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IDENTIFICATION OF BIOACTIVE COMPOUNDS IN CASSIA ALATA USING GC-MS AND THEIR POTENTIAL AGAINST MICROORGANISMS THAT CAUSE SKIN INFECTIONS

IDENTIFIKASI SENYAWA BIOAKTIF *CASSIA ALATA* MELALUI GC-MS DAN POTENSINYA TERHADAP MIKROORGANISME PENYEBAB INFEKSI KULIT

Ardian Khairiah[1]*, Olivia Agustin[2], Dasumiati[1]

[1]*Department of Biology, Faculty of Science and Technology, UIN Syarif Hidayatullah Jakarta, Ir. H. Djuanda St, No. 95, Ciputat, South Tangerang, Banten, Indonesia*

[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Padang State University, Prof. Dr. Hamka St, Air Tawar, Padang, West Sumatra, Indonesia.*

**Corresponding author:* ardian.khairiah@uinjkt.ac.id

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Abstract

Cassia alata is widely used in traditional medicine for treating skin infections, yet its antifungal properties and chemical constituents remain underexplored. This study aimed to identify bioactive compounds in *C. alata* leaves and evaluate their antioxidant and antimicrobial potential. Phytochemical analysis was performed using Gas Chromatography-Mass Spectrometry (GC-MS). Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Antimicrobial efficacy was tested against *Staphylococcus aureus, S. epidermidis, Malassezia furfur,* and *Trichophyton rubrum* at extract concentrations of 6; 9; 12; and 15%. GC-MS analysis revealed six major peaks, with myo-inositol identified as the dominant compound. The extract exhibited weak antioxidant activity, with an IC_{50} value of 174.9 ppm. However, significant antifungal activity was observed, particularly against *M. furfur* and *T. rubrum*, with the most effective inhibitory concentrations at 12% and 15%, respectively. Despite its modest antioxidant effect, *C. alata* leaf extract demonstrates promising antifungal potential, especially against common fungal pathogens associated with skin infections. These findings support the traditional use of *C. alata* and highlight its potential as a natural antifungal agent.

Keywords: Antifungal; Antimicrobial; *Cassia alata*; DPPH; GC-MS; Leaf; Skin infection

Abstrak

Cassia alata secara luas digunakan dalam pengobatan tradisional untuk mengatasi infeksi kulit, namun sifat antijamur dan kandungan kimianya masih belum banyak diteliti. Penelitian ini bertujuan untuk mengidentifikasi senyawa bioaktif dalam daun C. alata serta mengevaluasi potensi aktivitas antioksidan dan antimikrobanya. Analisis fitokimia dilakukan menggunakan Gas Chromatography-Mass Spectrometry (GC-MS). Aktivitas antioksidan diuji menggunakan metode penangkapan radikal 2,2-difenil-1-pikrilhidrazil (DPPH). Uji efektivitas antimikroba dilakukan terhadap Staphylococcus aureus, S. epidermidis, Malassezia furfur, dan Trichophyton rubrum dengan konsentrasi ekstrak 6; 9; 12; dan 15%. Hasil analisis GC-MS menunjukkan enam puncak utama, dengan senyawa dominan berupa mome inositol. Ekstrak menunjukkan aktivitas antioksidan yang lemah, dengan nilai IC_{50} sebesar 174,9 ppm. Namun, aktivitas antijamur yang signifikan diamati, terutama terhadap M. furfur dan T. rubrum, dengan konsentrasi hambat paling efektif masing-masing pada 12% dan 15%. Meskipun efek antioksidannya tergolong rendah, ekstrak daun C. alata menunjukkan potensi antijamur yang menjanjikan, khususnya terhadap patogen jamur penyebab infeksi kulit. Temuan ini mendukung penggunaan tradisional C. alata serta menunjukkan potensi pengembangannya sebagai agen antijamur alami.

***Kata Kunci**: Antijamur;Antimikroba; Cassia alata; Daun, DPPH; GC-MS; Infeksi kulit*

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INTRODUCTION

Local communities possess diverse ethnobotanical knowledge regarding the utilization of plants in their surrounding environment. This localized knowledge, shaped by various ethnic traditions, has led to a rich diversity in the use of plants, particularly for medicinal purposes. Medicinal plants are species recognized for their health-promoting properties, therapeutic potential, and the presence of bioactive compounds that may serve as precursors for synthetic drugs (Fitzgerald et al., 2020). Of the approximately 10,000 plant species recorded in Sumatra, around 7,500 have been documented as being used in traditional medicine. This highlights the vast potential of native flora as sources of pharmacologically valuable compounds, many of which remain scientifically underexplored (Chaachouay & Zidane, 2024). *Cassia alata*, a member of the *Fabaceae*, is one such plant that has been widely documented in traditional medicine, particularly for the treatment of skin-related ailments (Oladeji et al., 2020).

Skin diseases are commonly encountered in tropical countries such as Indonesia, where high humidity creates an ideal environment for the proliferation of pathogenic fungi and bacteria. These microbial agents often cause persistent or recurrent infections, making skin disorders both widespread and difficult to manage effectively (Hay et al., 2020). Consequently, local populations have developed ethnobotanical knowledge of medicinal plants traditionally used to treat various skin conditions, including tinea, ringworm, pityriasis versicolor, boils, acne, herpes, and others. Among these, *C.alata* is widely known across multiple ethnic groups in Indonesia for its therapeutic use in treating skin ailments (Astuti et al., 2024). Although its traditional use has been well documented, there remains a lack of scientific validation regarding its efficacy and bioactive constituents.

Cassia alata is a tropical plant species widely distributed across equatorial regions and traditionally used to treat various ailments, including leprosy, ringworm, eye infections, skin diseases, tinea versicolor, and liver disorders (Khairiah et al., 2017; Handayani et al., 2022). However, these studies do not provide detailed investigations into the specific phytochemical constituents responsible for its therapeutic effects, particularly in the treatment of skin infections. The traditional use of *C. alata* by local communities for managing skin diseases suggests the presence of bioactive compounds with potential pharmacological activity. Human skin infections are commonly caused by a variety of microbial pathogens, including bacteria (*Staphylococcus* spp., *Streptococcus* spp.), fungi (*Candida* spp., dermatophytes), and viruses (Grice & Segre, 2011; Alexander et al., 2020). A deeper understanding of the plant's chemical profile and its antimicrobial mechanisms is therefore critical for validating its medicinal efficacy and supporting its development as a natural therapeutic agent.

The utilization of *C. alata* remains largely confined to traditional knowledge, despite its potential as a valuable source of bioactive compounds for treating infections. Antimicrobial activity refers to the ability to inhibit or eliminate microbial pathogens, while antioxidant activity plays a crucial role in preventing cellular or tissue damage and promoting tissue repair.

According to Toh et al. (2023), secondary metabolites produced by plants are believed to exert antimicrobial effects by suppressing or inhibiting the growth of pathogens. In addition, these secondary metabolites often exhibit antioxidant properties, which can accelerate the healing of wounds and irritation caused by microbial infections of the skin. Antioxidants function by neutralizing free radicals and reactive molecules, thereby reducing oxidative stress. This mechanism supports the healing process of tissue damage commonly associated with skin infections (Abiya et al., 2018).

Phytochemical screening of *C. alata* has revealed the presence of various secondary metabolites with potential antimicrobial properties. Fatmawati et al. (2020) reported the isolation of several bioactive compounds from *C. alata* leaves, including flavon, flavonoids, glycosides, alatinone, alanonal, and β-sitosterol-β-D-glucoside. Additionally, compounds such as anthraquinones and naphthopyrone glycosides have also been identified in this species (Dewi et al., 2019).

Previous studies demonstrated that *C. alata* extracts at concentrations of 0.5,1; and 2% were capable of inhibiting the growth of *S. sobrinus*, a bacterium associated with oral infections. These findings highlight the necessity for further investigation into the chemical constituents of *C. alata* and their antimicrobial efficacy, including the determination of effective extract concentrations. Such

research is essential for validating the therapeutic potential of *C. alata* and supporting its development as a natural remedy for skin infections.

MATERIALS AND METHODS

This study was conducted from March to July 2022. Fresh leaf samples of *C. alata* were collected from the Paku Aji region, Tangerang, Indonesia, using a random-walk sampling method. Phytochemical analyses were performed at the Laboratory of Pharmacy, Faculty of Health Sciences, UIN Syarif Hidayatullah Jakarta, while antioxidant and antimicrobial assays were conducted at the Integrated Laboratory Center, UIN Syarif Hidayatullah Jakarta.

The experimental materials included fresh *C. alata* leaves, which were dried until moisture was completely removed, and then ground into a fine powder using a mechanical blender. The powdered material was stored in airtight containers for further analysis. Reagents and media used included HCl, 70% ethanol, physiological NaCl, sterile distilled water, amyl alcohol, Sabouraud Dextrose Agar (SDA), Mueller Hinton Agar (MHA), Nutrient Agar (NA), Potato Dextrose Broth (PDB), spiritus, ketoconazole, tetracycline, and sterile paper discs.

Microbial test organisms consisted of two bacterial isolates (*S. aureus* and *S. epidermidis*) and two fungal isolates (*M. furfur* and *T. rubrum*). This study employed a combination of survey and experimental methods. The survey aimed to identify potential bioactive compounds in *C. alata* and related species, while the experimental approach focused on evaluating the antimicrobial properties of the extract against pathogenic microorganisms.

Extraction Procedure

Leaves of *C. alata* were extracted via maceration using technical-grade chloroform. A total of 4 L of solvent was added to 2 kg of powdered leaves in two 2 L glass containers and left to stand for 24 hours. The chloroform extract was then concentrated using a rotary evaporator to obtain a viscous crude extract.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Chemical constituents of the *C. alata* leaf extract were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) at the Pharmacy Laboratory, Faculty of Health Sciences, UIN Syarif Hidayatullah Jakarta. A 1 µL sample was injected into a GC-MS equipped with a DB-SMS Agilent column (30 m × 0.25 mm, 0.25 µm film thickness). The oven temperature was programmed from 50 °C to 250 °C at a rate of 5 °C/min. Helium was used as the carrier gas at a pressure of 53.6 kPa with a total flow rate of 14 mL/min and a split ratio of 10:1. Compounds were identified using Wiley7 and NIST I47 mass spectral libraries.

Antioxidant Assay

The antioxidant activity of the crude extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A stock DPPH solution was prepared by dissolving 4 mg of DPPH powder in 100 mL of 96% ethanol. The absorbance of the control DPPH solution was measured at 515 nm using a UV-Vis spectrophotometer (400–800 nm range) to determine the maximum wavelength (λ max). The extract was tested at a concentration of 100 ppm and incubated for 30 minutes before absorbance was measured. The assay was conducted in duplicate. Antioxidant activity was expressed as percent inhibition.

Antimicrobial Assay

Antimicrobial activity was tested using a completely randomized factorial design. The first factor was the treatment type (negative control, *C. alata* extract, positive control with synthetic antimicrobials); the second factor was extract concentration (6, 9, 12, and 15%); and the third factor was microbial strain (bacterial: *S. aureus*, *S. epidermidis*; fungal: *M. furfur*, *T. rubrum*). Each treatment was performed in duplicate.

Sterile paper discs were placed on MHA (for bacteria) and SDA (for fungi) media, and 10 µL of extract solution at each concentration was applied. Plates were incubated at 37 °C for 24 hours

(bacteria) and at room temperature for 72 hours (fungi). Negative controls consisted of discs treated with 10 µL of 96% ethanol. Positive controls included tetracycline (for bacteria) and ketoconazole (for fungi). Antimicrobial activity was determined by measuring the diameter of inhibition zones.

RESULTS

The GC-MS chromatogram of the ethanol extract of *C. alata leaves* (Figure 1) revealed six dominant peaks, indicating the presence of six major bioactive compounds with retention times ranging from 5.245 to 11.512 minutes, each showing different levels of abundance and area percentage. The presence of active compounds in the methanol extract of *C. alata* was confirmed based on their respective retention times (RT). The first compound identified at a retention time of 5.245 minutes was ethyl aminomethylformimidate, suggesting its potential as a bioactive metabolite.

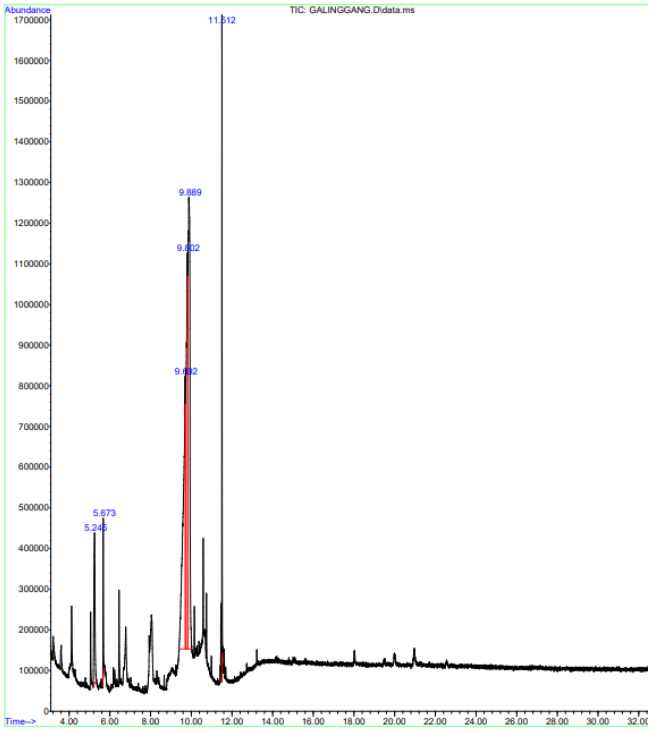


Figure 1. Gas Chromatography-Mass Spectrometry (GC-MS) chromatogram of the ethanol extract of *Cassia alata* leaves

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the ethanol extract of *C. alata* leaves revealed the presence of several chemical constituents, including ethyl aminomethylformimidate, 4H-pyran-4-one, 3-methylmannoside, mome inositol, and phytol acetate (Table 1). The peak at 9.887 minutes showed the highest area percentage (35.18%), identified as mome inositol, followed by another mome inositol peak at 9.800 minutes (25.57%). The dominance of these two peaks indicates that mome inositol is the principal compound in the *C. alata* leaf extract. Mome inositol belongs to the carbohydrate class of compounds known for their antimicrobial and antibacterial properties, supporting the ethnomedicinal use of *C. alata* in treating infectious skin diseases.

The identification of bioactive compounds, particularly those with antimicrobial potential, provides a scientific basis for the traditional use of *C. alata* in the treatment of fungal skin infections. The presence of phytol acetate, a diterpene known for its antifungal and anti-inflammatory activity, further supports the potential therapeutic application of the extract.

Mome inositol, the major compound identified in the ethanol extract of *C. alata*, has been previously reported to exhibit multiple biological activities, including anti-alopecic, anti-cirrhotic, anti-neuropathic, cholesterol-lowering, lipotropic, and sweetening properties. Another compound, n-hexadecanoic acid, also identified in the extract, is known to function as a 5-alpha-reductase inhibitor, hemolytic agent, and antioxidant. These findings are in line with previous observations that methanol

and ethyl acetate extracts have a strong capacity to yield a diverse range of active constituents responsible for significant pharmacological activities. Among other notable compounds, phytol, a major acyclic diterpene alcohol and a known precursor for vitamin E and K1, was also detected.

The antioxidant capacity of the ethanol extract of *C. alata* leaves was determined using the DPPH radical scavenging assay. The absorbance was measured at a wavelength of 515 nm, and the percentage of inhibition was calculated based on the decrease in absorbance compared to the blank control. The results are shown in Table 2. The IC_{50} value, which indicates the concentration of extract required to inhibit 50% of DPPH radicals.

Table 1. Gas Chromatography-Mass Spectrometry (GC-MS) identification of chemical components in the ethanol extract of *Cassia alata* leaves

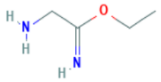
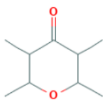
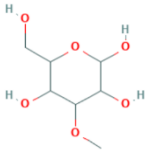
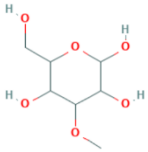
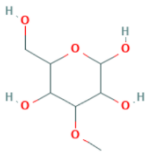
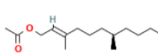
Peak	Retention time (RT, min)	Area (%)	Abundance	Chemical compound	Qual	Molecular form	
1	5,245	6.51	400,000	Ethyl aminomethylformimidate	35	$C_4H_{10}N_2O$	
2	5,673	2.21	500,000	4H-Phyran-4-one	94	$C_9H_{16}O_2$	
3	9,692	23.34	1,100,000	3-Methylmannoside	43	$C_7H_{14}O_6$	
4	9,800	25.57	1,150,000	Mome inositol	64	$C_7H_{14}O_6$	
5	9,887	35.18	1,250,000	Mome inositol	46	$C_7H_{14}O_6$	
6	11,512	7.19	1,700,000	Phytol, acetate	64	$C_{22}H_{42}O_2$	

Table 2. Antioxidant activity of *Cassia alata* leaf extract measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Concentration (ppm)	Absorbance (λ= 515 nm)	% Inhibition
(Control blank)	0.2410	-
15.63	0.2261	6.18
31.25	0.2199	8.76
62.50	0.1964	18.51
125.00	0.1475	38.80
250.00	0.0781	67.59
IC_{50}=	179.15	

The antioxidant activity of the ethanol extract of *C. alata* leaves was evaluated using the DPPH radical scavenging assay. The regression equation obtained from the concentration-inhibition curve was y= 0.2678x + 2.0246, with a correlation coefficient (r) of 0.9941, indicating a strong positive correlation (99.4%) between extract concentration and DPPH radical scavenging activity (Figure 2).

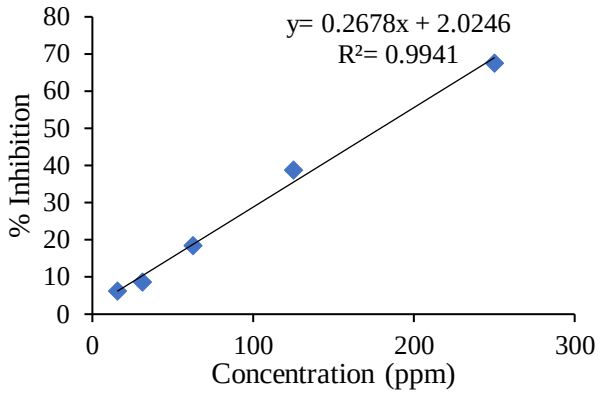


Figure 2. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *Cassia alata* leaf ethanol extract at various concentrations

The ethanolic extract of *C. alata* leaves was tested at concentrations of 6,9,12, and 15% against four microbial isolates, including two bacteria (*S. aureus* and *S. epidermidis*) and two fungi (*M. furfur* and *T. rubrum*). These microorganisms are known etiological agents of common tropical skin infections such as tinea versicolor, ringworm, athlete's foot, and acne. Antimicrobial activity was evaluated by measuring the diameter of inhibition zones around paper discs impregnated with the extract. As shown in Table 3, fungal pathogens exhibited greater sensitivity to the extract than bacterial strains, particularly at concentrations above 9%.

Table 3. Inhibition zone diameter (mm) of *Cassia alata* leaf extract against selected microorganisms

Extract concentration	Inhibition zone diameter (mm)			
	Bacteria		Fungi	
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Malassezia furfur*	*Trichophyton rubrum*
Control (+) *Tetracycline*	24.00	26.75	-	-
Control (+) *Ketoconazole*	-	-	3.00	2.00
Cassia alata 6%	4.50	2.50	8.00	7.50
C. alata 9%	3.50	2.50	10.50	10.00
C. alata 12%	2.50	2.25	11.50	10.50
C. alata 15%	2.25	3.25	11.00	11.50
Control (-)	-	-	-	-

Note: –= indicates no inhibition zone observed

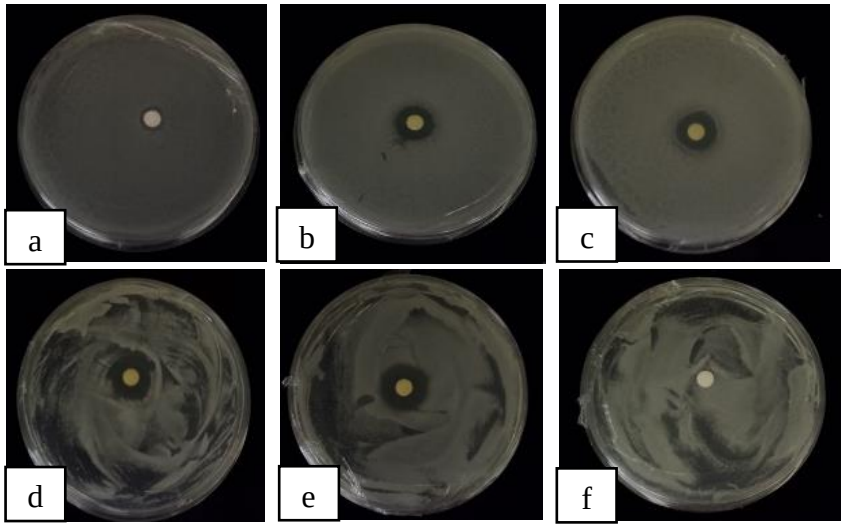


Figure 3. Inhibition zone diameter of *Malassezia furfur* following treatment with *Cassia alata* extract, control + (a), 6% (b), 9% (c), 12% (d), 15% (e), and control - (f)

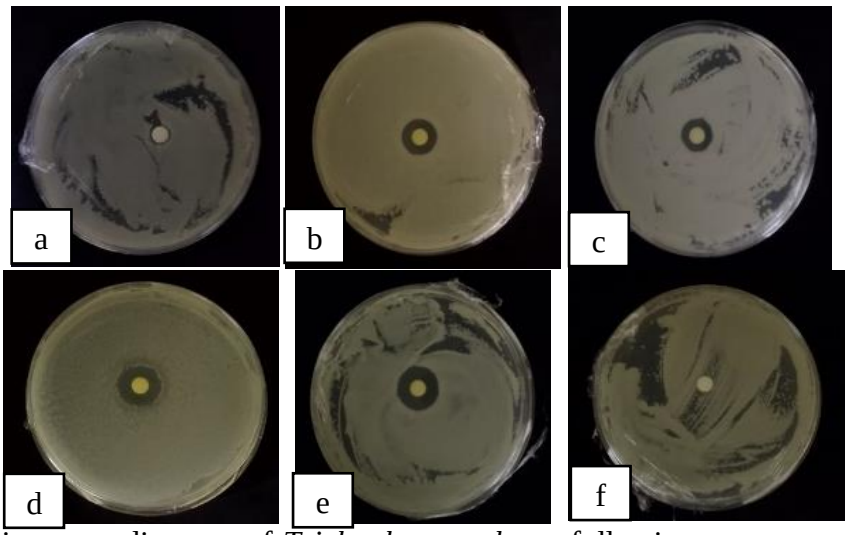


Figure 4. Inhibition zone diameter of *Trichophyton rubrum* following treatment with *Cassia alata* extract, control + (a), 6% (b), 9% (c), 12% (d), 15% (e), and control - (f)

The results of the assay demonstrated that *C. alata* extract exhibited a stronger inhibitory effect against dermatophytic fungi compared to its antibacterial activity. The most effective concentration in suppressing the growth of *S. aureus* was observed at 6% extract, whereas for *S. epidermidis*, the widest inhibition zone was achieved at 15% extract, with an average diameter of 3.25 mm. In contrast, the inhibition of fungal growth, particularly *M. furfur* and *T. rubrum*, was most pronounced at 15% extract concentration, yielding mean inhibition zone diameters of 11.00 mm and 11.50 mm, respectively. Tetracycline produced inhibition zones against *S. aureus* and *S. epidermidis* but showed no activity against fungi, whereas ketoconazole exhibited antifungal activity against *M. furfur* and *T. rubrum* but not against bacteria.

Observation of the inhibition zone diameters in both fungal isolates revealed a concentration-dependent response, in which higher extract concentrations produced wider inhibition zones (Figures 3 & 4). The largest mean inhibition zone against *M. furfur* was recorded at 11.50 mm with 12% *C. alata* leaf extract, whereas for *T. rubrum*, the maximum mean diameter of 11.50 mm was obtained at 15% extract concentration.

DISCUSSION

The GC-MS chromatogram of the ethanol extract of *C. alata* leaves (Figure 1) revealed six dominant peaks. These peaks reflect the chemical complexity of the extract, which may contribute to its observed antifungal activity. The identification of compounds through retention time and mass spectral matching provides preliminary insight into the possible mechanism of antimicrobial action, especially considering previous findings that alkaloid and amine derivatives, such as ethyl aminomethylformimidate can exhibit antimicrobial properties (Fatmawati et al., 2020), and alkanes, phenols, and sesquiterpenoids that possibly contributed to the antimicrobial anti-inflammatory activities (Toh et al., 2023).

The GC-MS analysis revealed several compounds identified in the *C. alata* leaf extract. The most abundant compound was mome inositol, approximately 35.18% of the total composition. Mome inositol belongs to the polysaccharide group and is known to possess antibacterial and antimicrobial properties (Jantapaso & Mittraparp-arthorn, 2022). Mome inositol has been reported to exhibit anti-alopecic, anti-cirrhotic, anti-neuropathic, cholesterol-lowering, lipotropic, and sweetening activities. n-Hexadecanoic acid acts as a 5-alpha-reductase inhibitor, as well as a hemolytic and antioxidant agent. These observations support the stronger extraction capacity of methanolic and ethyl acetate extracts, which are capable of yielding a wide range of active constituents responsible for multiple biological activities. Compounds identified through preliminary qualitative screening and GC-MS analysis are of considerable medical importance due to their unique structures and specific biological activities (Das et al., 2014).

Another major component detected at 9.692 minutes was 3-methylmannoside, accounting for 23.34% of the total area. This compound, a carbohydrate derivative, plays a crucial role in plant defense mechanisms and oxidative stress responses (Trouvelot et al., 2014). A compound detected at 11.512 minutes, phytol acetate, showed an area percentage of 7.19%, a major acyclic diterpene that serves as a precursor for vitamins E and K1. According to literature, phytol possesses antimicrobial, antioxidant, anticancer, anti-inflammatory, chemopreventive, and diuretic activities (Lee et al., 2016, Islam et al., 2020). Phytol has also been reported in species of the same genus, such as *C. italica* (Silva et al., 2014). The detection of phytol in related species such as *C. italica* further suggests that these diterpenes may represent conserved bioactive markers within the genus. Taken together, the presence of these compounds underscores the pharmacological relevance of *C. alata* as a promising source of natural agents with broad-spectrum biological functions.

The IC_{50} value, defined as the concentration of antioxidant required to inhibit 50% of DPPH radicals, was determined to be 179.15 ppm. According to Skrovankova and Mlcek (2025) lower IC_{50} value reflects stronger antioxidant activity, with compounds exhibiting IC_{50} values between 50–100 ppm classified as strong antioxidants. Therefore, based on this result, the antioxidant capacity of *C. alata* leaf extract is categorized as moderate to weak.

The antioxidant activity of a plant may be influenced by differences in the active components of the plant parts utilized, the geographical location of growth, climatic factors such as humidity, temperature, and air conditions, essential factors including soil nutrients, water, and light availability, as well as the concentration of extract applied (Maury et al., 2020).

In addition, drying of plant material is a critical step in preserving the stability of secondary metabolites. The primary purpose of drying is to prevent mold and fungal growth, reduce moisture content, inhibit enzymatic reactions, and facilitate the grinding of crude drugs into powder. In the case of *C. alata* leaves, drying was carried out using shade-drying until the leaves became brittle. This method was selected to protect the phytochemical constituents with antioxidant activity from degradation by direct sunlight. Tran et al. (2020) reported that drying processes significantly affect the phenolic and flavonoid content of crude drugs with antioxidant potential, as both antioxidant compounds and flavonoids are highly sensitive to light and heat exposure.

The inhibition zones formed against bacterial and fungal isolates are attributed to the presence of secondary metabolites with antimicrobial activity. One of the major constituents of *C. alata* extract, phytol, has been reported to exert antibacterial effects against *Staphylococcus aureus* by inducing cell membrane disruption, resulting in potassium ion leakage from bacterial cells (Colin et al., 2024).

The inhibitory effect was not derived from the solvent used during maceration. Ethanol 96%, employed as the negative control in this study, served solely as an organic solvent to extract secondary metabolites from plant tissues. Organic solvents do not contribute to the bioactivity of secondary metabolites against pathogenic bacterial species (Bitwell et al., 2023)

For bacterial isolates, tetracycline was used as the positive control and yielded the largest inhibition zone (Table 3). This was expected, since tetracycline is a broad-spectrum antibiotic capable of suppressing both Gram-positive and Gram-negative bacterial growth. In contrast, ketoconazole, used as the positive control against fungi, produced smaller inhibition zones compared with *C. alata* leaf extract. Ketoconazole, a widely prescribed antifungal drug for dermatophytosis, belongs to the azole class. Azoles, which are synthetic compounds categorized as imidazoles or triazoles, disrupt fungal cell membrane integrity by inhibiting ergosterol biosynthesis. Ketoconazole, the first clinically used oral azole (Gupta et al., 2015), acts by interacting with cytochrome P450-dependent C-14 alpha-demethylase, thereby preventing the demethylation of lanosterol into ergosterol (Hitchcock et al., 1990). This inhibition also interferes with the biosynthesis of triglycerides and phospholipids, while impairing oxidative and peroxidative enzyme activity, ultimately leading to the intracellular accumulation of toxic hydrogen peroxide concentrations (Yoon et al., 2021)

Inhibition zone observations showed that *S. aureus* was most susceptible at 6% extract concentration, whereas *S. epidermidis* required 15% extract to achieve maximum inhibition. Several factors influence antibacterial activity, including bacterial species, concentration of active compounds, extract diffusion capacity, and the structural characteristics of bacterial cell walls. Gram-

positive and Gram-negative bacteria differ significantly in their cell wall architecture, which determines antibacterial penetration and binding affinity (Silhavy et al., 2010).

Staphylococcus aureus is a Gram-positive bacterium, measuring 0.5–1.0 μm in diameter, non-spore forming, and non-motile, typically occurring in grape-like clusters (Kline et al., 2024). The outer cell wall of Gram-positive bacteria consists of a thick peptidoglycan layer without lipoprotein or lipopolysaccharide components (Silhavy et al., 2010). *S. aureus* is an opportunistic pathogen capable of causing a wide range of infections, from mild skin infections such as acne and food poisoning to severe systemic diseases (Kwiecinski & Horswill, 2020). The inhibition observed at 6% *C. alata* extract suggests its potential as a natural therapeutic agent for the treatment of skin infections.

Staphylococcus epidermidis is a Gram-positive, facultative anaerobic bacterium that constitutes part of the normal human flora, predominantly on the skin and, to a lesser extent, on mucosal surfaces (Severn & Horswill, 2022). It also contributes to the release of oleic acid through lipase activity, which has been implicated in acne development (Fournière et al., 2020). The inhibition zone observed with *C. alata* extract at the highest tested concentration (15%) highlights its potential application as a natural agent in the management of skin-related infections.

The differences in inhibition zones observed can be attributed to variations in fungal cell structure, the diffusion rate of the extract from the paper disk into the medium, and the diversity of compounds present in the extract (Mahardhika et al., 2021). Additional factors include the properties of the medium used, the molecular size and stability of the antimicrobial agents, the concentration of the active chemicals, incubation conditions, and the inoculum size (Li et al., 2017).

Malassezia furfur is a skin-associated fungus that causes pityriasis versicolor. Infected skin typically exhibits white to reddish-brown patches. Excessive sweating, moist environments, and limited awareness of personal hygiene and skin health are contributing factors to the growth of *M. furfur*. High temperature and humidity, as commonly found in Indonesia, further favor the proliferation of this fungus on human skin. The antifungal assay demonstrated that *C. alata* extract possesses strong potential as a natural candidate for topical formulations, such as ointments, in the treatment of skin infections.

Trichophyton rubrum is the most common causative agent of chronic dermatophytic infections of the skin and nails. Colony growth ranges from slow to rapid, with a soft texture, and the surface may appear whitish-yellow, light-colored, or even reddish-violet. Antifungal testing revealed that *C. alata* leaf extract effectively inhibited the growth of *T. rubrum*. The largest inhibition zone, measuring 11.50 mm, was observed at 15% extract concentration. These findings indicate that *C. alata* extract holds promise as a natural antifungal agent for the treatment of dermatophytoses.

An ideal antifungal agent exhibits selective toxicity, meaning it is harmful to the fungus but not to the host (Mazu et al., 2016). Based on their toxicological properties, antifungal agents may be classified as fungistatic, which inhibit fungal growth, or fungicidal, which inhibit and directly kill fungal cells. The mechanisms underlying antifungal activity vary, but generally include: (a) disruption of fungal cell wall components. Fungal cell walls are composed of chitin, cellulose, or a combination thereof, and interference with their synthesis compromises structural integrity; (b) damage to the fungal cell membrane. Since fungal membranes contain ergosterol, certain compounds can specifically target and disrupt this component; and (c) inhibition of protein and nucleic acid synthesis (Singulani et al., 2019). Proteins and nucleic acids (DNA and RNA) are essential for fungal cell survival, and inhibition of their synthesis leads to cell damage and death (Cordes et al., 2025).

CONCLUSION

Phytochemical screening indicate that the *C. alata* leaf extract is rich in secondary metabolites with potential antioxidant and antimicrobial properties, with mome inositol identified as the dominant constituent. The antioxidant assay of the methanolic extract demonstrated relatively weak radical-scavenging activity, with an IC_{50} value of 174.9 ppm. Antimicrobial testing indicated that *C. alata* leaf extract exhibited greater efficacy against fungal strains, with the highest activity observed at 15% concentration. These findings highlight the promising role of *C. alata* leaf extract as a natural candidate for the development of antifungal formulations.

Future studies are recommended to evaluate higher concentrations of *C. alata* leaf extract against Gram-negative bacteria and to further develop antifungal ointment formulations for the treatment of skin infections.

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