorghum ratoon leaves, which were obtained from the swamp land of the Faculty of Agriculture, Bengkulu University. ratoon leaves were chosen as the object of study because this part of the plant is believed to still actively produce secondary metabolites and allelochemical compounds after the main harvest, which may play a role in the allelopathy mechanism against other plants. The main tools used in this study included a drying oven, a blender, a sieve, a rotary evaporator, and a Gas Chromatography-Mass Spectrometry (GC-MS) instrument for chemical compound profiling.

Sample Preparation and Extraction

Sample preparation began by washing the sorghum *ratoon* leaves with running water to remove dust, dirt, and other contaminants. The samples were then dried in an oven at 70°C until they reached a constant weight. This drying process is carried out to reduce water content without damaging labile compounds sensitive to high temperatures. The leaves from the dried samples are then ground in a blender and filtered to a 40-mesh size. The reduction in particle size increases the material's surface area, enhancing contact between the sample and the solvent during the extraction process.

Extraction was performed using a stepwise cold maceration method, as this method is effective for extracting secondary metabolites without damaging the structural biological activity of bioactive compounds. The sample powder was soaked in solvent at a 1:10 (w/v) ratio. Maceration was carried out sequentially in n-hexane, ethyl acetate, and ethanol solvents for 3 x 24 hours with solvent replacement every 24 hours to optimize the extraction process (Tukiran et al., 2020; Roanisca et al., 2019). The maceration filtrate was filtered through Whatman No. 1 paper and concentrated in a rotary evaporator at 40°C until a thick extract was obtained. Before GC-MS analysis, the extract was stored at low temperature in a tightly sealed container to maintain compound stability.

GC-MS Analysis

The analysis of phytochemical compounds in sorghum leaf extract was performed using a Shimadzu QP-2010S GC-MS instrument in electron ionization mode at an energy of 70 eV (Rahmawati et al., 2022). This ionization mode was chosen because it produces characteristic and informative ion fragmentation in the compound identification process. Mass scanning was performed in the range of 50 to 650 m/z to identify volatile and semi-volatile compounds present in the extract (Yuan et al., 2017). A DB-5 MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness) was used as the stationary phase because it is suitable for separating complex organic compounds (Ferdosi et al., 2023).

The GC oven temperature program was set to 50°C, held for 2 minutes, then increased to 300°C at 10°C per minute and held for 10 minutes. The injector and MS interface temperatures were 280°C and 250°C, respectively. In this analysis, helium was used as the carrier gas at a flow rate of 1 mL/minute.

Sample injection was performed in 1:10 split mode, with an injection volume of 1 µL to maintain system stability and separation resolution (Othman et al., 2021). Compound identification was performed by comparing the mass spectra and retention times of chromatogram peaks with the NIST and Wiley libraries in the GC-MS system (Surahmaida et al., 2019). This process considered the ion fragmentation, retention index, and molecular weight of the compounds (Saragih, 2024).

Data Analysis

The composition of the chemical compounds in each extract was determined from the peak areas on the GC-MS chromatogram. The area corresponds to the relative proportion of each compound present in the sample (Rafi et al, 2018). This approach provides quantitative information on the secondary metabolite profile relative to a standard. Chromatogram analysis was used to identify and quantify the types and amounts of compounds, both dominant and minor, with potential allelochemical activity in sorghum leaf extracts (Rumanti & Saragih, 2023). A compound that has been successfully identified is then grouped based on its chemical structure and potential biological activity. This grouping serves as a reference for analysis to identify candidate allelochemical compounds that contribute to sorghum's bioherbicide activity (Surahmaida et al., 2019).

RESULTS AND DISCUSSION

Interpretation of Alkalochemical Compounds Based on GC-MS Analysis

Secondary metabolite profile

GC-MS analysis of ratoon sorghum leaf extract confirmed the presence of secondary metabolites that can be classified as potential allelochemicals. These metabolites include alcohols/phenols, fatty acids and their esters, amides, sterols, steroids, triterpenoids, and organometallic compounds. This diverse composition classifies ratoon sorghum leaves as a source of bioactive compounds with great Potential for the development of natural bioherbicides based on allelopathy. Allelochemical constituents are water-soluble organic acids, long-chain fatty acids, terpenoids, phenols, and phenolic acids. The phytotoxic Potential of these compounds varies and depends on their chemical structure and environmental solubility (Indarwati et al., 2023). Therefore, more polar fractions, especially those containing –OH or C=O functional groups, are likely to be more effective in inhibiting weeds, particularly during the germination and root development phases, due to the higher solubility and absorption of these fractions. The compounds identified in the ratoon leaf extract of sorghum plants are presented in Table 1, showing a diverse chemical profile that includes several constituents previously reported to be associated with allelopathic or phytotoxic activity.

Potential of polar compounds as allelopathic agents

Finding polar chemicals in sorghum ratoon leaf extracts is important for assessing their possible role in allelopathic activity, especially those related to alcohol and phenol. Their presence in the GC-MS profile offers chemical evidence of chemicals whose biological activities can be understood in reference to prior allelopathy investigations, rather than only demonstrating phytotoxic activity. Because of their comparatively polar properties, phenolic compounds and several alcohol-related metabolites have been linked to allelopathic interactions, which

may enhance their release and distribution in aqueous environments surrounding plant tissues (Septiani et al., 2019). Although the actual availability, concentration, and biological activity of the chemicals found in the current investigation were not explicitly assessed, this characteristic would be especially pertinent to weed control after the release of sorghum-derived metabolites.

Table 1. Compounds identified in the ratoon leaf extract of sorghum plants

No	Compound	Chemical Formula	Group	Content (%)	Retention Time (minutes)
1	(2E)-3,7,11,15-Tetramethyl- 2-Hexadecen-1-0L	C20H40O	Acyclic diterpenes	2.57	26,886
2	Hexadecanoic acid, ethyl ester	C18H36O2	Fatty acid	1.79	28,376
3	Hexadecanoic acid	$C_{16}H_{32}O_2$	Fatty acid	3.14	28,524
4	9,12-Octadecadienoic acid, ethyl ester	C20H36O2 C18H32O2	Fatty acid	2.71	29,431
5	Octadecanoic acid, Ethyl ester	C20H40O2 C18H32O2	Fatty acid	1.14	29,575
6	Methyl 19-methyl-eicosanoate	C22H44O2	Fatty acid	1.01	30,527
7	9-Octadecenamide, (Z) -	C18H35NO	Organic amide	1.26	30,720
8	Bis (6-Methylheptyl) Phthalate	C24H38O4	Diester	1.52	31,285
9	Bicyclo [3.1.1] heptan-3-one, 2,6,6-trimethyl-, (1.alpha., 2.alpha., 5.alpha.) -	C10H16O	Terpenoid	2.24	34,347
10	6-(3-Isopropenyl-3- Methyl-1-Cyclopropen-1- YL)-6-Methyl-2-Heptanone	C15H24O	Ketone	1.37	34,871
11	Campesterol	C28H48O	Steroids	5.42	36,864
12	Stigmasterol	C29H48O	Steroids	12.78	37,374
13	Norolen-12-ene	C29H48	Alicyclic hydrocarbons	8.09	38,291
14	3-Keto-urs-12-ene	C30H46O3	Triterpenoid	11.14	39,091
15	Benz [c] -Stigmastadiene-3-one	C29H50O C29H42O	Steroids	12.04	39,663
16	Stigmast-4-en-3-one	C29H48O	Steroids	9.86	40,760
17	Iron, (.eta.5-2,4-cyclopentadien-1-yl) hydrobis (trimethylphospine)	C12H21FeP C11H18FeP2	Organometallic	14.54	41,746

According to earlier research, phenolic and related polar chemicals can prevent sensitive plants' early root development and seed germination (Alridiwirsah et al., 2020). Because roots serve as an initial site of contact between germinating weeds and phytochemicals found in the surrounding growth medium, root growth is especially important in allelopathic interactions. Therefore, additional research may be necessary to determine the potential impacts of the polar elements found in the sorghum ratoon leaf extracts on target weed germination and early seedling development. However, the presence of these compounds in the GC-MS chromatogram does not prove that they reached phytotoxic amounts or inhibited the target weed's development. Only direct germination, root growth, seedling growth, and dose-response experiments can establish such a link.

Phenolic chemicals have been linked to changes in photosynthetic processes, water and nutrient intake, and plant enzyme activity at the physiological level (Xie & Wang, 2024). According to one theory, alterations in the integrity and permeability of the plasma membrane may have an impact on membrane-associated ATPase activity

and obstruct the movement of ions and water (Ziadaturrif'ah et al., 2019). Plant growth, nitrogen uptake, and stomatal control may all be impacted by disruptions in these systems. These mechanisms, however, should not be understood as experimentally proven effects of the chemicals found in the current sorghum ratoon leaf extracts, but rather as possible modes of action reported in earlier studies. The current GC-MS-based study did not examine membrane damage, ion leakage, photosynthetic efficiency, enzyme activity, or other physiological responses of the target weed.

Because chemicals that are more compatible with polar solvents may be easier to incorporate into water-based extraction or formulation methods, detecting polar constituents may also be significant for future bioherbicide research. However, polarity and solubility are not sufficient indicators of herbicidal effectiveness. Several variables, such as chemical identification, concentration, exposure duration, absorption, interactions between metabolites, weed species, and developmental stage, affect a target weed's biological response. Furthermore, even though some naturally produced compounds may exhibit advantageous traits such as comparatively quick disintegration and reduced environmental persistence, their environmental safety cannot be inferred exclusively from their natural origin (Anwar et al., 2021).

Therefore, rather than being verified phytotoxic agents, the polar chemicals found in sorghum ratoon leaf extracts should be considered potential allelochemical possibilities. Although their precise role in inhibiting the target plant has not yet been proven through experimentation, their identification offers a chemical foundation for explaining the possible allelopathic relevance of sorghum ratoon leaves. To determine whether these compounds are responsible for the observed or anticipated allelopathic effects and to assess their suitability for future bioherbicide development, more research combining GC-MS profiling with target-weed germination assays, seedling-growth tests, dose-response experiments, and physiological measurements is necessary.

Nonpolar compounds: steroids and triterpenoids from sorghum leaf extract

The extract also contains nonpolar compounds such as steroids and triterpenoids, which, although present in relatively high amounts, are limited in solubility due to their hydrophobic nature, rendering them ineffective when formulated as water-based biopesticides (Hasan et al., 2021; Amrullah et al., 2024). Therefore, the development of bioherbicides prioritizes polar compounds that can be easily dispersed by foliar spraying and can trigger physiological responses in target weeds (Khamare et al., 2022). However, the nonpolar fraction may still be contributory as an adjunct with possible synergistic effects or alternative toxicity pathways.

Induction of oxidative stress and activation of the antioxidant system

The dominance of phytotoxic components reinforces sorghum's position as an allelopathic plant that produces metabolites that inhibit weed growth (Hasan et al., 2021). Allelopathy often occurs due to the InductionInduction of oxidative stress caused by an increase in ROS, followed by the activation of the cellular antioxidant system (Bogatek & Gniazdowska, 2007). Under normal conditions, SOD, CAT, and POD neutralize ROS, but when ROS production exceeds defense capacity, oxidative stress occurs, triggering physiological damage and cell death (Stefanski et al., 2020). ROS also includes oxidized lipids, cellular DNA damage, and inactive enzymes (Concato et al., 2022; Gam et al., 2024), while increased antioxidant enzyme activity is an indicator of the response to this stress; failure to maintain redox balance ultimately leads to cell death in the target plant (Simbolon et al., 2020; Tucuch-Perez et al., 2025; Putri et al., 2023).

Potential Bioherbicide Implications of *ratoon* Sorghum Leaves

Overall, GC-MS analysis revealed the presence of allelochemicals with high phytotoxic Potential, with polar fractions (alcohols and phenolics) being the most relevant components for water-soluble bioherbicides due to their high solubility and rapid absorption. The nonpolar fraction (steroids and triterpenoids) is less efficient in water-based formulations but has the Potential to support allelopathy through synergistic contributions. These findings confirm *ratoon* sorghum leaves as a candidate source of natural, environmentally friendly, and sustainable bioherbicides.

Water-Soluble Polar Compounds (Alcohol-Phenol) as Potential Bioherbicides

Alcohol and phenol compounds, due to their -OH and C=O groups that increase the polarity of the compound, are prime candidates for water-soluble bioherbicides. Such polar compounds allow for easy mixing and produce even distribution in liquids. High solubility enhances contact and penetration within weed tissues, allowing the compounds to function as allelochemical growth inhibitors. Practical advantages include ease of spray application and relatively short environmental half-lives, as polar compounds rich in oxygen and nitrogen tend to degrade more rapidly and may be less likely to accumulate biologically than nonpolar compounds. The principle of "like dissolves like" explains how hydrogen bonding and dipole-dipole interactions enhance the solubility of polar compounds, which in turn supports their phytotoxicity. (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol (2.57%) is a terpenoid alcohol that has the potential to inhibit meristematic cell division in roots and disrupt hormonal balance, thereby inhibiting root and leaf development.

The hydroxyl group enhances moisture mobility and facilitates weed absorption. At the same time, the volatile nature of this compound can expand the distribution of the active compound around the target weed growth area. Another compound, 6-(3-Isopropenyl-3-Methyl-1-Cyclopropenyl)-6-Methyl-2-Heptanone (1.37%), is a slightly polar volatile ketone that can disrupt cell respiration and inhibit metabolic enzymes (Table 2).

Table 2. Candidate water-soluble bioherbicide compounds or growth inhibitor compounds

No	Identified compound	Classification	Concentration(%)	Allelopathy Potential
1	(2E)-3,7,11,15-Tetramethyl-2-Hexadecen-1-OL	Alcohol/	2,57	Allelochemical in nature, it can inhibit weed growth by disrupting hormones and cell metabolism.
2	6-(3-Isopropenyl-3-Methyl-1-Cyclopropenyl)-6-Methyl-2-Heptanone	Phenolic	1,37	Contains carbonyl groups that have the potential to act as physiological inhibitors of weeds.

The toxicity of the soak, caused by increased membrane permeability, is supported by allelopathic effects that inhibit key energy metabolism enzymes (Freitas et al., 2019), reducing the energy available for cell division and elongation (Hasan et al., 2021). Oxidized compounds, such as alcohols and ketones, are reported to exhibit higher phytotoxicity than nonpolar hydrocarbon liquids, consistent with a positive correlation between a compound's water solubility and its phytotoxicity (Chaimovitsh et al., 2016). Further mechanisms may include inhibition of mitochondrial electron transport in mitochondria, decreased ATP synthesis, and membrane peroxidation, leading to cell death (Cheng & Cheng, 2015; Hasan et al., 2022).

Although the primary objective of formulating water-soluble substances focuses on the polar fraction, the fatty acid fraction and its derivatives remain relevant, as they can disrupt membranes, cause electrolyte leakage, and induce redox imbalance via ROS and lipid peroxidation. The presence of phenolics and sorgoleone has also been reported to enhance allelopathy through increased membrane permeability and mitochondrial dysfunction that disrupts energy metabolism (Freitas et al., 2019; Liu, 2022).

Fatty acids, esters, and amides are the dominant fractions in sorghum leaf extracts, which are important for their phytotoxicity through membrane damage, photosynthesis disruption, and respiration suppression, consistent with the role of secondary metabolites as allelopathic essences in suppressing competitors (Anwar et al., 2021; Motmainna et al., 2024). The metabolite profile shows the dominance of this group, along with alkaloids, phenols, flavonoids, and coumarins, as additional allelochemical candidates (Wang et al., 2024). Plasma membrane damage inhibits water and ion absorption. It disrupts cell metabolism (Septiani et al., 2019), and this correlates with oxidative stress, which is the main driving force of weed growth suppression (Hasan et al., 2021). Esters and amides enhance these effects through changes in membrane permeability, hormonal disruption, and loss of cell viability (Amrullah et al., 2024; Anwar et al., 2023; Wang et al., 2024), so that all fractions exhibit synergistic toxicity (Ali et al., 2016; Anwar et al., 2021; Wang et al., 2024).

In Table 3, hexadecanoic acid (palmitic acid) (3.14%) is a saturated fatty acid that has the potential to increase membrane permeability and disrupt cell homeostasis. The ester of 9,12-octadecadienoic acid (2.71%) is a linoleic acid derivative that is more reactive and has the potential to increase oxidative stress and suppress photosynthesis and respiration, especially when membrane damage is accompanied by redox imbalance. Long-chain fats can also damage the membranes and cell wall structures of target organisms (Sogandi et al., 2019; Wahyuni et al., 2019). This is consistent with the properties of biosurfactants that enhance membrane function and inhibit certain enzymes (Hua et al., 2023; Toh et al., 2023) and, in some cases, alter physiological responses through metabolic involvement (Gam et al., 2024).

Table 3. Senyawa teridentifikasi yang kemungkinan bersifat fitotoksik

No	Identified Compound	Group	Concentration (%)	Reason for Not Being Prioritized
1	Hexadecanoic acid	Fatty acids	3,14	Causes damage to cell membranes and inhibits root growth.
2	Hexadecanoic acid, ethyl ester	Fatty acid esters	1,79	Toxic to young plant tissue.
3	9,12-Octadecadienoic acid, ethyl ester	Fatty acid esters	2,71	Inhibits respiration and photosynthesis in weeds.
4	Octadecanoic acid, ethyl ester	Fatty acid esters	1,14	Mildly phytotoxic, interferes with germination.
5	Methyl 19-methyl-eicosanoate	Fatty acid esters	1,01	Potentially inhibits weed growth through toxic lipid effects.
6	9-Octadecenamide (Z)	Fatty acid amides	1,26	Inhibits cell division and weed germination.
7	Bicyclo [3.1.1] heptanone	Volatile carbonyl compounds	2,24	Toxic through metabolic disruption.

Table 4. Compounds are not prioritized for water-soluble bioherbicides (purely hydrophobic).

No	Identified Compound	Group	Concentration (%)	Reason for Not Being Prioritized
1	Campesterol	Sterol/Kolesterol	5.42	Hydrophobic, insoluble in water
2	Stigmasterol	Steroid	12.78	Functions more as a structural metabolite, less effective as a water-soluble bioherbicide
3	Norolen-12-ene	Triterpenoid	8.09	Non-polar, difficult to absorb in solution applications
4	Benz[c]-Stigmastadiene-3-one	Steroid	12.04	Purely hydrophobic, not suitable for liquid formulations
5	Stigmast-4-en-3-one	Steroid	9.86	Heavy compound, low solubility
6	Iron organometallic compound	Kompleks organik	14.54	Not specific as a bioherbicide, potential for non-target compounds

Oleamide (1.26%) disrupts endogenous hormones, inhibits dividing meristematic cells, and reduces root elongation and biomass (El-Wakeel et al., 2024). Oleamide has also been reported to increase membrane permeability and disrupt the electron transport chain of cells (Agustini et al., 2022; Sukmawati et al., 2018), as well as modulate signaling and membrane integrity as a bioactive lipid (C. E. et al., 2014; Fatmawati et al., 2021; Fitri et al., 2023; Nogués et al., 2023). In the context of phytotoxicity, oleamide in the field can disrupt electron flow in Photosystem II and damage thylakoid membranes, resulting in decreased photosynthetic performance (Liu et al., 2022), consistent with

phytotoxic indications of electron transport disruption and decreased chlorophyll content (Hasan et al., 2022). Therefore, most fatty acids, esters, and amides emphasize this fraction as the primary driver of phytotoxicity due to membrane damage, oxidative stress, and disruption of cellular metabolism.

The extract from sorghum *ratoon* leaves also contains sterols, steroids, and triterpenoids that are heavy and hydrophobic. Therefore, their solubility, mobility, and distribution, as well as their components in water-based formulations, are also low. Although less suitable as the main active ingredient in water-soluble bioherbicides, this fraction remains relevant in the complex framework of allelopathy involving many metabolites (Barrales-Cureño et al., 2022). Some plant steroids, such as γ-sitosterol, which has been shown to dominate allelopathic plant extracts, have been reported to have the potential to inhibit the growth of target plants. However, confirmation of their specific activity still requires further isolation and bioactivity testing (Gam et al., 2024). Biologically, steroids/sterols play a role in membrane structure, hormonal regulation, and have antimicrobial and antioxidant activities (Tetti, 2014; Mulyadi et al., 2021), while interactions with phospholipid membranes can reduce membrane integrity and disrupt cellular homeostasis (Fauzia et al., 2018; Matias et al., 2018). For triterpenoids and saponins, the inhibitory effect on roots is partly due to meristematic tissue death and reduced biomass, which is thought to occur through interactions with membrane proteins and cytoplasmic lipids (Kostina-Bednarz et al., 2023; Almarie, 2020).

The fundamental limitation of this fraction is physical-chemical in nature: the long hydrocarbon structure causes the compound to be more soluble in non-polar solvents and difficult to formulate in water, in accordance with the principle of "like dissolves like" (Putra, 2019; Singapurwa et al., 2024; Syafira et al., 2025). Therefore, steroids and triterpenoids are often prioritized lower for water-soluble bioherbicides that require homogeneous dispersion and rapid diffusion (Adrianta et al., 2024; Sihombing et al., 2025). The discovery of dominant compounds (Table 3), such as stigmasterol (12.78%), benz[c]-stigmastadiene-3-one (12.04%), and campesterol (5.42%), shows a similar pattern: high content but hydrophobic, making them more suitable as supporting metabolites that require special formulation (Matias et al., 2018; Rochmat et al., 2017; Maulana et al., 2020; Sugijanto et al., 2020; Tensiska et al., 2020; Nurdiani et al., 2023; Syafira et al., 2025). Meanwhile, the presence of iron organomaterial (14.54%) is prioritized lower because it may be an analytical artifact or contamination, and the unbalanced solubility and mobility of the hydrophobic fraction reinforce that this group is not suitable for safe and effective water-soluble bioherbicides (Leksono et al., 2018; Marzuki et al., 2022). Thus, the development of water-soluble bioherbicides is more rationally directed toward polar fractions that are readily soluble in water, quickly distribute, and interact readily with target weeds (Adrianta et al., 2024; Sihombing et al., 2025).

CONCLUSION

GC-MS analysis of sorghum *ratoon* leaf extracts revealed the presence of allelochemicals with potential for the development of natural bioherbicides. Polar compounds derived from the alcohol and phenolic groups are prime candidates for water-soluble bioherbicides because they are readily soluble and directly contribute to inhibiting weed physiology. Conversely, fatty acid, ester, and amide constituents are the dominant phytotoxic fractions that disrupt weed cell membranes, suppress photosynthesis and respiration, and inhibit germination. Steroid and triterpenoid compounds are quite hydrophobic and, therefore, not a priority in water-soluble bioherbicide formulations due to their limited solubility and mobility in aquatic systems.

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AUTHORS CONTRIBUTIONS

E acted as the lead author responsible for research design, GC-MS analysis, data interpretation, and overall manuscript preparation and revision. H contributed to data collection and processing, supported analysis, and assisted in manuscript preparation and refinement. All authors have read and approved the final version of the manuscript and are responsible for the accuracy and integrity of the reported research.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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