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Phytochemical Screening, Antifungal & Antimicrobial Study of Ethanol Extract Of Gelinggang Leaf (*Senna alata* L. Roxb)

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ABSTRACT

Gelinggang is a powerful plant that has shown promise in treating skin infections caused by bacteria and fungi in traditional medicine. This study aimed to determine the class of secondary metabolites contained in the ethanol extract of gelinggang leaves and to determine its antimicrobial activity. Maceration with 96% ethanol yielded the extract, which was then tested for antibacterial activity using disc diffusion. We employed Staphylococcus aureus, Escherichia coli, and Candida albicans fungi for this antimicrobial activity study at 300, 600, and 1200 mg/ml. The findings of the phytochemical screening of the ethanol extract of gelinggang leaves confirmed the presence of secondary metabolites such as flavonoids, alkaloids, saponins, tannins, phenols, and steroids, confirming the validity of the research. The results of the antimicrobial activity test had the maximum apparent zone diameter for *Staphylococcus aureus* bacteria found at a concentration of 1200 with an evident zone diameter of 17.4 mm, and *Escherichia coli* was found at a concentration of 1200 with a diameter of 11.5 mm. *Candida albicans* fungus was present at a concentration of 1200 with a diameter of 14.8mm.

Keywords: Gelinggang; Secondary Metabolites; Disc Diffusion; Antimicrobial Activity

INTRODUCTION

Indonesia's tropical climate makes it a hotbed for several skin diseases. There is a significant issue with skin infections in the medical field. Its prevalence in developing countries ranges from 20% to 80%[1]. This is because of the fluctuating heat and moisture that characterises the tropics. It's easy for fungal, bacterial, and parasitic infections, among others, to flourish in the humid and hot air that permeates the region throughout the year[2].As one of the mega-diversity countries, Indonesia is home to several tropical plants with medicinal characteristics that have been used in traditional medicine for centuries. Ringinggang(Fig. 1) is a plant that has shown promise in treating skin conditions[3].The gelinggang plant (*Cassia alata* L. or often also called *Senna alata* L.) is widespread in the tropics of Indonesia[4].The Dayak Siang has long used Gelinggang leaves as a traditional medicine for treating various skin conditions, including ringworm, scabies, tinea versicolor, and acne[5].However, this plant is rarely employed in daily life in Indonesia, leading to its systematic extermination by locals[6].

Fig 1. Ringinggang(*Senna alata* L. Roxb)

In a study conducted by Nurlansiand coworkers[7], the antibacterial activity test of methanol extract and the ethyl acetate fraction of gelinggang leaves, the precise zone test results against Escherichia coli bacteria were 10.6 mm, and Salmonella typhi was 5.9 mm. The research was also undertaken by Lathifahand colleagues[8], who discovered a clear zone surrounding the extract-containing disc. Additionally, analyses of secondary metabolites in dry leaf extract by Asmahand coworkers[4] revealed the presence of alkaloids, phenols, and tannins. Meanwhile, the existence of steroid chemicals, terpenoids, saponins, flavonoids, phenols, and tannins was confirmed in a sample of fresh gelinggang leaves. This result, however, was compared

toresearch conducted by Bahiand coworkers[9], resulting in different secondary metabolites. Environmental conditions are a factor that affects secondary metabolite synthesis. The more heat and carbon dioxide present, the more secondary metabolites will be produced[10].
This has sparked an interest in testing the extract of gelinggang leaves for its antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli*, and fungi, as well as conducting a phytochemical screening study of the ethanol extract to identify secondary metabolites in gelinggang leaves collected in the city of Padang Pariaman.

METHODS

Chemicalsand sample preparations

All chemicals and reagents used were of analytical grade and were used as received without any further purification from Sigma-Aldrich. Gelinggang leaf samples were taken in Taluk Village, South Pariaman District and identified at theHerbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatra, Indonesia.
After collecting samples of gelinggang leaves, they were washed and sorted moist to eliminate any lingering contaminants, dried in the air, and finally broken up into manageable pieces using a simplicia cutter. After completely submerging the Simplicia, the maceration extraction method was carried out using a 96% ethanol solvent. One full day was spent soaking, with intermittent stirring. The resulting extract was collected, filtered, and evaporated in a rotary evaporator to yield a concentrated extract. Having obtained a yield, it was time to weigh it and log it.

Identification of Secondary Metabolite Content

Identifications of secondary metabolites, including alkaloids, flavonoids, phenol, saponin, tannin, terpenoid and steroids, were carried out using methods from the Ministry of Health of the Republic of Indonesia[11].

Identification of Phytochemical Compounds using TLC

TLC was used to identify chemical compounds using a silica gel plate F254 and ethyl acetate: n-hexane (2:1) eluent. A capillary tube is used to stain the extract 1.5 to 2 cm from the plate's bottom edge. The chromatography vessel is filled with plates. The developer solution in the vessel must reach the bottom edge of the absorbent layer without submerging the dots. The system places the vessel lid and waits for the mobile phase to propagate to the creepage distance limit. The plates were removed and dried in the air before being examined for spotting with visible light, shortwave UV (254 nm), and longwave UV (254 nm) (366 nm). The distance travelled by each spot from the spotting point is measured and recorded to calculate the Rf value.

Antimicrobial Test

The media Sabouraud Dextrose Agar (SDA) and Nutrient Agar (NA) were prepared. The sterilised NA and SDA media were poured into a petri dish and allowed to solidify.Colonies of the test organisms were isolated from agar slanted 1-2 oses, suspended in physiological sodium chloride (NaCl), and homogenised with a vortex in a clean test tube. 0.5% Mc Farland standard solution (9.5 ml of H2SO4 solution with 0.5 ml of 1% BaCl2 solution) was used to evaluate the concentration or turbidity.

The microbial test suspension was spotted onto sabouraud dextrose agar plates for fungi and nutrient agar medium for bacteria, each at a volume of 0.1 mL. Then, use a cotton bud to wipe the area until it is completely smooth. Three quantities of gelinggang leaf ethanol extract (75, 150, and 300 mg/mL) were evaluated.Additionally, ethanol extract fromgelinggang leaves was added to each medium, and sterile disc paper was added. A disc containing 30 g/ml of chloramphenicol was utilised as a positive control on nutrient agar, whereas a disc containing ketoconazole was employed as a control on sabouraud dextrose. At the same time, DMSO was used in the placebo group.The samples were then placed in an incubator and heated to 37 °Cfor bacteria for 24 hours and 27 °Cfor fungi for three days. The paper disc was inspected after incubation, and the inhibition zone was measured using a calliper. Ketoconazole 50 g/ml was employed as a fungal growth positive control. We used DMSO as a negative control which did not provide an inhibition zone for the tested bacteria and fungi.

The inhibition zone (mm) was measured for each ethanol extract concentration. To determine the size of the inhibition zone, a calliper is used to measure from the disc paper's outermost edge to the zone's outer boundary. The antibacterial inhibition zone is ranked in strength according to the following criteria: Weak inhibition is defined as a zone with a diameter of 5 mm, moderate inhibition as a zone of 5-10 mm, high inhibition as a zone of 10-20 mm, and potent inhibition as a zone of 20 mm[12].

RESULT AND DISCUSSION

This research utilised samples collected in Taluk Village, South Pariaman District, Padang Pariaman City, West Sumatra. The gill leaf is the cut-off piece of the plant that undergoes wet sorting to remove unwanted debris,soil and other impurities that stick to the leaves [13].The extract was made using the maceration technique. The maceration approach was selected because it can extract a large number of samples, is easier to perform, does not require

specialised equipment, and eliminates the risk of degradation of the active component under the effect of temperature because there is no heating step[14].The flavonoid compound is one such example; it belongs to a class of chemicals that is both heat-sensitive and prone to oxidation when exposed to high temperatures[15].The ethanol (96%) was used to macerate the samples. Because of its versatility, ethanol is frequently employed as a solvent, and it can dissolve a wide variety of molecules, including both inert and active polar ones.

The yield of the ethanol extract from the Gelinggang leaves was 5%, and the total amount collected was 20 g. the analysis of secondary metabolites showed positive results for alkaloids, flavonoids, saponins, tannins, phenols, and steroids. The qualitative analysis of these metabolites was different from previous studies. Bahi and coworkers[9] demonstrated the sample positive for alkaloids and steroids in n-hexane. Moreover, the research by Asmah and colleagues[4] showed positive for alkaloids, flavonoids, saponins, tannins, phenols, steroids, and terpenoids in methanol. Several internal and external factors influence the variation in research results. The same plant species can produce distinct secondary metabolite molecules depending on the growing conditions. The biotic environment (bacteria, viruses, fungi, and parasites) and the abiotic environment (climate, soil, and light) are to blame for this (light, temperature, humidity, Ph, nutrient content in the soil and altitude). Due to their close relationship with their surroundings, plants are susceptible to environmental influences that force them to generate secondary metabolites as a survival mechanism[16].

This study used polar silica gel F254 plates as the stationary phase and ethyl acetate: n-hexane (2:1) as the mobile phase as the eluent. The stains obtained in the TLC pattern under the UV lamp with a wavelength of 254 nm contained twocolours (Rf value of 0.4 & 0.3), while under the UV lamp with a wavelength of 366 nm, there were four stains (Rf value of 0.7;0.6;0.4;0.3).

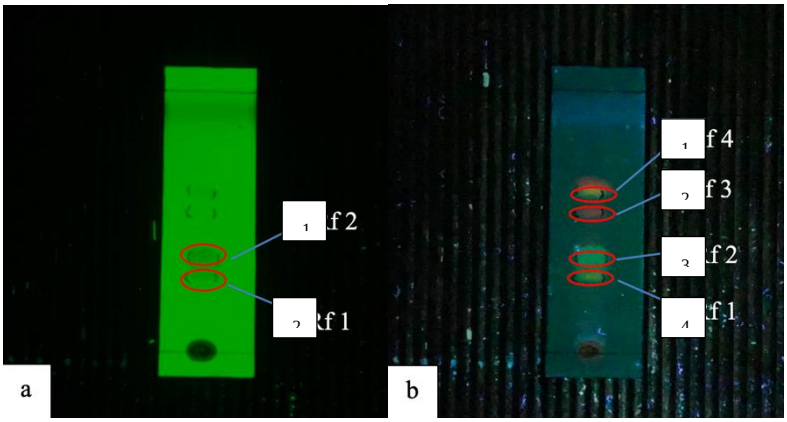

Fig 2. TLC pattern under the UV lamp 254 nm (a) 366 nm (b)

The disc diffusion method was utilised to examine the antibacterial activity of the sample because of its relative ease of use and reliable findings in detecting antibacterial activity. If there aren't any developing bacteria in the clear space around the disc, then the sample likely contains an action that prevents the spread of bacteria. The antibacterial activity test performed using this method is affected by factors such as the diffusion rates of the various chemicals and the varying responses of the microorganisms to the substances[17].

Concentrations of 1200, 600, and 300 mg/ml were used to test for antimicrobial activity. A chloramphenicol disc at 30 g/disk was utilised as a positive control for comparison in this study's antibacterial test. A ketoconazole disc at 50 g/disk was used for comparison in the study's antifungal test. The broad range of chloramphenicol makes it an efficient growth inhibitor for gram-positive and gram-negative bacteria[18]. Antifungal ketoconazole is effective because it blocks the production of ergosterol, a component of fungal cell membranes[19]. The solvent dimethyl sulfoxide was utilised as a negative control. Since dimethylsulfoxidewas not found to affect cell proliferation significantly, it did not confound the outcomes of the agar diffusion assay for detecting antibacterial activity.Dimethylsulfoxide is a solvent that dissolves practically any substance, including non-polar, semi-polar, and polar.

The antimicrobial and antifungal activity of the extract is shown in this table 1 and Fig. 3.

Table 1. Antimicrobial and antifungal activity studies

Microorganisms	**Concentrations**			**Positive control**
	300	**600**	**1200**	
Staphylococcus aureus	9.3 mm	14.9 mm	17.4 mm	21.3 mm
Escherichia coli	9.8 mm	10.8 mm	11.5 mm	10.7 mm
Candida albicans	11.4 mm	12.8 mm	14.8 mm	32.6 mm

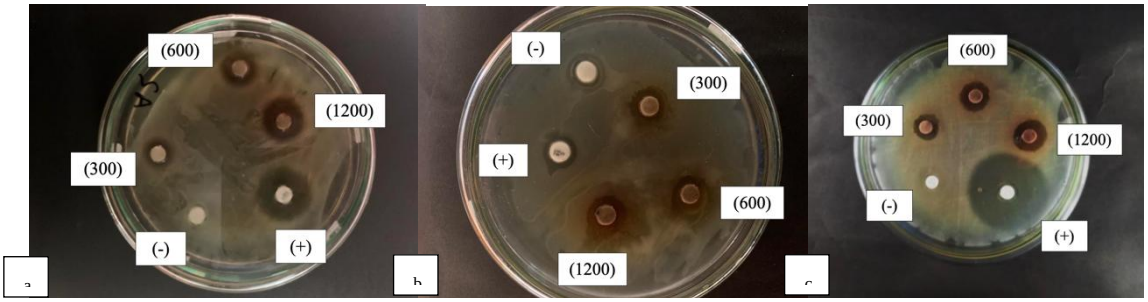

Fig 3. a: antimicrobial activity against *Staphylococcus aureus*. b. antimicrobial activity against*Escherichia coli c*. antifungal activity against*Candida albicans*

Several parameters, including the extract's concentration and the presence of antibacterial chemicals, affected the results of the antibacterial activity test. Some secondary metabolite components in gelinggang leaf extract are alkaloids, flavonoids, saponins, phenols, tannins, and steroids, which work together to create the clear zone. Secondary metabolites have antibacterial activity but do so via a unique mechanism.An examination of the alkaloids' antimicrobial properties revealed that they formed chelates with DNA and inhibited the topoisomerase enzyme. The creation of bacterial cell walls can be impeded by alkaloid chemicals, leading to the lysis and deformation of the cells they inhabit. As a result of interfering with DNA synthesis in fungi, alkaloids can inhibit their growth by inserting themselves between the cell wall and the fungi's genetic material[20].

Antibacterial flavonoid chemicals work by preventing nucleic acid production and cytoplasmic membrane function by interacting with several critical enzymes[21]. Additionally, lack of energy leads to cell lysis, and flavonoids can prevent aerobic metabolism[22].Fungi growth inhibition is due to the presence of flavonoid molecules. Flavonoids limit fungal growth by disrupting the ion channels and membrane permeability of fungal cells. Flavonoid compounds cause toxic effects on fungus because of the hydroxyl group they possess[23].

Foam can be generated by shaking a solution containing saponins, chemicals related to detergents. Saponins, chemically similar to detergents, can disrupt the permeability of bacterial cell membranes and lower the surface tension of bacterial cell walls. Because of cytoplasmic leaking, bacterial survival is compromised [24]. Saponins are antifungal because they impair the cell membrane's integrity, killing the fungus[25].Tannins block the enzymes reverse transcriptase and DNA topoisomerase, which results in antimicrobial effects. Tannins also affect intracellular protein transport by precipitating proteins in the cell membrane. To put it another way, this prevents the formation of bacterial cells[26]. With their ability to block the synthesis of chitin, which is employed in the production of cell walls in fungus, and damage cell membranes, tannins can also provide the ability as an antifungal with the mechanism provided by tannins[20].

The phenolic chemicals found in gelinggang leaf extract have been shown to disrupt the integrity of the bacterial cell wall, leading to membrane leaking and bacterial death. Phenol will cause the bacteria to release essential materials and replace them with phenol. Phenol is toxic to cells because it can denature proteins and nucleic acids, impede protein synthesis, and disrupt the cell's internal functioning mechanism and cytoplasmic membrane, perhaps leading to cell death. By disrupting mitochondrial function, which increases ROS production, and blocking the production of chitin, a key component of cell walls, phenol has an antifungal impact[27].Inhibiting bacterial development via the lipid membrane and sensitivity to steroid components that cause leaking in bacterial liposomes is how steroids work as an antibiotic[28]. Steroids' antifungal mechanism includes interacting with phospholipid membranes, which are permeable to lipophilic substances, resulting in diminished membrane integrity and a change in cell membrane shape, ultimately leading to cellular brittleness and lysis[29].

Each bacteria produced an extract with a distinct diameter of the clear zone and extract concentration. Distinctions in extract concentration and test microorganisms led to variations in clear zone diameter[30]. As the concentration rose, a more expansive, clean site formed. The extract's antibacterial component content is maximised at its highest concentration[31].The size of the transparentareacreated also varies depending on the test microorganisms utilised. The variation is due to test microorganisms having a slightly different cell wall composition. Gram-positive bacteria, including Staphylococcus aureus, have a single-layered cell wall, while gram-negative bacteria have a three-layered cell wall[32]. Gram-positive bacteria more readily absorb antibacterial chemicals due to the cell wall's simplified form[33]. Antibacterial chemicals will have a more challenging time penetrating gram-negative bacterial cells due to their cell wall's more complicated structure[34].

Candida albicans were grown at three doses on sabouraud dextrose agar to test for antifungal activity. The studies show that ethanol extract inhibits the growth of Candida albicans, suggesting that it has antimicrobial properties. Several antifungal secondary metabolite chemicals are linked to the plant's antimicrobial action.

CONCLUSIONS

Secondary metabolites in the ethanol extract of gelinggang leaves (Senna alata L. Roxb) were identified as flavonoids, alkaloids, saponins, tannins, phenols, and steroids, according to the results of this study. The zone of inhibition for the ethanol extract of gelinggang leaves against Staphylococcus aureus, Escherichia coli, and Candida albicans is very apparent.

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