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Analysis of Phytochemical Content of Weed Roots Gelagah Grass (*Saccharum Spontaneum* L.) and Antimicrobial Efficacy Test

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ABSTRACT
The purpose of this study was to determine the phytochemical content of the weed roots of gelagah grass (Saccharum spontaneum L.) and the antimicrobial efficacy test and to determine which type of solvent from the extract of the weed roots of gelagah grass gave the best inhibitory activity against microbial growth. The research design carried out is taking research samples from the field and analyzed in the laboratory. The weed roots of gelagah grass were taken from the Juanda area 2. The roots of the gelagah grass were macerated with ethanol and then concentrated using a rotary evaporator. Then it was continued by analyzing the content of the gelagah grass roots or ethanol phytochemical screening of the gelagah grass roots (alkaloids, phenolics, saponins, flavonoids, triterpenoids,) as well as the antimicrobial test (Propionibacterium acne). The results showed that: (1) the secondary metabolites contained in the crude ethanol extract of the weed root of gelagah grass were flavonoids, phenolics, and triterpenoids; (2) the results of the antimicrobial efficacy test showed that the crude ethanol extract of the gelagah grass roots with a concentration of 6, 8, 10, 12, 15%. can inhibit the growth of Propionibacterium acnes bacteria, respectively 8.99 mm; 9.44 mm, 9.77 mm (classified as moderate); 10.10 mm, and 10.66 mm (classified as strong); and (3) Minimum Inhibitor Concentration (MIC) of 6%.
Keywords: *Phytochemical Content, Antimicrobial Efficacy Test, Gelagah Grass*

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INTRODUCTION

Indonesia is a tropical country that has abundant wealth of various plants that can be used as traditional medicine, both from roots, stems, leaves, fruits and seeds. At this time the use of traditional medicine is well developed as an alternative to treat health problems in line with the tendency of people to return to nature. Medicinal plants will provide optimal results if consumed in moderation for medicinal purposes [1].
Efforts to find new drugs from natural ingredients for various diseases begin with conducting biological pharmacology screening on plants/plants that have been used empirically.
Gelagah (*Saccharum spontaneum* L.) or Reed Grass (English) is a tall perennial grass with deep roots and rhizomes that can grow well in marginal soils such as rocky areas, deserts and sandy loams where other plants cannot grow. Therefore, reeds can be considered as a drought tolerant, perennial grass. Gelagah have a high carbohydrate content, making the biomass of this species a suitable substrate for ethanol production [8].
Gelagah are widespread on all continents except Antarctica. This plant is found in Southeastern Europe, in the Ughari region of the Asian continent (Central Asia, West Asia, Arabia, China and East Asia), tropical Asia (India, Malaysia), Australia, the Pacific islands and Central America [11].
In Indonesia, gelagah are found in Sumatra, Java, Madura, Sulawesi, Kalimantan and on various other islands. Gelagah can grow from a height of about 0 - 1,700 meters above sea level. This grass likes areas with high rainfall of more than 1,500 mm per year, is easy to grow, so gelagah can become weeds on agricultural land [11].
The objectives of the study were: (1) to determine the phytochemical content of the weed roots gelagah grass (*Saccharum spontaneum* L.) and anti-microbial efficacy test, and (2) to obtain concentrations of the weed roots gelagah grass extract which is good for inhibiting microbial growth.

MATERIAL AND METHODS

A. Time and Place

This research was conducted from February 2021 to April 2021, starting from preparation to analysis results. The research location was at the Laboratory of Wood Properties and Product Analysis, Department of Agricultural Technology, Samarinda State Agricultural Polytechnic. Addressed at Jl. Samratulangi Keledang River, Samarinda Sebrang sub-District, Samarinda City, East Kalimantan.

B. Materials and Tools

The materials used in this study were gelagah weed roots, cotton, 95% ethanol, 10% ethanol, hydrochloric acid (HCl 2 N), dragendorff reagent, Liebermann-Burchard reagent, sulfuric acid reagent (H_2SO_4), 1% FeC13 reagent (iron III chloride), reagent $CHCl_3$ (chloroform), NaCl (sodium chloride), dilute acid (1% HCl), dilute hydroxide (1% NaOH), acetic anhydride $(CH_3CO)_2O$, nutrient agar, *Propionibacterium* acne bacteria, acetone, bacteria, and distilled water.

The tools used in this study were scissors, knives, blenders, separatory funnels, measuring cups, measuring cups, filter paper, *rotary vacum evaporator, shaker, autoclave, inkubator, HPCL*, refrigerators, ovens, closed containers, vials, dropper pipettes, macro and micro pipettes, column tubes, test tubes, *spatula*, *cutter, cork borer, petri dish*, Erlenmeyer tube as a measure, *hot plate*, scales, aluminum foil, cotton, plastic wrapping, tweezers, and stationery.

C. Research Activities

The research activities carried out were as follows: (1) field observations, (2) collection of research materials (gelagah grass weeds), (3) sample preparation, (4) extraction of secondary metabolites, (5) photochemical screening to test for the presence of metabolites secondary, (6) antimicrobial activity test, (7) preparation of positive control and negative control, (8) preparation of pure *Propionibacterium acne* bacterial culture, (9) antimicrobial activity test, (10) determination of Minimum Inhibitory Concentration or MIC (Greenwood, 1995), (11) data analysis, and (12) report preparation.

Extraction of secondary metabolites is made as follows: samples of gelagah weed roots that have been finely weighed first are then macerated with 95% ethanol solvent as much as 100 ml, after which they are tightly closed using aluminum foil and glued using cling warp so that the soaked extract does not evaporate , and leave it for 2 x 24 hours. After maceration for 2 x 24 hours, the extract was filtered using filter paper. The extract resulting from the filtering was evaporated with a rotary evaporator to obtain a crude extract.

Then the crude extract will be tested for phytochemicals to determine the type of secondary metabolite compounds contained in the crude extract. Furthermore, the antimicrobial activity test was carried out by measuring the diameter of the clear zone.

For antimicrobial testing, the crude extract was tested on *Propionibacterium acne* bacteria at various extract concentrations, namely 6%, 8%, 10%, 12% and 15%. This test also uses a positive control in the form of chloramphenicol and a negative control, namely acetone·

D. Data Collection

The research data collected were as follows: (1) results of phytochemical tests in the form of alkaloids, phenolics, saponins, flavonoids, and triterpenoids, and (2) results of antimicrobial activity tests.

E. Data Analysis

The data analysis technique used to test the antimicrobial activity is by measuring the diameter of the resulting clear zone. Davis Stout (1999) in Ardiansyah (2005) suggests that the provisions for antibacterial strength are as follows: an inhibition area of 20 mm or more means very strong, an inhibition area of 10-20 mm (strong), an inhibition area of 6-10 mm (medium), and an inhibition area of 5 mm or less means weak.

Table 1. Classification of Bacterial Inhibition

Barrier Area Diameter (DDH)	Growth Inhibition Response
>20 mm	Very strong
10 – 20 mm	Strong
6 – 10 mm	Medium
<5 mm	Weak

RESULTS AND DISCUSSION

A. Gelagah Grass Plants (*Saccharum spontaneum* L.)

Gelagah are grasses that can reproduce quickly and have deep and strong roots, this plant originates from India. The designations of this grass also vary in different areas, such as Galoga (Batak), Glagah (Java), Saraw and others. This plant can be found in open land with high rainfall, its distribution is in Indonesia and Southeast Asia. [10].

Gelagah grass can grow in a variety of environments, both tropical and non-tropical. Gelagah can reproduce generatively through seeds or vegetatively through stem cuttings. Gelagah are tall perennial grasses with deep roots and rhizomes that do well in marginal soils such as rocky areas, deserts and sandy flats, where no other plant can be grown or cultivated. Therefore, gelagah can be considered as a drought-tolerant, perennial grass. Gelagah have a high carbohydrate content, making the biomass of this species a suitable substrate for ethanol production [8].

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Figure 1. Gelagah Grass (*Saccharum spontaneum* L.)

B. Phytochemical Test Results

The results of the phytochemical test on the ethanol extract of gelagah grass weed roots showed that the extract contained several secondary metabolites as presented in Table 2 below.

Table 2. Phytochemical Test Results of Ethanol Extract of Gelagah Weed Roots

Etanol	Phytochemical Test				
	Alkaloids	**Phenolic**	**Saponin**	**Triter-penoid**	**Flavonoid**
	-	+	-	+	+
Note	Not formed it is orange to red-brown in color	Appear its a strong green, red, purple, blue or black color	There is no strong foam and it disappears if 1 drop of HCL 2N is added	formed is red or purple	formed it is brownish yellow

Keterangan :

\+ = Contains secondary metabolite compounds;

\- = Does not contain secondary metabolites

Based on the results of the phytochemical test on the ethanol extract of gelagah weed roots (Table 2), Three secondary metabolites were found, namely: phenolics, triterpenoids and flavonoids with the following characteristics:

1. Phenolic with the following characteristics: the appearance of a strong green, red, purple, blue or black color.
2. Triterpenoids with characteristics, namely: formation of red or purple color.
3. Flavonoids with characteristics, namely: the formation of a brownish yellow color.

The results of the phytochemical test on the ethanol extract of gelagah weed roots did not reveal other secondary metabolites such as alkaloids and saponins with the following characteristics:

1. Alkaloid with characteristics: does not form orange to brownish red color.
2. Saponins with the following characteristics: there is no strong foam and disappears when 1 drop of 2N HCL solution is added.

B. Antibacterial Activity Test Results

In the antibacterial activity test, the bacteria used was *Propionibacterium acne*. The results of the antibacterial activity test of the crude ethanol extract of gelagah grass weed roots for 24 hours are presented in Table 3.

Table 3. Results of Antibacterial Activity Test of Ethanol Extract of Gelagah Grass Weed Roots

Inhibition Models	Rate of Inhibition (mm)						
	Control (+)	Control (-)	Antibacterial Concentration				
			6%	8%	10%	12%	15%
Vertikal	16,00	0,00	8,33	8,66	9,00	9,33	9,66
Horizontal	20,00	0,00	9,33	10,00	10,33	10,66	11,00
Leaning	17,00	0,00	9,33	9,66	10,00	10,33	11,33
Amount	53,00	0,00	26,99	28,32	29,33	30,32	31,99
Average	**17,66**	**0,00**	**8,99**	**9,44**	**9,77**	**10,10**	**10,66**

Source: Processed Research Data (2021)

Based on Table 3 above, it can be explained that the antibacterial activity test showed that the crude extract of gelagah grass weed roots tested on *Propionibacterium acne* bacteria resulted in inhibition of the bacteria, namely as follows: (1) in the control treatment (+) there was inhibition of bacteria with vertical size, horizontal and oblique, respectively: 16.00; 20.00; and 17.00 mm with an average value of 17.66 mm; (2) in the control treatment (-) there was no bacterial inhibition; (3) at the concentration of 6; 8, and 10% there was inhibition of bacteria with vertical, horizontal and oblique sizes, respectively with a successive average of 8.99 mm; 9.44 mm and 9.77 mm were all classified as moderate, whereas in the administration of 12 and 15% there was inhibition of bacteria with vertical, horizontal and oblique sizes, respectively with an average of 10.10 mm and 10.66 respectively mm both are quite strong. This situation indicates that the greater the concentration of the crude extract of gelagah grass roots given the greater the inhibition of bacterial growth/development. This is because in the crude extract of gelagah grass weed root contains antibacterial compounds such as phenolics, flavonoids, and triterpenes. As stated by Darwis [3] that secondary metabolites such as triterpenoids, alkaloids, coumarins, steroids, and flavonoids are chemical compounds that generally have bioactivity capabilities that function as plant protectors from pests and diseases both for the plant itself and for its environment.

In general, the results of inhibition of bacteria at various antibacterial concentrations are presented in Figure 2. Based on Figure 2, it shows that the higher the concentration of the crude extract of gelagah grass weed roots, the greater the inhibition of *Propionibacterium acne* bacteria. This was explained by Dwidjoseputro [4] that the lower the concentration of antibiotics, the weaker the inhibitory power so that the formed zones will be smaller and conversely the higher the concentration of antibiotics, the larger the clear zone will be formed. Furthermore, it was stated by Prawata and Dewi [9] that the higher the concentration of an anti-bacterial substance, the stronger the anti-bacterial activity.

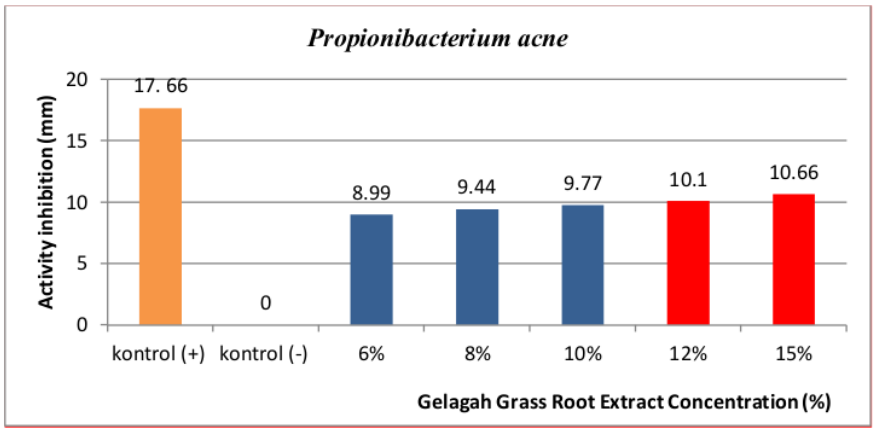

Figure 2. Bar chart of inhibition results at various antibacterial concentrations

Based on the research results it is known that the Minimum Inhibitory Concentration (MIC) value of gelagah grass weed root extract is found at a concentration of 6%, this indicates that gelagah grass weed root extract is effective for inhibiting the growth of *Propionibacterium acne* bacteria starting at a concentration of 6%.

CONCLUSIONS AND RECOMMENDATIONS

A. Conclusion

Based on the results of research and discussion it can be concluded as follows:

1. The secondary metabolite compounds contained in the crude ethanol extract of gelagah grass weed roots (*Saccharum spontaneum* L.) are flavonoids, phenolics, and triterpenoids.
2. Based on the results of the antimicrobial efficacy test showed that the crude ethanol extract of gelagah grass weed roots with concentrations of 6, 8, 10, 12, and 15%. can inhibit the growth of *Propionibacterium acne* bacteria, namely 8.99 mm; 9.44 mm, 9.77 mm (medium); 10.10 mm, and 10.66 (relatively strong).
3. The Minimum Inhibitory Concentration (MIC) value of gelagah grass weed root extract is at a concentration of 6%.

B. Suggestion

Based on the research results, several suggestions can be put forward, namely as follows:

1. To inhibit the growth of bacteria can be done by giving gelagah grass weed root extract with a concentration of 12-15%.
2. It is necessary to carry out further research using several other types of bacteria, especially bacteria that attack agricultural plants.
3. The research that has been carried out only uses ethanol extract in the form of crude extract, so further research is needed to examine more deeply the use of gelagah grass weed roots as an antibacterial drug.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6