

**Analysis Qualitative of Bioactive Compounds of Chromolaena odorata Leaves from Aceh Besar District**

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**Abstract**

*Chromolaena odorata* leaves have been used by the people of Aceh Besar as herbal medicine. The chemical compounds in the leaves of this plant act as antioxidants, anti-inflammatories, antibacterials and wound healers. The research objective was to identify phytochemical compounds of *C. odorata* leaves. The extraction method used is maceration and GC-MS analysis. The results showed that the yield percentage was 6.05%. The results of the analysis of phytochemical compounds obtained seven phytochemical compounds contained in *C. odorata*, namely compounds belonging to the class of flavonoids, phenolics, alkaloids, terpenoids, saponins, steroids and tannins. GC-MS results of *C. odorata* leaves have twenty metabolite secondary and divided into four groups (fatty acids and esters, terpenes and terpenoids, alcohols and phenols, steroids). It is recommended to be used this leaves as a natural pharmaceutical agent, particularly in the treatment of wounds. In the future, more research into biological characterization, isolation methods, and commercial studies as therapeutic possibilities should be planned.

**Keywords:** *C. odorata*, Rendemen, Maceration, GC-MS

**Introduction**

Indonesia has 9,600 reserves of medicinal plant germplasm that have the potential to be developed into medicinal plants. The use of medicinal plants from generation to generation is supported by the development of methods to determine the content of chemical compounds that can be used as medicine and cure diseases. The use of traditional medicine is traditionally considered safer and more affordable (Santi, 2022).

One of the plants used as medicine is kirinyuh (*C. odorata*). The leaves of this plant are often used by people in Aceh Besar as a wound medicine and antioxidant. This plant is cosmopolitan and can be found anywhere so it is very easy to get it when needed. The pharmacological effects obtained come from the chemical components contained in the leaves of the plant. Research on the phytochemical analysis of *C. odorata* leaves from Aceh Besar has not been carried out much. To study scientifically the important compounds found in *C. odorata* leaves, it is necessary to conduct research on qualitative analysis of the content of secondary metabolites in Aceh Besar. In addition, this study also examined the yield of *C. odorata* extract obtained from

maceration results. Determination of the yield is very important because in the process of preparing the drug, it must go through the extraction stage which will produce sufficient extract for the manufacture of drug products.

Based on the description above, this study aims to determine the yield of the ethanol extract of the leaves and to analyze the chemical components of the leaves of *C. odorata* qualitatively with GC-MS analysis. The GC-MS method was chosen because it is able to separate compounds in low concentrations. The combination of gas chromatography and mass spectrometry produces high quality chemical fingerprints (Santi, 2023)

**Methods**

*Materials*

The materials used in this study were *C. odorata* leaves, 70% ethanol, Mg, H<sub>2</sub>SO<sub>4</sub>, NaOH, mayer, wagner, dragendorf, aquades, Lieberman bouchart.

*Research procedure*

*Collection and preparation of plants material*



The research sample was *C. odorata* leaves obtained from the Ie Seum area of Aceh Besar district. The wet sorting process was carried out to separate the leaf samples from the impurities attached to the leaves. The next process is washing fresh leaves using running water and draining the water before drying the leaves at room temperature (36°C). The next stage is pollination of dry leaf samples.

#### Sample extraction

The extraction used is maceration. This process begins by putting 500 grams of *C. odorata* leaf powder into the maceration container, adding 1500 mL of 70% ethanol solvent. Soaking was carried out for three days and every day stirring was carried out and storage protected from sunlight. On the third day, it was filtered using filter paper and then re-macerated 2 times. The filtrate produced from this process was concentrated using a rotary evaporator at 40°C. The maserate which is still mixed with ethanol is heated over a waterbatch to get a thick extract.

#### Determination of leaf extract yield

The yield of *C. odorata* leaf extract was calculated using the formula:

$$\% \text{ leaf extract yield} = \frac{\text{weight of extract}}{\text{weight of dry}} \times 100$$

#### Phytochemical sceening

Phytochemical analysis was carried out by color test which included testing for flavonoids, alkaloids, tannins, saponins, terpenoids, steroids based on Harborne's method (1996).

#### GC-MS Analysis

Identification *C. odorata* metabolites by gas chromatography (Agilent Technologies 7890 Gas Chromatograph, California, USA) and Agilent 7890/5975 capillary column. GCMS has a film dimension with a length of 30 mm, a width of 0.2 mm and a height of 0.1 m with an ionization energy of 70eV through the electron collision mode (22). After injecting helium (1.2 mL/min.)

into the system, extract (5 L; 8:1 ratio) was injected. Constant temperatures for the ion and injector sources were established

at 230°C and 250°C, respectively. The oven was pre-heated to 80°C, with a thermal speed of 80-150°C/min, and then increased to 20-280°C/min increments. It was a term analysis.

The phytochemical spectrum of *C. odorata* leaves was compared with the mass spectrum listed in the W8NO8I database, available at <https://chemdata.nist.gov>. The presence of twenty phytochemicals was confirmed by peak (%), retention time (RT), molecular weight (MW). Compounds contained in the leaves of *C. odorata* can contribute to therapeutics (Sermakkani, 2011).

## Results

#### Leaf extract yield

The yield percentage of *C. odorata* leaf extract produced from 500 grams of dried simplicia was 4.96%.

#### Phytochemical screening

Identification of groups of chemical compounds that have potential as antioxidants, antibacterials and anti-inflammatories was carried out qualitatively based on the solubility properties of the compounds. The results of the color test analysis showed that the leaves of this plant contain flavonoids, alkaloids, saponins, phenols, terpenoids, saponins and tannins (Table 1).

#### GC-MS Analysis

GC-MS investigation of *C. odorata* leaves revealed the presence of twenty chemicals (Figure 1 and Table 2).

## Discussion

The extraction process used in this study is maceration which is a simple technique that allows ethanol to penetrate plant cell walls and enter the cell cavities containing active substances. This substance will later leave the cell due to differences in environmental concentrations inside and outside the cell (Wahyulianingsih et al, 2016).

The yield is the ratio of the amount of *C. odorata* leaf extract produced from the maceration process to the weight of the dry extract/simplicia. The yield value describes the number of bioactive compounds contained in the sample and has a positive correlation with pharmacological effects including anti-inflammatory and antibacterial.

**Table 1.** Results of the sample phytochemical screening of *C. odorata*

Compound	Reagen Test	Color Test	Conclusion
Flavonoid	HCL + Mg	Red/orange	+
Saponin	H <sub>2</sub> O	foam is formed	+
Tannin	Gelatin + H <sub>2</sub> SO <sub>4</sub>	Milky white precipitate	+
Phenolic	FeCl <sub>3</sub>	Blue green to black	+
Alkaloid	Wagner, Mayer, Dragendorff	Precipitate and reddish orange color	+
Steroid	Liebermann-Burchard	Green dark	+
Terpenoid		Brownish red	+

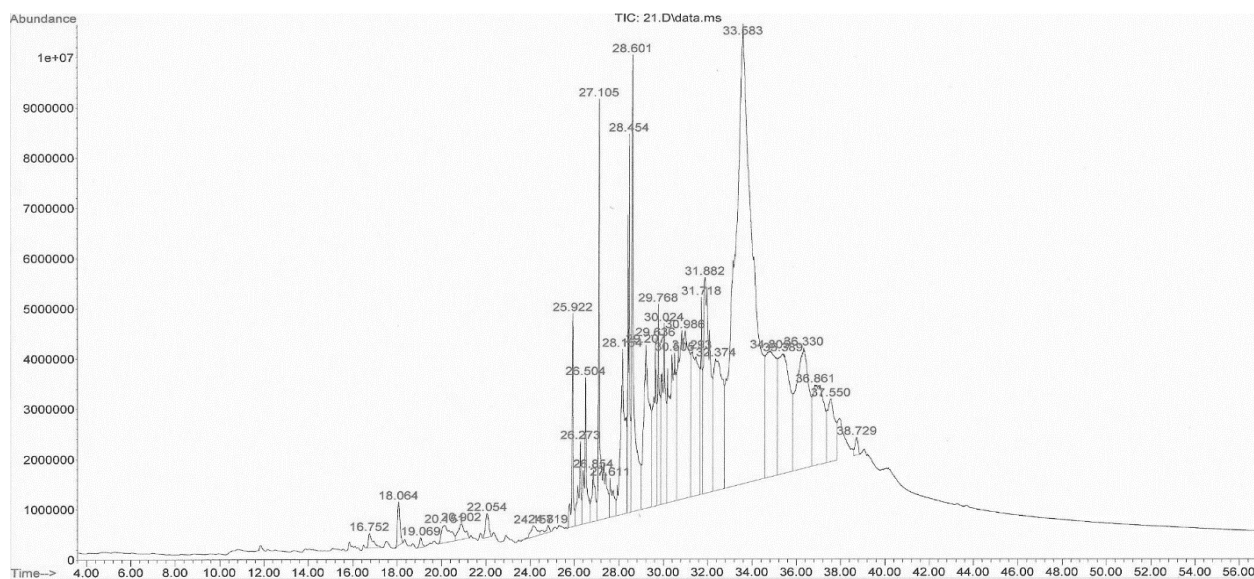


Figure 1. GC-MS chromatogram of *C. odorata*

Table 2. Phytochemicals identified in the *C. odorata*

Retention Time	Name	Molecular formula	Chemical group of compounds	Molecular Weight	Area %	Activity
27.107	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	fatty acids	270	2.55	Antibacterial, antifungal, antioxidant, pesticide, antiviral, anti-inflammatory, prevention and therapy for many disease (6)
28.155	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	fatty acids	256	2.65	Anti-inflammatory antioxidant, lubricant, hypocholesterolemic nematocidal, antiandrogenic flavor, pesticide, hemolytic 9.5-alpha reductase inhibitor potent mosquito larvicide (Thi, 2011)
28.451	9,12,15-Octadecatrienoic acid, methyl ester	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	fatty acids	292	2.05	Anti-inflammatory, antihistaminic, nematocidal, insectifuge, antieczemic, 5-alpha reductase-inhibitor, antiandrogenic, anticoronary, antiarthritic, antiacne, hepatoprotective hypocholesterolemic, cancer preventive (Zaman, 2020)
28.603	Phytol	C <sub>20</sub> H <sub>40</sub> O	terpene	296	3.47	Antioxidant, antinociceptive, antimicrobial, anti-inflammatory, diuretic and anticancer (Pejin, 2014)
29.210	9,12,15 Octadecatrienoic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	fatty acids	278	3.36	Anti-inflammatory, antioxidant, cancer preventive, hypocholesterolemic, hepatoprotective, anticoronary, insectifuge, nematocidal, antihistaminic, antiacne, 5-AlphaReductase-inhibitor, antieczemic, antiandrogenic (de Alencar, 2019)
29.637	Methyl eicosanoate	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	fatty acids	326	1.78	Antioxidant (Taiwo, 2008)
29.768	E,E-10,12-Hexadecadien-1-ol	C <sub>16</sub> H <sub>30</sub> O	terpene	238	1.47	Cox-1 inhibitor (Swetlin, 2020)
30.506	11,13-Dimethyl-12-tetradecen-1-ol acetate	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	terpene	282	3.60	Antioxidant (Santos, 2013; Zaman, 2020)
30.989	2-(7-heptadecyl yloxy) tetrahydro-2h-pyran	C <sub>22</sub> H <sub>40</sub> O <sub>2</sub>	flavonoid	336	6.67	No activity reported
31.292	Bicyclo [3.1.1] heptane, 2,6,6-trimethyl, [1R-(1.alpha.,2.alpha.,5.alpha.)]-	C <sub>10</sub> H <sub>18</sub>	terpene	138	3.92	Anti-tumor, chemo-preventive, hypocholesterolemic, sedative, analgesic, antibacterial, antiacne, anti-inflammatory, fungicide, insecticide, pesticide (Carvalho, 2020)
31.720	L-histidine amide, 5-oxo-l-	C <sub>11</sub> H <sub>16</sub> N <sub>4</sub> O <sub>5</sub>	fatty acids	155	1.17	Infertility, hyperplastic endometrial processes,

	prolyl-					endometriosis, and oncological diseases (Narsimha, 2015)
31.885	5-hydroxy-7-methoxy-2-(4-methoxyphenyl)-2,3-dihydro-4h-chromen-4-one	C <sub>17</sub> H <sub>16</sub> O <sub>5</sub>	flavonoid	300	4.99	Antimicrobial, antifungal (Mangoyi, 2015; Zahidah, 2017)
32.375	Farnesyl acetate	C <sub>17</sub> H <sub>28</sub> O <sub>2</sub>	terpene	264	4.91	Antimicrobial, antifungal (Kuz'mina, 2018)
33.582	Ethyl (e)-4-[(e)-2-phenylethenyl]-2-nonenoate	C <sub>19</sub> H <sub>26</sub> O <sub>2</sub>	fatty acids	176	28.88	No activity reported
34.802	2-(3-furyl)-6a, 10b-dimethyl-1-4a,5,6,6a,10,10a,10b-octahydro-2h-benzo[f]isochromene-4,7-dione	C <sub>19</sub> H <sub>22</sub> O <sub>6</sub>	fatty acids	346	4.74	No activity reported
35.388	Silane, dimethyl (3-methylphenoxy) ethoxy-	C <sub>11</sub> H <sub>18</sub> O <sub>2</sub> Si	fatty acids	210	5.12	No activity reported
36.333	Spiro [cyclopropane-1,2'(1'h)-phenanthrene]-1'4'(3'h)-dione,4'b,5',6',7',8',8'a,9',10'-octa hydroxy-2,4'b,7'-trimethyl-8-methylene-,[2's-2'.alpha.(r*), 3'.alpha., 4'b.beta., 7'beta., 8'a.alpha.,9'.beta., 10'.alpha.]]	C <sub>24</sub> H <sub>32</sub> O <sub>8</sub>	steroid	590	5.60	No activity reported
36.864	5,6,7-trimethoxy-2-(4-methoxyphenyl) chromen-4-one	C <sub>19</sub> H <sub>18</sub> O <sub>7</sub>	Fatty acids	342	3.30	Blood clotting factor activities, enhanced blood coagulation, modulate bacterial drug resistance (38)
37.553	Pyrano [2,3-c] pyrazol-6(1h)-one,1-(6-methoxy-2-benzo-thiazolyl)-3,4-dimethyl-[20]	C <sub>16</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub>	phenolic	240	1.65	No activity reported

Based on the yield of *C. odorata* leaf extract of 4.96%, it assumes that the yield produced is very small so that to produce the required extract requires a large number of samples. These results are in line with the study of Santi et al (2023) which explained that extracts with polar solvents can dissolve monosaccharides and oligosaccharides but not in non-polar solvents. This is supported by the research of Zhang et al (2011) who obtained an increasing yield with the addition of sample material and the amount of solvent. The yield of the extraction results will increase with the assumption that there is contact between the material matrix and the solvent so that it facilitates the penetration process into the material matrix and dissolves the target compound.

Phytochemical tests aim to identify secondary metabolites found in plant leaves. Based on qualitative tests, *C. odorata* leaf extract contains flavonoids, tannins, alkaloids, terpenoids, steroids and saponins.

A positive reaction to the presence of flavonoids is indicated by a change in the color of the solution to red or orange. This is due to the addition of magnesium powder or hydrochloric acid which forms H<sub>2</sub> bubbles which reduces the benzopiron nucleus in flavonoid compounds which causes the formation of red to orange colors (Setyowati et al, 2014).

In several developing countries, medicinal plants are becoming more important in basic health care for individuals and

communities. In several developing countries, medicinal plants are becoming more important in basic health care for individuals and communities. Secondary metabolism in plants is critical for the plant's survival in its environment. Furthermore, these substances may be responsible for the leaves' positive benefits on a variety of health-related indicators. Saponins, tannins, alkaloids, flavonoids, sesquiterpenes lactones, terpenoids, and phorbol esters are some of the elements that give plants their therapeutic characteristics. Some of them are synergistic, meaning they boost the bioactivity of other substances. Within a decade, a number of significant breakthroughs in analytical techniques, such as GC-MS, were made, making them potent tools for phytochemical separation, identification, and structure determination (Santi, 2015; Candra, 2017).

In Table 2, each active compound has a retention time (RT), molecular formula, chemical structure, molecular weight (MW), concentration (percent), and activity. These compounds are a source of information to identify new herbal medicines. GC-MS results *C.odorata* leaves were divided into four groups (fatty acids and esters, terpenes and terpenoids, alcohols and phenols, steroids). The fatty acid and ester groups consist of six compounds. Previous studies mentioned the function of fatty acids as the main energy provider during cell growth. In another study, fatty acids (n-hexadecanoic) in lemon balm leaves have antioxidant and alpha-reductase, hypocholesterolemic, and hemolytic activities. as well as other studies mention fatty acids (n-hexadecanoic) in *Aloe vera* leaves, and sidondo as an anti-

inflammatory, antioxidant, and antimicrobial (Essien, 2020; Sharafzadeh, 2011; Alrumman, 2018; Altemimi, 2017).

The terpenes and terpenoids group consists of four compounds, one of which is phytol. Previous research has stated that this compound plays a role in various biological properties. In addition, other studies have stated that phytol has anti-cancer, anti-inflammatory, antioxidant, diuretic, anti-allergic, immunostimulant activity. Another study that phytol decreases myeloperoxidase (MPO), as an anti-inflammatory, releases proinflammatory cytokines. Phytol is reduced interleukin (IL)-6, inhibits hyperalgesia, spinal COX-2 immunocent, reduces p38MAPK expression, and increased NFκB activity (Carvalho, 2020; Santos, 2013).

The alcohol and phenolic compounds identified in the *C. odorata* leaves are pirano[2,3-c]pyrazole-6 (1h)-one, and 1-(6-methoxy-2-benzothiazolyl)-3,4-dimethyl-. Previous studies have shown that pyrazole has no known specific function, but it inhibits the synthesis of interleukin IL-1-hijacked human immunodeficiency virus (HIV)-1 and has antibacterial, antihyperglycemic, anti-inflammatory, sedative/hypnotic, analgesic and antipyretic, and anti-cancer activities. The steroids in the EECL include spiro [cyclopropane-1,2'(1'h)-phenanthrene]-1'4' (3'h)-. compounds with biological activity related to antioxidant, anti-inflammatory, and antibacterial activities (Santos, 2013).

## Conclusion

In general, the results of this study show that GC-MS analysis identified 20 compounds. Some compounds in *C. odorata* are phytol (diterpenoid group), Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-,]1R-1.alpha.,2.alpha., 5.alpha.)]-( terpenes group), n-Hexadecanoic acid, 9,12,15 Octadecatrienoic acid (fatty acid group), Hexadecanoic acid, methyl ester (ester group) have wound healing and anti-inflammatory effect. Overall, *C. odorata* has the potential to be used as a natural pharmaceutical agent, particularly in the treatment of wounds. In the future, more research into biological characterization, isolation methods, and commercial studies as therapeutic possibilities should be planned. These compounds influence the physico-chemical soil properties in the le Seum.

## Conflict of Interest

No potential competing interest was reported by the authors.

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## Author contribution

Tahara responsibility for the manuscript submitted to Journal of Agromedicine. Aditya responsibility for read and revision the manuscript.

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