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Antibacterial activity and compound identification of *Eurya acuminata* leaf fractions against bacteria-causing skin infections

FESKAHARNY ALAMSJAH♥, ANTHONI AGUSTIEN, ARTHAULY YOPITA SINURAT, MUFIDHATUL MUQARRAMAH

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas. Limau Manis, Padang 25163, West Sumatra, Indonesia.
Tel./fax.: +62-812-6726277, ♥email: feskha@sci.unand.ac.id

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Abstract. *Alamsjah F, Agustien A, Sinurat AY, Muqarramah M. 2024. Antibacterial activity and compound identification of* Eurya acuminata *leaf fractions against bacteria-causing skin infections. Biodiversitas 25: 3441-3448.* Antibiotics previously used to treat skin infections have recently been ineffective due to pathogenic bacteria resistance, and the discovery of antibacterial agents derived from medicinal plants is a breakthrough. *Eurya acuminata* DC. is a member of the Pentaphylacaceae family and has efficacy values as a medicinal plant used in traditional medicines. This study aims to analyze the antibacterial activity of fractions (hexane, ethyl acetate, and water) of *E. acuminata* against bacteria causing skin infections and identify the chemical components of active fractions. A diffusion test was conducted for the antibacterial test. TLC (Thin Layer Chromatography) was used to determine the chemical components of the fractions. The fraction with the highest level of antibacterial activity was subjected to additional analysis using diffusion and LC-MS/MS (Liquid Chromatography-Mass Spectrometry/Mass Spectrometry). The antibacterial test showed that hexane, ethyl acetate, and water fractions were active against bacteria, with the ethyl acetate fraction having the highest activity. The TLC profile and the diffusion assay of the ethyl acetate fraction showed that spot number 4 with an Rf 1,00 had antibacterial activity. Identification of the compounds of the ethyl acetate fraction by LC-MS/MS resulted in identified compounds such as acacetin, kaempferol, peonidin, chrysoeriol, nobiletin, luteolin, and antroquinonol. This study demonstrates a new hope for further research into natural antibacterial agents, and it is a breakthrough with these phytochemicals.

Keywords: Antibacterial, ethyl acetate fraction, *Eurya acuminata*, hexane fraction, skin infections

INTRODUCTION

Skin infections are a public health concern that can result in morbidity, with an incidence rate of 4.8 skin infections per 100 person-years. It impacts the high incidence of ambulatory and inpatient settings, including abscesses and cellulitis (Miller et al. 2015). Infection on the skin and underlying soft tissue happens when bacteria interact with the host body, causing damage and various symptoms and clinical indicators (Lipsky et al. 2016). In older people, particularly diabetics, the skin recovery process slows down, increasing the risk of infections and inflammation becoming chronic (Smythe and Wilkinson 2023).

Some bacteria that cause skin infections include *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and MRSA (*Methicilin-resistant Staphylococcus aureus*). *P. aeruginosa* is an opportunistic pathogen that can produce destructive virulence factors that cause acute and chronic infections (Limoli and Hoffman 2019). *Staphylococcus epidermidis* is the most common CoNS (Coagulase-negative staphylococci) species found in clinical culture, and it's highly antibiotic-resistant (Morgenstern et al. 2016). *S. aureus* is a Gram-positive bacteria known as the predominant microorganism in wound infection. The methicillin-resistant strains of *S. aureus*, specifically MRSA, have led to an ongoing increase in infections (Norbury et al. 2016).

Antibiotics are one of the suggested therapies. However, a test for antibiotic susceptibility must be performed before using antibiotics. Many incidences of antibiotic resistance have resulted from the inappropriate use of antibiotics for clinical symptoms, such as misuse or without prescription. Antibiotics such as amoxicillin, penicillin, oxacillin, tetracycline, and erythromycin are no longer effective against the bacteria that cause wound infections (Mohammed et al. 2017). *S. aureus* and *P. aeruginosa* produce virulence factors, such as biofilms, which aid in the maintenance of infections and slow the healing of wound infections, which can also be affected by polymicrobial infection. It is recognized that this interspecies interaction provides a synergy that improves bacterial survivability, preventing disease eradication. The most commonly associated bacteria for polymicrobial infections were *S. aureus* and *P. aeruginosa* (Puca et al. 2021; Smith et al. 2020).

Because of the prevalence of antibiotic resistance, many studies must be conducted to identify new antibacterial agents from medicinal plants, such as *Eurya acuminata* DC. *E. acuminata* is a shrub or small tree similar to tea plants and belongs to the Pentaphylacaceae family (Heyne 1987; Hassler 2020). It can grow up to 15 m with lanceolate green leaves measuring 5.5-9.5×1.5-2.5 cm. The flowers are white to yellowish in the axillaries. The fruit is

small and has a smooth surface, is globose, and colored blue to black (Whitmore 1978; Wu et al. 2007). The leaves of *E. acuminata* effectively treat skin diseases, wound healing, dysentery/diarrhea, typhoid fever, and sore throats. For cuts and wounds, leaves paste is typically applied topically. Antibacterial compounds such as saponins, tannins, phytol, and β-sitosterol are present in the extract of *E. acuminata* leaves. However, these compounds have not been purified and tested against pathogens (Faisal et al. 2016; Kuncari 2011; Malewska 2014).

Ethanol is a suitable solvent for extracting chemical compounds from medicinal plants because it extracts antibacterial compounds (Alamsjah 2024). The ethanol extract from *E. acuminata* leaves has strong antibacterial activity against *P. aeruginosa* and *S. aureus* (Sinurat and Alamsjah 2022). According to Malewska (2014), a 70% ethanol extract of *E. acuminata* leaves inhibited *S. aureus*, MRSA, and MDRSA (*Multidrug-resistant S. aureus*) with Minimum Inhibitory Concentration (MIC) values of 625 µg/mL, as well as *Salmonella typhimurium* with MIC values of 2,500 µg/mL. Hexatriacontan-1-ol, a chemical obtained from *E. acuminata* leaves extracted using hexane, chloroform, and ethanol, shows antibacterial activity against *Candida albicans* with a MIC of 50 g/mL (Neipihoi et al. 2020). Meanwhile, the methanol-leaf extract of *E. acuminata* exhibited no antibacterial activity (Faisal et al. 2016).

The choice of extraction method and solvent can significantly affect the yield of bioactive compounds, making solvent selection a critical factor. Therefore, this study used ethanol as a solvent to extract chemical compounds of *E. acuminata*, and fractionation was carried out using solvents of varying polarity. This study aims to separate the active compounds from the *E. acuminata* leaf extract and determine their inhibitory effect on resistant pathogenic bacteria so that they can be used as an antibacterial.

MATERIALS AND METHODS

Sample collection

Samples were collected from the Biological Education and Research Forest, Andalas University, Indonesia. The plants were identified at the University's herbarium as *Eurya acuminata* DC.

Extraction and fractionation

The extraction and fractionation processes were carried out using modified methods by Al-Abd et al. (2015) and Kamdem et al. (2012). Fresh plant material was air-dried for one week, ground into a fine powder, and sieved with a 50 Mesh sieve. The fine powder was weighed. At room temperature, 800 g of the fine powder was macerated in 3.2 liters of ethanol for three days. The supernatant was filtered through the Whatman filter paper, while the residues were re-macerated for the second and third extraction. The dissolved parts were filtered and stored in a glass bottle each day. After the third extraction, the filtrates were filtered and evaporated until dry at 40°C using a rotatory vacuum evaporator to obtain the crude extract. For fractionation, 35 g of crude extract was dissolved in a 1:1 solution of ethanol and water. Then, this solution was added with hexane, ethyl acetate, and water separately at different fractionation times into a separatory funnel to obtain the hexane, ethyl acetate, and water fractions.

Microbial culture

The test bacteria used in this study were *P. aeruginosa* ATCC 27853, *S. epidermidis* ATCC 12228, *S. aureus* ATCC 25923, and MRSA ATCC 43300 obtained from Sumatran Biota Biotechnology Laboratory, Andalas University. Bacterial culture was taken using the inoculation needle and streaked onto a slanted Nutrient Agar (NA) medium. It was then incubated at 37°C for 24 hours. Test bacteria, aged 24 hours on slanted NA agar, were suspended in 0.9% sterile physiological NaCl solution to match the 0.5 McFarland turbidity standards (Gayathiri et al. 2018).

Antibacterial test of several fractions of *Eurya acuminata* leaves ethanol extract

NA media (25 mL) was poured into a petri dish and allowed to solidify. Then, the bacterial inoculum was spread evenly on the solid agar medium. Paper discs with a diameter of 0.5 cm were dipped in each fraction and placed on the media surface. It was incubated at 37°C for 24 hours until a clear zone was formed. The microbial-free area was measured using a vernier caliper, and the average was calculated (Bauer et al. 1966). Chloramphenicol was used as a positive control, and aquadest was used as a negative control. The analyses were carried out using 5 replications.

Phytochemical screening

Alkaloids

The extract (10 mL) was added with 1.5 mL of 2 N HCl, heated for 5 minutes, and then filtered. The filtrate was added 5 drops of Dragendorff reagent. The presence of a reddish-brown precipitate indicated a positive alkaloid result.

Flavonoids

As much as 1 mL of 70% ethanol, 0.1 g of Mg powder, and 10 drops of concentrated HCl were added to 1 mL of the extract, which was then homogenized. A pink to dark red solution indicates a positive result.

Steroids/terpenoids

5 mL of the extract was added with 2 mL of chloroform and 2 drops of anhydrous acid, then homogenized. Then, 2 drops of H_2SO_4 were added and homogenized. The presence of a blue color indicated the presence of steroids. A red-purple color indicated the presence of terpenoids.

Tannins

One milliliter (1 mL) of the extract, 3 mL of aquadest, and 3 drops of 10% $FeCl_3$ solution were mixed. The presence of a blue/green solution indicated the presence of tannins.

Saponins

The extract (1 mL) was added to 2 mL aquadest and shaken vigorously for 15 minutes. The appearance of foam for 10 minutes indicated the positive results of saponins.

Phenol

Five drops of 5% $FeCl_3$ were added to 1 mL of the extract. The presence of a dark green/bluish-black color indicated the presence of phenol content.

Anthraquinone

The extract (1 mL) was added to 10 mL of ammonia (NH_3) and shaken for 30 seconds. Pink, red, or purple hues indicated a positive result (Harborne 1987; Shaikh and Patil 2020).

Thin layer chromatography analysis

The TLC test used a modified method by Akpalo et al. (2020) on a 1x10 cm^2 silica plate (GF_{254}). Each fraction was spotted at a distance of ± 1 cm from the bottom edge of the plate using a capillary tube, then dried and eluted with the respective mobile phase of the compound group. The elution was stopped after the mobile phase movement reached the boundary line. The stains on the plate surface were examined under UV at 254 and 366 nm wavelengths. Then, the TLC plate was sprayed with 10% anisaldehyde-H_2SO_4. The Rf value was calculated, and the spots were observed on various mobile phases. The eluent was hexane: ethyl acetate: methanol with a ratio of 8:6:1 for the hexane fraction. In contrast, the ethyl acetate and water fraction ratio was 3:4:3 + 3 drops of formic acid.

Diffusion test of TLC spots

The diffusion test used a modified method by Bauer et al. (1966). The NA media were poured into a Petri dish, and 0.1 mL of inoculum was added, homogenized, and allowed to solidify. The TLC plate was dried in a laminar flow; the spot was scraped and then placed on the surface of the inoculated NA media. The media was then incubated at 37°C.

LC-MS/MS test

The test was carried out in the Forensic Laboratory Center, Bogor, Indonesia, using Ultra Performance Liquid Chromatography (UPLC) with an ES system (electrospray ionization) and a C_{18} column (1.8 µm 2.1x100 mm) HSS. The column temperature is 50°C, and the room temperature is 25°C. Water with 5 mM ammonium formate and acetonitrile with 0,05 % formic acid were used as the mobile phase. As many as 5 µL samples were injected and filtered using a 0.2 µm filter.

Data analysis

For the antibacterial test of several fractions, the diameter of the clear zone was measured and analyzed using SPSS, a one-way ANOVA test, and Duncan's test with a significance level of 5%. The collected data were transformed into tables.

RESULTS AND DISCUSSION

Antibacterial activity of several fractions of *Eurya acuminata* leaves ethanol extract

The ethanol fraction of *E. acuminata* leaves effectively inhibited the tested bacterial growth. The results of antibacterial activity revealed that the ethyl acetate fraction exhibited the highest zone of inhibition against the tested bacteria. At the same time, the hexane fraction has the lowest antibacterial activity and does not have an inhibitory zone against *S. epidermidis* and MRSA (Figure 1, Table 1).

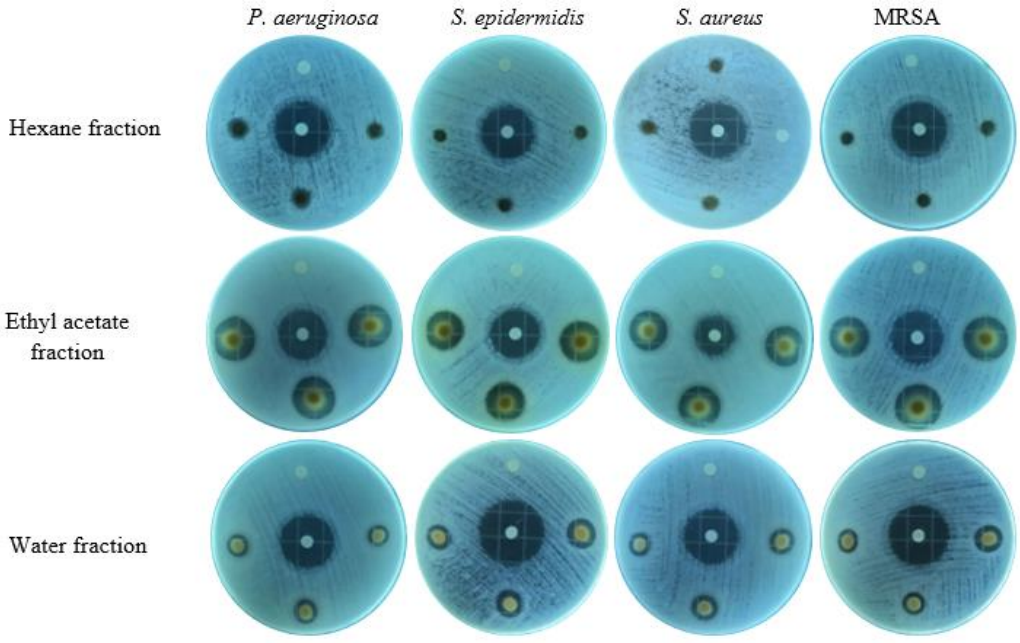

Figure 1. Antibacterial activity of several fractions of *Eurya acuminata* leaves ethanol extract. The positive control chloramphenicol was placed in the center of the petri dish

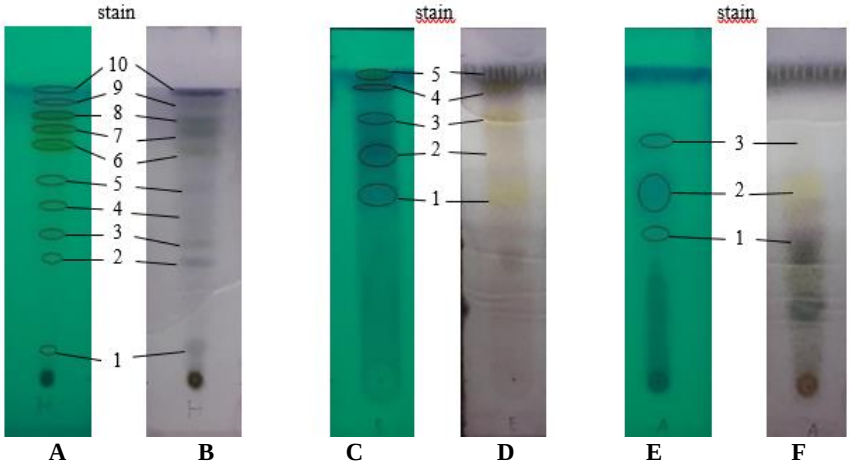

Figure 2. Chromatogram of hexane fraction observed: A. under UV at 254 nm, B. after sprayed with anisaldehyde-H_2SO_4, ethyl acetate fraction observed, C. under UV at 254 nm, D. after sprayed with anisaldehyde -H_2SO_4, water fraction observed under, E. UV at 254 nm, F. after sprayed with anisaldehyde-H_2SO_4

Table 2. Phytochemical compounds of the hexane, ethyl acetate, and water fractions of the *Eurya acuminata* leaf ethanol extract

Compound class	Type of solvent		
	Hexane	Ethyl acetate	Water
Alkaloids	-	-	-
Flavonoids	-	+	+
Phenol	+	+	+
Tannins	+	+	+
Saponins	-	+	+
Steroids	+	-	-
Terpenoids	-	+	+
Anthraquinone	-	+	-

Note: (+): compound detected; (-): compound undetected

Table 3. TLC separation of hexane, ethyl acetate, and water fractions of the *Eurya acuminata* leaf ethanol extract

Fraction	Spot	Rf Value	Color	After being sprayed with anisaldehyde
Hexane	1	0.07	Light yellow	Gray
	2	0.38	Light yellow	Gray
	3	0.47	Light yellow	Gray
	4	0.56	Light yellow	Gray
	5	0.64	Light yellow	Gray
	6	0.77	Yellow	Gray
	7	0.84	Yellow	Gray
	8	0.87	Yellow	Gray
	9	0.93	Purple	Purple
	10	0.97	Purple	Purple
Ethyl acetate	1	0.61	Purple	Yellow
	2	0.77	Purple	Yellow
	3	0.88	Purple	Yellow
	4	1.00	Purple	Purple
	5	1.03	Purple	Purple
Water	1	0.43	Purple	Purple
	2	0.58	Purple	Yellow
	3	0.80	Purple	White

Table 1. The average diameter of the inhibition zone of the hexane, ethyl acetate, and water fractions of *Eurya acuminata* leaf ethanol extract

Treatments	Average inhibition zone (mm)			
	P.aeruginosa	*S.epidermidis*	*S.aureus*	MRSA
Hexane	2.00^{b}	0^{a}	0.33^{b}	0^{a}
Ethyl acetate	13.46^{d}	13.03^{c}	13.53^{d}	12.39^{c}
Water	4.76^{c}	7.26^{b}	6.09^{c}	5.64^{b}
Control (+) chloramphenicol	18.68^{e}	19.33^{d}	19.33^{e}	17.31^{d}
Control (-) aquadest	0^{a}	0^{a}	0^{a}	0^{a}

Note: Numbers followed by different lowercase letters in the same column are significantly different at 5% DMRT

Phytochemical screening

The findings revealed that all *E. acuminata* leaf fractions contained antibacterial compounds. The ethyl acetate fraction contains six different classes of chemical compounds, and the hexane fraction contains only three different classes of chemical compounds (Table 2).

Chemical compound identification of several fractions of *Eurya acuminata* leaves by TLC

Light yellow, yellow, and purple spots were observed in the hexane fraction at UV 254 nm. After spraying with anisaldehyde-H_2SO_4, the grey spot confirmed the presence of steroid, whereas the purple spot indicated terpene. Three spots with purple color in the ethyl acetate fraction changed to yellow, indicating the presence of a flavonoid, while two spots remained purple, indicating the presence of a terpene. The water fraction had a purple spot indicating the presence of terpene, yellow indicating saponin, and no color (white) shown in the third spot. (Figure 2; Table 3).

Antibacterial activity of ethyl acetate fraction by diffusion method

The test was carried out on the ethyl acetate fraction with the most significant antibacterial activity. The formation of a clear zone in the stained area indicates antibacterial activity.

Table 4. Analysis of the identification of the ethyl acetate fraction of *Eurya acuminata* leaf ethanol extract

Retention time (minute)	$[M-H]^+$	Molecul formula $[M-H]^+$	Fragment ions	% Similarity	Compound
5.45	285.0760	$C_{16}H_{12}O_5$	179.1066; 271.0602	79.30	Acacetin
5.76	286.0659	$C_{15}H_{10}O_6$	111.5583; 197.2278	96.09	Kaempferol
6.24	505.1339	$C_{24}H_{24}O_{12}$	301.0705	96.14	Peonidin
7.81	301.0719	$C_{16}H_{12}O_6$	258.0529; 286.0481	99.53	Chrysoeriol
9.72	403.1396	$C_{21}HH_{22}O_8$	373.0923; 388.1152	97.11	Nobiletin
15.72	593.2776	$C_{34}H_{40}O_9$	533.2557	99.11	Luteolin
17.28	391.2845	$C_{24}H_{38}O_4$	121.0286	99.67	Antroquinonol

Identification of secondary metabolites of ethyl acetate fraction by LC-MS/MS

The compound identification was carried out using LC-MS/MS. The chromatogram of the ethyl acetate fraction showed seven dominant peaks. The compound spectrum in the chromatogram was compared with PubChem, HMDB (Human Metabolome Database), and MassBank data center libraries, and the result is shown in Table 4.

Discussion

Several fractions of *E. acuminata* showed antibacterial activity against the test bacteria, with the ethyl acetate fraction demonstrating a strong category. The antibacterial inhibition zone is categorized as follows: >10-20 mm (strong), 5-9 mm (moderate), 1-4 mm (weak), and no activity (Indranigrat et al. 2021). The ethyl acetate fraction of *E. acuminata* leaves contains flavonoids, phenols, tannins, saponins, terpenoids, and anthraquinones. These metabolites have their respective activities inhibiting bacterial growth, working synergistically as antibacterial agents (Álvarez-Martínez et al. 2020).

Flavonoids are phenolic compounds that function as antimicrobials by forming complex compounds with extracellular proteins; this action disrupts the integrity of membranes and cell walls. Phenols can bind to proteins through hydrogen bonds, damaging the protein structure (Silva and Fernandes 2010). Tannins inhibit bacterial cell wall synthesis and disrupt cell permeability. Additionally, the o-dihydroxyphenyl groups in tannins can chelate iron, which is essential for bacterial growth (Farha et al. 2020).

Saponins contain hydrophilic and lipophilic molecules that reduce surface tension in aqueous solution, causing structural alterations in biological macromolecules. It disrupts cell permeability and can potentially lead to bacterial mortality (Xue et al. 2020). Terpenoids can cause bacterial cell wall lysis by reacting with porins (transmembrane proteins) in the bacterial membrane, forming strong polymeric bonds that disrupt cell wall permeability (Setiaji et al. 2024). On the other hand, quinone compounds will bind to proteins and form complexes with amino acids, thus disrupting bacterial cell metabolism and causing proteins to lose their function (Silva and Fernandes 2010).

The antibacterial activity of the water fraction of *E. acuminata* against *S. epidermidis, S. aureus,* and MRSA is classified as moderate, while activity against *P. aeruginosa* is classified as weak. *P. aeruginosa* is classified as a resistant bacteria and produces beta-lactamases, such as Extended-Spectrum β-Lactamases (ESBLs) and Metallo-β-Lactamases (MBLs), that can hydrolyze beta-lactam rings (antibiotics) also remove antibiotic substances from cells using an efflux pump (Hosu et al. 2021). In addition, the compounds extracted from the water fraction of *E. acuminata* leaves may have low-intensity antibacterial properties or lack the potential to kill bacteria. Furthermore, high evaporation temperatures cannot be used with a rotary evaporator in the solvent evaporation process because some compounds are unstable at high temperatures. This condition makes it difficult to evaporate the water from the water fraction, so the tested extract still contains water that results in low concentration of extract and low antibacterial activity (Yang et al. 2017)

The hexane fraction possesses weak growth inhibition activity against *P. aeruginosa* and *S. aureus* and no growth inhibition against *S. epidermidis* and MRSA. The non-polar hexane fraction only contains steroids as non-polar compounds, affecting a small inhibitory zone. Additionally, the non-synergic action of the secondary metabolites in fraction affected the inhibitory zone. In addition, antibacterial activity is also influenced by the potential of an antibacterial substance in the tested solution and the bacterial sensitivity against the antibacterial substance (Gorlenko et al. 2020).

Table 1 shows that the antibacterial activity of chloramphenicol was categorized as strong antibacterial. Chloramphenicol is a bacteriostatic and broad-spectrum antibacterial that inhibits bacterial protein synthesis (Dinos et al. 2016). The ethyl acetate and chloramphenicol fractions both produced strong antibacterial activity. This condition shows that the ethyl acetate fraction has antibacterial activity similar to chloramphenicol in inhibiting bacterial growth. The water fraction did not produce antibacterial activity against the test bacteria, as indicated by the absence of an inhibition zone.

The TLC analysis showed spots with different Rf values and colors. Steroids showed gray spots, while terpenoids showed purple spots (Gerlach et al. 2018). After being sprayed with anisaldehyde-H_2SO_4 and heated, the yellow color on TLC was identified as flavonoids (Seo et al. 2016). Saponin is indicated by purple spots, which turn invisible after sprayed with anisaldehyde-H_2SO_4 (Karthika et al. 2014).

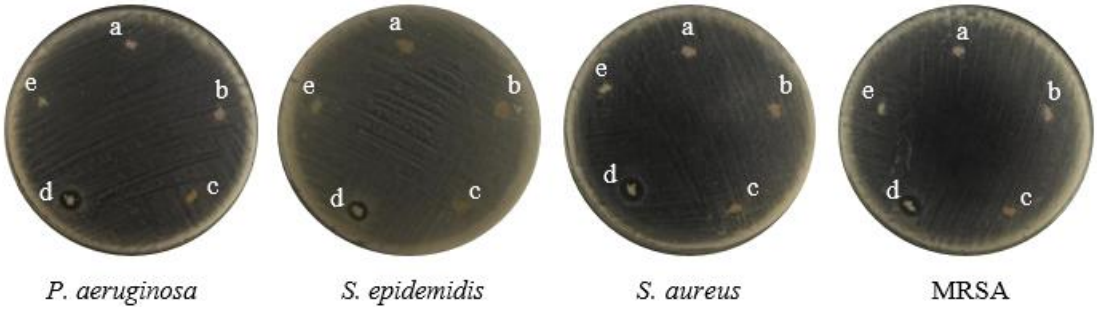

Figure 3. Antibacterial assay of TLC spots of the ethyl acetate fraction by diffusion test: a. 1st spot, b. 2nd spot, c. 3rd spot, d. 4th spot, e. 5th spot

Figure 3 shows that the 4th spot was suspected to be a terpenoid due to color changes after spraying using anisaldehyde-H_2SO_4 (Saifudin 2014). Terpenoids can cause cell membrane damage, inhibit protein synthesis, and impair enzyme function. Terpenoids appear to play a role in inhibiting Quorum Sensing (QS), the communication pathway between cells, which prevents biofilm creation. Terpenes such as carvacrol and thymol can effectively inhibit QS and prevent *Acinetobacter baumannii* biofilm formation (Tapia-Rodriguez et al. 2023).

LC-MS/MS analysis revealed peaks at 285.0760, 179, and 271 m/z, similar to Acacetin (Table 4). Acacetin ($C_{16}H_{12}O_5$), has a parent ion of 285 m/z and a fragment ion of 270, 242, 213, 193, 153, 133, and 118 m/z. Acacetin can inhibit the growth of pathogenic microbes such as MRSA, *S. aureus, P. aeruginosa, E. coli, Enterococcus faecalis*, and some species of Bacillus (Wojakowska et al. 2013; Cha et al. 2014; Komape et al. 2014; Joseph et al. 2015).

Furthermore, Table 4 identified a compound with a parent ion of 286.0659 m/z, followed by fragment ions of 111.5583 m/z and 197.2278 m/z at a retention time of 5.76 as kaempferol. Kaempferol ($C_{15}H_{10}O_6$) obtained from the *Arabidopsis thaliana* methanol extract has a molecular weight of 287 g/mol with fragment ions of 241, 213, 197, 165, and 111 m/z (Doerfler et al. 2014). Kaempferol can be an antimicrobial. These compounds can inhibit the growth of *S. aureus, P. aeruginosa,* and *E. coli* (Cruz et al. 2020). In addition, kaempferol has anticancer activity against brain, breast, liver, colon, prostate, pancreas, and kidney cancer (Imran et al. 2019b).

At a retention time of 6.24 minutes, the $C_{24}H_{24}O_{12}$ formula compound, with a parent ion of 505.1339 m/z and a fragment ion of 301.0705 m/z, was identified as Peonidin. Peonidin is a magenta pigment that is commonly found in berries. Peonidin shows the same molecular ion of 301 m/z (Xu et al. 2012). Peonidin showed molecular ions at 301, 286, and 258 m/z. Peonidin inhibits the growth of *E. coli, Micrococcus luteus, Klebsiella pneumoniae, Haemophilus influenzae,* and *Candida albicans* with a MIC value of >400 µg/mL (Kammerer et al. 2003; Bhat et al. 2022).

Chrysoeriol compounds exhibit 301.0719 m/z parent ion molecules and 258.0529 and 286.0481 m/z fragment ions at a retention time of 7.81 minutes. Chrysoeriol has a $C_{16}H_{12}O_6$ formula with a 300 g/mol molecular weight. This molecule contains one methoxy group at C3' and three hydroxyl groups at C5, C7, and C4' (Chan et al. 2022). Chrysoeriol is found in *E. ciliata, Lonicera japonica, Aspalathus linearis,* and *Medicago sativa*. Chrysoeriol inhibits the growth of pathogenic bacteria *S. aureus* and MRSA (Bashyal et al. 2019).

The compound with a retention time of 9.72 minutes and had parent ions 403.1396 m/z and fragment ions 373.0923 and 388.1152 m/z was identified as nobiletin. According to Yao et al. (2012), nobiletin peaks at 403.2 and 373.0 m/z, close to the fragment ions in this study. The molecular weight of nobiletin is 402.4 g/mol with the $C_{21}H_{22}O_8$ formula, commonly found in *Citrus.* Nobiletin inhibits the growth of *P. fluorescens* and *P. aeruginosa* (Kumar et al. 2011).

At the retention time of 15.72 minutes, the compound with $C_{34}H_{40}O_9$ formula, 593.2776 m/z molecular ions, and 533.2557m/z fragment ions were identified as luteolin. Luteolin derivative compounds show 593, 533, 287, and 153 m/z ionic molecules. Luteolin is mainly found in legumes, Scrophulariaceae, and vegetables such as celery, broccoli, and leeks. This compound has the potential as an anticancer for various types of cancer and antibacterial activity against *S. aureus* with a MIC value of 64 µg/mL (Imran et al. 2019a; Qian et al. 2020; Sinan et al. 2020).

Antroquinonol compounds exhibit ionic molecules at 391.2845 and 121.0286 m/z at a retention time of 17.28 minutes. Antroquinonol with the molecular formula $C_{24}H_{38}O_4$ has a similar molecular, 391.2845 m/z. This compound has an anticancer activity which was effective against MCF-7 and MDA-MB-231 (*human breast carcinoma*) (Wang et al. 2018). Antroquinonol can potentially inhibit cell proliferation and growth of some cancer cells. This compound is commonly found in *Antrodia camphorate* (Ho et al. 2014).

The study demonstrated that the hexane, ethyl acetate, and water fractions of *E. acuminata* leaves exhibit antibacterial activity against *P. aeruginosa, S. epidermidis, S. aureus,* and MRSA, with the ethyl acetate fraction showing the largest inhibition zone. The TLC profile and bioautography of the ethyl acetate fraction at the 4th stain (Rf 1.00) had antibacterial activity against all test bacteria. LC-MS/MS identified compounds in the ethyl acetate fraction identified as acacetin, kaempferol, peonidin, chrysoeriol, nobiletin, and luteolin, which all exhibit antibacterial properties, and antroquinonol, which has anticancer activity. *E. acuminata* leaves have the potential to be further developed as a new antibacterial drug agent.

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