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Antimicrobial Properties of Medicinal Plants in the Lower Area of Ie Seu-um Geothermal Outflow, Indonesia

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Abstract

The lower area of the Ie Seu-um manifestation, located in Ie Seu-um village, Aceh Besar District, harbors several medicinal plants that exhibit potential for the treatment of infectious diseases. This study aims to assess the secondary metabolite content and in vitro antimicrobial activity against *Staphylococcus aureus, Escherichia coli, and Candida albicans* of medicinal plants inhabiting the geothermal region. Medicinal plants, namely *Pluchea indica* (L.) Less., *Acrostichum aureum* L., *Acacia mangium* L., and *Calotropis gigantea* (L.) Dryand., were collected within a range of 100-150 meters from the hot springs in the lower area. Methanol extracts of these medicinal plants underwent phytochemical screening and were tested for antimicrobial activity using the Kirby-Bauer disc diffusion method at a concentration of 50%. The results of phytochemical screening demonstrated positive variations in alkaloids, flavonoids, saponins, steroids, triterpenoids, and tannins for each medicinal plant. The antimicrobial activity of the methanol extracts noticeably inhibited the growth of *S. aureus* compared to *E. coli* and *C. albicans*. The largest inhibition zones were observed for the leaf part of *A. mangium* (12.70 ± 2.30 mm) against *S. aureus*, the aerial part of *A. aureum* (11.57 ± 2.01 mm) against *E. coli*, and the aerial part of *P. indica* (9.89 ± 1.11 mm) against *C. albicans.* Based on the research findings, medicinal plants originating from the lower area of the Ie Seu-um manifestation exhibit potential as antimicrobial agents, particularly against gram-positive bacteria.

1. Introduction

Seulawah Agam is a volcano in Aceh Province, Indonesia that is estimated to have geothermal energy potential of more than 160 MW [1]. Geographically, Seulawah Agam is mostly located in a protected forest area where a variety of biodiversity exists. The existence of the mountain gave rise to several points of geothermal manifestation, one of which is found in Ie Seu-um village, Mesjid Raya subdistrict, Aceh Besar district. This manifestation, known as the Ie Seu-um manifestation, is a hot spring situated in the outflow geothermal zone of Mount Seulawah Agam [2, 3].

Geothermal manifestations support the growth of vegetation and enable adaptation to extreme environmental conditions [4, 5]. The average temperature of the soil surface in the Ie Seu-um manifestation area is approximately 39 to 42°C [6]. The environmental conditions resulting from hydrothermal alteration around the Ie Seu-um manifestation will impact the biosynthetic pathway of secondary metabolites in these plants. Numerous studies have demonstrated that many secondary metabolites play a role in the plant's adaptation system to its surroundings and exhibit antimicrobial activity [7–9].

On the other hand, the world is currently facing a health problem characterized by the emergence of numerous cases of antimicrobial resistance caused by bacteria, fungi, viruses, and protozoa [10, 11]. The increasing incidence of resistance among pathogenic microorganisms against antimicrobial agents is primarily attributed to the inappropriate use of these agents in treating various infectious diseases [12]. These conditions have encouraged many researchers to explore and discover new antimicrobial agents by investigating the potential of plants and other natural resources [13, 14].

Based on a preliminary survey conducted in the Ie Seu-um manifestation area, it was found that the local community continues to utilize traditional medicines derived from plants in the vicinity of the geothermal site to treat various diseases. However, there is limited research data available on the medicinal properties of plant species in this area. Considering the significant potential of plants as a source of antimicrobial agents, this study aims to explore the in vitro antibacterial and antifungal activities of selected medicinal plants from the lower area of the Ie Seu-um manifestation.

2. Materials and Methods

2.1. Sample Collection

Plant materials were collected from the lower area of the Ie Seu-um manifestation (outflow geothermal zone) located in Ie Seu-um Village, Mesjid Raya Subdistrict, Aceh Besar District at coordinates 05°32'50''N - 95°32'45''E and 05°32'45"N - 95°32'50"E with an altitude of approximately 89 m above sea level (Figure 1). The lower area of the Ie Seu-um manifestation is 100-150 m away from the mineral-rich hot springs and has several medicinal plants that have adapted to the unique environmental conditions with an average ground temperature of 27 to 40^{o}C. The plant samples were collected in a random and representative manner using the quadrat sampling method, employing squares of predetermined sizes as sampling units.

2.2. Authentication of Medicinal Plants

The plant species were identified at the Botany Laboratory of the National Research and Innovation Agency (BRIN), Bogor Regency with identification number of 267/IPH.1.02/If.07/II/2016.

2.3. Preparation of methanol extract of medicinal plants

The collected plant materials were air-dried for 4 weeks and ground into a fine powder using a home grinder. Each part of the powdered plant materials was extracted using the cold maceration method. After macerating 100 grams of each part of a dry weight of medicinal plant in 500 mL of methanol for five days and stirring occasionally each day, the resulting mixture was filtered using Whatman filter paper No. 1 (Whatman Ltd). The filtrate was evaporated at 40°C using a rotary evaporator (BÜCHI Labortechnik AG) to obtain a viscous methanol extract. The yield of the crude extracts obtained was determined by using the following equation:

$$Percentage\ Yield\ (\%) = \frac{C}{S} \times 100\%$$

where C represent the dry weight of concentrated plant extract (g) and S represent the dry weight of a part of medicinal plants (g).

2.*4. Phytochemical Screening*

Preliminary phytochemical qualitative analysis was conducted using standard procedures to identify the presence of biologically active compounds. The analysis focused on determining the presence of alkaloids, flavonoids, saponins, steroids, triterpenoids, and tannins in the sample [2].

2.5. Preparation of Extract Concentration

The concentration of the crude extract of the plant materials, used for testing antimicrobial activity, was 50% for each sample of medicinal plants. The crude extract was dissolved in distilled water with the assistance of sonication for 20 minutes at 40°C to obtain the desired test concentration [15]. Subsequently, 20 µL of the test extract concentration was applied to a blank disc paper with a diameter of 6 mm.

2.6. Antimicrobial Activity Testing

The microbial isolates used as bioindicators in this research were *Staphylococcus aureus, Escherichia coli, and Candida albicans*. These isolates were collected from the

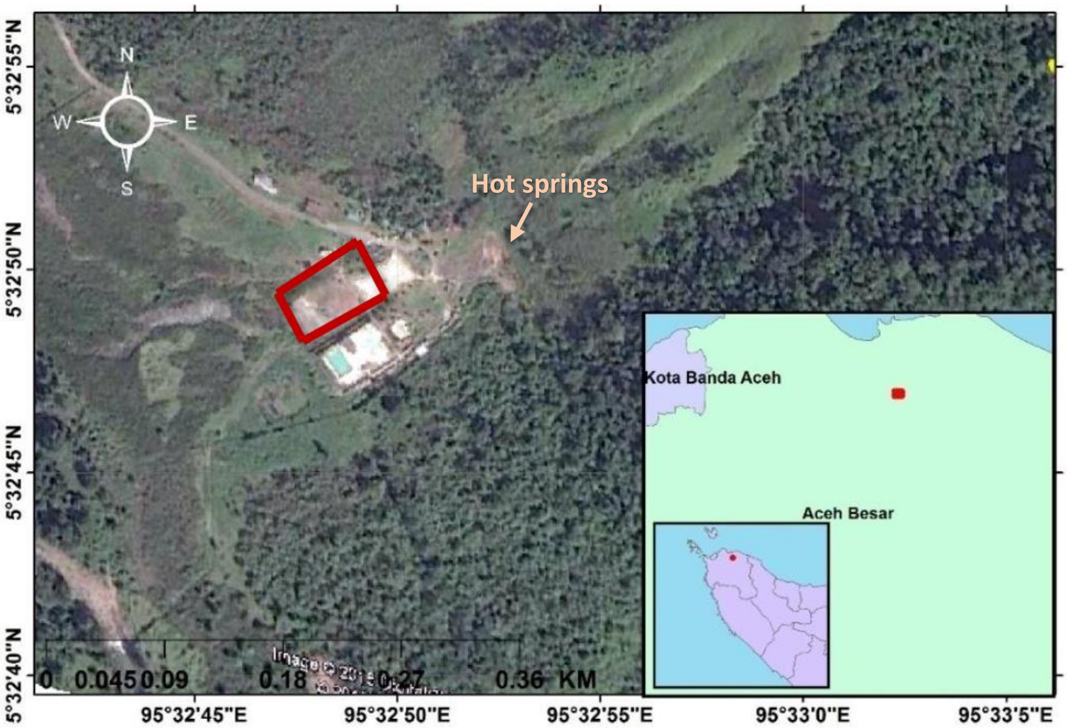

Figure 1. Location of the lower area of the Ie Seu-um manifestation (geothermal outflow zone).

Microbiology Laboratory of the Faculty of Medicine, Universitas Syiah Kuala, which have undergone rejuvenation and reidentification according to standard protocols [6]. The antimicrobial activity test was performed using the disk diffusion method (Kirby-Bauer). The turbidity of the microbial isolate suspensions was adjusted to the 0.5 McFarland standard, resulting in a final inoculum of 1.5 x 10^8 CFU/mL for bacteria and 2.5 x 10^6 CFU/mL for fungi [16, 17]. The bacterial suspensions of *S. aureus* and *E. coli* were each inoculated onto the surface of Mueller Hinton Agar (MHA), while *C. albicans* was inoculated onto the surface of Sabouraud Dextrose Agar (SDA) in Petri dishes using a sterile swab. The microbial isolate suspensions were then allowed to be absorbed by the agar for 15 minutes at room temperature. The Petri dishes that had been inoculated with microbial isolates were then placed with discs containing a 50% concentration of the methanol extract from each part of medicinal plants. The positive controls used were Gentamicin 10 µg (Oxoid) disk for bacterial isolates, Nystatin 100 U (Oxoid) disk for fungal isolate, and the negative control was a blank disc soaked in distilled water. The Petri dishes were incubated at 37°C for 24 hours with the lids inverted, and the inhibition zones (in mm) of bacterial and fungal growth were observed by measuring the diameter of the clear zones formed around the disks using a vernier calliper [18]. The antimicrobial activity testing was conducted with three repetitions to determine the diameter of the inhibition zones.

Figure 2. The medicinal plant species in the lower area of the Ie Seu-um manifestation.

3. Results and Discussion

3.1. Authentication and Extraction Yields of Medicinal Plants

In the lower area of the Ie Seu-um manifestation (outflow geothermal zone), numerous medicinal plants were observed, dispersed throughout the manifestation. Sample collection plots were established in a 50 x 50 meters size at various points within that area, leading to the identification of two tree species and two shrub species (Figure 2).

In Table 1, the authentication of plants in the lower area of the Ie Seu-um manifestation consists of the following species: *Pluchea indica* (L.) Less. (Compositae), *Acrostichum aureum* L. (Pteridaceae), *Acacia mangium* L.

Table 1. Ethnobotanical data and the percent yield of the methanol extracts of the medicinal plants from the lower area of the Ie Seu-um manifestation.

Scientific name	Family	Parts used	% Yield of extract	Locally used to treat [6]
Pluchea indica (L.) Less.	Compositae	Aerial	7.75	Lack of appetite, asthma, rheumatism, toothache, and fever.
Acrostichum aureum L.	Pteridaceae	Aerial	5.94	Inflammation, ulcers, constipation, asthma, and sore throats.
Acacia mangium L.	Leguminosae	Leaf	8.98	Fever, premature ejaculation, fever, and hypertension.
		Bark	10.35	Diarrhea, diabetes, and itching.
Calotropis gigantea (L.) Dryand.	Apocynaceae	Leaf	5.35	Canker sores, boils, scabies, and fever.
		Stem	6.14	Dyspepsia, canker sores, and itching.

Table 2. Preliminary phytochemical screening of methanol extracts of the medicinal plants from the lower area of the Ie Seu-um manifestation.

Scientific name	Parts used	Qualitative phytochemical					
		Alkaloids	Flavonoids	Saponins	Steroids	Triterpenoids	Tannins
Pluchea indica (L.) Less.	Aerial	+(ve)	-(ve)	+(ve)	-(ve)	+(ve)	+(ve)
Acrostichum aureum L.	Aerial	-(ve)	+(ve)	-(ve)	+(ve)	+(ve)	+(ve)
Acacia mangium L.	Leaf	+(ve)	+(ve)	-(ve)	-(ve)	+(ve)	+(ve)
	Bark	+(ve)	+(ve)	+(ve)	+(ve)	-(ve)	+(ve)
Calotropis gigantea (L.) Dryand.	Leaf	+(ve)	-(ve)	-(ve)	+(ve)	+(ve)	+(ve)
	Stem	-(ve)	+(ve)	-(ve)	-(ve)	+(ve)	-(ve)

Note: +(ve): Positive; -(ve): Negative.

(Leguminosae), and *Calotropis gigantea* (L.) Dryand. (Apocynaceae). These plant samples have been traditionally used by the local community to treat various diseases. Cold maceration method was employed for extraction, and the highest yield was obtained from the crude extract of *A. mangium* bark at 10.35%, while the lowest yield was obtained from the crude extract of *C. gigantea* leaves at 5.35%.

3.2. Phytochemical Screening and Antimicrobial Effect from Methanol Extract of Medicinal Plants

Identification of phytochemicals qualitatively from the methanol extracts of medicinal plants revealed the presence of different secondary metabolite groups including alkaloids, flavonoids, saponins, steroids, triterpenoids, and tannins (Table 2). All medicinal plants contain tannin compounds, except for the extract from the stem bark of *C. gigantea*. Positive triterpenoid content was found in all samples of medicinal plant extracts, except for the extract from the leaves of *A. mangium*. Several studies have shown that phytochemicals such as alkaloids, flavonoids, polyphenols, and terpenoids play an important role as broad-spectrum antimicrobials against *S. aureus, E. coli,* and *C. albicans* [19–22].

The phytochemical compounds present in plants can be influenced by various factors, such as differences in climate, habitat, soil nutrient conditions, and the timing of plant sampling [23]. The geothermal area exhibits extreme conditions of temperature, acidity, alkalinity, and mineral content in comparison to other areas in general [24]. These conditions are suspected to affect the qualitative and quantitative phytochemical content found in plants living in the area [25, 26].

The antimicrobial effect of the medicinal plants showed different potential activities against pathogenic bacteria and fungi at a concentration of 50% (Table 3). In this study, all medicinal plants actively inhibited the growth of *S. aureus,* with the largest inhibition zone of 12.70 ± 2.30 mm from the methanol extract of the leaf part of *A. mangium*. On the other hand, the methanol extract of *A. mangium* had no activity against *E. coli.* The largest inhibition zone against *E. coli* was observed to be 11.57±2.01 mm from the methanol extract of the aerial part of *A. aureum*. Some medicinal plants were not active in inhibiting fungal growth, except for the methanol extract of aerial part of *P. indica*. which showed activity in inhibiting *C. albicans* with an inhibition zone of 9.89±1.11 mm. Based on the research, only the methanol extract of the aerial part of *P. indica* showed antibacterial and antifungal activity. The positive control used for evaluating antibacterial activity was Gentamicin 10 µg, which exhibited strong activity against *S. aureus* and *E. coli*. Meanwhile, the positive control for evaluating antifungal activity was Nystatin 100 U, which showed strong inhibition against the growth of *C. albicans*.

Table 3. Antimicrobial activity of the methanol extract of medicinal plants from the lower area of the Ie Seu-um manifestation.

Scientific name	Parts used	Diameters of inhibition zones ± SD (mm)					
		S. aureus		*E. coli*		*C. albicans*	
		Ext.	GM	Ext.	GM	Ext.	NS
Pluchea indica (L.) Less.	Aerial	12.10 ± 1.32	25.52±0.80	8.15±1.55	19.50± 0.77	9.89±1.11	18.90±2.36
Acrostichum aureum L.	Aerial	10.12 ± 0.80	25.03±1.02	11.57±2.01	19.10±2.01	0±0	18.55±2.66
Acacia mangium L.	Leaf	12.70 ± 2.30	25.52±1.82	0±0	18.89±2.33	0±0	18.01±1.92
	Bark	11.98 ± 1.78	24.95±0.56	0±0	18.35±2.41	0±0	18.55±2.66
Calotropis gigantea (L.) Dryand.	Leaf	11.95 ± 1.23	24.90±1.21	10.52±2.23	18.55±1.78	0±0	18.90±2.36
	Stem	10.87 ± 1.56	25.34±1.02	9.30±1.78	18.39±2.03	0±0	18.55±1.04

Note: Ext.: Extract of plant part at 50% concentration; GM: Gentamicin 10 μg; NS: Nystatin 100 U

The available data on the antimicrobial activity of crude extracts from medicinal plants found in the Ie Seu-um area was limited. Several studies have reported that the crude extract of *P. indica* exhibits antimicrobial effects against *S. epidermidis, S. aureus, Enterococcus faecalis, Fusobacterium nucleatum*, and *C. albicans* at concentrations ranging from 10% to 90% [27–30]. *A. aureum* exhibits strong antibacterial activity, which is dependent on the extraction solvent used. However, there have been no significant reports indicating significant antifungal activity for *A. aureum*. The methanol extract of *A. aureum* showed a bactericidal effect at a concentration of 25 mg/ml against *Pseudomonas aeruginosa* [31]. Several species of the genus Acacia have been reported to have antibacterial and antifungal activity, but *A mangium* does not exhibit significant activity against fungi [32]. The modification of leaf and flower extracts of *C. gigantea* (L.) into nanoparticle forms has enhanced the antimicrobial effectiveness against *S. aureus, E. coli,* and *C. albicans* [33].

The antimicrobial activity of methanol plant extracts from the Ie Seu-um area is generally more effective in inhibiting *S. aureus* compared to *E. coli* and *C. albicans*. Structurally, Gram-negative bacteria like *E. coli* have more complex cell walls compared to Gram-positive bacteria, which may potentially hinder the activity of phytochemical compounds from the methanol plant extracts. A study reported that the phosphodiester bond in the lipopolysaccharide structure of *E. coli* can inhibit the penetration of several types of antibiotics, leading to resistance [34]. In contrast to bacterial cells, the fungus *C. albicans* has a eukaryotic cell structure with more complex cellular regulation compared to prokaryotic cells in bacteria [35]. Several factors in *C. albicans* can survive exposure to phytochemicals from plants, including inhibiting the penetration of active substances and overexpression of active transport of phytochemical content out of cells by efflux pumps [36]. In this study, the utilization of laboratory microbial isolates enabled these microbes to develop resistance against specific phytochemicals derived from plants, thereby influencing the diameter of the resulting inhibition zone.

4. Conclusions

The Ie Seu-um manifestation (Outflow Geothermal Zone) provides several medicinal plants that can adapt to extreme environments and potentially possess various pharmacological activities. This study presented an overview of the phytochemical content and *in vitro* antimicrobial effects of the medicinal plants found in this manifestation. The evaluation of the phytochemical content of the crude extracts of these medicinal plants showed varied results in terms of alkaloids, flavonoids, saponins, steroids, triterpenoids, and tannins. The antimicrobial effect of the methanol extract of medicinal plants at a concentration of 50% was more active in inhibiting *S. aureus* compared to *E. coli and C. albicans*. Based on the research, only the aerial part extract of *Pluchea indica* (L.) Less. showed antifungal effect against *C. albicans* with a zone of inhibition diameter of 9.89±1.11 mm. However, further research is required to assess the impact of different concentrations on the resulting antimicrobial activity and to quantitatively characterize the phytochemical content of medicinal plants in the Ie Seu-um manifestation.

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