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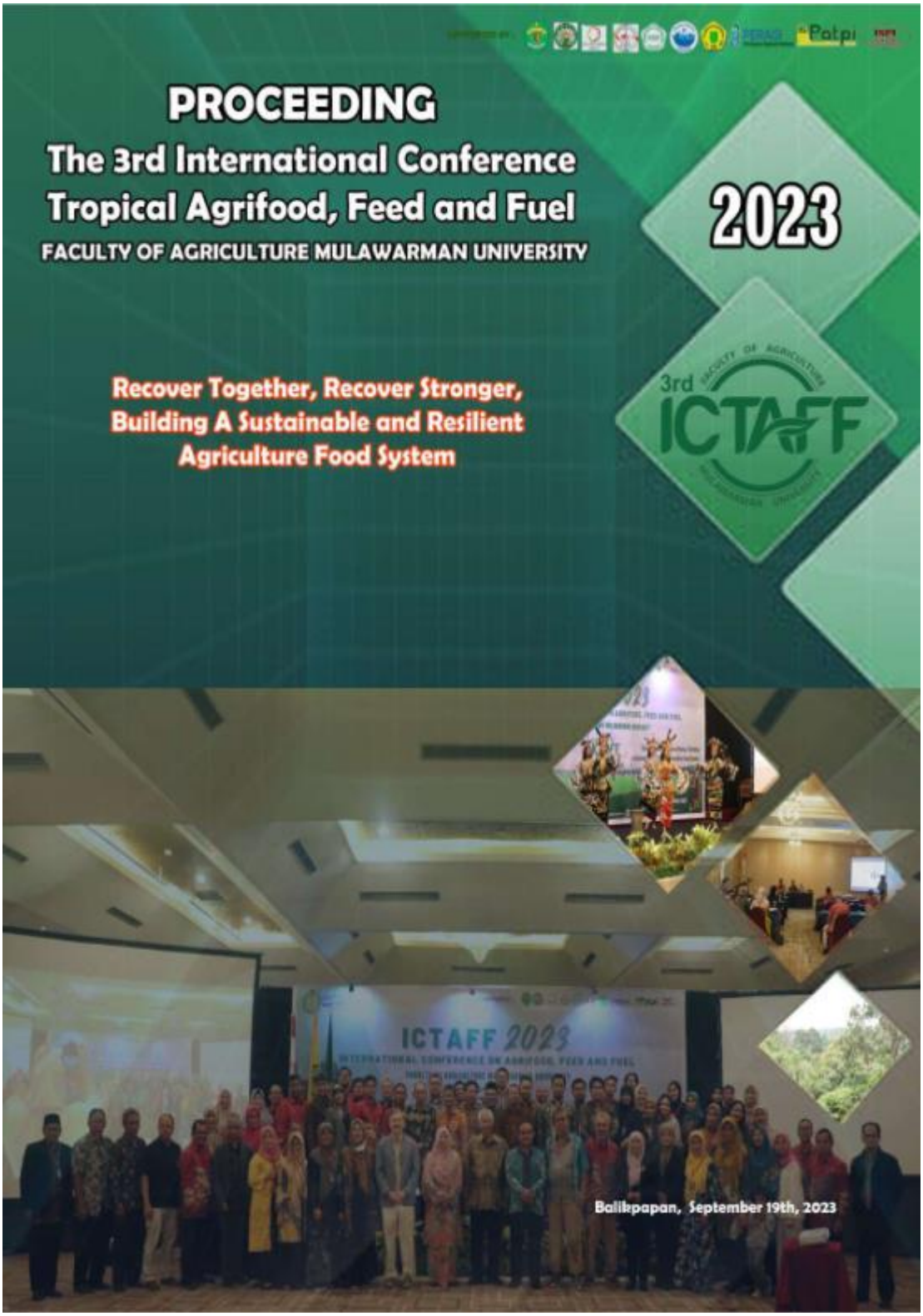

PROCEEDING BOOK

INTERNATIONAL CONFERENCE
ON TROPICAL AGRIFOOD, FEED, &FUEL(ICTAFF) 2023

"Recover Together, Recover Stronger,
Building A Sustainable and Resilient Agriculture Food System "

September, 19-20th 2023

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Faculty of Agriculture, Mulawarman University
Samarinda, East Kalimantan

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Table of Content

Phytochemical Analysis and Efficacy Test of Mangrove Leaf Extract *(Rhizophora apiculata)* from Marang Kayu District, Kutai Kartanegara Regency, East Kalimantan Province Against *Propionibacterium acnes* Bacteria

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ABSTRACT

Mangroves have several compounds that can be used as antibacterials, one of which is *Rhizophora apiculata*. Samples of the *R. apiculata* type of mangrove were taken from Marang Kayu District, Kutai Kartanegara Regency, East Kalimantan Province. The aim of this study was to determine whether the *R. apiculata* mangrove leaf extract takenfrom Marang Kayu District, Kutai Kartanegara Regency, East Kalimantan Province had an antimicrobial effect on Propionibacterium acnes Bacteria. Research methods included taking leaf samples, drying and grinding, maceration and extraction, phytochemical tests, sterilization of tools and materials, bacterial culture, preparation of test media and antibacterial activity tests. Observations were made after 1x24 hours of incubation period. The clear zone formed around the wells was measured horizontally and vertically. The diameter of the inhibition zone is measured in millimeters (mm). The results of research conducted on oil mangrove leaves (*Rhizophora apiculata*) obtained the content of phytochemical compounds in the form of tannins, flavonoids, saponins, alkaloids, carotenoids and steroids.Antibacterial tests on P. acne showed that the inhibition zone became larger as the concentration of the extract increased. The inhibition zone at 20% concentration was 17 mm. The smallest inhibition value (MIC) at a concentration of 2.5%is 9.50

Keywords: Mangroves, leaf extract, phytochemical compounds, concentration of the extract, diameter of the inhibition zoner

INTRODUCTION

Indonesia is a maritime country consisting of more than 17,000 islands with the second longest coastline in the world, which is 99,083 km / 61,567 miles long. (Countries by Coastline 2023). It has large climate variations that support a variety of vegetation that grows from coastal areas to mountainous areas. Mangrove forests have high economic and ecological value. The economic functions of mangrove forests include providing wood, leaves as raw materials for medicines and so on. Ecological function as a provider of nutrients for aquatic biota, spawning and nurturing place for various kinds of biota, preventing abrasion, raging hurricanes and tsunamis, absorbing waste, preventing sea water intrusion and so on (Halidah, 2014).

On most of Indonesia's coastlines, various types of mangroves grow. These mangroves form a forest ecosystem whose width ranges from several meters to several kilometers. Ordinary people call mangroves mangroves. In fact, mangroves and mangroves are different things. Mangrove is the local name for Rhizophora sp., one of the mangroves that exist in nature. So, mangroves are not necessarily mangroves, but mangroves are definitely mangroves. Rhizophora is a type of mangrove.

Mangroves are typical plants found in river estuaries and coastal areas which are influenced by sea tides. Most of the mangrove plants are useful as food and medicine (Purnobasuki, 2004 in Henny, et al., 2017). Mangroves have compounds such as alkaloids, flavonoids, phenols, terpenoids, steroids andsaponins which are called secondary metabolite compounds, these compounds are used as fish poisons and antimicrobials (Kordi, 2012 in Senoaji and Muhamad Fajrin Hidayat 2016).

Acne is a disease that often occurs on the surface of the skin on the face, neck, chest and back. Acne appears when the skin's oil glands are too active, so that the skin pores become blocked by excessive fat deposits (Sawarkar, 2010 in Virsa Handayani 2016). If the deposits are mixed with sweat, dust and other dirt it will cause fat deposits with black spots on them which are called blackheads.

Bacteria that cause acne include Propionibacterium acnes and Staphylococcus epidermis. Propionibacterium acnes is a normal flora of the Pilosebaceous glands of human skin, this bacterium causes acne by producing lipase which breaks down free fatty acids from skin lipids. The genome of this bacterium has been sequenced and research shows that several genes can produce enzymes to shed skin and proteins, which may be immunogenic (activate the immune system) (Pramasanti, 2008).

Acne treatment is usually done with antibiotics and chemicals such as sulfur, resorcinol, salicylic acid, tetracycline, erythromycin and clindamycin. However, these drugs also have side effects such as

antibiotic resistance and skin irritation. Based on this, it is necessary to carry out research to look at the formulation and antibacterial potential of natural plants in Indonesia, not only because the side effects are relatively low but also because of the adequate bioavailability of natural ingredients.

The results of research conducted by Mutik et al., in the 2022 Tropical Marine Journal, show that Rizophora apiculata leaf extract from the waters of Jepara's Awur Bay contains several bioactive compounds such as: alkaloid, flavonoid, phenolic and saponin bioactive compounds in methanol solvent; alkaloid, phenolic and steroid compound groups in ethyl acetate solvent; while the alkaloid and steroid compound groups are in n-hexane solvent. *R. apiculata* leaf extract in the three solvents did not show any antibacterial activity against MDR bacteria (*Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus* dan *Bacillus subtilis*). Based on this, the author wants to conduct research on the activity test of mangrove leaf extract of the *Rhizophora apiculata* species taken from pond areas in Marang Kayu District, Kutai Kartanegara Regency, East Kalimantan Province.

MATERIAL AND METHODS

This research was carried out at the Wood Properties and Product Analysis Laboratory, Samarinda State Agricultural Polytechnic campus, majoring in Forest Products Technology (THH), starting from February 21 2023 to March 24 2023, including activities in the field and experiments in the laboratory.

Material

The research tools and materials used in this study are essential for conducting experiments and gathering data effectively. These tools include scissors, a blender, a dropper pipette, aluminum foil, a rotary evaporator, a spectrophotometer, a laminar airflow hood, an analytical balance, an autoclave, a measuring cup, a hole punch/well-making tool, tweezers, a spoon, an ose needle, test tubes, Erlenmeyer flasks, beaker glasses, micro pipettes, Petri dishes, a fume hood, stationery, a mobile camera, and a computer for data input and processing. Additionally, the materials used in this research consist of oil from Mangrove leaves (Rhizophora apiculata), Propionibacterium acne bacteria, cotton swabs, filter paper, 95% alcohol for maceration, 10% ethanol as a solvent and negative control, nutrient agar (Nutrient Broth, agar, glucose) as a bacterial growth medium, Pb acetate, acetone, hydrochloric acid (HCl 2N), NaOH, CH3COOH, Mayer's reagent, Dragendorff's reagent, Bouchardat's reagent, $CHCl_3$ (chloroform), NaCl (sodium chloride), and chloramphenicol as a positive control. Aquades was used as a liquid for making agar media and other purposes.These tools and materials were crucial for conducting experiments, analyzing samples, and ensuring the accuracy of the research results. They allowed for precise measurements, controlled conditions, and proper documentation of research activities.

Research Implementation

The research conducted in this study is a laboratory experimental research focused on evaluating the antibacterial activity of Mangrove Oil (Rhizophora apiculata) leaf extract against Propionibacterium acne bacteria. The evaluation was performed in vitro using the well diffusion method, and the diameter of the inhibition zone was measured to determine the antibacterial efficacy.

Research Procedure

Sample Preparation

Samples of Oil Mangrove (Rhizophora apiculata) leaves were collected manually from the vicinity of a pond near Bunga Putih Village, Marang Kayu District, Kutai Kartanegara Regency, East Kalimantan Province.

Preparation of Simplisa and Extraction of Secondary Metabolite Compounds

The collected leaves were dried indoors for four days and then finely ground using a blender. This resulted in finely powdered Mangrove Oil (Rhizophora apiculata) leaves suitable for extraction.

Extraction Process

A 50-gram sample of the finely powdered Mangrove Oil (Rhizophora apiculata) leaves was weighed. This sample was then subjected to maceration using 95% alcohol as the solvent for two rounds of 24-hour extraction with occasional stirring. After the extraction process, the mixture was filtered through filter paper, and the solvent was evaporated using a rotary evaporator at 40°C, yielding a crude ethanol extract.

Phytochemical Testing

The crude ethanol extract underwent phytochemical testing to identify the types of secondary metabolite compounds it contained. The tests conducted included Tannin, Flavonoid, Saponin, Alkaloid, Carotenoid, Steroid, and Triterpenoid tests.

Antimicrobial Activity Test

- Sterilization of Tools and Materials
- Propionibacterium acne bacteria were cultured to create a pure bacterial culture.
- Test media were prepared.
- Positive control (+) was created using 0.0030 mg of chloramphenicol dissolved in 5 ml of 10% ethanol.
- Negative control (-) was prepared using 10% ethanol.
- Test solutions were prepared, and Minimum Inhibitory Concentration (MIC) was determined.

Observation and Data Collection

After a 24-hour incubation period, observations were made by examining the clear zone formed around the well and measuring its diameter horizontally and vertically. The diameter of the inhibition zone was measured in millimeters (mm) using a ruler, calculated as the overall diameter minus the hole diameter of 7 mm. The diameter of the clear zone was then categorized based on its antibacterial inhibitory strength using Davis and Stout's classification.

Data Analysis

The analysis of antimicrobial activity involved measuring the diameter of the clear zone around the wellhole. According to Davis and Stout (1971), antibacterial strength categories were defined as follows: a clear zone diameter of over 20 mm indicated very strong antibacterial activity, 10-20 mm indicated strong activity, 5 mm indicated moderate activity, and less than 5 mm indicated weak activity.

Table 1. Classification of Bacterial Inhibitions (Davis and Stout, 1971)

No	Resistance Area Diameter	Response To the Inhibition of *Propionibacterium acnes* Growth
1	> 20 mm	Very strong
2	10-20 mm	Strong
3	5-10 mm	Moderate
4	< 5 mm	Weak

RESULT AND DISCUSSION

Mangrove Leaf Extract Oil (*Rhizophora apiculata*)

In this study, Mangrove Leaf Extract Oil (*Rhizophora apiculata*) was obtained through the maceration method, involving the use of 50 grams of dry sample and 95% alcohol solvent, resulting in the extraction of 13.14 grams of extract in a paste-like form. The extract displayed a dark green color and a thick paste-like texture. The maceration method was chosen for its ability to efficiently extract active compounds through soaking without the application of heat, thus minimizing the risk of damaging unstable or heat-sensitive components (Dean, 2009). Phytochemical tests were conducted to identify secondary metabolite compounds within the plant, providing initial insights into its potential biological activity. The analysis revealed that the leaves of Oil Mangrove *(Rhizophora apiculata)* contained various phytochemical compounds, including tannins, flavonoids, saponins, alkaloids, carotenoids, and steroids.

Antibacterial Activity

The assessment of antibacterial activity against Propionibacterium acne involved measuring the diameter of the inhibition zone around each well on agar plates after 24 hours of incubation.

Figure 1. Inhibitory zone of mangrove leaf extract Mangrove Oil

Information:

+	: Positive Control
A	: Negative Control
B	: Concentration 2,5 %
C	: Concentration 5 %
D	: Concentration 10 %
E	: Concentration 15 %
F	: Concentration 20 %

The results, presented in Figure 1, displayed varying inhibition zone diameters corresponding to different extract concentrations. Notably, a concentration of 20% demonstrated the highest inhibition against the bacteria compared to other concentrations. This variation in inhibition zone diameter can be attributed to differences in the concentration of active compounds within each extract, with higher concentrations yielding more significant antibacterial effects (Lingga et al., 2016).

Phytochemical Testing

The crude ethanol extract underwent phytochemical testing to identify the types of secondary metabolite compounds it contained. The tests conducted included Tannin, Flavonoid, Saponin, Alkaloid, Carotenoid, Steroid, and Triterpenoid tests.

Tannin

The results of tannin analysis of oil mangrove (*R. apiculata*) leaf extract were declared positive. This is characterized by the formation of a yellow precipitate when the sample extract is reacted with a 1% Pb Acetate solution. In the world of medicine, tannins are used to treat diarrhea, stop bleeding, and treat hemorrhoids (Noviyanty, et al. 2019). Tannin is a bioactive compound which is included in the polyphenol group (Wrasiati et al., 2011) and plays a role in defense against microorganisms (Anggraito et al, 2018 in Akasia, 2021). Tannins can dissolve in alcohol solvents based on phytochemical tests. The results of tannin testing on Oil Mangrove (R. apiculata) leaves showed positive results as demonstrated by the formation of brownish yellow precipitates. This condition occurs because tannin compounds are polar so they can dissolve in alcohol which also has polar properties. This is in accordance with Prabowo's (2014) statement which states that tannin compounds have many OH groups so that tannins which are polar can dissolve in polar solvents so they can be extracted well.

Flavonoids

Flavonoids were detected in the leaves of Oil Mangrove (*R. apiculata*) characterized by the formation of a yellow color when dilute NaOH solution was added and the yellow color disappeared again when dilute HCl was added. The existence of flavonoids in plants was also stated by Waluyo, 2013 in Noviyanty, et al. 2019 that flavonoids are a group of polyphenol compounds that are naturally found in fruits, vegetables, nuts, seeds, flowers, leaves, skin, trees, etc. Flavonoids are a group of aromatic compounds which include polyphenols and contain antioxidants.

Flavonoids function as growth regulators, photosynthetic process regulators, antimicrobial and antiviral substances (Endarini, 2016). This compound is usually produced by plant tissue as a response to infection (Endarini, 2016). These results show that alcohol can dissolve flavonoid compounds. This is reinforced by Markham's (1988) statement that flavonoids have bonds with sugar groups which cause flavonoids to dissolve more easily in polar solvents.

Saponins

Saponin testing on Oil Mangrove (*R. apiculata* leaves was declared positive. Saponin is a type of chemical compound that is abundant in various plant species. This compound is an amphipathic glycoside which can produce foam if shaken vigorously in the solution and the foam is stable and does not disappear easily. Saponin has glycosyl which functions as a polar group and is active so that when shaken with water, saponin can form micelles. In the micelle structure, the polar groups face outward while the non-polar groups face inward. This condition looks like foam, therefore in this analysis the ability of the sample to form foam is looked.

Alkaloids

The results of the research showed that the oil mangrove (*R. apiculata*) leaf extract contained alkaloids as indicated by the presence of a white precipitate after adding Dragendorf's reagent. The alkaloid content in plants can be used in many ways, including in medicine. Plants are considered the oldest source of alkaloids, and some of the most widely known alkaloids, such as morphine, quinine, strychnine, and cocaine, come from plants (O'Connor, 2010). In general, alkaloids are often used in medicine (Harborne, 1996). Alkaloids can function as antioxidants, this is supported by antioxidant test research (Hanani et al., 2005). According to Priyanto (2012), the levels of alkaloids produced by green plants are not the same in all tissues and at each stage of growth.

Carotenoids

Carotenoids are very important natural substances, this is because some carotenoids can be converted into vitamin A, where these pigments are often found in plants together with chlorophyll (Apriyantono, 1989). The results of the phytochemical test for rubberonioid on oil mangrove (*R. apiculata*) leaf extract showed positive results. Carotenoids are pigments that are yellow, orange to red in color (Gross, 1991). This pigment is found in many vegetables and fruits, and is also found in fungi, bacteria, animals and humans (Gross, 1991).

Steroids

In the analysis of steroids and triterpenoids in plants, they can be tested using the Liebermann-Buchard method which will give a red or purple color for terpenoids and a green or blue color for steroids. This test is based on the ability of triterpenoid and steroid compounds to form color in the presence of concentrated H2SO4 in glacial acetate solvent to form an orange color (Marlinda, 2012). The results of the steroid analysis of Mangrove Oil (*R. apiculata*) leaf extract were positive for containing steroids because the solution turned green.

Antibacterial Activity

The antibacterial activity assessment against P. acne bacteria revealed inhibition zones ranging from 9.50 mm to 17 mm. This inhibition of bacterial growth was attributed to damage to the structural components of the bacterial cell membrane caused by the bioactive compounds present in the mangrove extract, including steroids, saponins, flavonoids, and tannins. It's worth noting that the concentration of the extract significantly influenced the antibacterial activity, with higher concentrations yielding larger inhibition zones (Zuhud, 2001).

Proposed Improvements

To fully harness the potential of Oil Mangrove (*Rhizophora apiculata*) leaves, it is recommended to explore various solvents for extraction and extend antibacterial testing to a broader spectrum of bacteria. Additionally, conducting antioxidant testing could further elucidate the extract's potential health benefits. When conducting antibacterial assays, it is advisable to use freshly cultured bacteria to ensure the accuracy of results. It is essential to maintain the sterility of research tools to prevent contamination and uphold result integrity.

CONCLUSION

In the extract of Mangrove Oil (*Rhizophora apiculata*) leaves collected from the pond area around Bunga Putih Village, Marang Kayu District, Kutai Kartanegara Regency, we identified various secondary metabolite compounds, including flavonoids, saponins, carotenoids, tannins, alkaloids, and steroids. Notably, terpenoids were absent in the extract. This Mangrove Oil (*Rhizophora apiculata*) leaf extract

exhibits potential as an antibacterial agent, with a minimum inhibitory concentration (MIC) of 2.5% against P. acne. Among the concentrations tested, the highest inhibitory effect on the growth of P. acne bacteria was achieved at a concentration of 20%, resulting in an inhibition zone measuring 17 mm. The observed differences in inhibition zone diameters can be attributed to varying levels of bioactive compounds within the *R. apiculata* mangrove leaf extract. As Dewi (2010) explained, higher extract concentrations contain more active compounds, directly influencing the diameter of the inhibition zone formed in bacterial tests. This suggests the extract's potential as a valuable antibacterial agent against P. acne, which warrants further investigation and development.

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