



GC-MS analysis of active compounds from ethanolic extract of *Jatropha gossypifolia* L. and antibacterial activity against *Pseudomonas aeruginosa*

[Análisis GC-MS de compuestos activos del extracto etanólico de *Jatropha gossypifolia* L. y actividad antibacteriana frente a *Pseudomonas aeruginosa*]

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Abstract

Context: *Pseudomonas aeruginosa* is an opportunistic Gram-negative bacterium characterized by rapid growth and antibiotic resistance, posing a significant global health challenge. *Jatropha gossypifolia* L. has demonstrated considerable potential in addressing drug resistance in pathogens.

Aims: To explore the potential bioactive compounds contained in various parts of *J. gossypifolia* and to evaluate their antibacterial properties against *P. aeruginosa*.

Methods: An ethanolic extract of *J. gossypifolia* (roots, stem bark, leaves, fruits) was analyzed by GC-MS and screened for antibacterial activity against *P. aeruginosa*.

Results: Phytochemical tests revealed saponins, flavonoids, and phenolics in all parts. GC-MS identified 9-cis-retinal in roots, N-methoxy-formamide in bark, and phytol in leaves. Disc-diffusion assays demonstrated concentration-dependent inhibition, with root extract exhibiting the largest inhibition zone (21.3 ± 0.8 mm at 20%). MIC and MBC values for all extracts were 1% (v/v).

Conclusions: These findings suggest that root-derived compounds of *J. gossypifolia* may serve as leads for novel antibacterial agents.

Keywords: antibacterial compounds; geothermal le Seum; *Jatropha gossypifolia*; *Pseudomonas aeruginosa*.

Resumen

Contexto: *Pseudomonas aeruginosa* es una bacteria gramnegativa oportunista que se caracteriza por su rápido crecimiento y resistencia a los antibióticos, lo que representa un importante desafío para la salud mundial. *Jatropha gossypifolia* L. ha demostrado un potencial considerable para abordar la farmacoresistencia en patógenos.

Objetivos: Explorar los posibles compuestos bioactivos presentes en diversas partes de *J. gossypifolia* y evaluar sus propiedades antibacterianas contra *P. aeruginosa*.

Métodos: Se analizó un extracto etanólico de *J. gossypifolia* (raíces, corteza del tallo, hojas y frutos) mediante cromatografía de gases-espectrometría de masas (GC-MS) y se evaluó su actividad antibacteriana contra *P. aeruginosa*.

Resultados: Las pruebas fitoquímicas revelaron saponinas, flavonoides y compuestos fenólicos en todas las partes. La GC-MS identificó 9-cis-retinal en las raíces, N-metoxi-formamida en la corteza y fitol en las hojas. Los ensayos de difusión en disco demostraron una inhibición dependiente de la concentración, siendo el extracto de raíz el que presentó la mayor zona de inhibición ($21,3 \pm 0,8$ mm al 20%). Los valores de CMI y CBM para todos los extractos fueron del 1% (v/v).

Conclusiones: Estos hallazgos sugieren que los compuestos derivados de la raíz de *J. gossypifolia* podrían servir como precursores para nuevos agentes antibacterianos.

Palabras Clave: compuestos antibacterianos; geothermal le Seum; *Jatropha gossypifolia*; *Pseudomonas aeruginosa*.

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INTRODUCTION

Over the past decade, the rise in antibiotic resistance has emerged as a significant challenge in global public health (Ferri et al., 2017). *Pseudomonas aeruginosa*, a major cause of this issue, emphasizes the urgent need for the development of novel therapeutic strategies (Chatterjee et al., 2016). This pathogen poses a considerable challenge in clinical treatment worldwide due to its inherent resistance to multiple antibiotics and its ability to generate biofilms, which hinders and changes the interaction mechanism of antibiotics. These biofilms not only protect the bacteria from antimicrobial agents but also enhance their persistence in hospital environments, posing a severe risk to immunocompromised patients. Therefore, addressing the multifaceted resistance mechanisms of *Pseudomonas aeruginosa* is crucial for enhancing treatment outcomes and protecting vulnerable patient populations (Yin et al., 2022).

In the pursuit of combating antibiotic resistance, natural resources, particularly medicinal plants from unique environments such as geothermal areas, offer promising solutions. Geothermal regions, like the one in Ie Seum, Aceh, are characterized by high temperatures, fluctuating pH levels, and diverse mineral compositions. These harsh conditions foster the growth of plants with exceptional adaptive mechanisms, leading to the production of unique bioactive compounds. *Jatropha gossypifolia* L., found in this geothermal region, has emerged as a plant of significant interest due to its potential antibacterial properties (Doudi et al., 2021). Geothermal environments induce plants to produce distinctive secondary metabolites with potential pharmacological benefits (Fakri et al., 2023). The harsh thermal and chemical stresses in these areas drive *J. gossypifolia* to adapt by generating metabolites with potent antibacterial activity. Still largely unexplored, these compounds could be pivotal in developing new treatments to overcome antibiotic resistance. Innovative strategies may be unlocked through further investigation of these plants and their bioactive compounds (Munira et al., 2022).

J. gossypifolia, which is also commonly known as the bellyache bush (Kolawole et al., 2016), is a species of flowering plant in the *Euphorbiaceae* family. This shrub, which typically grows to a height of 2-4 meters, is notable for its striking reddish-purple, cotton-like leaves, a characteristic reflected in its specific epithet "*Gossypifolia*." The plant belongs to the order *Euphorbiales* and the genus *Jatropha*, which includes various species known for their medicinal properties and sometimes toxic nature. *J. gossypifolia* produces small, bright red flowers and seeds that are highly

toxic if ingested. It thrives in well-drained soils, often found in tropical and subtropical regions, particularly in disturbed areas like roadsides and abandoned fields. Despite its toxicity, the plant has been traditionally used in folk medicine to treat various ailments, though its use requires caution. Additionally, the oil extracted from its seeds has been studied for potential applications in biofuels, showcasing the plant's diverse yet hazardous properties (Cavalcante et al., 2020).

Research on the antibacterial potential *J. gossypifolia* from geothermal Ie Seum Aceh has essential implications in the medical field to overcome antibiotic resistance to pathogens such as *P. aeruginosa*, environmental conservation, and local economic development (Okoh et al., 2016). The discovery of new antibacterial compounds could provide an alternative to synthetic antibiotics and enrich scientific knowledge. In addition, exploration and utilization of *J. gossypifolia* support geothermal ecosystem conservation efforts and provide economic benefits to local communities by developing plant-based products (Idroes et al., 2019). On the other hand, *J. gossypifolia* is a plant that grows in tropical areas and has long been used in traditional medicine. Traditional antibiotics are becoming increasingly ineffective against antibiotic-resistant pathogens, such as *P. aeruginosa*. Thus, there is an urgent need to develop novel therapeutic strategies, such as utilizing plant-derived compounds with antibacterial properties (Chatterjee et al., 2016). This plant has been reported to have various pharmacological properties, including antibacterial activity (Dahlan et al., 2023). The Ie Seum geothermal region in Aceh, which is the natural habitat of this species, offers unique conditions that may contribute to the plant's secondary metabolite profile, which in turn may affect its antibacterial properties (Doudi et al., 2021).

Research on *J. gossypifolia* has revealed significant antibacterial properties against *P. aeruginosa*, a pathogen often resistant to antibiotics (Khalifa et al., 2025). The study identified various active components, including alkaloids, terpenoids, flavonoids, and saponins. Further analysis showed that geothermal environmental conditions could influence and increase the production of secondary metabolites in these plants, offering additional potential for developing new antibacterial agents (Suryawati et al., 2025). The results of this study emphasize the importance of *J. gossypifolia* as a potential source of novel antibacterial agents, highlighting the need for biodiversity preservation and the development of plant-based pharmaceuticals. Through the examination and conservation of botanical species that possess potential therapeutic activi-

ties, our comprehension of natural ecosystems and their contribution to the fight against antibiotic resistance can be expanded.

The primary objective of this investigation was to identify and evaluate the active components in plant extracts and their potential to induce cell damage in *P. aeruginosa*, thereby contributing to the discovery of therapeutic alternatives to combat infections caused by resistant bacteria. The urgency of the research lies in addressing the growing threat of antibiotic resistance, exploring new treatment methods, and harnessing the potential of natural resources, such as *J. gossypifolia*, to develop effective antibacterial agents. Therefore, this research was conducted to answer the following research questions: 1) What are the variations in secondary metabolites present in different parts of *J. gossypifolia* L. growing in the Ie Suuem geothermal area? and 2) Which plant part of *J. gossypifolia* contains secondary metabolites with the highest antibacterial activity against *P. aeruginosa*?

MATERIAL AND METHODS

Chemicals

Chemicals, including ethanol 96%, Mg, HCl, amyl alcohol, sodium chloride, FeCl₃, sulphuric acid, and all reagents for phytochemical screening, were purchased from Sigma-Aldrich and used as received without further purification. Antibacterial properties testing was performed on *P. aeruginosa* PA01.

Plant material

This study used *J. gossypifolia* plants cultivated from the Ie Seum geothermal area, Aceh Besar, Aceh, Indonesia (5°32'50.42"N; 95°32'48.76"E), collected in July 2023. Plant species were identified at the Biosystematics Laboratory of the Department of Biology, FMIPA Syiah Kuala University, No. 268/UN11.1.8.4/TA.00.03/2023. A sampling of roots, stem bark, leaves, and fruits from the red castor plant was carried out in the morning in a geothermal area in Ie Seum Aceh Besar, Aceh, Indonesia (Fig. 1). The root sample from the central root part or root branch was collected, the bark of the stem was taken from an old gray stem, the leaves sample was collected from the third to sixth leaves of the shoot, and the green fruit was selected. The 3-5 cm diameter of the plants was selected as a sample.

Extraction and GC-MS evaluation

Each part of the plant material, including roots, stem bark, stems, leaves, and fruits, was thoroughly washed, drained, and air-dried for 7-14 days to reduce the water content, then mashed into a powder

weighing 1 kg. Extraction was carried out by the maceration method, in which 100 g of each powdered sample was placed into a jar and mixed with 96% ethanol at a ratio of 1:10 (w/v) for 3 × 24 hours. During the maceration process, the solvent was stirred occasionally and changed every 24 hours. The solution was filtered using Whatman filter paper No. 42 and the solvent was then removed with a rotary vacuum evaporator at 50°C to obtain a concentrated extract. This concentrated extract was then subjected to phytochemical screening, GC-MS analysis, and antibacterial bioactivity testing.

Analysis of chemical compounds was performed using Gas Chromatography-Mass Spectroscopy (GC-MS), with each extract from a plant part analyzed using a GC-MS spectrometer QP2000A at 70 eV. GC-MS analysis, which combines Gas Chromatography for compound separation and Mass Spectroscopy for compound detection based on fragmentation patterns, is performed to identify compound types and content qualitatively and quantitatively. A sample of 1 µl of plant extract was injected into a glass column (25 m × 0.25 mm in diameter with a stationary phase thickness of 0.25 µm, at an oven temperature set from 70-270°C with a temperature increase rate of 10°C/min. Helium, 99.99% with a flow rate of 10 mL/min, a pressure of 12 kPa (absolute pressure) was used as a carrier gas, a split ratio of 1:50, a total flow rate of 10 mL/min, and an MS scan range (m/z 40-500) (Gani et al., 2023).

Phytochemical screening

Phytochemical tests were carried out on all parts of *J. gossypifolia*, including roots, stem bark, leaves, and fruits, by employing the standard procedure as outlined in Table 1 (Yusuf et al., 2021).

Antibacterial assay

This study involved making MHA media dissolved in 1 L of aqueous solution and sterilized via autoclave at 121°C and 15 lbs pressure for 15 min for antibacterial testing of *P. aeruginosa* using the disc diffusion method. Extracts with concentrations of 1%, 5%, 10%, and 20% were applied to 6 mm diameter disc paper and incubated at 37°C for 24 hours. The positive control used the antibiotic ciprofloxacin, while the negative control used aquadest. Antibacterial activity is indicated by the formation of a clear zone around the disc, with measurements of the diameter of the clear zone to determine the inhibitory power categories: weak (6-10 mm), medium (11-20 mm), strong (21-30 mm), and very strong (>30 mm) (Soraya et al., 2020).

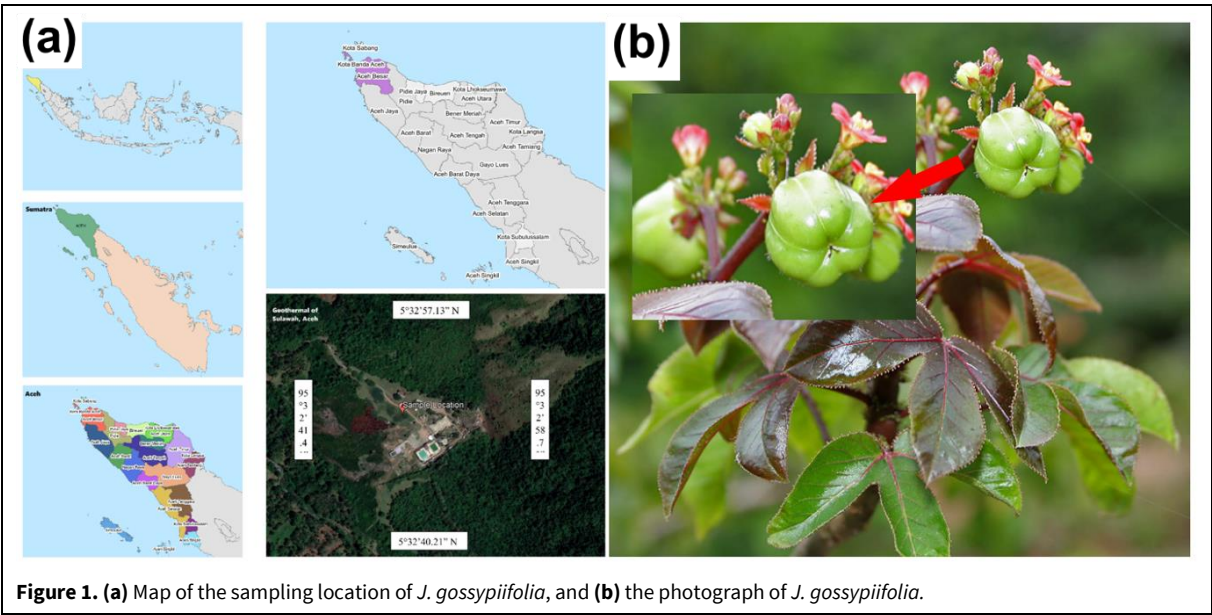


Table 1. Phytochemical screening of *J. gossypifolia*.

Secondary metabolite	Testing procedure
Flavonoid	Mg was added to 0.5 mL of HCl and amyl alcohol, and the mixture was added to 2 mL of extract. The positive result was indicated by red-purple color formation.
Tannin	A gelatin solution 1% was mixed with sodium chloride into 2 mL of the extract. A white precipitate indicated a positive result.
Saponin	A solution of HCl 0.1 was mixed into 2 mL of extract and shaken vigorously vertically for 15 s. The persistent foam with a height of around 1 cm was a positive sign of saponin.
Alkaloid	Hydrochloric acid was dissolved in 2 mL of extract, and then the standard tests, including Mayer's test, Wagner's test, and Dragendorff's test, were performed.
Steroid and terpenoid	Carr Price reagent was added to 2 mL of the extract. The presence of steroids was indicated by a green color formation. While a positive terpenoid was indicated by a red solution.

Statistical analysis

The inhibition of *P. aeruginosa* by *J. gossypifolia* extracts from various parts of the plant was statistically examined using one-way ANOVA, followed by HSD Tukey's test at a significance level of 0.05. The analysis was performed using IBM SPSS v.25.

RESULTS AND DISCUSSION

The interaction between active components in *J. gossypifolia* and *P. aeruginosa* is explored, offering fresh perspectives for the development of antibacterial medications. Utilizing a cross-disciplinary approach that integrates phytochemistry, microbiology, and bioinformatics, the research aims to leverage the distinct biodiversity of geothermal areas to discover novel therapeutic strategies, while concurrently promoting the preservation and sustainable exploitation of natural resources. Different parts of *J. gossypifolia*, including root, stembark, leaf, and fruit, were collect-

ed and underwent several physical treatments and chemical tests, including phytochemical screening, GC-MS analysis, and antibacterial assay. Table 2 reports phytochemical results performed on *J. gossypifolia* from the Ie Seum geothermal region, Aceh, Indonesia, revealing a promising diversity of secondary metabolites from four extract sources: roots, stembark, leaves, and fruits. The study successfully detected the presence of compounds used in specific reagents such as alkaloids, steroids, terpenoids, saponins, flavonoids, phenolics, and tannins, each of which has a unique distribution in different parts of the plant. Alkaloids were found exclusively in the leaves, indicating the specialization of this part of the plant in producing such compounds. Steroids were identified in the stembark of stems and leaves, indicating the potential of both parts to provide bioactive components, while roots and fruits do not contain steroids. Terpenoids, known for their diverse biological activity, were detected in roots and fruits,

Table 2. Phytochemical screening of ethanolic extract of *J. gossypifolia* from Ie Seum Geothermal Area Aceh, Indonesia.

No	Secondary metabolite	Reagent	Source extract			
			Root	Stembark	Leaf	Fruit
1	Alkaloid	Mayer	-	-	+	-
		Wagner	-	-	+	-
		Dragendorff	-	-	+	-
2	Steroid	Liebermann Burchard	-	+	+	-
3	Terpenoid	Liebermann Burchard	+	-	-	+
4	Saponin	Shuffling	++	+	+	-
5	Flavonoid	HCl and Mg	+	+	+	+
6	Phenolic	FeCl ₃	+	+	+	+
7	Tannin	Gelatin + H ₂ SO ₄	-	+	-	-

indicating diversification of this plant's biological functions and pharmaceutical applications. Saponins, present in almost all parts of the plant except the fruit, confirm their widespread presence in *J. gossypifolia*. Flavonoids and phenolics, compounds with significant antioxidant and therapeutic benefits, are found evenly throughout the extract source. Tannins, found only in stembark and known for their astringent properties, provide additional insight into the specificity of the distribution of bioactive compounds in plants.

Table 2 presents current research on *J. gossypifolia* from the geothermal area of Ie Seum Aceh, highlighting the prominent diversity of secondary metabolites and the biochemical adaptations of plants to their unique geothermal environment. The analysis revealed that all extracts contained flavonoids and phenolic compounds. Among the various plant parts, alkaloid metabolites were detected exclusively in the leaf extracts, confirmed positive by Mayer's, Wagner's, and Dragendorff's reagents. Steroidal compounds were identified in both the stembark and leaf extracts, while terpenoids were present in the root and fruit extracts. Saponins were predominantly found in the root extract, though they were also detected in the stembark and leaf at lower concentrations. In contrast, tannins were solely identified in the stembark and were entirely absent in the other plant parts. The widespread presence of saponins throughout the plant, except for fruit, and flavonoids and phenolics in all parts of the plant, confirms their potential in medical and pharmaceutical applications, including antioxidant and therapeutic properties. Previous studies have also supported the biological activity of *J. gossypifolia*, including anti-inflammatory, analgesic, and antibacterial properties, which are attributed to its richness in secondary metabolites (Cavalcante et al., 2020).

Félix-Silva et al. (2014b) showed that leaf extract of *J. gossypifolia* has significant analgesic and anti-inflammatory effects in animal models, which highlights this extract's potential in treating inflammatory and pain conditions. Furthermore, Okoh et al. (2016) explored the antimicrobial activity of the extract of *J. gossypifolia*. They found that flavonoid and terpenoid compounds in the extract contributed to antibacterial effects against various strains of bacteria, including some resistant to antibiotics. The study by Othman et al. (2019) also emphasizes the importance of isolating and characterizing bioactive compounds from plants in developing new therapies against resistant bacteria. They suggest that secondary metabolites of plants, such as alkaloids and saponins, may provide a source of new antibacterial compounds that are effective against resistant pathogens such as *P. aeruginosa* (Oyetayo and Temenu, 2023).

Fig. 2 and Table 3 depict the dominant active compounds possessed by *J. gossypifolia* originating from the Ie Seum geothermal area based on the GC-MS analysis. The data revealed a diversity of active compounds that have the potential for pharmacological use, found in the roots, stembark, leaves, and fruits of plants. The chromatographic analysis identified unique compounds such as retinal, 9-cis-, 2-cyclopenten-1-one, 3-methyl-2-(2,4-pentadienyl)-, (Z)-, and 1-(1,3-dimethyl-but-1,3-dienyl)-3,7-trimethyl-2-oxa-bicyclo[3.2.0] hept-3-ene in roots. Previous studies have proven that the cyclopentene derivatives found in the mangrove extract, particularly those with hydroxyl substituents, have shown inhibitory activity against various fungi, such as *Candida albicans* and *Cladosporium cucumerinum* (Buatong et al., 2011). In addition, some active compounds identified in the stembark, such as phytol and sitosterol, have beneficial effects on human health by reducing plasma cholesterol levels and have anti-

inflammatory, antidiabetic, and anticancer activities (Miras-Moreno et al., 2016). Another study revealed that the squalene found in the *J. gossypifolia* leaf extract exhibited various biological activities, including immunity enhancement, hypolipidemic, antioxidant, antitumor, antibacterial, and detoxification effects (Huang et al., 2009). In fruit, n-hexadecanoic acid (palmitic acid) is identified as a compound with potential antibacterial activity (Sopandani et al., 2020). Although not all compounds have direct antibacterial activity, this diversity promises synergies that can enhance therapeutic effects, signaling the importance of further research to explore the medical and thera-

peutic applications of *J. gossypifolia*, connecting its natural resources to develop new drugs.

Table 3 shows chromatographic analysis to map chemical diversity in different parts of the *J. gossypifolia* plant, from root to fruit, identifying compounds with significant potential for pharmacological applications. At the root, retinal, 9-cis- is recognized as a substantial component with therapeutic potential for eye disorders. The stembark contains formamide, N-methoxy- and acetone, 1-[4-(dimethylaminoethoxy) phenyl]-, showing potential as solvents and synthetic precursors, although their antibacterial effects require further research.

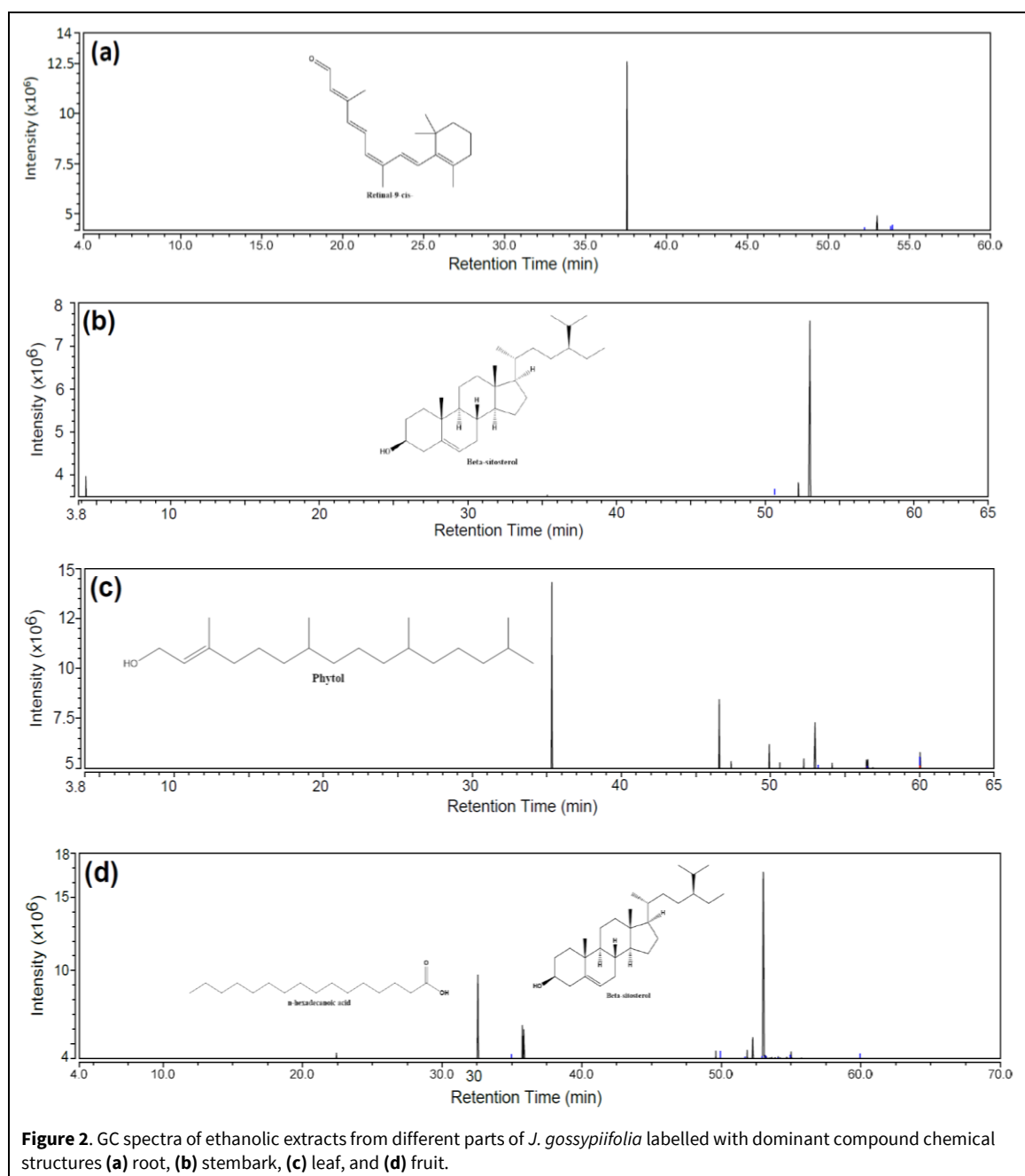


Figure 2. GC spectra of ethanolic extracts from different parts of *J. gossypifolia* labelled with dominant compound chemical structures (a) root, (b) stembark, (c) leaf, and (d) fruit.

Table 3. The active compound content of *J. gossypifolia* from the Ie Seum Geothermal Area, Aceh, Indonesia.

No	Retention time (min)	Compound name	Area % (tot. ion current)	Source
1	37.57	Retinal, 9-cis-	52.14	Root
2	38.96	2-Cyclopenten-1-one, 3-methyl-2-(2,4-pentadienyl)-, (Z)-	6.71	
3	40.02	1-(1,3-Dimethyl-but-1,3-dienyl)-3,7-trimethyl-2-oxa-bicyclo[3.2.0] hept-3-ene	11.90	
4	4.30	Formamide, N-methoxy-	6.66	Stembark
5	19.69	Acetone, 1- [4-(dimethylaminoethoxy)phenyl]-	10.57	
6	22.39	1,3-Dioxane-2-propanol, 2-methyl-	5.01	
7	35.34	Phytol	13.89	
8	50.64	α -Tocopheryl acetate	7.79	
9	52.24	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3 α ,5 α)-	9.11	
10	53.00	Sitosterol	35.87	
11	22.56	1-Dodecanamine, N,N-dimethyl-	7.75	Leaf
12	35.34	Phytol	22.33	
13	46.57	Squalene	10.65	
14	47.36	9-Octadecenoic acid (Z)-, 2,3-bis(acetyloxy)propyl ester	5.93	
15	49.92	9-Octadecenoic acid (Z)-, 2,3-bis(acetyloxy)propyl ester	6.65	
16	22.43	1-Dodecanamine, N,N-dimethyl-	7.03	Fruit
17	32.56	n-Hexadecanoic acid	14.19	
18	35.75	(Z)-18-Octadec-9-enolide	6.13	
19	35.85	trans-13-Octadecenoic acid	8.56	

The leaves are rich in compounds such as 1-dodecanamine, N,N-dimethyl-, phytol, and squalene, with phytol showing significant antibacterial activity. The fruit contains n-hexadecanoic acid (palmitic acid), known for its ability to disrupt bacterial cell membranes, promising as a powerful antimicrobial. These findings confirm the pharmacological potential of *J. gossypifolia* to be used to develop new drugs as antibacterial agents.

Formamide, N-methoxy-, and acetone, 1-[4-(dimethylaminoethoxy)phenyl]-, where these two compounds show potential as solvents and precursors of synthesis. Although their antibacterial effects have not yet been fully revealed, their potential in the chemical industry suggests that further research could open up new applications, including possible antimicrobial activity (Raut and Karuppayil, 2014). In addition, phytol, found in chlorophyll, has been known for its antimicrobial activity against various pathogens, including *P. aeruginosa*. Python can disrupt the integrity of bacterial cell membranes, induce cell damage, and limit bacterial growth (Aunjum et al., 2020). With its antioxidant potential, squalene may also provide additional therapeutic benefits, although its antibacterial mechanisms are not yet fully under-

stood. Palmitic acid is known to disrupt bacterial cell membranes, causing cell damage and death. It offers promise as a powerful antimicrobial, with studies showing its effectiveness in inhibiting the growth of various bacteria (Desbois and Smith, 2010).

Fig. 3 illustrates the antibacterial test of extracts from various parts of *Jatropha gossypifolia* L., including roots, bark, leaves, and fruit, against the bacterium *P. aeruginosa*. Ciprofloxacin was used as a positive antibiotic control in this test. Fig. 3a shows clear zones indicating bacterial inhibition by each substance, with the positive control showing a significantly larger clear zone compared to the samples tested. Precise measurements indicate that the root extract has the highest inhibitory effect on the bacteria, followed by extracts from the leaves and fruit, with the bark extract showing the smallest inhibition zone. The roots, in particular, act as the first line of defense, where these metabolites accumulate in higher concentrations. This is likely an evolutionary adaptation, as roots are constantly in contact with soil microbes, including potential pathogens. Terpenoids, for example, are well-known for their role in disrupting microbial cell membranes, while saponins can cause cell lysis in various pathogens. The enhanced production

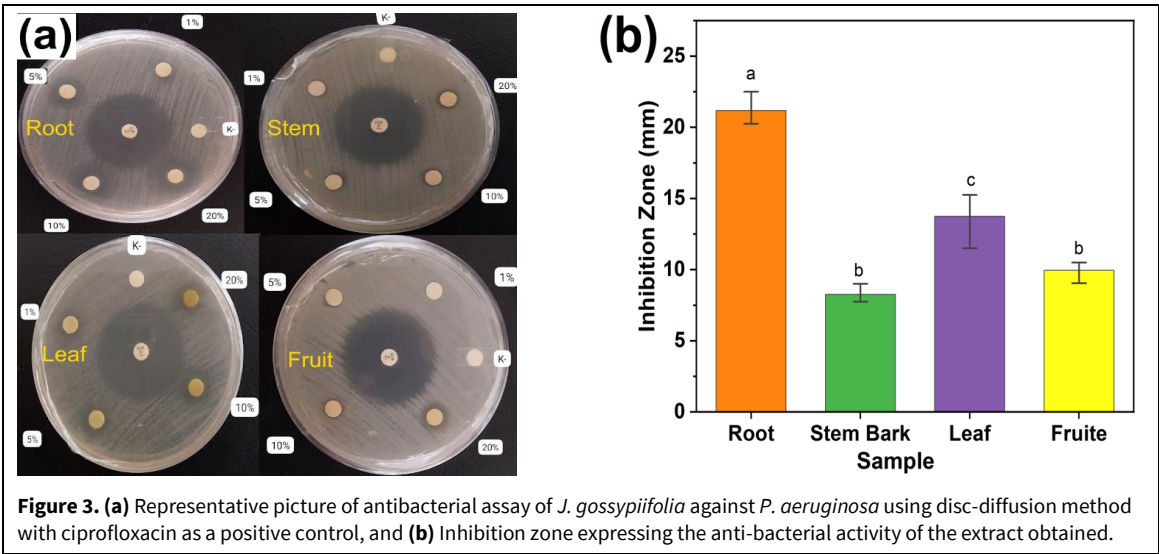


Figure 3. (a) Representative picture of antibacterial assay of *J. gossypifolia* against *P. aeruginosa* using disc-diffusion method with ciprofloxacin as a positive control, and (b) Inhibition zone expressing the anti-bacterial activity of the extract obtained.

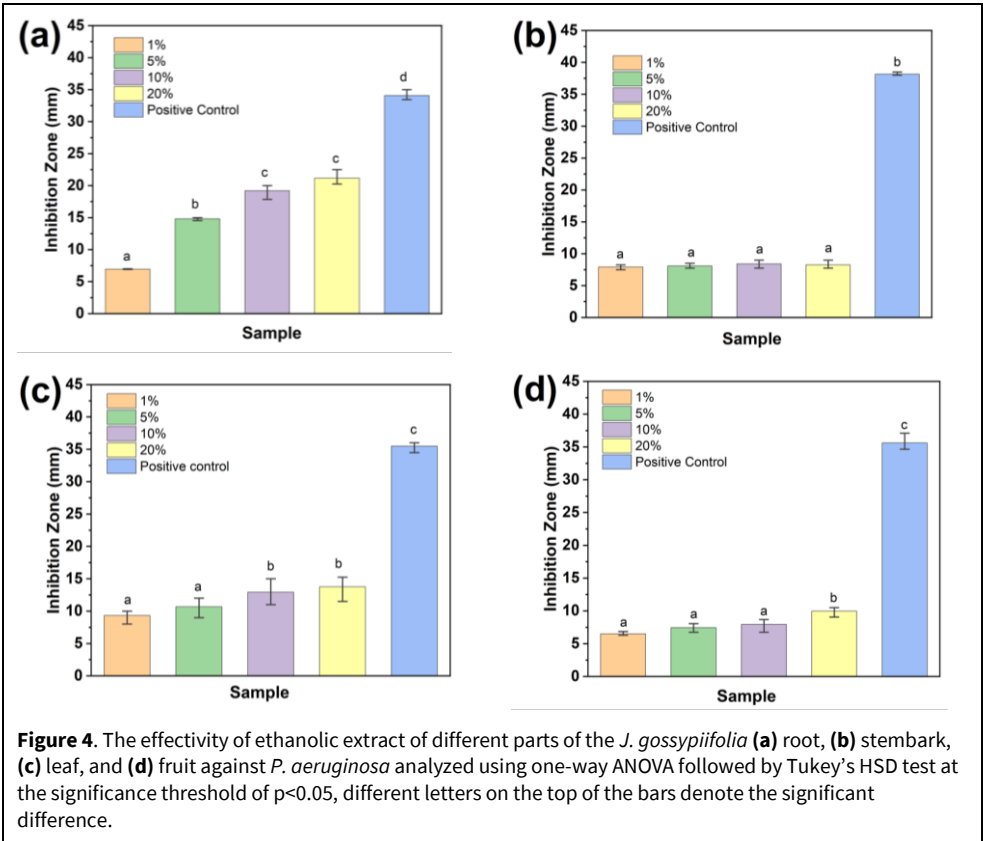


Figure 4. The effectivity of ethanolic extract of different parts of the *J. gossypifolia* (a) root, (b) stembark, (c) leaf, and (d) fruit against *P. aeruginosa* analyzed using one-way ANOVA followed by Tukey's HSD test at the significance threshold of $p < 0.05$, different letters on the top of the bars denote the significant difference.

of these metabolites in *J. gossypifolia*'s roots and leaves suggests a targeted defense strategy, enabling the plant to effectively manage and mitigate external pathogenic threats. Further analysis of how each part of the plant material extract differs in its activities against *P. aeruginosa*, ANOVA analysis of each extract was performed, and can be observed in Fig. 4.

Fig. 4 illustrates the notable effectiveness of *J. gossypifolia* in combating the bacterium *P. aeruginosa*, which is notorious for its resistance to multiple anti-

biotics. The study assessed the bacterium's growth inhibition using different extract concentrations (1%, 5%, 10%, and 20%) and measured the effect based on the size of the inhibition zones formed around the extract applications on growth media. The findings indicate considerable differences in the inhibitory effects of plant parts, including the roots, stembark, leaves, and fruits, on *P. aeruginosa*. The analysis results indicate that the bacterial inhibition zone expands as the concentration of the extract increases;

however, the root extract exhibits a significantly greater increase in the inhibition zone compared to extracts from other plant parts. This finding suggests that the roots of *J. gossypifolia* contain higher levels of secondary metabolites and other compounds with antibacterial properties. This conclusion is further supported by phytochemical screening (Table 2), which reveals that the root extract has a higher content of saponins and terpenoids compared to other extracts. Terpenoids and saponins are well-documented for their critical roles in protecting plants from soil and airborne pathogens (Shakeel et al., 2025). The plant's defense mechanisms against pathogens are most pronounced in the roots and leaves (Erb, 2012). Therefore, in general, secondary metabolites with pathogen-protective abilities are more abundant in these two parts, as evidenced by the more progressive bacterial inhibition observed in the roots and leaves of *J. gossypifolia*, as illustrated in Fig. 4a and c. In addition to phytochemical screening, GC-MS analysis to explore other chemicals that potentially play a significant role in inhibiting bacterial growth has also been carried out. The analysis reveals that some dominant chemicals were detected in each part of the extract (Table 3).

Retinal, 9-cis-, the main component in roots of *J. gossypifolia* is primarily known for its role in the visual cycle (Félix-Silva et al., 2014a; 2014b). 9-cis-Retinal is one form of retinal (vitamin A aldehyde). Regarding the antibacterial role of 9-cis-retinal, there has been a lot of research on the antimicrobial properties of various vitamin A derivatives and related compounds, specifically preventing eye infections by bacteria (Sekharan and Morokuma, 2011). Vitamin A and its derivatives affect the immune system and can increase defenses against microbial infections (Huang et al., 2018). Research on the antimicrobial properties of 9-cis-retinal suggests that retinoids may have direct antimicrobial effects against certain microorganisms, including bacteria that cause eye infections (Pechère et al., 2002). The exact mechanisms can vary, including disrupting microbial cell membranes or intervening in microbial metabolism. In the context of 9-cis-retinal, specific research may have explored how this isomer, in particular, contributes to those mechanisms in preventing bacterial eye infections (Blomhoff and Blomhoff, 2006). 9-cis-Retinal, besides playing a role in the vital function of vision and regulating gene expression, also shows potential in preventing bacterial development. Other retinoid derivatives might contribute to antimicrobial activity through several mechanisms: integrating into microbial cell membranes to disrupt function and increase their permeability, impairing microbial viability; strengthening the host's immune response, including increasing phagocytic activity; reducing the expression or activity of

bacterial virulence factors, limiting their ability to cause disease; and trigger the expression of genes involved in antimicrobial responses, including the production of antimicrobial peptides (Chen et al., 2023; Sun, 2012; Zasada and Budzisz, 2019).

Root *J. gossypifolia* showed a significant increase in inhibitory power at higher concentrations, indicating the presence of bioactive compounds that effectively disrupt bacterial protein synthesis. Research by Battaglia et al. (2023) supports these findings, suggesting that certain plant root extracts contain alkaloids and terpenoids that have potential as inhibitors of protein synthesis in bacteria. This strategy is important because protein synthesis is essential for bacteria to survive and multiply. Although the antibacterial activity of bark extract is relatively limited, the consistency of its inhibitory power across all concentrations suggests the presence of compounds with specific activity that may act through inhibition mechanisms that have not yet been fully revealed. The research results by Nenaah et al. (2015) found that flavonoids and saponins in plant bark have potential as antimicrobial agents through inhibition of cell wall formation or modification of bacterial cell membranes.

The leaf extract showed increased antibacterial activity from weak to moderate at higher concentrations, which can be attributed to its phenolic and flavonoid content. Research by Matsuura and Fett-Neto (2015) has shown that flavonoids can interfere with bacterial DNA and RNA synthesis and inhibit critical enzyme function. This strategy allows leaf extracts to block bacterial development by interfering with genetic replication and transcription. The increased antibacterial activity of fruit extracts at high concentrations confirms the potential of bioactive compounds in inhibiting the growth of *P. aeruginosa*. Verma et al. (2017) found that fruit extracts from some plants contain fatty acids and terpenoids that can damage bacterial cell membranes or interfere with energy metabolism pathways.

The minimum inhibitory concentration (MIC) assay was conducted using four different concentrations: 1%, 5%, 10%, and 20%. The results indicated that bacterial growth of *Pseudomonas aeruginosa* was successfully inhibited at a concentration of 1%. No bacterial growth was observed following the addition of active compounds extracted from various plant parts, as well as in the positive control (chloramphenicol). Furthermore, the minimum bactericidal concentration (MBC) was also determined to be 1% for each extract, confirming that this concentration was not only sufficient to inhibit bacterial growth but also to kill the bacteria (Table 4).

Table 4. Minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of different parts of *J. gossypifolia* extract against the growth of *P. aeruginosa*.

Test material	Replication	Concentration (%)				Control	
		20	10	5	1	Positive	Negative
Root	1	-	-	-	-	-	+
	2	-	-	-	-	-	+
	3	-	-	-	-	-	+
Fruit	1	-	-	-	-	-	+
	2	-	-	-	-	-	+
	3	-	-	-	-	-	+
Leaves	1	-	-	-	-	-	+
	2	-	-	-	-	-	+
	3	-	-	-	-	-	+
Stembark	1	-	-	-	-	-	+
	2	-	-	-	-	-	+
	3	-	-	-	-	-	+

These findings are significant, as both MIC and MBC data highlight the potent antibacterial activity of the plant extracts. Determining the MIC and MBC is crucial for evaluating the efficacy of active compounds, optimizing the minimum effective dose, and reducing the risk of toxicity (Kowalska-Krochmal & Dudek-Wicher, 2021). Additionally, comparing these results with a positive control provides valuable insight into the relative effectiveness of the extracts compared to established antibiotics (Gajic et al., 2022). The low MIC and MBC values observed in this study underscore the potential of these active compounds to be developed into cost-effective and efficient therapeutic agents, particularly against *P. aeruginosa*, a pathogen commonly associated with nosocomial infections. These findings support the importance of continued research and development of natural product-based antimicrobial formulations.

Strategies involving active compounds in *J. gossypifolia*, sourced from its roots, stembark, leaves, and fruits, demonstrate a comprehensive method to hinder the growth of *P. aeruginosa*, showcasing a multifaceted tactic in combating antibiotic resistance (Carels, 2009). Extracts from different parts of this plant offer a different spectrum of antibacterial activity, which can be attributed to the unique chemical composition and diverse mechanisms of action against the vital processes of bacteria (Ramalho et al., 2018).

Research on *J. gossypifolia* in the geothermal ecosystem of Ie Seum Aceh opens new insights into the potential of this plant in fighting resistant bacteria

such as *P. aeruginosa*. The variation in inhibitory power found in extracts from roots, stembark, leaves, and fruits indicates the diversity of bioactive compounds that can be used as antibacterial agents. This discovery emphasizes the importance of conserving Ie Seum Aceh's geothermal ecosystem for the sustainability of research and development of nature-based medicines. Strategies for the future include increased research and development, ecosystem-based management, education and public awareness, multi-stakeholder collaboration, and sustainable ecotourism development. This integrated and sustainable approach is vital to overcoming antibiotic resistance and preserving the Ie Seum region's unique biodiversity as a valuable asset for the medical and pharmaceutical worlds.

J. gossypifolia is rich in secondary metabolites, with its primary constituents being alkaloids in its leaves, steroids in both the stembark and leaves, and terpenoids in the roots and fruits. Saponins are widespread across the plant except in the fruit, while tannins are specifically located in the stembark, along with flavonoids and phenolics. Compounds such as 9-cis-retinal in the roots, N-methoxy-formamide in the stembark, as well as N,N-dimethyl-1-dodecanamine, and phytol in the leaves, are predicted to exhibit bioactivity by blocking peptidoglycan glycosyltransferase and hindering the synthesis of DNA, RNA, and proteins in bacteria. The effectiveness against *P. aeruginosa* is observed to escalate with the increasing concentration of the extract.

CONCLUSION

This study reveals that each part of *Jatropha gossypifolia* L. contained slightly different compounds based on the phytochemical screening and GC-MS analysis. Antibacterial assay confirmed that ethanolic extracts of root, stem bark, leaf, and fruit of *J. gossypifolia* possess antibacterial activities against *P. aeruginosa*, where the root extract shows an enormous activity compared to other extracts.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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GENERATIVE ARTIFICIAL INTELLIGENCE (AI)

The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the preparation, writing, or editing of this manuscript. All content was developed solely by the authors, who take full responsibility for its accuracy and integrity.

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AUTHOR CONTRIBUTION:

Contribution	Munira M	Saidi N	Ginting B	Suhartono S
Concepts or ideas	x	x		
Design	x			
Definition of intellectual content		x		
Literature search	x			
Experimental studies	x			
Data acquisition		x		
Data analysis			x	x
Statistical analysis			x	x
Manuscript preparation	x			
Manuscript editing	x			
Manuscript review	x	x	x	x

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