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Original Article

The Potential Antibacterial Effect of Papaya Leaf Extract (Carica papaya L) and Miana Leaf Extract (Coleus scutellarioides L) as Adjuvant Therapy for Rifampicin-Resistant Tuberculosis

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ABSTRACT

The adhesion of Rifampicin-resistant TB to neutrophils plays an essential role in colonization. Several active compounds in papaya leaf and Miana leaf (Coleus scutellarioides L) are believed to regulate or prevent the formation of bacterial colonies. The purpose of this study was to determine the anti-bacterial effectiveness of extracts of papaya leaf (Carica Papaya L.) and Miana leaf (Coleus scutellarioides L) against bacterial isolates of Rifampicin-Resistant TB strain (RR). This research method is a in vitro laboratory experiment, and extracts of papaya leaf and Miana leaf (50, 25, 12,5%) were tested as anti-bacterial using the M-TB susceptibility test using the Proportion Method. The results showed the anti-bacterial ability of papaya leaf extract against bacterial isolates of the MTBC-Resistant Rifampicin strain at a concentration of 50% with a resistance percentage value of 0% so that it was included in the Sensitive category, but at a concentration of 25% the resistance percentage value was 42.86% % and a concentration of 12.5%, the percentage value of resistance is 42.86% so that it is included in the category of resistance (Resistant > 1% and Sensitive < 1%). The anti-bacterial ability of miana leaf extract against bacterial isolates of the MTBC-Resistant Rifampicin strain at a concentration of 50% with a resistance percentage value of 5.33%, at a concentration of 25%, with a resistance percentage value of 17.14%, and at a concentration of 12.5%, with a resistance percentage value of 100%, so all are included in the resistant category. The Conclusion 50% papaya leaf extract inhibits the formation of Rifampicin-resistant MTBC-resistant bacterial colonies, allowing its usage as a substitute ingredient in Rifampicin-resistant MTBC-resistant medications.

Keywords: Extract, Papaya Leaf, Miana Leaf, Antibacterial, Rifampicin-Resistant TB Strains.

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INTRODUCTION

The introduction should briefly place the Tuberculosis is a disease that can be passed from one person to another. This disease often occurs in tropical areas such as Indonesia because the air is a lot of dust and the temperature is warm and humid so microbes can thrive caused by the bacterium Mycobacterium tuberculosis [1,2]. These bacteria are spread in the air through the sprinkling of the patient's saliva, for example, when talking, coughing, or

sneezing; however, the transmission of TB requires close and long contact with the patient, not as easy as the spread of the flu [3].

According to WHO, one person is infected with tuberculosis every second. After India, Indonesia is the second largest country with the most pulmonary TB cases globally. The total number of TB cases in 2016 was 351,893 people, most of whom were people of productive age (25-34 years) [4]. Tuberculosis is curable, and patients must routinely take antibiotics, typically recommended for six months. Antibiotics are compounds generated by microorganisms that prevent or destroy the growth of other microbes. In developing nations, however, the rise of antibiotic- and drug-resistant bacterial species is a significant concern [5].

Drug-resistant tuberculosis is TB caused by Mycobacterium tuberculosis which has developed immunity to anti-tuberculosis drugs (OAT). MDR-TB is drug-resistant tuberculosis (RO-TB) to at least two of the most potent anti-TB medications, isoniazid (INH) and rifampicin, or accompanied with immunity to other first-line anti-TB drugs like ethambutol, streptomycin, and pyrazinamide [6]. Indonesia ranks eighth among the 27 nations with the highest prevalence of MDR-TB globally [5]. The grouping of TB-resistant TB is as follows, bacterial strains of TB-Monoresistant, TB-Polyresistant, TB-Multi Drug Resistant (MDR), TB-Extensively Drug Resistant (XDR), and TB-Rifampicin Resistant (RR) [7, 8].

One of the factors causing resistance to OAT is that patients do not comply with doctor or health worker recommendations, do not regularly take OAT alloys, have impaired drug absorption, and stop treatment unilaterally prematurely. Patients stop treatment unilaterally because they cannot stand the side effects of each OAT drug [5,6]. The side effects of OAT drugs require us as health workers to look for alternatives to natural ingredients that are commonly consumed by people who do not have side effects.

Papaya and miana leaf extract can be used as a substitute for medicinal herbs to reduce adverse effects [9,10]. Papaya and miana leaf extracts include antimicrobial components, including flavonoids, alkaloids, and tannins [11–16]. The membrane will be damaged by lipophilic flavonoids, increasing permeability and interfering with bacterial metabolism [17,18]. Alkaloids can impede the formation of peptidoglycan in bacteria, resulting in the incomplete formation of the cell wall layer and bacterial mortality [9,10,19]. The antimicrobial effect of tannins can inactivate microbial adhesives, inactivate hydrolytic enzymes such as proteases and carbohydrates, and inhibit enzymes in envelope transport proteins [10,20,21]so that the compounds in papaya leaf extract and miana leaf extract can influence or inhibit the growth of bacterial colonies [10,22,23]. Therefore, researchers want to know the role of Papaya Leaf Extract (Carica papaya L), Miana (Coleus scutellarioides L) as Antibacterial in Rifampicin-resistant TB.

METHOD

This research is a laboratory experimental. It was started by preparing samples of papaya leaf extract and miana leaf extract with concentrations of 50, 25, and 12.5% , respectively, from 100% thick extract. Then make, preparation of 75% extract from 100% extract of papaya leaf and miana leaf, 7.5 mL of extract plus 1 mL of DMSO, homogenized using a vortex plus 1.5 mL of aqua dest, homogenized again using a vortex so that 75% extract is available. Preparation of 50% extract with 8 mL of 75% extract + 4 mL of sterile distilled water, homogenized with the help of a vortex, obtained 12 mL of 50% extract. Preparation of extract 25 with 3 mL of 50% extract + 3 mL of sterile distilled water, homogenized with the help of a vortex, obtained 6 mL of 25% extract. Preparation of 12.5% extract with 1.5 mL of 50% extract + 4.5 mL of sterile distilled water, homogenized with the help of a vortex, obtained 6 mL of 12.5% extract. Furthermore, the provision of MTBC-RV bacteria as a bacterial control strain from pure M-TBC strains that are sensitive to 4 types of M-TB drugs and the provision of M-TBC-RR bacteria that are resistant to rifampin was successfully isolated from patients examined at the Provincial Health Laboratory. West Java.

Then, the sensitivity test of Mycobacterium tuberculosis (M.tb) was carried out to determine the sensitivity status of M-TBC bacteria to anti-TB drugs. This sensitivity test is needed to determine the most appropriate anti-M-TB drug choice for the patient. The M-TB susceptibility test was carried out using the conventional method, which requires a

relatively long time (2 - 3 months). The data obtained are primary data by examining according to the procedures carried out at the Microbiology Laboratory Health Laboratory of West Java Province, Sederhana Bandung Street, namely M-TBC Sensitivity Test Proportion Method The standard procedure according to the Ministry of Health, two types of extracts were simultaneously tested for sensitivity to control MTBC bacteria (sensitive to various OATs) RV and to Rifampicin-resistant M-TB bacteria M-TB RR with bacterial strengths of 10-3 and 10-5 dilutions were simultaneously tested for sensitivity to 4 anti-MTBC drugs namely Isoniazid, Rifampicin, Ethambutol, Streptomycin.

The new MTBC colony growth readings can be observed after incubation on day 28 and wait for it to be re-read on day 42 to observe whether or not there is the certainty of colony growth from the test bacteria MTBC-RV (control) and MTBC-RR in contact with Isoniazid, Rifampicin, Ethambutol, Streptomycin and extracts of papaya and miana leaf with concentrations of 50%, 25%, and 12.5%, respectively.

The data obtained were from the sensitivity values of MTBC bacteria to the leaf extracts tested. To determine the difference in sensitivity of each concentration using the T-test and Bonferroni post hoc test with a significance level of 95%.

Ethical approval the research proposal was approved by the Health Ethics Commission of the Poltekkes Kemenkes Banten. Aspects of

research use the general principles of research ethics in humans, namely: Respect for human dignity, beneficence, and justice. Number of the ethical protocol: 05/KEPK/POLKESTEN/IV/2021.

RESULTS

Table 1. Antibacterial test results of papaya leaf extract and Miana leaf against MTBC-RR.

No	Code	Reading Results 10-3	Scale	Reading Results 10^{-5}	Scale	Resistance
1	Rifampicin 50% (Control)	2+	3	0	0	
		2+	3	2 colony	1	
2	papaya leaf -50%	0	0	0	0	sensitive *
		0	0	0	0	
3	Rifampicin 25% (Kontrol)	3+	4	1+	2	
		3+	4	1+	2	
4	Papaya Leaf -25%	2+	3	1+	2	Resistance
		2+	3	1+	2	
5	Rifampicin 12,5% (Kontrol)	3+	4	1+	2	
		3+	4	1+	2	
6	Papaya Leaf -12,5%	2+	3	14 colonies	1	Resistance
		2+	3	16 colonies	1	
7	Rifampicin 50% (Kontrol)	1+	2	0	0	
		2+	3	4 colonies	1	
8	Miana Leaf 50%	2 colonies	1	0	0	Resistance
		8 colonies	1	0	0	
9	Rifampicin 25% (Control)	3+	4	1+	2	
		3+	4	1+	2	
10	Miana Leaf 25%	12 colonies	1	0	0	Resistance
		1+	2	2 colonies	1	
11	Rifampicin 12,5% (Control)	2+	3	1+	2	
		2+	3	1+	2	
12	Miana Leaf 12,5%	2+	3	13 colonies	1	Resistance
		2+	3	17 colonies	1	

Explanation	Number of Colonies	Reporting	Scale
	Tidak ada pertumbuhan	Negative	0
	1-19 Colony	Write down the amount (write the number of colonies)	1
	20-100 Colony	1+ (Count the number of colonies)	2
	100-200 Colony	2+	3
	200-500 Colony	3+ (almost confluent)	4
	>500 Colony	4+ (confluent)	5

Interpretation:

Resistance

$$\frac{Number\ of\ colonies\ in\ media\ containing\ drug,extract}{(Number\ of\ colonies\ in\ control)}\ X\ 100\% = \%\ Resistance$$ [1]

Resistance ≥ 1%

Sensitive < 1%

The antibacterial test results between papaya leaf extract and Miana leaf against the rifampicin-resistant TB test bacteria showed antibacterial ability, marked by reduced growth of the test bacterial colonies. The test results at several concentrations of papaya leaf and miana leaf showed sensitivity to Rifampicin-Resistant TB bacteria in papaya leaf samples with a concentration of 50%.

Table 2. Colony Count Reading Results in MTBC-RR Control.

Control	Reading Results			
	I	R	E	S
(10^{-3})	0	2+	0	0
(10^{-3})	0	2+	0	0
(10^{-5})	0	10 colonies	0	0
(10^{-5})	0	1+	0	0

The number of colonies in the MTBC-RR control at 10-3 dilution was 2+, meaning 100-200 colonies, an average of 150 colonies.

Table 3. Antibacterial effectiveness of papaya leaf extract against MTBC-RR.

Papaya Leaf Extract

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
$RV10^{-3}$	K	50%	-3.00000*	0.00354	0.000	-3.0172	-2.9828
		25%	-4.00500*	0.00354	0.000	-4.0222	-3.9878
		12,5%	-4.00000*	0.00354	0.000	-4.0172	-3.9828
$RV10^{-5}$	K	50%	-0.50000	0.35355	1.000	-2.2151	1.2151
		25%	-2.00000*	0.35355	0.029	-3.7151	-0.2849
		12,5%	-2.00000*	0.35355	0.029	-3.7151	-0.2849
$RR10^{-3}$	K	50%	0.00000	0.00354	1.000	-0.0172	0.0172
		25%	-3.00000*	0.00354	0.000	-3.0172	-2.9828
		12,5%	-3.00500*	0.00354	0.000	-3.0222	-2.9878
$RR10^{-5}$	K	50%	0.00000	0.00354	1.000	-0.0172	0.0172
		25%	-2.00500*	0.00354	0.000	-2.0222	-1.9878
		12,5%	-1.00000*	0.00354	0.000	-1.0172	-0.9828

The mean difference is significant at the 0.05 level.

Statistical test results using the Bonferroni test showed that a concentration of 50% papaya extract inhibited growth at dilutions of MTBC-RR bacteria 10-5 and 10-3, marked with a significance of 1,000. Thus there is no difference between the sensitivity of the MTBC-RR bacteria to papaya leaf extract with anti-MTBC drugs as a comparison. MTBC-RR bacteria have the same sensitivity to 50% papaya leaf extract and anti-MTBC drugs.

Table 4. Antibacterial effectiveness of Miana Leaf extract against MTBC-RR.

Miana Leaf Extract							
Dependent Variable			**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
$RV10^{-3}$	K	50%	-2.50000*	0.35355	0.013	-4.2151	-0.7849
		25%	-4.00000*	0.35355	0.002	-5.7151	-2.2849
		12,5%	-3.00000*	0.35355	0.006	-4.7151	-1.2849
$RV10^{-5}$	K	50%	-0.50000	0.35355	1.000	-2.2151	1.2151
		25%	-2.00000*	0.35355	0.029	-3.7151	-0.2849
		12,5%	-2.00000*	0.35355	0.029	-3.7151	-0.2849
$RR10^{-3}$	K	50%	-1.00000	0.35355	0.285	-2.7151	0.7151
		25%	-1.50000	0.35355	0.079	-3.2151	0.2151
		12,5%	-3.00000*	0.35355	0.006	-4.7151	-1.2849
$RR10^{-5}$	K	50%	0.00000	0.35355	1.000	-1.7151	1.7151
		25%	-0.50000	0.35355	1.000	-2.2151	1.2151
		12,5%	-1.00000	0.35355	0.285	-2.7151	0.7151

*The mean difference is significant at the 0.05 level.

Statistical test results showed that 50% miana extract concentration inhibited growth in MTBC-RV 10-5 dilution (p = 1,000), meaning there was no statistical difference. Statistical test results showed that 50% and 25% miana extract concentrations inhibited growth in the dilution of MTBC-RR bacteria 10-3 (p = 0.285; 0.079), meaning there is no difference with statistics. Statistical test results showed that at concentrations of miana extract, 50% and 25% inhibited growth in dilution of MTBC RR bacteria 10-5 (p = 1.000) means that there is no difference in statistics

DISCUSSION

Drug-resistant tuberculosis (RO-TB) is still a danger to tuberculosis (TB) control and a serious global public health concern in many nations [24,25]. In 2019, it was anticipated that 3.3% of newly diagnosed TB patients and 17.7% of previously treated TB patients would be drug-resistant. In Indonesia, an estimated 2.4% of all new TB patients and 13% of cured TB patients have resistant tuberculosis, with a total incidence of 24,000 cases, or 8.8/100,000 population [5].

The management of TB RO patients has been carried out in Indonesia since 2009. The treatment results of TB RO patients from 2009–2017 still show a trend of decreasing treatment success rates, increasing dropout rates, and increasing patient mortality rates [5]. To prevent patients from dropping out of treatment due to the side effects of TB drugs, researchers are trying to find alternative drugs derived from natural ingredients commonly consumed by the public that do not have side effects. The plants used were papaya and miana leaf extract [9–12,17,21,23,26].

The results demonstrated that 50% papaya leaf extract inhibited the growth of MTBC-Rifampicin-resistant bacterial colonies with a resistance value of 0%; therefore, the content of papaya leaf extract can be used as an alternative material for anti-Rifampicin-resistant MTBC drugs because it falls under the category of Sensitive. As papaya leaf extracts with concentrations of 25% and 12.5% and miana leaf extracts with concentrations of 50, 25%, and 12.5% demonstrated resistance to

Rifampicin-resistant MTBC bacterium.

One of the plants that can be used to inhibit bacterial growth is papaya leaf extract. The content in papaya leaf extract in the form of flavonoids, carpain alkaloids, papain enzymes, and tannins can inhibit bacterial activity [10,14–22].

This study demonstrates the potential of papaya leaf extract as an alternative medicine to lessen the adverse effects of anti-tuberculosis drugs based on its capacity to prevent the growth of resistant MTBC bacteria during an in vitro trial. Flavonoids, saponins, tannins, carpain alkaloids, and papain enzymes could suppress bacterial colonization of MTBC-RR [10,14–22].

Previous research revealed that papaya leaf flavonoids inhibit Gram-positive bacteria. Gram-positive bacteria's inhibitory action inhibits the function of the bacterial cell wall as a shape-determinant and protects cells against osmotic lysis. By interfering with the permeability of the bacterial cell wall and causing cell lysis, flavonoids can suppress the growth of Staphylococcus aureus [10,15,18].

Because these compounds' presence can diminish the cell wall's surface tension, saponins can inhibit the growth of bacteria. The wall will lyse or break down when interacting with the bacterial cell wall. Saponins interfere with the surface of the cell wall; when the surface is disrupted, antibacterial substances can easily enter the cell and disrupt the metabolism of bacteria, resulting in their eventual demise [10,27].

By causing bacterial protoplasm to coagulate, tannin compounds can restrict bacterial growth. Tannins serve as antibacterial agents by binding to proteins to prevent the production of cell walls. Tannins are inhibited because saponin and flavonoid chemicals lyse bacterial cell walls, allowing tannin compounds to easily enter bacterial cells and coagulate bacterial cell protoplasm; as a result, the cells are unable to carry out living activities, and their growth is inhibited or even stopped [10,18,14,20,27]. Papain, a proteolytic enzyme, also inhibits the growth of Gram-positive and Gram-negative bacteria through its bactericidal and bacteriostatic properties [9,10,11].

This study has limitations because the active substance in papaya leaf extract and the mechanism involved in suppressing the MTBC RR bacterial colony are unknown. However, the findings of this investigation reveal that papaya leaf extract can effectively inhibit the formation of Rifampicin-resistant MTBC bacterial colonies.

CONCLUSION

The study results indicate that 50% of papaya leaf extract can inhibit the formation of Rifampicin-resistant MTBC bacterial colonies, allowing its content to be utilized as an alternative ingredient for Rifampicin-resistant MTBC-resistant medications. Additional study is required for antibacterial papaya leaf extract concentrations of TB drug-resistant MTBC other than Rifampicin > 50%, examining the immunomodulator of papaya leaf extract in experimental animals, and producing papaya leaf extract capsules as anti-MTBC-Rifampicin resistance.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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