

IDENTIFICATION OF CHEMICAL COMPOUNDS AND ANTIBACTERIAL ACTIVITY TEST OF *Kirinyuh* LEAF EXTRACT (*Chromolaena odorata* L.) FROM *Ie Seum* GEOTHERMAL AREA, REGENCY OF ACEH BESAR, INDONESIA

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ABSTRACT

This research aims to identify and compare the chemical components of *Chromolaena odorata* leaf extract grown in the geothermal and outer areas of the geothermal source site. The antibacterial activity of the plant extracts was also identified. The plant materials were collected from the *Ie Seum* Geothermal area and *Blangkrueng* region, approximately 31 km from the geothermal area. All samples were extracted using three different solvents: ethanol, n-hexane, and ethyl acetate. A phytochemical test was performed to identify the secondary metabolites, volatile components were identified using GC-MS, and the inhibition zone method was used to assess the antibacterial activity of the extracts against *S. aureus*. The ethanol extract and ethyl acetate from both areas contained phenolic, flavonoid, and steroid, while the n-hexane extracts from both areas' steroid groups were identified. In addition, GCMS data revealed that the plant material sample from the geothermal contained more chemical constituents compared to the sample from the geothermal outer area. The extract from geothermal also shows better antibacterial activity against *S. aureus*, and statistical analysis indicates a significant difference in antibacterial activity among solvents and between the origin of the samples.

Keywords: *Kirinyuh*, *Chromolaena odorata* L, Antibacterial Activity, Geothermal.

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INTRODUCTION

A geothermal system is a hydrothermal system that refers to the transportation and circulation of water in the deep crust. The hot fluid that causes convection to rise to the soil surface produces elemental deposits. Thus, the geothermal environment is unique, characterized by high temperatures, high soil moisture, high acidity, and unusual concentrations of minerals and trace elements.¹ Distinguished elements and minerals resulted in unique vegetation compared to its surroundings. The flora that grows in the geothermal area is greatly influenced by the heat generated by the reservoir and transported through water flow. In Indonesia, there are more than 265 geothermal locations spread along the volcanic route stretching from the islands of Sumatra, Java, Bali, Nusa Tenggara, Sulawesi, and Maluku, as well as non-volcanic areas such as Kalimantan and Papua. One of the geothermal areas that have not been intensively studied is the *Ie Seum* Geothermal area located in the subdistrict of *Mesjid Raya*, *Aceh Besar* District, Aceh Province, Indonesia (Fig.-1). Hidayat² reported that there are 23 families of 34 species with a total number of 534 individuals scattered in the geothermal area, which is divided into herbaceous, shrubs, poles, and trees. Of the four levels of plants, the most commonly found are plants at the herb and shrub levels. Herbs and shrubs quickly grow and thrive in shaded environmental conditions with sufficient sunlight. One of the most found plants is *kirinyuh* (*Chromolaena odorata*). *Kirinyuh* commonly called Siamese weed, is one of the broad-leaved weeds. *Kirinyuh* not only grows in dry land or mountains but can also grow in swamps and other wetlands. *Kirinyuh* is an easy-to-grow and very invasive plant. The seeds of *kirinyuh* are easily dispersed widely by

wind and evolve quickly in a favorable environment. In addition, this weed is also known as a "marginal crop," which can still grow well in less fertile areas or in areas that are not suitable for other crops to grow. Therefore, in the low soil fertility areas, with strong winds blowing, and no human intervention (vacant land), a large *kirinyuh* population would be found. This weed also thrives in the coastal areas of Aceh and mountainous and geothermal areas. The *Kirinyuh* leaf water extract contains antibacterial substances that can inhibit the growth of bacteria that cause rot in vegetables. *C. odorata* is also reported to contain antibacterial chemical compounds against pathogens. This weed extract is also antifungal against *Aspergillus niger* and *Drechslera hevea*. *Kirinyuh* extract may also be able to inhibit the growth of *S. aureus*. Therefore, this study tested the *kirinyuh* extract against the growth of *S. aureus* bacteria. In this study, ethanol, ethyl acetate, and n-hexane were used as solvents according to different levels of polarity.

EXPERIMENTAL

Material and Methods

All chemicals, including ethanol, n-hexane, ethyl acetate, HCl, NaOH, and reagents for photochemical screening, were purchased from Sigma Aldrich (Singapore) and used without further purification. Deionized water was purchased from a local pharmacy and used without further purification.

Plant Material and Sample Collection

Kirinyuh leaves were collected from 3 sampling points in the geothermal area and three spots in the outer geothermal area. The samples taken were three leaves from the shoots of the *kirinyuh* plant. *Chromolaena odorata* L was identified at the Biology Laboratory Herbarium, Syiah Kuala University, Banda Aceh. The plant material was cultivated from the area of *Ie seum* geothermal at the coordinate of 5°32'50.6"N 95°32'53.6"E. while the sample from outside the geothermal area was obtained at the coordinate of 5°35'22.4"N 95°22'14.0"E. The outer area of the geothermal where the sample was obtained is located approximately 31 km away from the geothermal location, as depicted in Fig.-1.

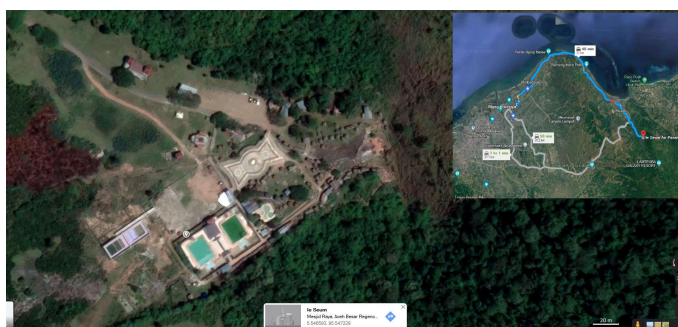


Fig.-1: Google Map View of *Ie Seum* Geothermal Area and the Outer Geothermal Area of Sample Collection, Aceh Besar Regency, Indonesia

Sample Extraction and Evaporation

Kirinyuh leaf sample was extracted by the maceration method. The sample was soaked in ethanol, n-hexane, and ethyl acetate solvent for 72 hours with stirring every 24 hours. The solvents were replaced twice and then filtered using filter paper. All the macerate was collected and thickened with a rotary evaporator at a temperature of 40°C and pressure of 20±0.5 kPa; the remaining filtrate was evaporated using a rotary evaporator (Buchi, Switzerland) in a water bath until a thick extract was obtained.

Chemical Detection Methods

Qualitative analysis of secondary metabolic constituents was performed, including phenolic, flavonoid, steroid, terpenoid, saponin, tannin, and alkaloid tests. The volatile components were assessed and separated using Gas Chromatography-Mass Spectroscopy (GC-MS) method.

Antibacterial Activity Test

The Kirby-Bauer method or the disc diffusion method was used for the antibacterial test. On top of 3 Petri dishes, 20 mL of HHA (Mueller Hinton Agar) media was poured into each petri dish and allowed to harden. Then the *Staphylococcus aureus* bacterial suspension was swabbed using a cotton swab over the hardened media. The media was divided into seven areas and then placed on disc paper soaked in deionized water

(control), ethanol extract, ethyl acetate extract, and n-hexane extract of *kirinyuh* leaves both from the geothermal area and outside the geothermal area. All Petri dishes were incubated at 37°C for 24 hours, and bacterial growth was observed in each treatment. The diameter of the inhibition zone was measured using a Vernier caliper.³

RESULTS AND DISCUSSION

C. odorata has been used as a traditional medicine in several countries such as Vietnam, Cambodia, and Indonesia. This plant extract is often used for wound healing and inflammation treatment. The aqueous extract of the leaves of *C. odorata* can also be used to treat diarrhea, malaria, and diabetes. Research reports that *C. odorata* contains antidiabetic, antioxidant, antimicrobial, anti-cataract, antihelmintic, and wound-healing active compounds. Several secondary metabolites in *C. odorata* leaf extract include alkaloids, saponins, tannins, flavanols, flavonoids (eupatiline), chalcones, and phenolic acids as ferulic acid and protocatric acid have also been reported.⁴ Methanol extract from *C. odorata* leaves was also reported to contain secondary metabolites, including alkaloids, tannins, steroids, terpenoids, flavonoids, and cardiac glycosides.⁵ In this study, the leaves of *C. odorata* were tested for their secondary metabolite content. The test results showed that the extract of *C. odorata* with three different solvents positively contained alkaloids, flavonoids, steroids, saponins, and phenolic compounds. Phytochemical test result is depicted in Table-1.

Table-1: Phytochemical Screening of *Chromolaena odorata* L. Leaf Extracts

No	Secondary Metabolites test	Ethanol extract (Geothermal)	Ethyl Acetate (Geothermal)	N-Hexane Geothermal	Ethanol (Outside Geothermal)	Ethyl Acetate (Outside Geothermal)	N-Hexane (Outside Geothermal)
1	Phenolic	+++	+	-	+++	++	-
2	Flavonoids	+++	++	-	+++	+++	-
3	Steroid	++	++	+++	+	++	+++
4	Terpenoid	-	-	-	-	-	-
5	Saponin	+	-	-	-	-	-
6	Tannins	-	-	-	-	-	-
7	Alkaloid						
	Dragendorff	-	-	-	+	+	-
	Mayer	-	-	-	+	+	-
	Wagner	-	-	-	+	+	-

+ =positive result, - = negative result

Table-1 shows phytochemical screening test results of *Kirinyuh* plant leaves obtained from geothermal and external geothermal areas using three different solvents, namely ethanol (polar), ethyl acetate (semi-polar), and n-hexane (non-polar). The test results revealed that both areas' ethanol and ethyl acetate fractions were positive for phenolic compounds, flavonoids, and steroids, and the geothermal ethanol fraction also contained saponins. Meanwhile, alkaloids were only detected in the ethanol and ethyl acetate fractions outside the geothermal area. The test results for the n-hexane fraction showed that both *kirinyuh* leaf extract from the geothermal area and outside the geothermal area positively contains steroids. This result is coherent with the nature of n-hexane, which has a dielectric constant of 1.89 at 20° C. The dielectric constant value is the reference for the polarity of each solvent, with a value of 1.89, making n-hexane a very non-polar solvent, so it is commonly used to extract fats and other non-polar compounds, one of which is steroids which are a group of non-polar secondary metabolites. Phytochemical screening is a qualitative test commonly used for the initial detection of secondary metabolites contained in a sample of natural products using appropriate reagents and solvents. Each solvent can dissolve different components depending on their polarity. Therefore, the negative test results shown do not necessarily indicate the absence of a particular compound, for example, terpenoid group compounds which are generally non-polar compounds that show negative results in each extract (Table-1), but based on the GC-MS results, a number terpenoid compounds such as caryophyllene, copaene, and germacrene present in almost all fractions. Because most terpenoid compounds are volatile, the sample handling process such as evaporation significantly affects the content of terpenoid compounds; if it is evaporated for too long, the volatile compounds would also evaporate along

with the solvent that is accommodated in the evaporation process. The three dominant constituents of each extract are tabulated in Table-2.

Table-2: Three Major Constituents of *Chromolaena odorata* L. Extracts from the Geothermal Area and Outside the Geothermal Area

Geothermal area			Outside geothermal area		
Ethanol (22)*	Ethyl acetate (15)*	N-Hexane (18)*	Ethanol (12)*	Ethyl acetate (13)*	N-Hexane (11)*
1-Heptatriacotan- ol (26.7%)	Hexadecanoic acid, methyl ester (29.5%)	Copaene (11.0%)	Hexadecanoic acid, methyl ester (33.6%)	Hexadecanoic acid, methyl ester (31.1%)	Hexadecanoic acid, methyl ester (27.6%)
Delta-Cadinene (7.8%)	9,12-Octadecadienoic acid (Z, Z)-, methyl ester (17.5%)	Caryophyllene (19.7%)	9,12-Octadecadienoic acid (Z, Z)-, methyl ester (16.8%)	9,12-Octadecadienoic acid (Z, Z)-, methyl ester (16.6%)	9,12-Octadecadienoic acid (Z, Z)-, methyl ester (17.8%)
1-Dodecanol, 2-hexyl- (6.6%)	9-Octadecenoic acid, methyl ester, (E)- (41.7%)	delta-Cadinene (17.6)	9-Octadecenoic acid, methyl ester, (39.2%)	9-Octadecenoic acid methyl ester (40.8%)	9-Octadecenoic acid, methyl ester, (44.6%)

* Total components detected in each extract

Table-2 shows that *kirinyuh* leaf extract from geothermal areas contains relatively more volatile compounds¹³ than *kirinyuh* leaves from outside geothermal¹⁵. All extracts from outside geothermal showed the same dominant chemical content as the ethyl acetate extract of *kirinyuh* leaves from geothermal areas, namely alkyl ester compounds. Meanwhile, ethanol extract and n-hexane extract from geothermal areas showed different chemical constituents. Alcohol-derived compounds were found to be more dominant in the ethanol extract, while the n-hexane extract showed the dominance of terpenoid compounds. The structure of several dominant compounds in *kirinyuh* leaf extract can be seen in Fig.-2.

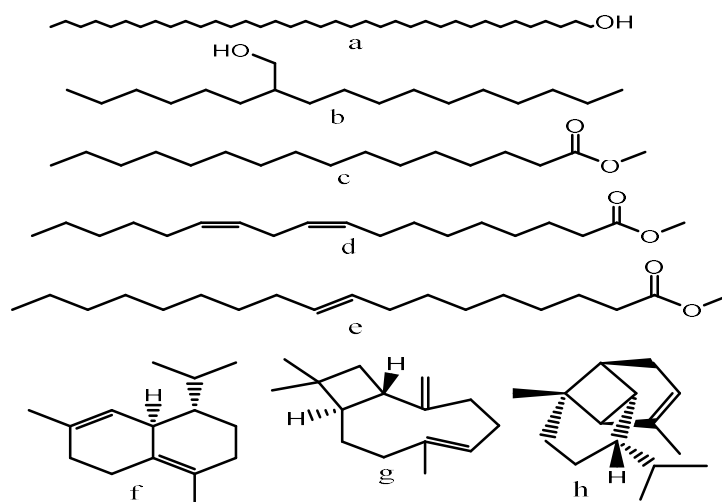


Fig.-2: Chemical Structures of Significant Components Detected in *Chromolaena odorata* L from Both Geothermal and Outside Geothermal Areas using GC-MS (a) 1-heptatriacotan-ol, (b) 1-Dodecanol, 2-hexyl-, (c) Hexadecanoic acid, methyl ester, (d) 9,12-Octadecadienoic acid (Z, Z)-, methyl ester, (e) 9-Octadecenoic acid, methyl ester (E), (f) Delta-Cadinene, (g) Caryophyllene, (h) Copaene

Figure-1 shows the structure of dominant compounds found in *kirinyuh* leaf extract from both geothermal and non-geothermal areas. The most dominant compound in the ethanol extract of *kirinyuh* leaves from geothermal areas is 1-Heptatriacotan-ol. Based on a recent study, the compound known to be contained in *Vernonia guineensis* Benth was reported to have excellent antibacterial activity.⁶ Meanwhile, copaene and caryophyllene are compounds that are already very popular in the medical world, especially in the treatment

of tumors, cancer, and aromatherapy. In addition, sesquiterpene compounds are also widely used as antibacterial, antifeedant, and anti-malarial compounds.⁷⁻¹¹ In addition, several essential compounds were also found in the leaf extract of *C. odorata*. Some of these critical compounds include trans-caryophyllene (RT=13.59 minutes), alpha-Guaiene (RT=13.98 minutes), and alpha-Humulene (RT=14.27 minutes), which are naturally occurring bicyclic sesquiterpene compounds that make up most essential oils, especially clove⁷, Cannabis sativa¹², rosemary¹³, and hops oils.¹⁴ These compounds are usually found as a mixture of iso-caryophyllene (a cis double bond isomer) and humulene. Both compounds are famous because of their rarity in nature.

Antibacterial Activity

The antibacterial properties of *C. odorata* leaf extract was tested against *S. aureus* bacteria. *S. aureus* is an opportunistic pathogenic bacterium found on several human organs' skin and mucosal surfaces. *S. aureus* can infect healthy individuals with a 30 to 50% chance, and up to 60% of hospital-acquired infections occur through catheter-associated urinary tract infections.

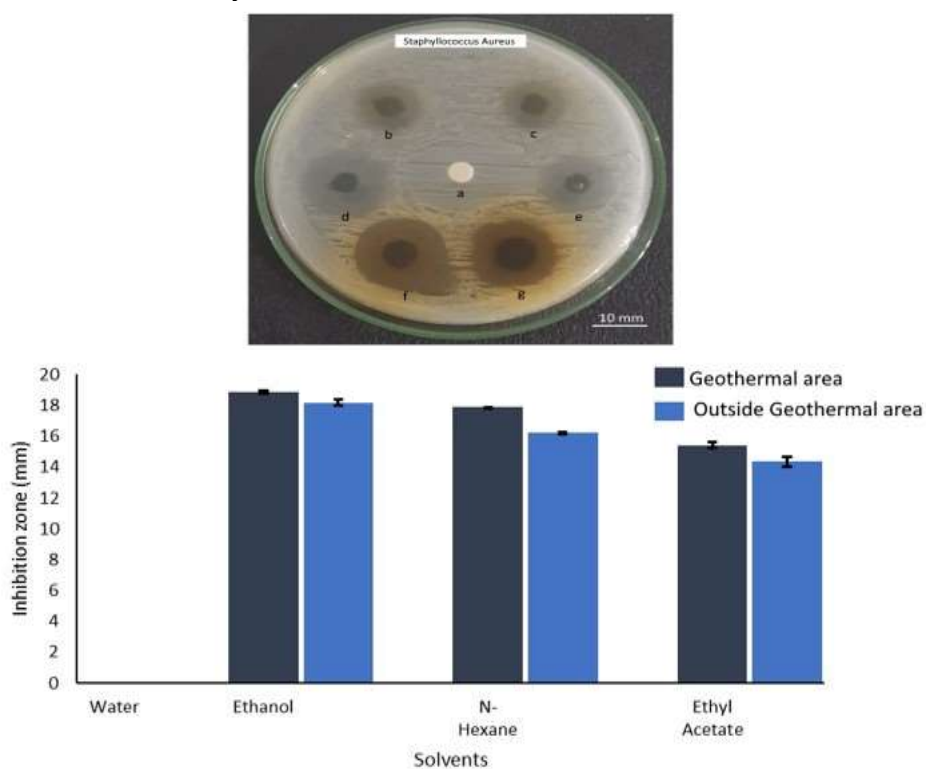


Fig.-3: Inhibition Zone Around the Sample Using Three Different Solvents (a) water (standard), (b) ethyl acetate geothermal area, (c) ethyl acetate outside geothermal area, (d) n-hexane geothermal area, (e) n-hexane outside geothermal area, (f) ethanol geothermal, (g) ethanol outside geothermal area, and mean diameter (mm) of inhibition zone for each sample against *S. aureus*. Data are expressed as Means \pm SE (n = 3).

Based on the inhibition test against *S. aureus*, the ethanol extracts showed the most significant inhibiting ability against the bacterium, followed by n-hexane and ethyl acetate. The test also revealed that the *kirinyuh* leaf cultivated in the geothermal area generates a better inhibition zone than the samples obtained from outside the geothermal area. Further statistical analysis using ANOVA indicates that the inhibition zone between the two groups and between three different solvents are significantly different ($p < 0.001$). The inhibition zone test is the systematic method used to study the activity of the natural product against bacteria.¹⁵ Using plant extracts with antimicrobial activity is beneficial in healing wounds¹⁶ or infections caused by bacteria such as *S. aureus*. *C. odorata* has excellent potential to inhibit the growth of *S. aureus* bacteria. The test results show that the inhibition of *C. odorata* leaf extract from geothermal and outside geothermal areas is in the range of 14 to 20 mm. If the material has an inhibition area of more than 20 mm, it is categorized as robust activity; if the inhibition area ranges from 10 to 20 mm, it means a substantial

antibacterial effect; 5-10 = moderate, whereas if the inhibition area is smaller or equal to 5 mm, the antibacterial activity is classified as weak. Based on the results of the antibacterial test in Fig.-3, the ethanol extract, n-hexane, and ethyl acetate of *kirinyuh* leaves show strong activity in inhibiting *S. aureus* bacteria.

CONCLUSION

Based on GC-MS data, it is revealed that the sample of *C. odorata* leaf from the geothermal area contained more constituents compared to the sample from the geothermal outer area. The extract from geothermal also shows better antibacterial activity against *S. aureus*, and the statistical analysis indicates a significant difference in antibacterial activity among three different solvents and between the two origins of the sample.

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