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PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF ETHANOLIC EXTRACT OF *Chromolaena odorata* LEAVES

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Abstract

Chromolaena odorata leaves are empirically known to act as anti-inflammatory and other pharmacological effects. In this study, ethanol extracts from *Chromolaena odorata* leaves were analyzed using phytochemical screening and gas chromatography-mass spectrometry (GC-MS) to identify phytochemical constituents. Phytochemical tests showed the presence of flavonoids, alkaloids, tannins, saponins, and phenolics. GC-MS analysis of ethanol extracts from *Chromolaena odorata* leaves detected the presence of seven phytochemical compounds, namely Ethyl (E)-4-(2-phenylethylamino)but-2-enoate; 2h-pyran 2-(7-heptadecynyloxy) tetrahydro; 3,7,11-Trimethyldodeca-2,6,10-trienyl acetate; Bicyclo(3.1.1)heptane, 2,6,6-trimethyl-,(1alpha,2beta,5alpha)-11,13-Dimethyl-12-tetradecen-1-yl acetate; (2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol, and 9,12,15-Octadecatrienoic acid. This study identified several constituents that are biologically active compounds through GC-MS. These phytocompounds have potential as herbal medicines due to pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, antibacterial, and anticancer.

Keywords: *Chromolaena odorata* leaves, extract, screening phytochemical, GC-MS, pharmacological activity

INTRODUCTION

The World Health Organization (WHO) defines traditional medicine as the knowledge, skills, and practices that differ across cultures and are used to treat or prevent physical or mental illnesses, based on theories, personal experiences, and beliefs (World Health Organization, 2023). Phytochemicals, also known as active secondary metabolites, are abundant in medicinal plants and are commonly used in traditional medicine (Santi & Candra, 2025). Only 2%, or approximately 350,659 medicinal plant species worldwide, have been scientifically studied, and their phytochemical components and bioactivities beneficial for improving public health are known. With population growth, inadequate drug availability, rising medical costs, side effects of various synthetic drugs, and the emergence of drug resistance, people are turning to nature to utilize plant substances as remedies for various human health problems. Substances derived from natural sources are essential in both traditional and modern medicinal practices (Santi et al., 2025).

WHO has taken appropriate steps in conducting research to discover new and effective therapeutic compounds from plants. Among the many traditionally used plants, *Chromolaena odorata* is known worldwide and has been used for thousands of years for its various therapeutic properties. *Chromolaena odorata* is often used traditionally to treat wounds, fever, malaria, and digestive problems. This plant is used as a hemostatic and anti-inflammatory agent by extracting its juice and applying it to wounds. Furthermore, the antioxidant, antibacterial, and antidiabetic activities of *Chromolaena odorata* leaves have been reported to be related to their secondary metabolites (Santi, Zakaria, et al., 2023). Various studies have identified phytochemical compounds, including flavonoids, terpenoids, and phenolics. Some have documented distinct phytochemical compounds from *Chromolaena odorata* grown in Indonesia. However, testing of the phytochemical compounds of *Chromolaena odorata* in Aceh, particularly in Banda Aceh, remains limited. Furthermore, varying extraction, separation, purification, and quantification methods can impact the identification of phytochemicals from medicinal plants. Furthermore, agroecological conditions significantly influence the phytochemical characteristics of plants from different regions

(Candra et al., 2024; Santi, Siregar, et al., 2023; Santi & Candra, 2024). This study aimed to determine the secondary metabolites from ethanol extracts of *Chromolaena odorata* leaves through qualitative phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

LITERATURE REVIEW

Indonesia is a highly biodiverse nation, home to 30,000 plant species distributed across its land, placing it second globally, just after Brazil. Many plants are recognized for their diverse advantages, with around 1,000 species exhibiting therapeutic traits (Candra et al., 2023; Candra & Santi, 2017; Santi, 2025a). Phytocompounds, bioactive substances obtained from plants, have gained prominence in both traditional and contemporary medicine because of their various healing attributes. Natural substances like alkaloids, flavonoids, terpenoids, tannins, and phenolic acids provide various pharmacological effects, such as anti-inflammatory, antioxidant, antimicrobial, anticancer, and cardioprotective activities. The healing potential of these plant compounds is still being investigated, resulting in the creation of new medications and therapies that utilize their inherent properties. This expanding research highlights the significance of phytocompounds in enhancing healthcare and presents a hopeful path for finding new therapeutic agents (Nelly et al., 2024; Santi, 2015; Santi et al., 2022).

Investigating the active compounds in *Chromolaena odorata* is essential to furnish scientific data about its traditional applications as an anti-inflammatory, wound healing, antidiabetic, antioxidant, antibacterial, anticancer, and hepatoprotective agent. Numerous studies have found chemical substances in papaya leaves, such as saponins, tannins, alkaloids, flavonoids and glycosides, anthraquinones, steroids, and resins (Candra et al., 2025; Santi, Siregar, et al., 2023). Additional studies on the chemical constituents in *Chromolaena odorata* leaves have been carried out to pinpoint active chemical substances, employing both straightforward phytochemical analysis and Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS technique is commonly employed for analyzing medicinal plants, as it can effectively separate compounds that are present together in low concentrations. Gas chromatography is a method for separating the elements of a mixture over time, whereas a mass spectrometer determines the composition of those elements. The beneficial blend of gas chromatography's rapidity, sensitivity, and resolution, alongside the mass spectrometer's superior selectivity for molecule analysis, provides insights into their chemical weight and structure. The exceptional separation abilities of GC-MS generate high-quality chemical fingerprints that are very beneficial in clarifying the connection between the chemical components of herbal plants and their pharmacological impacts (Santi et al., 2025; Santi & Candra, 2023; Tahara et al., 2024).

Prior studies have discovered numerous active substances in the leaves of herbal plants via GC-MS analysis. Earlier studies employing GC-MS have discovered leaf chemical constituents such as kaempferol, 5,7-dimethoxycoumarin, flavonoids, protocatechuic acid, caffeic acid, chlorogenic acid, p-coumaric acid, and coumarin. Other research indicates that GC-MS analysis unveiled a range of chemical compounds, such as tetramethyl-2-hexadecen, stigmasterol, oleic acid, sitosterol, tocopherol, butyl 9,12,15-octadecatrienoate, n-hexadecanoic acid. The other research, GC-MS analysis of *Chromolaena odorata* leaves showed the detection of pregeijerene, germacrene D, alpha-pinene, beta-caryophyllene, vestitenone, beta-pinene, delta-cadinene, geijerene, bulnesol, and trans-ocimene, vitamin E, oleic acid, (2R, 3S)-2, 3-Dimethylmalate, choleste-5-en-3-one, and phytol (Eze & Jayeoye, 2021; Olawale et al., 2022).

Numerous studies have identified differences in secondary metabolites, probably due to the plant's growth environment and factors like humidity, pH, temperature, and the soil's chemical and physical characteristics (Candra et al., 2024). This research focuses on identifying the active compounds in *Chromolaena odorata* leaf extract through phytochemical tests and GC-MS analysis, as there remains insufficient information about the active compounds in Banda Aceh. This study's uniqueness stems from the first investigation into the chemical makeup of *Chromolaena odorata* leaves, a topic that has not received extensive attention prior. Employing GC-MS, this research offers an in-depth examination of the phytochemical profile of the plant, uncovering a diverse array of bioactive compounds and emphasizing the plant's potential for medicinal uses. Consequently, this study acts as a foundational analysis for additional exploration into the therapeutic possibilities of *Chromolaena odorata*.

METHOD

Research Design and Chemicals

This research employed qualitative techniques, specifically GC-MS for identifying phytochemical compounds. The chemicals used for this study were analytical grade.

Leaf Sample Collection

Chromolaena odorata leaves were collected on January 20, 2026, from the banks of the Krueng Aceh River, Banda Aceh. Leaf selection was based on the following criteria: green, not too young or old, unblemished, free from caterpillars or other pests, and free from mold. The harvested leaves were then wet sorted to remove impurities such as gravel, leaves from other plants, and even soil introduced during harvesting. The leaves were then cleaned by washing them under running water until clean. The next step was to chop the leaves into smaller pieces to facilitate the drying process. The leaves were dried in a place away from direct sunlight to ensure even drying.

Sample Preparation and Extraction

Dried *Chromolaena odorata* leaves (1000 grams) were ground into a fine powder using a blender. *Chromolaena odorata* leaf extract was prepared using a maceration method using 96% alcohol. The crushed leaves were placed in a glass bottle and ethanol was added at a 1:5 ratio, stirring until thoroughly mixed. The mixture was then left for three days, stirring occasionally, until evenly distributed. After three days, the extract was filtered using a glass funnel lined with filter paper. The filtrate was collected in a sample bottle, and the leaf pulp was soaked again in 96% ethanol for two days, also stirring during this time. Filtration was carried out after two days, and the filtrate was added to the sample bottle. The extract was passed through Whatman No. 41 filter paper and concentrated at 60ºC under vacuum with a rotary evaporator to remove the ethanol.

Examination of Qualitative Phytochemical Compounds

Ethanol extracts were employed to qualitatively identify different secondary metabolites through standard procedures. Employing a standard analytical method, initial qualitative phytochemical analysis was performed to assess the presence or absence of secondary metabolites in ethanol leaf extracts. For qualitative phytochemical analysis, 0.5 g of plant extracts was mixed with 100 mL of ethanol to create a stock solution concentration of 5 mg/mL.

Test for Alkaloids

The extract was placed into a test tube containing about 2 mL. Wagner's reagent was used, then 2 mL of diluted 1% HCL was added, and the mixture was incubated for 15 minutes. Alkaloids are indicated by the formation of a reddish-brown precipitate. A stock solution of Wagner's reagent was prepared by mixing iodine (1.3 g) and potassium iodide (2.0 g) in 100 mL of distilled water. The blend was stored in an amber container.

Test for Tannins

A test tube with 5 mL of extract was added a few drops of freshly prepared 10% lead acetate. A yellow sediment indicates the presence of tannins. Ten milligrams of lead acetate were mixed with 100 mL of distilled water to create a 10% solution. A lead acetate test was utilized for this procedure.

Test for Saponin

The extract was mixed with distilled water (1:10 mL) and vigorously shaken for 30 seconds prior to being left at room temperature. After 30 minutes, a foam resembling honeycomb on the surface shows the presence of saponins.

Test for Flavonoid

Two milliliters (2 mL) of dilute NaOH were incorporated into 3 mL of extract in test tubes. Flavonoids are evident when a vivid yellow hue appears. 4.25 g of NaOH was mixed in 50 mL of distilled water to create the diluted NaOH solution.

Test for Phenolic Compounds

Three drops of this mixture were incorporated into 3 mL of each extract, together with a combination of 10% ferric chloride and 10% ferrocyanide. The orange-brown hue of the precipitate signifies the existence of phenolics.

Ten milligrams of ferric chloride and ten milligrams of ferrocyanide were mixed in 100 mL of distilled water to prepare a working solution, and a test for ferric chloride was conducted.

GC-MS Analysis

GC-MS is essential for analyzing unidentified components obtained from plants. GC-MS analysis of *Chromolaena odorata* extract was conducted using an Agilent Technologies GC system model GC-7890A/MS-5975C (Agilent Technologies, Santa Clara, USA) fitted with an HP-5MS column (30 m length × 250 µm diameter × 0.25 µm film thickness). The spectroscopic detection through GC-MS utilized an electron ionization method employing high-energy electrons (70 eV). Helium gas (99.995%) was utilized as the carrier gas at a flow rate of 1 mL/min. The starting temperature was established between 50 and 150 °C, with a ramp rate of 3 °C/min and a duration of about 10 minutes. Ultimately, the temperature was raised to 300 °C at a pace of 10 °C/min. A microliter of 1% extract diluted with each solvent was injected in splitless mode. The proportions of chemical compounds found in each *Chromolaena odorata* extract were represented as percentages derived from the peak areas observed in the chromatogram. Mass spectra from GC-MS were analyzed with the National Institute of Standards and Technology (NIST) database, holding more than 62,000 patterns. Unknown component spectra were matched against the known component spectra archived in the NIST library. The components of the test material were identified in terms of their names, molecular weights, and structures, and the findings were organized into a table.

RESULTS AND DISCUSSION

Taxonomy and Morphology

The taxonomic assessment performed at the Biology Laboratory of Syiah Kuala University in Banda Aceh recognized *Chromolaena odorata* (Table 1). Taxonomic identification was considered essential to validate the correctness of the plant's traits.

Table 1. Taxonomy of *Chromolaena odorata*

Domain	Flowering plant
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	Chromolaena
Species	*Chromolaena odorata*

Each plant has unique morphological characteristics, particularly in leaf shape. In fact, variations in leaf shape are often referred to as "fingerprints" because they are so specific and serve as a key tool for botanists in identifying plant species. Leaf shape of *Chromolaena odorata* findings showed that the leaves are oval, measuring 6-10 cm in length and 3-6 cm in width at the base (Figure 1).

Figure 1. *Chromolaena odorata* leaves

Phytochemical Screening Test

The qualitative evaluation of various phytochemical constituents in ethanolic extracts of *Chromolaena odorata* leaves was conducted and is presented in Table 2. Plants offer an abundant supply of bioactive compounds (flavonoids, alkaloids, sponins, and tannins) that can be beneficial and can be used to develop new chemotherapeutic drugs. In both conventional and contemporary medicine, they are utilized to treat numerous diseases and control different pathogenic organisms. Scientists globally are investigating the application of pharmacologically active compounds derived from herbal plants. Herbal remedies are utilized by 80% of the global population because of their effectiveness, affordability, non-addictive properties, and absence of adverse effects. Phytochemical substances are categorized as secondary metabolites that are found naturally in plants. So far, significant advancements have been achieved in the detection and functional analysis of bioactive compounds relevant to medicine (Santi, Siregar, et al., 2023).

Table 2. Phytochemical analysis of *Chromolaena odorata* leaves

Phytochemical constituents	Result
Flavonoids	Present
Alkaloids	Present
Saponins	Present
Tannins	Present
Phenolics	Present

Qualitative screening plays a crucial role in identifying the phytochemical compounds found in herbal plants. This process is an easy initial requirement before conducting an in-depth phytochemical analysis. Using this approach, we detection of flavonoids, alkaloids, phenolics, saponins, and tannins in *Chromolaena odorata* leaf extract verifies the medicinal significance of this plant. Alkaloids possess antibacterial, antiviral, anticancer, antifungal, and antimalarial effects, whereas saponins exhibit insecticidal, anthelmintic, anticancer, antiviral, antibacterial, and antifungal effects. Likewise, flavonoids possess antibacterial, anti-aging, antiallergic, anti-inflammatory, anticancer, and antiviral effects, whereas phenolic compounds exhibit antioxidant and antibacterial characteristics (Santi et al., 2022).

Table 1 shows that *Chromolaena odorata* leaves contain active biochemicals such as alkaloids, flavonoids, phenols, tannins, and saponins. Magar's research reported that phytochemical tests on *Chromolaena. odorata* leaves found flavonoids, alkaloids, tannins, and phenolics. However, no saponins were found. Meanwhile, Ishak's research only found tannins and alkaloids, except for the presence of saponins, flavonoids, or phenols. The varying results from these studies may be due to factors such as soil type, chemical, and physical properties, climate conditions, and the identification techniques used (Candra et al., 2024; Ishak et al., 2023; Magar et al., 2023). On the other hand, our findings revealed the presence of alkaloids, flavonoids, saponins, tannins, and phenolics. The efficiency of solvents in isolating phytochemicals serves as a preliminary signal of medicinal qualities, and the existence of bioactive compounds in *Chromolaena odorata* confirms the medicinal importance of this plant. Alkaloids exhibit antibacterial, antiviral, anticancer, antifungal, and antiprotozoal characteristics, whereas saponins demonstrate insect-repellent, antiparasitic, anticancer, antiviral, antibacterial, and antifungal characteristics. Likewise, flavonoids possess antibacterial, anti-aging, anti-allergic, anti-inflammatory, anticancer, and antiviral properties, whereas phenolic compounds exhibit antioxidant and antibacterial effects (Candra et al., 2025; Santi, Siregar, et al., 2023).

GC-MS Analysis of *Chromolaena odorata* Leaves

GC-MS examination of the ethanol extract from *Chromolaena odorata* leaves produced seven chemical compounds. The three compounds with the greatest percentage content were Ethyl (*E*)-4-(2-phenylethylamino)but-2-enoate (28.88%), 2h-pyran 2-(7-heptadecynyloxy) tetrahydro (6.67%), and 3,7,11-Trimethyldodeca-2,6,10-trienyl acetate (4.31%). A comprehensive tabulation of GC-MS analysis for ethanol extracts is presented in Table 3. GC-MS analysis represents an initial step in comprehending the characteristics of active principles in medicinal plants and identifying if a plant species contains specific compounds or groups of compounds. GC-MS spectral analyses validate the existence of key components through their retention times. The heights of the peaks reflect the relative amounts of the components found in the extract. The phytoconstituents are characterized and identified by comparing the mass spectra of the components with the NIST library.

Table 3. GC-MS analysis of *Chromolaena odorata* leaves

RT	Name of the Compounds	Molecul Formula	MW (g/mol)	% Peak Area	Pharmacological Effect
33.582	Ethyl (*E*)-4-(2-phenylethylamino) but-2-enoate	$C_{19}H_{26}O_2$	284.41	28.88	Antioxidant, antimicrobial effect, breaking down bacterial resistance
30.989	2h-pyran 2-(7-heptadecynyloxy) tetrahydro	$C_{22}H_{40}O_2$	336.56	6.67	Anti-inflammatory, radical-scavenging activity
32.375	3,7,11-Trimethyldodeca-2,6,10-trienyl acetate	$C_{17}H_{28}O_2$	264.4	4.31	antioxidant, anti-inflammatory, antimicrobial properties, along with possible chemopreventive (anti-cancer) effects
31.292	Bicyclo(3.1.1)heptane, 2,6,6-trimethyl-, (1alpha,2beta,5alpha)-	$C_{10}H_{18}$	138.25	3.92	Anti-inflammatory properties, antimicrobial properties, antitumor potential
30.506	11,13-Dimethyl-12-tetradecen-1-yl acetate	$C_{18}H_{34}O_2$	282.5	3.6	Antimicrobial properties, antioxidant
28.603	(2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	3.47	Healing of wounds, antioxidant properties, pain-relief, antimicrobial effects, anti-inflammatory, diuretic, and anticancer.
29.210	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278.4	3.36	Antioxidant, anti-inflammatory, antimicrobial

In this research, the preliminary evaluation of secondary metabolites from leaf extracts of *Chromolaena odorata* was conducted using spectrometry and chromatography techniques. GC–MS is utilized to identify functional groups and locate diverse bioactive therapeutic compounds present in medicinal plants, offering the benefit of being among the most efficient and precise methods for detecting numerous compounds, such as alcohols, alkaloids, nitro compounds, long-chain hydrocarbons, organic acids, steroids, esters, and amino acids, while only a small quantity of plant extract. consequently, this research utilized the GC–MS method to identify and detect phytochemical compounds present in the leaves of *Chromolaena odorata* (Santi et al., 2022; Santi, Siregar, et al., 2023).

Chromolaena odorata contains seven organic compounds. Compounds 9,12,15-octadecatrienoic acid; Ethyl (E)-4-(2-phenylethylamino) but-2-enoate are grouped into fatty acids and esters, which are useful as primary energy providers during cell growth. Furthermore, (2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol; Bicyclo(3.1.1)heptane, 2,6,6-trimethyl-, (1alpha,2beta,5alpha)- are grouped into terpenes and terpenoids, which play important roles in various biological properties such as anti-inflammatory, anticancer, antioxidant, diuretic, anti-allergic, immunostimulant, anti-trypanosomal, antimicrobial activity, and cholesterol-lowering effects (Santi, 2025b; Santi, Siregar, et al., 2023; Santi, Zakaria, et al., 2023). Due to the presence of these essential components, the ethanol extracts of *Chromolaena odorata* may possess significant therapeutic value.

CONCLUSION

From the findings of this study, it can be inferred that the biological functions of the recognized phytocomponents serve anti-microbial, anti-inflammatory, and anti-cancer purposes. Consequently, Chromolaena odorata is suggested as a source of phytopharmaceutical worth.

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