

Profiling Essential Oil Chromolaena Odorata Leaves Using GC-MS Hydrodistillation

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ABSTRACT

Indonesia, as an agrarian country, possesses a wide diversity of medicinal plants that are potential sources of essential oils, including Siam weed leaves (*Chromolaena odorata* L.), which are known to be rich in secondary metabolites and traditionally used as antimicrobial and wound-healing agents. This study aimed to profile the chemical components of essential oil from Siam weed leaves obtained by hydrodistillation as baseline data to support the exploration of its pharmacological potential. The essential oil was extracted using the hydrodistillation method with a Clevenger apparatus, then analyzed by Gas Chromatography–Mass Spectrometry (GC-MS) to identify volatile compounds based on retention time, mass spectra, and percentage peak area. The analysis revealed 40 volatile compounds belonging to monoterpene, sesquiterpene, aromatic ether, and other cyclic compound classes, with major components including α -pinene, β -pinene, limonene, β -caryophyllene, germacrene D, and δ -cadinene, which are known to exhibit antimicrobial, anti-inflammatory, and antioxidant activities. These findings indicate that hydrodistillation is effective for obtaining essential oil from Siam weed leaves and that its terpenoid-dominated chemical profile supports further development as a natural resource for pharmaceutical applications and plant-based antimicrobial products.

Keywords: Chromolaena; Hydrodistillation; Essential; GC-MS; Bioactive

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INTRODUCTION

Indonesia is an agrarian country with abundant biological natural resources, including various spice and medicinal plants that have the potential as sources of essential oils. Essential oils are vegetable oils obtained from various parts of plants, such as leaves, flowers, fruits, seeds, stems, bark, roots, or rhizomes. Essential oils are characterized by being volatile and producing a distinct aroma, and they are widely utilized in the pharmaceutical, cosmetic, food, and aromatherapy industries due to their content of various bioactive compounds (1).

Chromolaena odorata Linn (L.), previously known as *Eupatorium odoratum* L., belongs to the kingdom Plantae, family Asteraceae, genus *Chromolaena*, and species *Chromolaena odorata* L (2). Traditionally, this plant has long been used by communities to treat various diseases, ranging from diabetes, soft tissue wounds, and skin infections to diarrhea and malaria (3). This traditional use is supported by the high content of secondary metabolites in the leaves of *Chromolaena odorata*, particularly phenolic and flavonoid compounds, which have been shown to possess various pharmacological activities (3)(4)(5).

Various studies have demonstrated the bioactivity potential of *Chromolaena odorata* extracts. Leaf extracts of *Chromolaena odorata* have been reported to inhibit the growth of all tested clinical isolates of *Streptococcus aureus* and also exhibit significant antibiofilm activity (6). In addition, extracts and isolated flavonoid compounds from *Chromolaena odorata* have also demonstrated antimycobacterial activity against *Mycobacterium tuberculosis* and several other *Mycobacterium* species, along with hepatoprotective effects on rifampicin-induced HepG2 cells (4). Phenolic extracts from the aerial parts of *Chromolaena odorata* have also been reported to exhibit antioxidant and antifibrillogenic activities, as well as being cytocompatible with human red blood cells and HEK-293 cells (5).

In addition to extracts, the essential oil of *Chromolaena odorata* has also attracted attention due to the diversity of volatile compounds it contains. The chemical profile of the essential oil from the stems and leaves of *Chromolaena odorata* originating from Vietnam shows a predominance of α -pinene, β -pinene, geijerene, germacrene D, and (E)-caryophyllene, with anti-inflammatory, cytotoxic activity against several cancer cell lines, antioxidant, and antidiabetic effects (7). The chemical composition of the essential oil from *Chromolaena odorata* leaves from central Vietnam, dominated by α -pinene, geijerene, germacrene D, and β -caryophyllene, contributes to a wide range of biological activities, such as larvicidal, repellent, fumigant, molluscicidal, anticholinesterase, and antimicrobial effects, thereby demonstrating its potential as a source of natural bioactive compounds (8). Furthermore, the sesquiterpene compound caryophyllene oxide present in the essential oil of *Chromolaena odorata* leaves has been reported to exhibit sedative activity in mice through a central nervous system depressant mechanism (9).

Based on its bioactivity potential and the complexity of its volatile components, the extraction process is a crucial step in obtaining *Chromolaena odorata* essential oil with appropriate chemical characteristics. Various extraction approaches have been developed to improve the efficiency of essential oil yield, one of which is the hydrodistillation method. Hydrodistillation is the most commonly used essential oil extraction method due to its simplicity; however, both the yield and quality of the resulting oil are strongly influenced by several process factors, such as particle size, the ratio of water to plant material, and the addition of electrolytes (10). Several modified methods, such as electromagnetic heating-assisted hydrodistillation, have also been developed to improve the yield and quality of essential oils obtained from various plant materials (11). This method is widely used for essential oil extraction due to its relative simplicity, cost-effectiveness, and ability to extract volatile compounds without the use of organic solvents. In addition, hydrodistillation can produce essential oils of good quality and is more environmentally friendly compared to extraction methods that employ organic solvents (12).

Essential oils obtained through the extraction process subsequently need to be characterized to determine their chemical composition and constituent volatile compounds. Accurate identification of the chemical profile of essential oils requires sensitive and specific analytical techniques. Gas

chromatography–mass spectrometry (GC-MS) is one of the most established and widely used metabolomics techniques for identifying and quantifying both volatile and non-volatile compounds in plants (13)(14). This technique enables comprehensive characterization of essential oil components, both for chemical purposes and for elucidating the basis of the biological activities exhibited by a plant extract.

Although various biological activities of *Chromolaena odorata* have been widely reported, studies on the chemical characteristics of *Chromolaena odorata* leaf essential oil, particularly those obtained through the hydrodistillation method and analyzed using GC-MS, remain relatively limited. This lack of information highlights the importance of characterizing the volatile components of essential oils in order to obtain a more comprehensive understanding of the relationship between their chemical profiles and their bioactivity potential. Therefore, this study aims to profile the chemical constituents of essential oil from *Chromolaena odorata* leaves obtained through hydrodistillation extraction using GC-MS analysis as baseline data to support the exploration of its pharmacological potential and further development of natural product utilization.

METHODS

Hydrodistillation

The extraction of essential oil from *Chromolaena odorata* leaves was carried out using the hydrodistillation method with a Clevenger-type apparatus. The dried and ground leaves of *Chromolaena odorata* were placed into a round-bottom flask, followed by the addition of distilled water until the material was fully submerged. The mixture was heated so that the volatile components in the material were carried along with water vapor into the condenser and subsequently condensed into a distillate. The obtained distillate was collected, after which the essential oil was separated from the hydrosol and stored in a dark bottle for further analysis (15).

Analysis of Essential Oil using GC-MS

The essential oil sample was injected into the GC instrument at the inlet, where it was carried by a carrier gas into the column for separation. The separation of compounds occurs due to different interactions between the mobile phase and the stationary phase within the column. Compounds with lower affinity for the stationary phase will elute first, whereas compounds that interact more strongly will elute at a later time. The separated compounds are subsequently detected, producing a chromatogram. Compound identification is subsequently performed using mass spectrometry through the analysis of mass spectra, retention time (Rt), and molecular weight, thereby enabling the determination of the chemical profile of volatile constituents in *Chromolaena odorata* leaf essential oil (16).

The chemical components of the essential oil were characterized using a gas chromatography–mass spectrometry (GC-MS) instrument (Shimadzu QP2010 SE). The essential oil sample was injected into the gas chromatography system to separate volatile components based on differences in volatility and their interactions with the stationary phase of the column. The separated components were then introduced into the mass spectrometer detector to generate molecular fragmentation patterns.

Compound identification of the essential oil constituents was performed by comparing the obtained mass spectra with reference mass spectral libraries in the GC-MS system. The relative composition of each component was determined based on the percentage peak area (% area) obtained from the chromatogram.

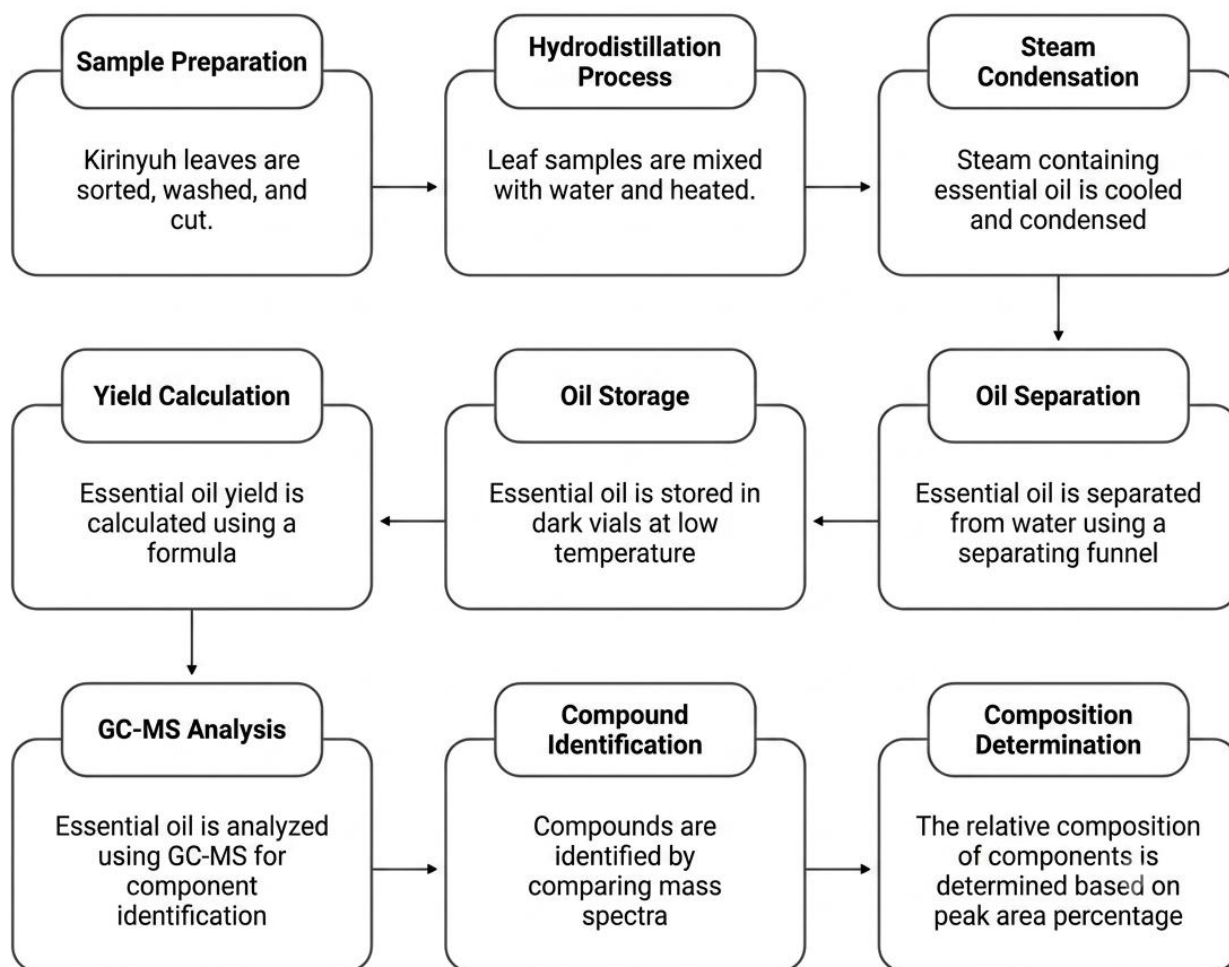


Figure 1. Research Stages Flowchart

RESULTS

GC-MS analysis of *Chromolaena odorata* leaf essential oil revealed that the sample has a complex volatile profile composed of various compounds, including monoterpenes, sesquiterpenes, and other classes of compounds.

Based on the peak distribution in the chromatogram, the essential oil of *Chromolaena odorata* leaves is not dominated by a single major component, but rather by several main constituents with relatively significant contributions. This pattern suggests that the plant's biological activity is likely the result of synergistic interactions among various volatile compounds. This condition is commonly observed in plant essential oils, where pharmacological effects are not determined solely by a single major compound, but rather by interactions among their constituent components.

These results also indicate that the analytical method used is adequate for separating and identifying the major volatile compounds in the sample. The consistency among retention time data, mass spectra, and library matching strengthens the validity of compound identification. Therefore, the obtained GC-MS data can serve as a basis for further studies, both for the isolation of specific compounds and for more targeted biological activity testing of *Chromolaena odorata* leaf essential oil.

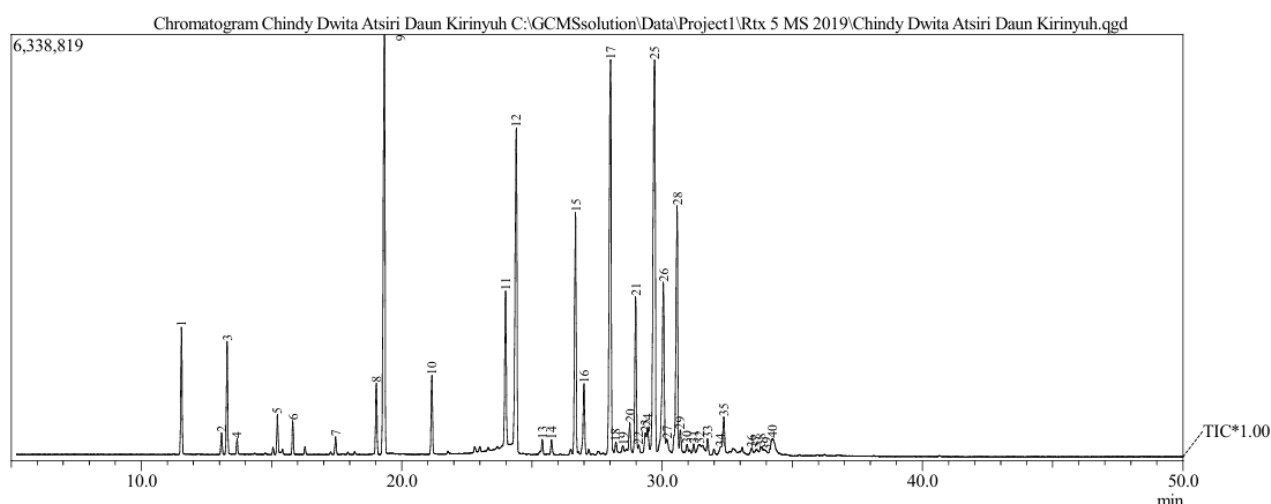


Figure 2. Graph Chromatogram Chromolaena odorata Leave

Table 1. GC-MS analysis results of Chromolaena odorata leaf essential oil

Line#	R.Time	Compound Name	SI	Molecular Formula	Class of compounds
1	11.541	alpha-Pinene (α -PINENE)	97	C ₁₀ H ₁₆	Monoterpene hydrocarbons
2	13.081	Sabinene (SABINENE)	95	C ₁₀ H ₁₆	Monoterpene hydrocarbons
3	13.292	beta-Pinene (α -Pinene)	97	C ₁₀ H ₁₆	Monoterpene hydrocarbons
4	13.677	Myrcene (MYRCENE)	96	C ₁₀ H ₁₆	Monoterpene hydrocarbons
5	15.226	l-Limonene (l-LIMONENE)	95	C ₁₀ H ₁₆	Monoterpene hydrocarbons
6	15.815	trans- β -Ocimene (TRANS-.BETA.-OCIMENE)	95	C ₁₀ H ₁₆	Monoterpene hydrocarbons
7	17.458	1-1,2,3-Trimethylbicyclo[2.2.1]-2-heptanol	95	C ₁₀ H ₁₈ O	Oxygenated monoterpenes (alcohol)
8	19.024	Cyclohexene, 3,4-diethenyl-3-methyl-	92	C ₁₁ H ₁₆	Alicyclic hydrocarbon/non-terpenoid
9	19.334	Cyclohexene, 3,4-diethenyl-3-methyl-	94	C ₁₁ H ₁₆	Alicyclic hydrocarbon/non-terpenoid
10	21.158	Methyl Chavicol (METHYL CHAVICOL / Estragole)	97	C ₁₀ H ₁₂ O	Fenilpropanoid
11	23.988	Benzene, 1-methoxy-4-(1-propenyl)-(trans-Anethole)	96	C ₁₀ H ₁₂ O	Fenilpropanoid
12	24.397	Pregeijerene	92	C ₁₂ H ₁₈	Trisnor-Sesquiterpene
13	25.397	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-	94	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
14	25.750	α -Cubebene (.alpha.-Cubebene)	93	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
15	26.673	Copaene	95	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
16	26.998	β -Elemene (.beta.-elemene)	94	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
17	28.021	trans-Caryophyllene	96	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
18	28.223	Germacrene-D (GERMACRENE-D)	94	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
19	28.487	Alloaromadendrene	91	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
20	28.755	α -Cubebene (.alpha.-Cubebene)	90	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
21	28.984	α -Humulene (α -Caryophyllene)	97	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
22	29.102	Germacrene-D	88	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
23	29.357	δ -Cadinene	83	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
24	29.441	Naphthalene	89	C ₁₅ H ₂₄	Senyawa aromatik
25	29.711	Germacrene-D (GERMACRENE-D)	95	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
26	30.047	Bicyclogermacrene	91	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
27	30.180	Cis-Farnesol	87	C ₁₅ H ₂₆ O	Oxygenated Sesquiterpene (alcohol)
28	30.575	δ -Cadinene (.DELTA.-CADINENE)	96	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
29	30.699	Calamenene	88	C ₁₅ H ₂₂	Sesquiterpene aromatic

30	30.958	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	95	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
31	31.212	Calacorene (CALACORENE) & α-Calacorene (.ALPHA.-CALACORENE)	92	C ₁₃ H ₁₆ / C ₁₅ H ₂₀	Sesquiterpene aromatic
32	31.437	Elemol	93	C ₁₅ H ₂₆ O	Oxygenated Sesquiterpene (alkohol)
33	31.743	Germacrene B	83	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
34	32.233	1H-Cycloprop[e]azulen-7-ol / Spathulenol	78	C ₁₅ H ₂₄ O	Oxygenated Sesquiterpene (alkohol)
35	32.368	Caryophyllene oxide (CARYOPHYLLENE OXIDE)	93	C ₁₅ H ₂₄ O	Oxygenated Sesquiterpene (epoksida)
36	33.433	Diepi-α-cedren I	85	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
37	33.618	Elemol	85	C ₁₅ H ₂₆ O	Oxygenated Sesquiterpene (alkohol)
38	33.799	1-Naphthalenol	86	C ₁₅ H ₂₆ O	Oxygenated aromatic compounds
39	33.992	Sulfuric Acid	79	C ₁₈ H ₃₂ O ₃ S	Inorganic acid
40	34.241	Cyclohexanemethanol	88	C ₁₅ H ₂₆ O	Alicyclic alcohol / non-terpenoid

GC-MS analysis of *Chromolaena odorata* leaf essential oil revealed a complex volatile compound profile dominated by monoterpene and sesquiterpene groups. Several major peaks were detected at retention times of 19.334, 24.397, 28.021, 29.711, and 30.575 minutes, which were identified as Cyclohexene, Pregeijerene, trans-Caryophyllene, Germacrene-D, and δ-Cadinene, respectively.

The presence of these compounds indicates that *Chromolaena odorata* leaf essential oil possesses significant bioactivity potential. Thus, the GC-MS identification results support the use of *Chromolaena odorata* leaves as a natural resource that may be further developed in the pharmaceutical and health fields.

Table 2. GC-MS analysis results of *Chromolaena odorata* leaf essential oil showing major peaks

No	Retention time (minutes).	Identified compounds	Compound
1	19.334	Cyclohexene	Alicyclic hydrocarbon
2	24.397	Pregeijerene	Trisnor-Sesquiterpene
3	28.021	Caryophyllene	Sesquiterpene hydrocarbons
4	29.711	Germacrene-D	Sesquiterpene hydrocarbons
5	30.575	δ-Cadinene	Sesquiterpene hydrocarbons

DISCUSSION

The essential oil of *Chromolaena odorata* leaves was obtained through the hydrodistillation method by boiling the leaf samples in water, allowing volatile compounds to evaporate together with water vapor. The vapor was then condensed into an oil–water mixture, after which the essential oil was separated and stored in a dark glass bottle. This method is widely used due to its simplicity, cost-effectiveness, and lack of requirement for organic solvents, although heat exposure may affect the stability of certain volatile compounds.

The essential oil of *Chromolaena odorata* is subsequently analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) to identify the profile of its constituent volatile compounds. GC-MS analysis produces a chromatogram showing multiple peaks at different retention times, reflecting the presence of various chemical components in *Chromolaena odorata* leaf essential oil. Each peak represents a specific compound separated according to its physicochemical properties within the chromatographic column. Compound identification is carried out based on the mass fragmentation patterns generated by the mass spectrometer, as well as by matching the obtained mass spectra with reference data in the spectral libraries available in the GC-MS software. Through this matching process, the compounds contained in the essential oil can be identified more accurately based on the degree of similarity between their mass spectra and retention times.

Based on the GC-MS analysis of *Chromolaena odorata* (L.) leaf essential oil obtained through the hydrodistillation method, 40 volatile compounds were identified, belonging to monoterpenes, sesquiterpenes, aromatic ethers, and other cyclic compounds. The chromatogram shows multiple peaks within a retention time range of 11–34 minutes, indicating the presence of various constituents of *Chromolaena odorata* leaf essential oil. This diversity of compounds suggests that the essential oil has a complex chemical composition and may exhibit a wide range of biological activities.

For the monoterpene group, the identified compounds include α -pinene (2.63%), sabinene (0.44%), β -pinene (2.39%), myrcene (0.30%), limonene (0.86%), and trans- β -ocimene (0.68%). Monoterpenes are volatile compounds with relatively low molecular weights that contribute to the characteristic aroma of essential oils. α -Pinene and β -pinene are known to exhibit antimicrobial and anti-inflammatory activities, while limonene has been reported to possess antioxidant activity and potential anticancer properties(17). The presence of monoterpene compounds indicates the general characteristics of essential oils derived from aromatic plants(18).

The sesquiterpene group is the most abundant class of compounds found in *Chromolaena odorata* leaf essential oil. The identified compounds include trans-caryophyllene (12.49%), germacrene-D (14.48%), α -copaene (6.24%), bicyclogermacrene (6.00%), α -caryophyllene or humulene (3.93%), α -cubebene (0.35% and 0.90%), calacorene (0.33%), and δ -cadinene (0.69% and 7.34%). Caryophyllene and humulene have been widely reported to exhibit anti-inflammatory, analgesic, antimicrobial, and antioxidant activities(19). In addition, sesquiterpene compounds are known to play a role in the plant's natural defense system against microbial and herbivore attacks. The dominance of sesquiterpenes in *Chromolaena odorata* leaf essential oil indicates its high potential for bioactivity.

In addition to terpenoids, GC-MS analysis also identified a phenylpropanoid compound, methyl chavicol (estragole), at 1.73%. This compound is known to exhibit antimicrobial, antioxidant, and insecticidal activities, thereby potentially contributing to the biological activity of *Chromolaena odorata* leaf essential oil. The presence of methyl chavicol together with various terpenoid compounds indicates that the essential oil has a diverse chemical composition and may produce synergistic pharmacological effects.

GC-MS analysis also revealed the presence of other cyclic compounds, such as Cyclohexene, 3,4-diethenyl-3-methyl- (12.03%), along with several other cyclic compounds in

lower amounts. The presence of these compounds contributes to the chemical characteristics and aroma of *Chromolaena odorata* leaf essential oil. Overall, GC-MS profiling results indicate that the hydrodistilled *Chromolaena odorata* leaf essential oil is composed of monoterpenes, sesquiterpenes, aromatic ethers, and other cyclic compounds, which have potential to be developed as natural resources for pharmaceutical, health, aromatherapy, and antimicrobial applications. The variation in compound composition obtained may be influenced by environmental factors, geographic location, plant age, harvesting time, and the extraction method used.

The GC-MS profile of *Chromolaena odorata* leaves shows a complex mixture of volatile compounds, with a predominance of terpenoid groups. Based on the previously analyzed peak data, the main identified compounds include Cyclohexene, Pregeijerene, β -Caryophyllene, Germacrene-D, and δ -Cadinene. The presence of these compounds indicates that the volatile fraction of *Chromolaena odorata* leaves in the analyzed sample is consistent with essential oils from aromatic plants rich in sesquiterpenes and other compounds(14).

The identification of β -caryophyllene, Germacrene-D, and δ -cadinene further supports the interpretation that this *Chromolaena odorata* leaf sample is rich in bioactive sesquiterpenes. These compounds have been repeatedly reported in the literature on *Chromolaena odorata* from various regions, both as major constituents and as characteristic chemical markers(20). β -Caryophyllene is widely recognized for its anti-inflammatory and antimicrobial activities and its pharmacological synergy with other sesquiterpenes. In this context, the presence of these three compounds is particularly important, as it supports the notion that the bioactivity of *Chromolaena odorata* is not solely dependent on other constituents, but is also strongly influenced by its sesquiterpene fraction.

Trans-anethole, also identified in this dataset, adds an interesting dimension to the chemical interpretation of the sample. This compound is not consistently reported as a dominant constituent in all studies of *Chromolaena odorata*. Therefore, the presence of trans-anethole suggests a possible specific characteristic of the local sample, enriching the chemotaxonomic information of this plant(21).

When compared with the literature, the observed pattern shows good agreement. A recent comprehensive review concluded that *Chromolaena odorata* contains a wide range of volatile compounds(3). Mechanistic reviews have also confirmed that the biological activities of this plant are multicomponent in nature and are highly dependent on the actual phytochemical composition of the extract or essential oil being evaluated (22). Studies on *Chromolaena odorata* leaf essential oil have even demonstrated notable bioactivities, such as sedative, antimicrobial, repellent, larvicidal, and certain enzyme-inhibitory activities, all of which are associated with the presence of major terpenoids and their derivatives(7).

Biologically, the dominance of sesquiterpenes in these results provides a plausible basis for explaining the traditional use of *Chromolaena odorata* as a wound-healing and antimicrobial plant. Sesquiterpenes also contribute to the modulation of inflammation and the regulation of more complex biological responses(20). Thus, the GC-MS results are not merely descriptive but also pharmacologically relevant, as they demonstrate the relationship between compound identity and potential biological activity.

CONCLUSION

GC-MS analysis of the essential oil from *Chromolaena odorata* leaves obtained through hydrodistillation successfully identified 40 volatile compounds belonging to the monoterpene, sesquiterpene, aromatic ether, and other cyclic compound classes. The chemical profile of this essential oil was not dominated by a single major compound, but rather by several major constituents that contributed significantly, including trans-caryophyllene, germacrene-D, δ -cadinene, pregeijerene, and cyclohexene. The sesquiterpene group was the most dominant class

of compounds, known for its anti-inflammatory, analgesic, antimicrobial, and antioxidant activities. These findings indicate that hydrodistillation is an effective method for extracting essential oil from *Chromolaena odorata* leaves, and its terpenoid-rich chemical profile supports its further development as a natural resource for pharmaceutical, health, aromatherapy, and antimicrobial applications.

The presence of these bioactive compounds also provides scientific support that strengthens the traditional use of *Chromolaena odorata* as a wound-healing and antimicrobial agent. The GC-MS data obtained may serve as baseline data for further research, both in the isolation of specific compounds and in more targeted biological activity testing of *Chromolaena odorata* leaf essential oil.

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Conflicts of Interest

The authors declare no conflict of interest related to this study.

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