

## Effect of Bay Leaf (*Syzygium polyanthum*) Extract on Antioxidant Activity, MDA Levels, and Liver Histopathology Feature of Ethambutol Induced Wistar Rats

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### Abstract

Bay leaf extract (*Syzygium polianthum*) is one herbal element that may be used to lessen liver function issues, lessen symptoms of nausea, vomiting, discomfort and improve adherence and the effectiveness of tuberculosis treatment. The objective of this study was to determine the effect of bay leaf extract on antioxidant activity, Malondialdehyde (MDA) levels, and liver histopathology of ethambutol-induced Wistar rats. This research is a laboratory experiment. Antioxidant activity of Bay Leaf Extract was evaluated by comparing vitamin C with spectrophotometry methods. White Wistar rats were separated into 6 groups and used to test the label of MDA and livers histopathology. Group 1 serves as the control group and received DMSO (placebo); Group 2 was received ethambutol 50mg/kg BW; Group 3 was received ethambutol and silymarin with dose 50mh/kg BW. Group 4-6 had been given ethambutol and extra ethanol extract of bay leaves of 75,150, and 300mg/kg BW, respectively. The results of the spectrophotometry showed that the Bay Leaf Extract had antioxidant activity comparable to that of vitamin C, with an IC<sub>50</sub> of 11.4 g ± (4.4%). There was a significance difference in each group's MDA levels (p=0.002). Although there was no significant difference in the liver histopathology of treated rats (p>0.05). While bay leaf extract significantly lowers MDA levels in ethambutol-induced Wistar rats, it has no discernible impact on the liver histopathology of ethambutol-induced Wistar rats. Bay leaf extract possesses antioxidant activity comparable to vitamin C.

### Keywords

Antioxidant, Histopathology, Malondialdehyde, *Syzygium polyanthum*.



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## INTRODUCTION

Tuberculosis (TB) is a serious infectious disease in the world. According to a 2015 report by the World Health Organization (WHO), tuberculosis is still the 10<sup>th</sup> leading cause of death globally. In Indonesia, the prevalence of TB in 2014 was 297 per 100,000 population. In 2014, 9.7 million new cases of TB were discovered, with approximately 480,000 people progressing to multi-drug resistant (MDR) TB (1). It is reported that only 161,365 people (82.8%) that experienced to cure rates for lung disease tuberculosis patients from 194.853 pulmonary tuberculosis patients in Indonesia and only 14,964 cases (7.7%) received complete treatment (2). Isoniazid (H), Rifampicin (R), Pyrazinamide (Z), Ethambutol (E) are the first-line standard therapy for TB. Long-term use of anti-tuberculosis therapy drugs, especially Isoniazid, Rifampicin and Ethambutol, tends to have side effects of hepatotoxicity, gastrointestinal disorders, neurological disorders, and hypersensitivity reactions. Impair liver function (hepatotoxicity) is one of the most serious and common side effects of anti-tuberculosis (3,4).

Based on previous study, states that bay leaf (*Syzygium polyanthum*) was utilized to treat of gout, gastritis, diarrhea, high cholesterol, type 2 diabetes mellitus and hypertension. Saponins, triterpenoids, flavonoids, polyphenols, alkaloids, tannins,

and essential oils made up of sesquiterpenes, lactones, and phenol are among the chemical compounds found in this leaf (5). Plants produce flavonoids, which are polyphenol secondary metabolites, in a variety of plant components, including fruit, seeds, leaves, stems, and flowers. Flavonoids are classified into eight classes as flavones, anthocyanidins, flava, flavanones, flavonolignans, isoflavones, isoflavones, and chalcones (6). According to another study, the active ingredients in Propolis from South Sulawesi are phenolic compounds like flavonoids which have been demonstrated to have hepatoprotective effects in mice. The results showed that the total polyphenol and flavonoid test of propolis contained 1.1% and 2.7%, respectively. In addition, the antioxidant activity test showed an IC<sub>50</sub> (The half maximal inhibitory concentration) value of 9849 ppm and Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) analysis supported phenolic compounds in propolis from South Sulawesi (7). According to a prior study, the phenolic extract ingredient in bay leaves has an antioxidant activity (8). We hypothesis that bay leaf extract significantly affects Malondialdehyde (MDA), antioxidant and histological characteristics of the liver. This study aimed to determine the effect of ethanol extract from bay leaf on antioxidant activity, MDA levels, and liver histopathology of ethambutol-induced Wistar rats.

## MATERIALS AND METHODS

### Preparation of 70% ethanol extract of bay leaves

The bay leaves dried for 7 days at room temperature, sorted, and mechanically ground into a coarse powder of *S. polyanthum*. A total of 1000 g of *S. polyanthum* powder were macerated in 7000 mL of ethanol 70% for 4 hours, then the supernatant was progressively filtered through filter cloth, cotton and Whatman paper no 1. A vacuum rotary evaporator was then used to evaporate the solution at a regulated temperature of 40°C while operating at a reduced pressure of 204 mbar. The extraction procedure was repeated three times after the remaining material was recovered.

### Determination of dosage

Previous research has used mice at doses of 1250mg/kg BW, 2500mg/kg BW, and 5000mg/kgBW to investigate the acute toxicity of bay leaf extract. No experimental animals perished from either the control group or the treatment group, according to the findings of observations made over the course of fourteen days of treatment. This suggests that test animals can still receive a single oral dose of an ethanolic extract of bay leaves up to the maximal dose (5000mg/kg BW), or nearly 200 times the usual human dose, without the animals dying (9). As a result, the maximum dose in this investigation was set at 300 mg/kg BW, or

10% of the acute toxicity limit. In this study, bay leaf ethanol extract was administered at doses of 75 mg/kg BW, 150 mg/kg BW, and 300 mg/kg BW.

### Treatment

The implementation of this study has been approved and issued a certificate of ethics from the Universitas Nahdlatul Ulama Surabaya ethics committee with the number 242/UC/KEPK/UNUSA/2020.

Wistar rats weighing about 200 gr were obtained from Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya. Rats were adapted for one week and fed regular animal pellets with water ad libitum. The animals were grouped into six groups. Each group consisting of 6 male rats. Group I (normal control): Rats were merely given food, water, and a placebo in place of any form of treatment for each mouse. Group II (positive control): Ethambutol was given to this group in combination with a dose of 50 mg/kg BW of rats. Group III: Ethambutol and antihepatotoxic medicines (silymarin) were given to this group in doses of 50 mg per kg BW of rats. Group IV: This group received 75 mg/kg of a 70% ethanol extract of bay leaf together with 75 mg/kg of ethambutol. Group V: 150 mg/kg of the 70% ethanol extract of *S. polyanthum* (EESP) and ethambutol were given to this group. Group VI: 300 mg/kg of the 70% ethanol extract of *S. polyanthum* (EESP) and ethambutol were given to this group.

### **Determination Malondialdehyde (MDA)**

Plasma MDA levels were measured according to the Wills Method (10). The 200  $\mu$ L plasma sample was added 1 mL 20% trichloro acetate (TCA) and 2 mL 0.67% thiobarbituric acid (TBA). The solution was well mixed until homogeneous and heated on a water bath for 10 minutes. After cooling the solution, centrifuge it at 3000 rpm (1 rpm = 1/60 Hz) for 10 minutes. The pink colored filtrate was measured at a wavelength of 532 nm using a UV-VIS spectrophotometer. MDA levels calculated using standard MDA curves with concentrations 0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 nmol/mL, respectively (11).

### **Liver Histopathology**

Wistar rats' liver tissue were dissected from the body and fixed in 10% formalin solutions, then dehydrated it in gradually ethanol solution in stages (50-100%). Tissue were cleaned with xylene and embedded in paraffin. Using a rotary microtome, sections were cut at a nominal thickness of 5–6  $\mu$ m and stained with hematoxylin and eosin to observe microscopic histopathological alterations and their severity. The microscopic observation was observed by a single observer, performed by an anatomy and histology lecturer from the medical faculty. The parameters of liver damage observed are cell degeneration consisting of hydropic generation, fat degeneration, necrosis, and congestion. They were scored

as follows: 1) if no damage was visible in the field of view magnification at 100x and 400x; 2) if the percentage of damage was visible at or below 10% in the field of view at 100x and 400x; and 3) if the percentage of visible damage was between 10% and 30% in the field of view at 100x and 400x (12).

## **RESULTS**

This research is an experimental study which uses experimental animals that are treated with Ethambutol as an analogue of tuberculosis patients receiving Ethambutol of the antioxidant activity of bay leaf extract (*S polianthum*) using the DPPH (2,2 diphenyl-1-picrylhydrazyl). The Scavenging Assay method was carried out at the Testing Service Unit of the Faculty of Pharmacy, Airlangga University. The antioxidant activity test of the bay leaf extract was carried out with a comparison of Vitamin C. The reading of the results using the spectrophotometric method showed that the bay leaf extract (*S polyanthum*) had antioxidant activity comparable to Vitamin C, with an  $IC_{50}$  of 11,4  $\mu$ g $\pm$  (4,4%).

The lower the  $IC_{50}$  value were indicated the greater the antioxidant activity in reducing free radicals. These findings suggest that bay leaf extract has a powerful, comparable to vitamin C ability to decrease free radicals. The antioxidant activity of polyphenolic compounds can be formed in the neutralization reaction of free radicals or

the termination of the chain reaction that takes place because flavonoids, which are polyphenolic compounds, have the capacity to donate hydrogen atoms to free radical molecules.

### Malondialdehyde (MDA) Test

IBM SPSS Statistics 25 are used to analyze data in this study. The One-Way ANOVA test was carried out and achieved a

significance value (Sig) of 0,002 (Table 2). The difference between the average MDA levels in each group is indicated by the significance value of 0.05. Table 1-4 showed the result for the data analyzed. According to the results of these experiments, giving the bay leaf extract to Wistar rats after they had been given ethambutol to induce MDA levels had a substantial impact.

**Table 1.** Test of Normality of *Malindialdehyde* (MDA)

		Shapiro-Wilk		
MDA Levels	Sample Group	Statistic	df	Sig.
	Group 1: Normal Control	0.935	4	0.625
	Group 2: Positive Control	0.963	6	0.840
	Group 3: Ethambutol + Silymarin	0.947	6	0.713
	Group 4: EESP 75 mg/kg	0.910	6	0.343
	Group 5: EESP 150 mg/kg	0.980	5	0.932
	Group 6: EESP 300 mg/kg	0.801	4	0.105

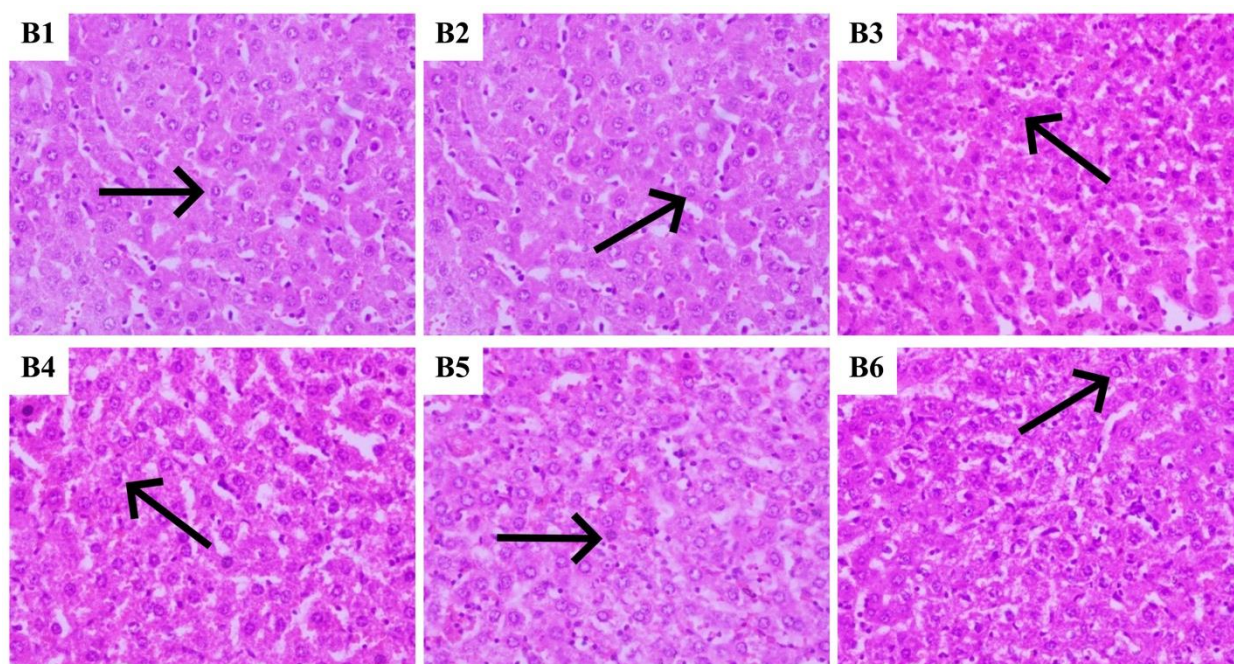
**Table 2.** One-Way ANOVA test of *Malindialdehyde* (MDA)

	Sum Of Squares	df	Mean Square	F	Sig.
Between Groups	1047221.735	5	209444.347	5.446	0.002
Within Groups	961546.200	25	38461.848		
Total	2008767.935	30			

### Histopathological Test of Wistar Rats

Histopathological feature showed no liver organ changes from all the groups. No abnormalities between hepatocyte among groups was observed. Statistical test was performed using the Kruskal Wallis method because the data were not normally distributed, then the Asymptotic significance 2-tailed (Asymp. Sig value) was 0.106. The

Asymp. Sig value >0,05 indicates that there is no significant difference in the liver histopathology of treated rats. This result indicated that the administration of the bay leaf extract did not have a significant effect on the liver histopathology of Wistar rats which are induced by ethambutol. There is no statistically detected difference between the groups in our study



**Figure 1.** Liver Histopathology of Wistar rats, hepatocytes are showed representatively by arrows in each figure; B1 (Normal control), B2 (Positive control), B3 (ethambutol + silymarin), B4 (EESP 75mg/kg), B5 (EESP 150mg), B6 (EESP 300mg).

**Table 3.** Tests of Normality Liver Histopathology of Wistar Rats

Histopatological Score	Shapiro-Wilk			
	Sample Group	Statistic	df	Sig.
	Group 1: Normal Control	0.630	4	0.001
	Group 2: Positive Control	0.822	6	0.091
	Group 3: Ethambutol + Silymarin	0.853	6	0.167
	Group 4: EESP 75 mg/kg	.	6	.
	Group 5: EESP 150 mg/kg	0.881	5	0.314
	Group 6: EESP 300 mg/kg	0.630	4	0.001

**Table 4.** Kruskal-Wallis Test of Wistar Rats Induced Ethambutol and Ethanol Extract of Bay Leaf

Histopathological Score	
Kruskal-Wallis H	9.085
df	5
Asymp. Sig	0.106

## DISCUSSION

### Antioxidant properties of bay leaf (*S polianthum*)

Indonesia is renowned for having high level of biodiversity, including a variety of

medicinal herbal plants. One of the plants that are often used in herbal medicine is bay leaf. One of the many functions of the active ingredients in bay leaves is as an antioxidant. Earlier studies utilizing the DPPH technique

demonstrated that the antioxidant activity of bay leaf extract was 68.55%. (13). The antioxidant activity of bay leaf extract demonstrates the variety of chemicals that contribute to the antioxidant process in preventing the formation of free radical (14). In this case, the form of chemicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) as free radical compounds. The findings of this study suggest that bay leaf extract has a robust, comparable to vitamin C ability to decrease free radicals. This is in consistent with the study conducted by Widyawaty et al., (15), which demonstrated that the antioxidant activity of the ethanolic extract of bay leaf had values of 12,65 ppm and 48,98 ppm by the DPPH and ABTS methods, respectively, and flavonoids and phenol totals of 15,666 QE/g and 206 GAE/g, respectively. With an IC<sub>50</sub> value under 50 ppm, it can be said that the ethanolic extract of *S. polyanthum* belongs to the very powerful antioxidant group.

### Malondialdehyde Levels

Research on oxidative stress and redox signaling have long used the measurement of MDA content as a lipid peroxidation marker, notably in those studies focusing on plant responses to abiotic and biotic challenges (16). The present study suggests that elevated serum levels of MDA and cortisol are strongly associated with major depressive disorder. The elevations of MDA and cortisol in serum level arise independently in the

blood and they may act as biomarkers for major depressive disorder (17). The significant value of 0,05 in this study demonstrates that there are differences in the average MDA levels between the groups. Based on the results of these experiments, it can be deduced that giving bay leaf extract to Wistar rats after they had been given ethambutol to generate MDA had a considerable impact on their levels of MDA. This finding was related to the possible antioxidant activity of bay leaf.

### Liver Histopathology

The first-line adjunctive anti-tuberculosis drug ethambutol is only used in conjunction with drugs like isoniazid and rifampicin. Ethambutol is nearly always combined with isoniazid, rifampicin, or other anti-tuberculosis medications, so it is impossible to estimate how frequently ethambutol alone will produce serum aminotransferase increases. The addition of ethambutol to isoniazid, rifampicin or Pyrazinamide does not appear to increase the rate of transient ALT elevations during therapy. In addition, ethambutol is a rare cause of acute, symptomatic liver damage as well (4). It has been demonstrated that the flavonoid components in bay leaves can inhibit the rise in IgG. The highest IgG inhibition was observed at 100 ppm, reaching 1.01 g/mL as opposed to the 57.19 g/mL of normal controls. Along with its flavonoid components, the bay leaf also contains a

chemical called eugenol, which has a good affinity of  $-6.3$  kcal/mol for inhibiting COVID-19 proteases (18). Additionally, high-nitric oxide macrophage production from bay leaf extract can boost the immune system (19,20). The liver histology of the treated rats in this investigation did not significantly differ from that of the control rats, indicating that the administration of bay leaf extract did not significantly affect the liver histopathology of Wistar rats induced by ethambutol as show in Figure 1. This outcome could be attributed to ethambutol's hepatotoxicity, which is not significant when administered without the addition of isoniazid or other anti-tuberculosis medications.

## CONCLUSIONS

The bay leaf extract has antioxidant activity comparable to vitamin C, has a

significant effect on MDA levels in ethambutol-induced Wistar rats, but does not have a significant effect on liver histopathology of ethambutol-induced Wistar rats. We suggest for the future study to add another anti-tuberculosis drugs that primarily caused hepatotoxic.

## AUTHOR CONTRIBUTIONS

All authors contributed equally to this work (conceived and designed the analysis; collected the data; contributed data or analysis tools; performed the analysis; wrote the paper).

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## CONFLICT OF INTEREST

There is no conflict of interest.

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