



Phytochemical screening and antibacterial activity of ethnomedicinal plants from Gayo Lues Highland, Indonesia

[Cribado fitoquímico y actividad antibacteriana de plantas etnomedicinales del altiplano de Gayo Lues, Indonesia]

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Abstract

Context: Investigations of phytochemical screening and antibacterial activity were carried out on traditional medicinal plants collected in the highlands of Gayo Lues, Aceh Province, at an elevation of 1,000 meters above sea level (m.a.s.l.).

Aims: To evaluate the antibacterial activity of plants chosen based on the interview results with the traditional healers (n = 5) in Gayo Lues.

Methods: Ethanolic maceration was performed on the 12 identified ethnomedicinal plants and qualitatively screened for the phytochemical contents. Antibacterial activities against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* were tested for each extract based on the disc diffusion method, and MIC was determined using cephazolin as a reference drug.

Results: The phytochemical screening of twelve plant species showed secondary metabolites class steroids, terpenoids, flavonoids, phenols, alkaloids, and saponins. Evaluation of the antibacterial activity of the extract (10 mg/mL) yielded inhibition zone ranges of 9.8 ± 0.26 to 21.87 ± 0.47 mm for *E. coli* and 8.93 ± 0.9 to 23.97 ± 0.68 for *S. aureus*. The ethanolic extract of *Garcinia macrophylla* Mart stem barks showed the highest antibacterial activity, where at the lowest concentration (1.25 mg/mL), the inhibition zones were found to be 19.2 ± 0.61 and 20.72 ± 0.44 mm for *E. coli* and *S. aureus*, respectively. The MIC was found to be 1.25 mg/mL.

Conclusions: This study concludes that the twelve plant species are worthy of further investigation for novel antibacterial agent exploration.

Keywords: antibacterial; *Escherichia coli*; ethnomedicinal plant; *Garcinia macrophylla*; phytochemical; *Staphylococcus aureus*.

Resumen

Contexto: Se llevaron a cabo investigaciones de cribado fitoquímico y actividad antibacteriana en plantas medicinales tradicionales recolectadas en las tierras altas de Gayo Lues, provincia de Aceh, a una altitud de 1.000 metros sobre el nivel del mar (m.s.n.m.).

Objetivos: Evaluar la actividad antibacteriana de plantas elegidas basándose en los resultados de las entrevistas con los curanderos tradicionales (n = 5) de Gayo Lues.

Métodos: Se realizó una maceración etanólica de las 12 plantas etnomedicinales identificadas y se analizó cualitativamente su contenido fitoquímico. Se probó la actividad antibacteriana de cada extracto contra *Escherichia coli* ATCC 25922 y *Staphylococcus aureus* mediante el método de difusión en disco y se determinó la CMI utilizando la cefazolina como fármaco de referencia.

Resultados: El cribado fitoquímico de doce especies de plantas mostró una clase de metabolitos secundarios de esteroides, terpenoides, flavonoides, fenoles, alcaloides y saponinas. La evaluación de la actividad antibacteriana del extracto (10 mg/mL), arrojó rangos de zona de inhibición de $9,8 \pm 0,26$ a $21,87 \pm 0,47$ mm para *E. coli* y de $8,93 \pm 0,9$ a $23,97 \pm 0,68$ para *S. aureus*. El extracto etanólico de la corteza del tallo de *Garcinia macrophylla* Mart mostró la mayor actividad antibacteriana, ya que a la concentración más baja (1,25 mg/mL) las zonas de inhibición fueron de $19,2 \pm 0,61$ y $20,72 \pm 0,44$ mm para *E. coli* y *S. aureus*, respectivamente. La CMI fue de 1,25 mg/mL.

Conclusiones: Este estudio concluye que las doce especies de plantas son dignas de una mayor investigación para la exploración de nuevos agentes antibacterianos.

Palabras Clave: antibacteriano; *Escherichia coli*; fitoquímico; *Garcinia macrophylla*; planta etnomedicinal; *Staphylococcus aureus*.

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INTRODUCTION

Indonesia is a country that belongs to the mega biodiversity pool with tropical rainforest vegetation (Wasis, 2012). Aceh is one of its provinces with a climate of high rainfall (McCarthy, 2002), while Gayo Lues District is a highland area (\pm 1,000 meters above sea level—masl) enriched with the biodiversity of fauna and flora. The paramount of the Gayo Lues District is the Leuser Ecosystem, which has been popularly known as the lung of the world (Djufri, 2015). This district was covered by hilly and mountainous areas, hence the name Gayo Lues Highland. This area is far from the urban area and can be reached by land where access is difficult to travel by transportation (BPS, 2015). In addition, Gayo Lues has a limited number of hospitals, and their facilities are minimal. Hence, the locals utilize plants from the surrounding environment for disease management (Fitrianti et al., 2012; Lastari and Agustina, 2018; Rosita and Wani, 2018). About 80% of the global population has used plants as traditional medicines (Hosseinzadeh et al., 2015). The potential of plants as a source of medicine is due to the active role of secondary metabolites contained in plants (Barnes, 2010; Lipkovskaya et al., 2014; Takos and Rook, 2013). Many consider that plant-based medicines are less toxic, minimal side effects and cost-effective. Antibacterial activity is the most common bioactivity observed in medicinal plants.

Multiple investigations have been carried out to explore the antibacterial potentials of plant extracts. This is because bacterial infection diseases have been perceived as a significant threat to human health, with a high number of annual deaths recorded (Jones et al., 2008). Moreover, the demand for new antibacterial agents is currently high since the development of multidrug-resistant bacteria caused by the improper use of antibiotics (Pulingam et al., 2022). Of many cases of bacterial infection or intoxication, *Escherichia coli* and *Staphylococcus aureus* have been reported to be predominant (Frickmann et al., 2019). These two pathogenic bacteria are responsible for hygiene quality-related diseases such as diarrhea (Barreto de Deus et al., 2017). In a review article, antibacterial plant extracts have been recognized as being almost as effective as synthetic drugs (Kadhim et al., 2016).

Herein, we have reported the results from antibacterial activity screening against *E. coli* and *S. aureus* of ethnomedicinal plants from Gayo Lues Highlands (geographically located at 3°40'24"-4°40'26" N and 96°43'24" - 97°55'24" E Longitude) (BPS, 2020). The sampling location generally has an elevation above sea level of >700 masl (Istiawan and

Kastono, 2019). We first retrieved information about the ethnomedicinal plant by interviewing the traditional healer in Gayo Lues. Only plants rarely used or underreported were included to add novelty to our study.

MATERIAL AND METHODS

Materials

The supporting instruments for research were a rotary evaporator (Hidolph Laborota 4003 Control), autoclave (Llenado-Filling), shaker (Edmund Buhler D-7454 Bodelshausen), spectrophotometer (Spectro 20 D Plus Spectrophotometer), and an incubator (Lab-Line Instruments Ultra-Clean "100").

The bacteria (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923) were obtained from the Microbiology Laboratory, Faculty of Pharmacy at the University of Sumatera Utara (USU), Medan, Indonesia. This research was run with the following materials: Lactose Broth (LB), distilled water, Muller Hinton Agar (MHA), dimethyl sulfoxide (DMSO) 99.9%, NaCl, paper disc (6 mm), cephalosporin 30 µg (disc 6 mm), ethanol 96%, Whatman filter paper grade 41 (Merck, Darmstadt, Germany), Liebermann-Burchard's reagent, NaOH, Shinoda's reagent, FeCl₃, Dragendorff's reagent, Wagner's reagent, and Mayer's reagent. Otherwise stated, all chemicals used in this research were analytical grade and purchased from Merck (Selangor, Malaysia).

Interview and sample collection

Plant specimens were collected based on the interview results from traditional healers (n = 5) located across different sub-districts in Gayo Lues Highland. The interview was conducted by asking close-ended questions and note-taking to record the data. The questions included the name of the medicinal plants, their medicinal benefits, and how to prepare and administer them. The medicinal plants should be uncommonly used by the local community (for example, *Murraya koenigii*, a medicinal plant but commonly used as one of the local spices, hence was not enlisted). In addition, medicinal plants should be used individually without concoction. After that, the sample collection began in September 2016. Sampling locations were sub-districts in Gayo Lues, namely Blangkejeren, Dabun Gelang, Blang Pegayon, and Putri Betung. The map visually indicating the sampling locations is presented in Fig. 1, while the coordinates have been presented in Table 1. The specimens were identified by a botanist at Research Center for

Biology at the Indonesian Institute of Sciences (LIPI), Bogor, Indonesia.

Extraction

Different parts of one plant sample another were used according to information obtained from the healers; for instance, the extraction of *Artocarpus elasticus* Reinw ex Blume was only carried out on its stem barks while *Lonicera pulcherrima* Ridl - on its leaves

(Table 2). The already chopped leaves or stem barks from the respective plant were air-dried at room temperature ($25 \pm 1^\circ\text{C}$) for 7 days. Each dried sample weighed 170 g was macerated with hot ethanol 96% (1.7 L) (initial temperature of 60°C) and left in a sealed container for three days at room temperature. After the separation, all filtrates were subsequently concentrated using a rotary evaporator.

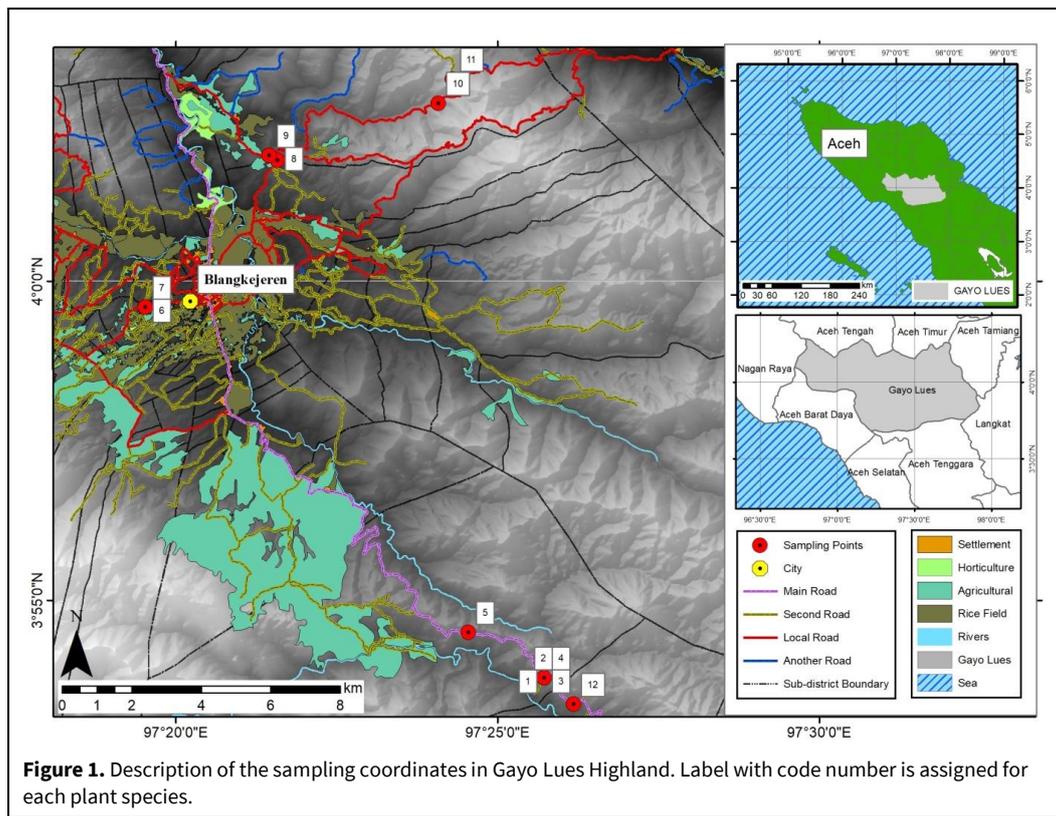


Figure 1. Description of the sampling coordinates in Gayo Lues Highland. Label with code number is assigned for each plant species.

Table 1. Location of the coordinates of sampling points in Gayo Lues Regency.

Plant code	Coordinates		Elevation (m.a.s.l.)	Subdistrict
	Latitude	Longitude		
1	3° 53' 47.544" N	97° 25' 43.536" E	1268	Blangkejeran
2	3° 53' 47.544" N	97° 25' 43.536" E	1268	Blangkejeran
3	3° 53' 47.544" N	97° 25' 43.536" E	1268	Blangkejeran
4	3° 53' 47.544" N	97° 25' 43.536" E	1268	Blangkejeran
5	3° 54' 30.276" N	97° 24' 32.868" E	1285	Blangkejeran
6	3° 59' 35.988" N	97° 19' 31.296" E	997.9	Blang Pegayon
7	3° 59' 35.988" N	97° 19' 31.296" E	997.9	Blang Pegayon
8	4° 1' 54.012" N	97° 21' 34.632" E	1033.2	Dabun Gelang
9	4° 1' 58.692" N	97° 21' 27.036" E	1038.4	Dabun Gelang
10	4° 2' 47.724" N	97° 24' 4.896" E	1456	Dabun Gelang
11	4° 3' 10.548" N	97° 24' 18.144" E	1624.4	Dabun Gelang
12	3° 53' 22.74" N	97° 26' 10.86" E	1239.4	Putri Betung

Table 2. Weight and percent yield extracted from traditional medicinal plants used in Gayo Lues.

Plant code	Name	Part	Weight (g)	Yield (%)
1	<i>Artocarpus elasticus</i> Reinw ex Blume	Stem bark	5.80	3.41
2	<i>Cinnamomum iners</i> Reinw. ex Blume	Stem bark	3.70	2.18
3	<i>Lonicera pulcherrima</i> Ridl.	Leaf	7.10	4.18
4	<i>Orophea corymbosa</i> (Blume) Miq.	Leaf	7.80	4.59
5	<i>Macaranga diepenhorstii</i> (Miq.) Mull. Arg.	Stem bark	6.90	4.06
6	<i>Bischofia javanica</i> Blume	Stem bark	3.30	1.94
7	<i>Stemonurus malaccensis</i> (Mast.) Sleumer	Stem bark	4.10	2.41
8	<i>Glochidion philippicum</i> (Cav.) C. B. Rob.	Stem bark	4.70	2.76
9	<i>Homalanthus populneus</i> (Geiseler) Pax	Stem bark	6.50	3.82
10	<i>Piper aduncum</i> L.	Leaf	6.10	3.59
11	<i>Garcinia macrophylla</i> Mart.	Stem bark	5.20	3.06
12	<i>Cinnamomum porrectum</i> (Roxb.) Kosterm	Stem bark	4.80	2.82

Phytochemical test

Secondary metabolites for steroids, terpenoids, flavonoids, phenols, alkaloids, and saponins of the extracts were identified using phytochemical screening methods. The steroids and terpenoids test were analyzed with Liebermann-Burchard's reagent, the flavonoids were observed by adding NaOH and Shinoda's reagent, the phenols were confirmed to the FeCl₃, the alkaloids were indicated by adding the Dragendorff's, the Wagner's and Mayer's reagents and the saponins were offered to a firm of formation foam (Tiwari et al., 2011).

Antibacterial assay

Bacteria *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used for antibacterial testing as representatives of Gram-negative and Gram-positive bacterial strains, respectively. The subcultures method was done to inoculate each strain of bacteria, as suggested previously (Khan et al., 2013). A volume of 20 mL of LB medium was prepared for each bacterium. The bacterium was taken from agar media stock and added to each LB medium. The bacterium in LB media was regenerated overnight at 37°C. The fresh bacterium was suspended in 10 mL NaCl 0.85% until an absorbance came to be 0.5-0.8 (to afford 1 × 10⁸ CFU/mL) on OD₆₀₀ by a spectrophotometer (Ngule and Ndiku, 2014).

The antibacterial activities were carried out by the disc diffusion method (Mostafa et al., 2018). A 25 mL of MHA solution was poured into a petri dish glass (size 150 × 15 mm) for screening assay of the ethanolic extract of the plants. Furthermore, new *E. coli* and *S.*

aureus were smeared over the petri dish agar media. The paper discs containing extracts (dose of 10 mg/mL), positive control of cephazolin (30 µg/mL), and negative control of DMSO (99.9%) were placed on the top of the growth media. Incubation was carried out at 37°C for 24 h. The inhibition zones were measured by a caliper in millimeters (mm) to indicate antibacterial activity.

The most effective extract was determined the MIC value by the disc diffusion method. A volume of 15 mL of MHA was placed into a Petri dish glass (size of 100 × 15 mm) and spread with fresh bacteria. The paper discs contained different concentrations (15, 12.5, 10, 5, 2.5, and 1.25 mg/mL), DMSO as a negative control and cephazolin (30 µg/mL) as a positive control were sited on top of the media. The incubation time and temperature were set at 24 h and 37°C, respectively. A measurement of the inhibition zone (mm) was done by using the calipers. The MIC was calculated as suggested by a previous study (Mostafa et al., 2018), where the value represents the lowest concentration of the extract to inhibit the bacterial growth following 24 h incubation.

Data analysis

The obtained data were managed using Microsoft Excel 2010 software (Microsoft Corp., Redmond, WA). All data were presented as means ± standard deviation (SD) in the antibacterial assay. The statistical significance of differences was analyzed on GraphPad Prism 9 based on ANOVA and post hoc Tukey. Statistical significance was considered reached if p<0.05.

RESULTS AND DISCUSSION

Medicinal plants suggested by the local healers

The interview results with the local healers in Gayo Lues Highland across four sub-districts (Blangkejeren, Dabun Gelang, Blangpegayon, and Putri Betung) have been presented (Table 3). All healers were above 50 years old, 54 years old being the youngest, and 74 years old being the oldest. The healers identified 12 medicinal plants: terap, rangut galang, jerig, mata kule, tampu, aging, selupik, bebelo, medang kanis, tingkem, toyol uten, gerupel, which were characterized as *Artocarpus elasticus* Reinw ex Blume, *Cinnamomum iners* Reinw. ex Blume, *Orophea corymbosa* (Blume) Miq., *Lonicera pulcherrima* Ridl., *Macaranga diepenhorstii* (Miq.) Mull. Arg., *Glochidion philippicum* (Cav.) C. B. Rob., *Homalanthus populneus* (Geiseler) Pax, *Piper aduncum* L., *Garcinia macrophylla* Mart., *Bischofia javanica* Blume, *Stemonurus malaccensis* (Mast.) Sleumer, and *Cinnamomum porrectum* (Roxb.) Kosterm, respectively. These plants are from families *Annonaceae* (n = 1), *Caprifoliaceae* (n = 1), *Clusiaceae* (n = 1), *Euphorbiaceae* (n = 2), *Lauraceae* (n = 2), *Moraceae* (n = 1), *Phyllanthaceae* (n = 2), *Piperaceae* (n = 1), and *Stemonuraceae* (n = 1). The commonly utilized part is their stem barks or leaves in treating various diseases, including diarrhea, skin diseases, fever, and diabetes. Many preparation methods

included boiling the plant and administering it by drinking (oral) or making the plant paste by crushing and applied on the skin.

A dozen plants in this study have been used by many people in Gayo Lues highland from generation to generation to treat diseases caused by bacteria. They are *Artocarpus elasticus* Reinw ex Blume, *Bischofia javanica* Blume, *Glochidion philippicum* (Cav.) C. B. Rob., *Cinnamomum iners* Reinw. ex Blume, *Cinnamomum porrectum* (Roxb.) Kosterm, *Garcinia macrophylla* Mart., *Stemonurus malaccensis* (Mast.) Sleumer, *Homalanthus populneus* (Geiseler), *Macaranga diepenhorstii* (Miq.) Mull. Arg., *Lonicera pulcherrima* Ridl., *Orophea corymbosa* (Blume) Miq., and *Piper aduncum* L. Our search of scientific document database suggested that the previous ethnomedicinal plants were underreported. Therefore, to compare with published reports, we searched for their families, where the results have been presented in Table 4. The bioactivity of plants under the same family could be similar to their mutually shared secondary metabolites among the family members (Wink, 2003). Most of the studies, however, did not discuss the chemical content and the value of the antibacterial activity. Therefore, the results of our work could be novel findings for reporting the antibacterial activities of the ethnomedicinal plants from Gayo Lues Highland.

Table 3. Interview results with healers in Gayo Lues Highland.

Healer (age, years old)	Sub-district address	Medicinal plant		Traditional medicine			
		Local name	Latin name (Family)	Part	Disease	Stock or preparation	Administration
A (54)	Blangkejeren	Terap	<i>Artocarpus elasticus</i> Reinw. ex Blume (Moraceae)	Stem bark	Diarrhea	Brew	Oral
					Facial spots	Paste	Topical
					Diabetes	Brew	Oral
		Rangut galang	<i>Cinnamomum iners</i> Reinw. ex Blume (Lauraceae)	Stem bark	Diarrhea	Brew	Oral
					Damaged skin	Brew, paste	Oral, topical
					Inflammation	Brew	Oral
		Jerig	<i>Orophea corymbosa</i> (Blume) Miq. (Annonaceae)	Leaf	Fever	Crushed-squeezed	Oral
					Nausea	Crushed-squeezed, paste	Oral, respiratory
					Stomachache	Brew	Oral
					Wound	Paste	Topical
					Skin diseases	Brew, paste	Oral, topical
					Diabetes	Brew	Oral

Table 3. Interview results with healers in Gayo Lues Highland (continued...)

Healer (age, years old)	Sub-district address	Medicinal plant		Traditional medicine								
		Local name	Latin name (Family)	Part	Disease	Stock or preparation	Administration					
B (56)	Blangkejeren	Mata kule	<i>Lonicera pulcherrima</i> Ridl. (Caprifoliaceae)	Leaf	Wound	Paste	Topical					
					Inflammation	Brew	Oral					
					Stomachache	Brew	Oral					
					Flu	Crushed-squeezed	Oral					
					Diabetes	Brew	Oral					
					Oral hygiene	Brew	Gargle					
					Diarrhea	Brew	Oral					
		Tampu	<i>Macaranga diepenhorstii</i> (Miq.) Mull. Arg. (Euphorbiaceae)	Stem bark	Wound	Paste	Topical					
					Furuncle	Paste	Topical					
					Bruise	Paste	Topical					
					Swelling	Paste	Topical					
					Damaged skin	Brew, paste	Oral, topical					
					C (58)	Dabun Gelang	Aging	<i>Glochidion philippicum</i> (Cav.) C. B. Rob. (Phyllanthaceae)	Stem bark	Common cold	Fresh	Chew
										Diarrhea	Brew	Oral
Diabetes	Brew	Oral										
Hypercholesterolemia	Brew	Oral										
Wound	Paste	topical										
Selupik	<i>Homalanthus populneus</i> (Geiseler) Pax (Euphorbiaceae)	Stem bark	Diarrhea	Brew			Oral					
			Wound	Paste			Topical					
			Furuncle	Paste			Topical					
			Bruise	Paste			Topical					
			Swelling	Paste			Topical					
Bebelo	<i>Piper aduncum</i> L. (Piperaceae)	Leaf	Damaged skin	Brew, paste			Oral, topical					
			Cold and fever	Crushed-squeezed			Oral					
			Diarrhea	Brew			Oral					
			Wound	Paste			Topical					
Medang kanis	<i>Garcinia macrophylla</i> Mart. (Clusiaceae)	Stem bark	Diabetes	Brew	Oral							
			Diabetes	Brew	Oral							
			Wound	Paste	Topical							
			Obesity	Dried-powder	Oral							
			Anti-carcinogenic	Brew	Oral							
			Skin diseases	Brew, paste	Oral, topical							
			Sprue	Brew	Gargle							
			Hypertension	Brew	Oral							

Table 3. Interview results with healers in Gayo Lues Highland (continued...)

Healer (age, years old)	Sub-district address	Medicinal plant		Traditional medicine			
		Local name	Latin name (Family)	Part	Disease	Stock or preparation	Administration
D (73)	Blangpegayon	Tingkem	<i>Bischofia javanica</i> Blume (<i>Phyllanthaceae</i>)	Stem bark	Diarrhea	Brew	Oral
					Diabetes	Brew	Oral
					Common cold	Brew	Oral or topical
					Wound	Paste	topical
E (74)	Putri Betung	Gerupel	<i>Cinnamomum porrectum</i> (Roxb.) Kosterm (<i>Lauraceae</i>)	Stem bark	Diarrhea	Brew	Oral
					Diabetes	Brew	Oral
					Hypercholesterolemia	Brew	Oral
					Wound	Paste	Topical

Table 4. The profiles of the traditional medicinal family plants used in Gayo Lues Highland.

Plant family	Secondary metabolites	Uses as traditional medicine	Reference
<i>Annonaceae</i>	Alkaloids, flavonoids, terpenoids, steroids, lignans, acetogenins, phenols	Malaria, wounds, respiratory infections, skin diseases, diarrhea, dysentery, fever, chills, cancer, hepatitis	(Attiq et al., 2017; Harahap et al., 2022; Zhao et al., 2019)
<i>Caprifoliaceae</i>	Saponins, flavonoids, coumarins, phenolics, alkaloids, terpenoids	Infectious diseases, wounds, inflammation, hypotension, sedation, fever	(Chen et al., 2012; Guvenalp et al., 2012)
<i>Clusiaceae</i>	Flavonoids, phenols, tannins, saponins, terpenoids, alkaloids	Inflammation, oxidative stress, microbial infection, cancer, obesity	(Che Hassan et al., 2018; Hemshekhar et al., 2011; Utami, 2016)
<i>Euphorbiaceae</i>	Terpenoids, flavonoids, tannins, coumarins, polyphenols, alkaloids, steroids, saponins	Diarrhea, inflammation of the respiratory tract, swelling, wounds, ulcers, bruises, skin diseases, suppuration, tumors, asthma, cough, dysentery	(MagadulaJoseph, 2014; Pascal et al., 2017; Rizk, 1987)
<i>Lauraceae</i>	Alkaloids, phenylpropanoids, polyphenols, terpenoids, steroids, flavonoids	Toothache, oral infections, diarrhea, cardiovascular disease, oxidative stress	(Espineli et al., 2013; Hammid et al., 2016; Nabavi et al., 2015; Oliveira Filho et al., 2015; Saetan et al., 2016)
<i>Moraceae</i>	Alkaloids, flavonoids, phenols, tannins, terpenoids, steroids, saponins	Stomach pain, ulcers, asthma, ringworm, diabetes, gallstones, wounds, liver cirrhosis, hypertension, skin diseases	(Jagtap and Bapat, 2010; Somashekhar et al., 2013)
<i>Phyllanthaceae</i>	Terpenoids, tannins, alkaloids, flavonoids, phenols, steroids	Hair stimulant, wounds, diarrhea, dysentery	(Ajaib and Khan, 2012; Aththorick and Berutu, 2018; Huang et al., 2015; Jambak et al., 2019)
<i>Piperaceae</i>	Alkaloids, flavonoids, steroids, terpenoids, saponins, phenols	Insecticides, asthma, bronchitis, hay fever, inflammation, sores, diarrhea, dysentery, nausea, ulcers, genito-urinary infections	(Batan et al., 2018; Bouzada et al., 2009; Chahal et al., 2011; Kartika et al., 2019; Pacheco et al., 2014; Torres-Pelayo et al., 2016)
<i>Stemonuraceae</i>	Terpenoids, alkaloids, tannins	Hypertension, asthma, stomachache	(Potgieter et al., 2016)

Table 5. The phytochemical screening of the traditional medicinal plant extract.

Plant	Secondary metabolites					
	Steroid	Terpenoid	Flavonoid	Alkaloid	Phenol	Saponin
<i>A. elasticus</i>	-	+	+	+	+	+
<i>C. iners</i>	-	+	+	+	+	+
<i>L. pulcherrima</i>	+	+	+	+	+	+
<i>O. corymbosa</i>	+	+	+	+	+	+
<i>M. diepenhorstii</i>	-	+	+	+	+	+
<i>B. javanica</i>	+	+	+	+	+	-
<i>S. malaccensis</i>	+	+	+	+	+	+
<i>G. philippicum</i>	+	+	+	+	+	+
<i>H. populneus</i>	+	+	+	+	+	+
<i>P. aduncum</i>	+	+	+	+	+	+
<i>G. macrophylla</i>	+	+	+	+	+	+
<i>C. porrectum</i>	-	+	+	+	+	-

(+): present; (-): absent.

Phytochemical profiles of the ethnomedicinal plants

The obtained weight and yield of the extract of each plant specimen have been presented in Table 2. Yield percentages ranged from 1.94 to 4.59%, with *B. javanica* having the lowest percentage and *O. corymbosa* having the highest. As for the phytochemical class screening, the results have been presented in Table 5. *L. pulcherrima*, *O. corymbosa*, *S. malaccensis*, *G. philippicum*, *H. populneus*, and *G. macrophylla* were found positive containing all phytochemical classes (steroids, terpenoids, flavonoids, alkaloids, phenols, and saponins). Several plant extracts, such as those from *A. elasticus*, *C. iners*, *M. diepenhorstii*, and *C. porrectum* were either negative containing steroids or saponins.

The phytochemical results revealed that the compound groups of terpenoids, flavonoids, alkaloids, and phenols were present in the extracted plants. Comparing our findings (Table 5) with those reported (Table 4), we conclude that the plant species contains the same predominant phytochemical classes under the same family. The previous phytochemicals have been associated with bacteriostatic and bactericidal activities of plant extracts (Cowan, 1999; Harahap et al., 2022).

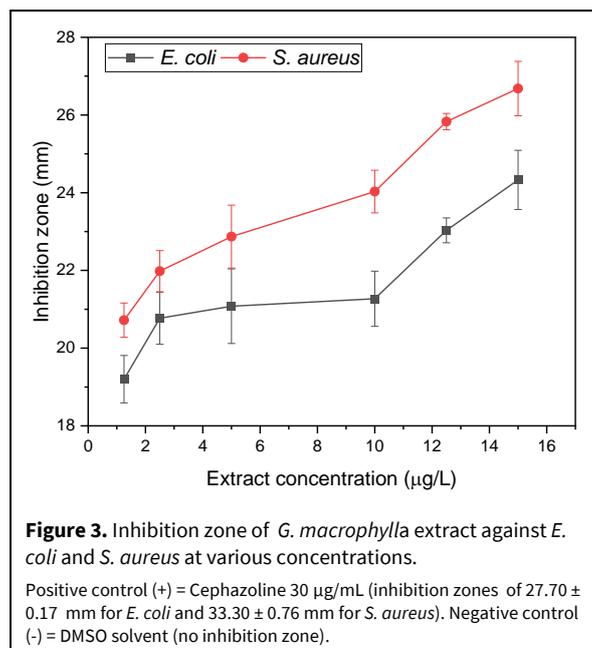
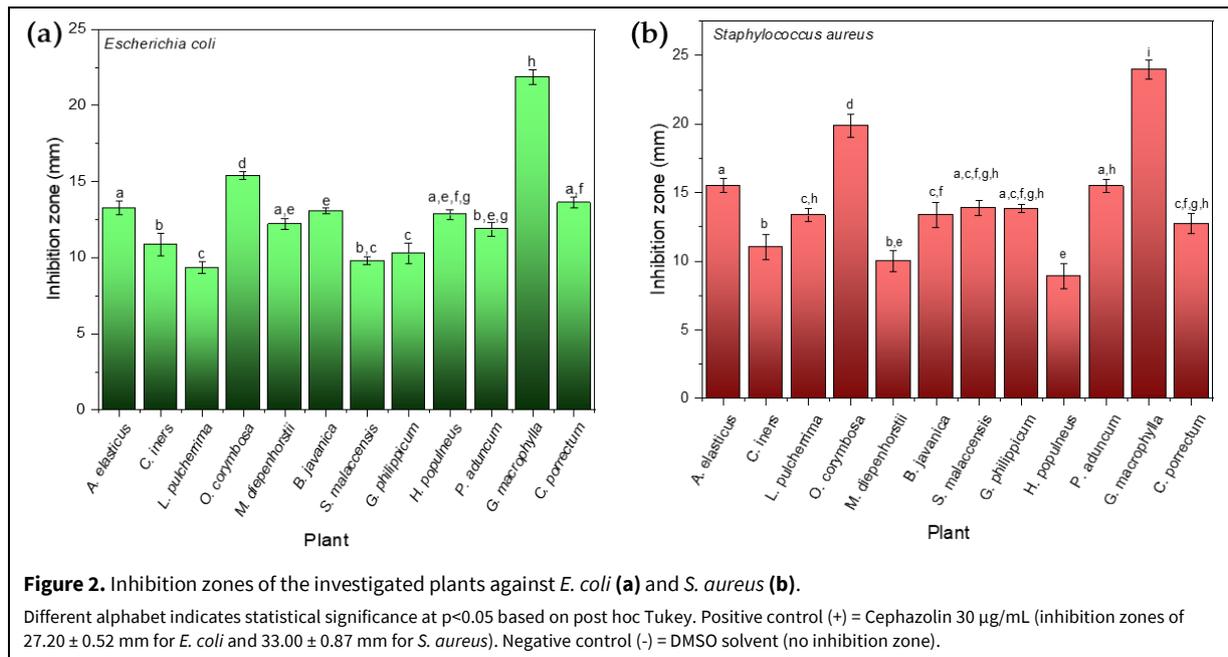
Antibacterial activity of ethnomedicinal plants

The plant species were investigated for evaluate their antibacterial activity against a strain bacteria Gram-negative (*E. coli* ATCC 25922) and Gram-positive (*S. aureus* ATCC 25923). The results of the

antibacterial activity of these plant extracts with a dose of 10 mg/mL have been presented in Fig. 2. From the highest to the lowest, *G. macrophylla*, *O. corymbosa*, and *A. elasticus*, were the top three bacterial growth inhibitor against both *E. coli* and *S. aureus*. Mostly, the extracts were more potent against Gram-positive *S. aureus* than that of Gram-negative *E. coli*, except in the case of *C. porrectum*, *H. populneus*, and *M. diepenhorstii*. Ethanolic extract from *G. macrophylla* stem barks achieved inhibition zones of 21.87 ± 0.47 and 23.97 ± 0.68 mm for *E. coli* and *S. aureus*, respectively. Further investigation was carried out using the extract from *G. macrophylla* stem barks due to its high antibacterial activities.

Inhibition zones against *E. coli* ATCC 25922 or *S. aureus* ATCC 25923 observed following the diffusion of *G. macrophylla* with concentration variation (1.25–15 mg/mL) have been presented in Fig. 3. At all concentrations, the activity was always higher in *S. aureus* as opposed to *E. coli*. Increasing trend of inhibition zones was found to be dependent to the concentration of *G. macrophylla* extract. At concentration of 15 mg/mL, the inhibition zones against *E. coli* and *S. aureus* were 24.33 ± 0.76 and 26.68 ± 0.7 mm, respectively. Meanwhile, at the lowest concentration, the inhibitions zones of 19.2 ± 0.61 and 20.72 ± 0.44 mm were obtained against *E. coli* and *S. aureus*, respectively. Herein, the MIC of the extracts against both *E. coli* and *S. aureus* was found to be 1.25 mg/mL.

The secondary metabolite content of the plant extract can act as an antibacterial. The steroids,



terpenoids and saponins have been demonstrated to disrupt and damage the cell membrane of bacteria (Doğan et al., 2017; Francis et al., 2002; Jasmine et al., 2011). The antibacterial actions of flavonoids have been displayed to inhibit nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, and the porins on the cell membrane (Xie et al., 2015). The antibacterial mechanism of alkaloids has been investigated through inhibitions of nucleic acid synthesis and the work of enzymes (Cushnie et al., 2014). The phenols have shown a role in inhibiting growth by denaturing and coagulating proteins (Sabbini, 2016).

Based on the interview results, the plants were used to treat diseases among the local community in Gayo Lues Regency. These diseases could be associated to bacterial infections such as diarrhea and wound infections. About 80% of the area of Gayo Lues District are a forest area. Therefore, all of these plants are easily found in the forest surrounding to community settlements (Tetra Tech ARD, 2013). Some plants with the same family group in Gayo Lues, namely *Annonaceae*, *Euphorbiaceae*, *Phyllanthaceae*, *Clusiaceae*, *Lauraceae*, *Moraceae*, and *Piperaceae* were previously reported as a source of medicine in the

management of diseases caused by bacteria (Elliott and Brimacombe, 1987).

The bioactive test results on the growth of *E. coli* and *S. aureus* demonstrated that the plant extract tested were in the zone of moderate activity inhibition based on the classification of antimicrobial strength as weak, moderate, and strong activity (indicated by inhibition zones of <12, 12-20, and >20 mm, respectively) (Shaaban et al., 2017). The extract of *C. iners* on both bacteria showed weak antibacterial activity. Although the extract of *C. iners* stem barks was weak in inhibiting the microbial growth, it is often used as medicine by the Gayo Lues community. This plant could be found around residential areas on house fences (Buru et al., 2014). The extract of *G. macrophylla* showed strong activity against the *E. coli* and *S. aureus*. Therefore, evaluations of its antibacterial activity using various concentrations of 1.25, 2.50, 5.00, 10.00, 12.50, and 15.00 mg/mL against *E. coli* and *S. aureus* were performed. At the lowest concentration (1.25 mg/mL), the extract had moderate and strong activities against *E. coli* (19.20 ± 0.61 mm) and *S. aureus* (20.72 ± 0.44), respectively. This activity could suggest *G. macrophylla* role in managing healing diarrhea, dysentery and external and internal wounds.

CONCLUSION

The ethanol extract from the uncommonly used ethnomedicinal plants from Gayo Lues Highlands showed weak to strong inhibitory activity against *E. coli* bacteria ATCC 25922 and *S. aureus* ATCC 25923. The extract from *G. macrophylla* stem barks was revealed to possess the strongest antibacterial activity among other extracts. The study concludes these ethnomedicinal plant species have the potency in the field of medicine to overcome bacterial infectious diseases.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Cane HPCA	Musman M	Yahya M	Saidi N	Darusman D	Nanda M	Rizki DR	Puspita K
Concepts or ideas	x	x	x	x	x			
Design	x	x				x	x	x
Definition of intellectual content	x	x	x	x	x			
Literature search	x	x	x	x	x		x	x
Experimental studies	x						x	x
Data acquisition	x						x	x
Data analysis	x	x	x	x	x	x	x	x
Statistical analysis	x	x	x	x			x	x
Manuscript preparation						x		
Manuscript editing						x		
Manuscript review	x	x	x	x	x	x	x	x

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