ORIGINAL ARTICLE

Exploration of Potential Moraceae as an Antimicrobial Agent for Coliform Bacteria

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ABSTRACT

Introduction: Extract plants can be used as a treatment strategy for bacterial infections. Plants have potential as an antimicrobial and antioxidant, with a variety of molecules that can protect the human body from pathogens. Coliform bacteria are a major causative agent of public health problems, such as gastroenteritis. Therefore this research was conducted to know the potential of several types of plants as an alternative antibacterial agent. **Methods:** The study was conducted using the disc diffusion method with extract plants of the Family Moraceae. The extract of leaf and bark plants (*Artocarpus sericicarpus, Artocarpus anisophyllus* and *Artocarpus dadah*) was carried out by maceration method with DMSO. The measurement inhibition zone was calculated with Disc Diffusion Method. **Results:** The highest inhibition zone was formed in *Artocarpus anisophyllus* againts *E. aurogenosa* (8.17 + 0.19 mm) in concentration equal to 10,000 ppm with moderate category. Further results showed that *Artocarpus sericicarpus, Artocarpus anisophyllus* and *Artocarpus dadah* can inhibit *Klebsiella pneumonae*, *E. aurogenosa* and only *Artocarpus dadah* can inhibit *E. coli*, with weak category. While *Artocarpus sericicarpus*, *Artocarpus anisophyllus* can inhibit all bacteria with a concentration of 10,000 ppm extract. **Conclusion:** This study emphasizes the importance of involving parents in the obesity intervention programs for children.

Keywords: Antimicrobial, Artocarpus, Coliform, Disc diffusion

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INTRODUCTION

Bacterial resistance and limited use of drugs have a significant influence on the effectiveness of treatment with antibiotics. New antimicrobial therapy is needed in the field of medicine. Current research tends to look for alternative agents as antibacterial for disease-causing bacteria. Many studies have shown that the plant extract has potential as an antibacterial agent. Thus, plant extracts can be used as an alternative therapy for bacterial infections (1,2,3).

It is known that pathogenic bacteria are resistant to several antibiotics, and this is a determining factor for treatment, especially for patients with decreased immunity or immunocompromised (4). The usage of plant extracts as an antibacterial is developed to find effective antibacterial with a minimum resistance side effect in the future. In addition, garlic extracts (*Allium sativum*) have activity against Gram-negative, including *Escherichia, Salmonella, Klebsiella*, and *Proteus* (5). Another experiment showed that the extracts of *Moringa oleifera* and *Metricaria recutita* can inhibit for *Klebsiella* spp. and *E. coli* (6). Medicinal plants have the potential to be antimicrobial and antioxidant, with a variety of molecules that can protect the human body from pathogens and cell oxidation. This is closely related to secondary metabolite compounds contained in these plants (7).

Groups of coliform bacteria are the causative agent of major public health (8). Coliform group bacterial agents, including *Escherichia coli, Klebsiella* sp., and *Enterobacter* sp. give several infections such as gastroenteritis, urinary tract infections, and other infectious diseases (9). The bacteria that produce ESBL are the most common drug-resistant bacteria in hospitals. One of the ESBL groups of bacteria that are *Escherichia coli, Klebsiella pneumonia*, and *Klebsiella oxytoca* (10). Antibiotic resistance occurs because of genetic changes in bacteria and research has to develop new antibiotics. The emergence of antibiotic resistance in several coliforms has prompted further research to

find suitable therapy against resistant strains. Thus, that is important to explore various medicinal plants that have the potential as antimicrobials against coliform bacteria.

MATERIALS AND METHODS

Plant Extraction

The plants used in this study were from the Moraceae family (*Artocarpus anisophyllus, Artocarpus dadah, and Artocarpus sericicarpus*). All plants were collected from Balikpapan Botanical Garden, East Kalimantan. Fresh leaf and bark from all plants were extracted each by maceration ultrasonic using 90% n-hexane absolute with the sample and solvent ratio of 1:4, in 3 x 2 minutes. The extraction was repeated with Dichloromethane in 3 x 2 minutes, and with methanol in 3 x 2 minutes. Each repetition was filtered to separate the extract from the residue. Extracts methanol maceration results of each sample were concentrated using a rotary evaporator. The concentrated extract obtained was dried at room temperature.

Stock Preparation of Plant Extracts

The extract solution with 1% DMSO (dimethyl sulfoxide) (Sigma-Aldrich) was used as a stock solution and absolute methanol was used as a solvent. Concentrations of each plant extract used in this study were 5000 ppm and 10,000 ppm. Methanol was also used as a control to evaluate the test results.

Bacterial Strains and Culture Condition

Coliform bacteria (*Escherichia coli, Klebsiella pneumoniae, and Enterobacter aurogenosa*) were selected as standard strains from Microbiology Laboratory, Universitas Airlangga, Surabaya. Bacterial cultures were cultured on MCA (Mac Conkey Agar) and NA (Nutrient Agar) (MERCK). Bacterial cells in the suspension were measured according to standard 0,5 Mac Farland.

Disc Diffusion Method

The disc diffusion method is used for the antibacterial test. The fifteen ml of Mueller Hinton Agar (MERCK) (pH ± 7.2) was poured into a sterile Petri dish for media preparation. The bacterial suspension (standard 0.5 Mac Farland) was poured in each base plate by swab method using sterile cotton (Himedia). The extract used was 100 µl of each test sample and then immersed in sterile paper discs diameter 6 mm and the discs were being air-dried before being placed onto the inoculated medium plate. After placing paper discs into the inoculated medium plate, incubation was performed at ± 37˚C for 24 hours. The control antibiotic used was Ampicillin with a concentration of 20 mg/disc. The zone of inhibition formed a clear zone around the disc and, in this zone, bacterial growth was inhibited. The calculated inhibition zone diameter (mm) was then compared with the antibacterial inhibition category based on the inhibition zone diameter (<8 mm: weak, 8-11 mm: moderate, 11-20 mm: strong,> 20 mm: very strong) (11). The experiment was carried out with three repetitions.

Statistical Analysis

The data used were the mean diameter of the inhibition zone ± SEM. The results were analyzed by Microsoft excel ver. 2013.

RESULTS

Antibacterial Activity of Plants

Antibacterial activity was determined based on the measured inhibition zone diameter after 24 hours of incubation (Table I). Six-part extract of plant tested against three bacterial (*E. aurogenosa, K. pneumonia*, and *E. coli*) strains showed significant inhibitory activity below 10,000 ppm (Table I-II). All the extracts including *A. sericicarpus, A. Anisophyllus,* and *A. dadah* showed different inhibitory activity against bacteria. In contrast, treatment with the leaves extract of *A. sericicarpus* showed no inhibitory activity for all bacterial strains. Further results showed that the leaves extract of *A. anisophyllus* can inhibit *K. pneumonia* and *E. aurogenosa*. and only the leaves extract of *A. dadah* can inhibit *E. aurogenosa* activity with a concentration of 10,000 ppm extract (Table I).

Table I : Antibacterial activity of different leaves plant extracts with well diffusion assay

Plant species	Concentration	*K. pneumonia*		*E. aurogenosa*		*E. coli*	
		Inhibition zone (mm)	inhibition category	Inhibition zone (mm)	inhibition category	Inhibition zone (mm)	inhibition category
A. sericicarpus	5000 ppm	-	-	-	-	-	-
	10000 ppm	-	-	-	-	-	-
A. anisophyllus	5000 ppm	-	-	-	-	-	-
	10000 ppm	6.92 ± 0.77	Weak	8.17 ± 0.19	Moderate	-	-
A. dadah	5000 ppm	-	-	-	-	-	-
	10000 ppm	-	-	6.55 ± 0.28	Weak	-	-
Methanol		-	-	-	-	-	-
DMSO		-	-	-	-	-	-

Data are means of three replicates (n = 3) with standard deviations

Table II : Antibacterial activity of different bark plant extracts with well diffusion assay

Plant species	Concentration	*K. pneumonia*		*E. aurogenosa*		*E. coli*	
		Inhibition zone (mm)	inhibition category	Inhibition zone (mm)	inhibition category	Inhibition zone (mm)	inhibition category
A. sericicarpus	5000 ppm	-	-	-	-	-	-
	10000 ppm	6.52 ± 0.82	Weak	6.72 ± 0.2	Weak	-	-
A. anisophyllus	5000 ppm	-	-	-	-	-	-
	10000 ppm	7.39 ± 0.67	weak	7.25 ± 0.40	Weak	-	-
A. dadah	5000 ppm	-	-	-	-	-	-
	10000 ppm	-	-	6.87 ± 0.92	Weak	6.32 ± 0.14	Weak
Methanol		-	-	-	-	-	-
DMSO		-	-	-	-	-	-

Data are means of three replicates (n = 3) with standard deviations

Meanwhile, the treatment with bark extract of *A. sericicarpus* showed inhibitory activity for *K. pneumonia* and *E. aurogenosa*. The bark of *A. anisophyllus* can inhibit *K. pneumonia* and *E. aurogenosa*. Then, the bark extract of *A. dadah* can inhibit the activity of *E. aurogenosa* and *E. coli* with a concentration of 10,000 ppm extract (Table II). Therefore, the results showed that only the bark extract of *A. anisophyllus* showed moderate antibacterial activity (>8 mm) against *E. aurogenosa* (8.17 + 0.09 mm). However, leaves extract (*A. sericicarpus, A. Anisophyllus*, and *A. dadah*) and bark extract (*A. sericicarpus* and *A. dadah*) extracts have no adequate antibacterial activity (> 8 mm) against 3 strains of *E. aurogenosa, K. pneumonia*, and *E. coli*. The results showed that bactericidal activity was directly proportional to the extract concentration.

Based on the classification of the inhibition zone according to Pelczar and Chan (11), plant extracts with a concentration of 5000 ppm and 10.000 ppm were classified in the weak inhibition category (<8 mm). The plant groups were bark extracts for A. *sericicarpus* against K. *pneumonia* and E. *aurogenosa*, A. *anisophyllus* against K. *pneumonia*, A. *dadah* against E. *aurogenos*a and E. *coli* (Table I). Other groups for leaves extract are A. *anisophyllus* against K. pneumonia and E. aurogenosa, A. dadah against E. *aurogenosa* (Table II). Meanwhile, the bark extract of A. *anisophyllus* extract with a concentration of 10.000 ppm was included in the moderate category (8-11 mm).

The media used for antimicrobial tests is Mueller-Hinton Agar (MERCK). Mueller-Hinton Agar is the standard medium for testing antibiotic sensitivity. This media was used due to its contents such as cation concentration, thymidine content, sulfonamide inhibitors, trimethoprim, and tetracycline. Cation concentrations are related to media pH (7.2 ± 0.2), and they do not affect antibacterial activity in the medium. The bacterial control treatment with methanol and DMSO was not affected.

DISCUSSION

New research on antibacterial agents deserves attention. New antibiotics are a solution to reduce antibiotic resistance to pathogenic bacteria. This study focus on the use of extract of plants that are available in nature and have potential as medicine, without side effects. Inappropriate use of antibiotics is the main cause of bacterial antibiotic resistance. These results may cause ineffective use of antibiotics in the long term (12,13). The *A. sericicarpus, A. Anisophyllu,s* and *A. dadah* are plants of the Famili Moraceae. The leaves of *A. anisophyllus* are applied externally as a treatment for boils and itch. The latex of *A. dadah* was reported to have disinfectant properties and is applied to wounds. The danger of all the plants are unknown (14).

The results of the six extracts showed antibacterial activity against 3 types of bacteria. Based on observations, each plant extract with a concentration of 10,000 ppm had the inhibition effect on the growth of *E. aurogenosa, K. pneumonia* and *E. coli* bacteria. The results showed that each plant had a different potential to inhibit bacterial growth. Based on the measurement, leaves extracts of *A. anisophyllus* had potential as an antimicrobial agent better than other plants. Meanwhile, other plant extracts had a low inhibition effect on the selected bacteria. The concentration used in this study was still categorized as low in use, and plant had a different potential for inhibitory bacteria activity. According to Ariyanti *et al* (15), the higher concentration of antimicrobial substances, the greater ability to control and kill microorganisms. This is related to one or more active compounds in the extract.

The results of the study by Kuete *et al*. (16) that the determination of MIC showed that crude extract and *Ficus polita* were able to prevent the growth of the eight microorganisms tested, namely Providencia smartii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Stahylococcus aureus, Salmonella typhi, Escherichia coli and, Candida albicans. The crude extract was recorded

at 50%. The result of another study, Adeniyi *et al* (17) have explored the potential of *Ficus vogelii* leaves as an antimicrobial. The extract with n-Hexane showed better activity than Ethanol, at the highest concentration (100 mg / mL), against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, and *Bacillus subtilis*.

Plants have the potential to produce secondary metabolites that act as antimicrobial substances. Each class of compounds has different effects in inhibiting bacterial growth. The environmental factors that influence bacterial growth were nutrition, temperature, pH, and humidity. It is necessary to determine the specific antimicrobial substances in used plant extracts. Therefore, the determination of concentration can be reduced to optimize the function as an antibacterial agent. The mechanism of inhibiting bacterial growth by antibacterial compounds can be in the form of cell wall damage by inhibiting or changing the formation. Besides, changes in the permeability of the cytoplasmic membrane can cause cell lysis. Other mechanisms include changes in protein and nucleic acid molecules, inhibition of enzyme work, and inhibition of nucleic acids and protein synthesis (11).

The potency of antibacterial agents can be seen by the presence of a clear zone. Inhibition zone diameter is influenced by several factors such as diffusibility of antimicrobial substances, antibiotic concentration, nature, and medium composition, presence of inhibitors or stimulants, pH, and incubation time (18). The increasing concentration of the antimicrobial substance can increase the active compounds that function as antibacterial so that the ability of antimicrobial substances in killing bacteria is also greater (19). Several species of the Genus *Artocarpus* (Moraceae) have been investigated for their natural ingredients. Secondary metabolites isolated from the Genus *Artocarpus* consist of terpenoids, flavonoids, stilbenoids, arylbenzofuran, neolignans, and the Diels-Alder adduct. The flavonoid group is the most common compound found in the Artocarpus plant, and the compounds are reported for their antimicrobial (20).

The results from this study provided that the used plant extracts had antibacterial activity against *E. coli*, *K. pneumonia,* and *E. aurogenosa.* More research is needed on these topics because of its promising findings. Preliminary phytochemical analysis showed that the plants included in the Moraceae Famili contained flavonoids and terpenoids. Bioactive compounds such as flavonoids have been reported as antibacterial, and they are used by plants for protection against antimicrobial activity (21).

CONCLUSION

Our research showed the potential of Moreaceae (A. sericicarpus, A. *anisophyllus*, and A. *dadah*) as an antimicrobial still needs further confirmation, specifically for the A. *anisophyllus* plant with moderate category that still needs to be developed as an antimicrobial. The concentration of minimum inhibitory of several used plant extracts was different from the used bacteria in the study. Furthermore, vivo research is needed to determine and explain the bioactive compound to develop new antibacterial drugs.

ACKNOWLEDGEMENT

All authors express gratitude toward the Team of Microbiology for supporting the effort in helping to prepare all necessary steps for microbial identification.

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