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Serum IL-10 levels in acute coronary syndrome Wistar mice model with Salam leaf extract (*Syzygium polyanthum*) administration

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Abstract
Coronary Heart Disease (CHD) has been one of the main causes of death in the world. *Syzygium polyanthum* or locally known bay leaf (*Daun salam*) is a plant that has been well known by the community. At present, *S. polyanthum* leaves are reported to have anti-inflammatory, antioxidant, antidiabetic, antimicrobial, antihypertensive, antitumor, antidiarrheal, acetyl cholinesterase inhibition, and lipase inhibitory activity. This study was to prove the effect of bay leaf extract (*Syzygium polyanthum*) on serum interleukin (IL)-10 levels in the heart of Wistar Strain Mice Model with Acute Coronary Syndrome (ACS). This is an experimental study to compare the IL-10 level in ACS mice with and without bay leaf extract administration, on day 1, 4, 7, and 14. The result showed that the treatment group has the higher levels of IL-10, which shows the potential advantages of bay leaf extract administration in patients with ACS.

Keywords: Acute coronary syndrome, bay leaf, interleukin-10, *Syzygium polyanthum*

1. Introduction
Coronary heart disease (CHD) is one of the main causes of death in developed and developing countries, including Indonesia. The disease progresses over the years, especially when diagnostic tools become available [1]. *Riset Kesehatan Dasar* (Riskesdas) 2018 in Indonesia showed that the prevalence of cardiovascular disease increases with age [2].
IL-10 is an immunoregulatory cytokine produced by numerous cells, including activated monocytes, macrophages, and lymphocytes. In animal studies, IL-10 has anti-inflammatory properties and protective role, both in the formation and stability in atherosclerotic lesions. A study conducted in ACS patients suggests that IL-10 biological action demonstrates a protective role both in atherogenesis and plaque susceptibility [3]. Elevated IL-10 levels at baseline were a strong and independent predictor of reduced risk of death and myocardial infarction at 6 months of follow-up [4].
S. polyanthum, known as bay leaf (*Daun salam*) also known as *salam* in Java, Madura, and Sunda, *kastolam* in Kangean and Sumenep, *manting* in Java, and *meselengan* in Sumatra [5]. It is well known as spice or flavoring because of its distinctive aroma. Bay leaves are also used for alternative medicine because these plants are easily obtained and considered efficacious.
Kusuma *et al.* found carbohydrates, tannins, alkaloids, steroids, triterpenoids, and flavonoids, while mature fruits contain saponins, carbohydrates, tannins, alkaloids, triterpenoids, and flavonoids in the raw leaves and fruits of *S. polyanthum* [6].
S. polyanthum leaves are reported to have anti-inflammatory, antioxidant, antidiabetic, antimicrobial, antihypertensive, antitumor, antidiarrheal, acetylcholinesterase inhibition, and lipase inhibitory activity [7]. Hasan *et al.*, showed a decrease in levels of C-reactive protein and Myeloperoxidase showed that *S. polyanthum* leaf extract may decrease the inflammation of the heart caused by myocardial infarction [8]. They also showed that there were significant increases in IL-10 level since day 1 in ACS model mice having *S. polyanthum* leaf extract [8].
The purpose of this study was to analyze the serum IL-10 levels in acute coronary syndrome Wistar mice with the administration of Salam leaf extract (*Syzygium polyanthum*)

2. Materials and Methods
This was a posttest-only control group design experimental study. IL-10 levels was measured through plasma. Due to ethical reasons, this study used an experimental animal model of ACS to determine the effect of giving bay leaf extract (*S. Polyanthum*). The Wistar strain white rat (*Rattus norvergicus*, sp) was used in this study. The mice were then grouped into a control and an experimental group.

The control group was provided because of the high mortality risk of ACS model used and to reduce treatment bias on the same subject. The research was conducted at the Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Brawijaya, Malang during November 2019 - February 2020.

The number of samples for each treatment group is 4 samples. The procedure in this study was similar with the previous study by Hasan *et al.* [8]. First, the bay leaves were gathered from one location only. Then, they were washed and dried naturally under direct sunlight or aerated and were stored in a suitable container. By means of maceration, the leaf ethanol extract was prepared by weighing 300 g of simplified bay leaf powder, then put in a closed container (macerator), adding 3 liters of 70 percent ethanol. For the first 6 hours, it will then need to be stirred and allowed to stand for 18 hours, stirred periodically. It was subsequently filtered with cotton and filter paper, and the filter (macerate I) was collected. The waste obtained was returned to the container of the macerator, and then 70% ethanol was applied up to half of the volume of extraction I. Then by soaking, stirring, settling and filtering to obtain macerate II, the maceration process was repeated. The next phase was to combine macerate I and macerate II, then steam the macerate at a temperature of 40°C using a Rota vapor tool, until a dense extract was obtained. In a plastic pot, inject the dense extract and then mark it. The extract was dissolved into a suspension solution of 0.5 percent carboxyl methyl cellulose sodium (CMC-Na) and administered (using a syringe) through a nasogastric tube. The same volume of normal saline solution was given through a nasogastric tube (using a syringe) to the control group.

The sample will be randomly divided into 8 selected groups (4 as control groups and 4 others as treatment groups, and will be examined on day 1, 4, 7, and 14). The first group did not receive any intervention and was examined for the serum IL-10 on day 1. The second group received *S. polyanthum* leaf extract and was examined for the serum IL-10 on day 2. The third group did not receive any intervention and was examined for the serum IL-10 on day 4. The fourth group received *S. polyanthum* leaf extract and was examined for the serum IL-10 on day 4. The fifth group did not receive any intervention and was examined for the serum IL-10 on day 7. The sixth group did not receive any intervention and was examined for the serum IL-10 on day 14. The last group received *S. polyanthum* leaf extract and was examined for the serum IL-10 on day 14. The serum IL-10 levels were examined from blood samples using the enzyme-linked immunosorbent assay (ELISA) method.

The ANOVA was used to find significant mean differences between groups of samples, with p value that was considered significant was <0.05. Variable which was significant will be analyzed using post-hoc test.

3. Results and Discussion

These were 32 mice which were divided into 8 groups. Serum IL-10 levels were collected and analyzed according to the corresponding group. We conducted Lavene's test of equality of error variance, resulted in 0.138 significance value which indicates that the data were eligible to one-way ANOVA test. Details were presented in table 1.

There is an increase in IL-10 levels along with the duration of examination following the onset of ACS induction (Table 1). The highest average IL-10 levels was found in both groups on the 14th day. This study also showed that the treatment group had a higher IL-10 value compared to the control group. One-way ANOVA statistical test results yield a p-value <0.001 which shows the statistical significance of this result.

Table 1: The effect of *S. polyanthum* extract on serum IL-10

Group	Serum IL-10 (ng/mL) (mean ± SD)	p
Control		<0.001*
Day 1	5.00±1.73	
Day 4	7.45±0.57	
Day 7	8.67±2.78	
Day 14	11.94±4.03	
Treatment		
Day 1	12.34±3.65	
Day 4	19.32±3.12	
Day 7	31.93±9.38	
Day 14	33.63±4.82	

*one-way ANOVA

Following the significant result of the one-way ANOVA, we conducted a post-hoc test using Turkey HSD method to determine which of the groups has significant difference. The result was showed in Table 2.

Table 2: Post-hoc analysis between control and treatment group (p-value)

	Treatment Group			
Control Group	**Day 1**	**Day 4**	**Day 7**	**Day 14**
Day 1	0.51	0.02*	0.00*	0.00*
Day 4	0.87	0.07	0.00*	0.00*
Day 7	0.96	0.13	0.00*	0.00*
Day 14	1.00	0.50	0.01*	0.00*

* Value < 0.05 considered significant

Post-hoc analysis showed that most of the differences between control and treatment group were significant at day 7 and day 14, suggesting that the effect of the treatment began to appear at day 7.

Coronary atherosclerosis, also referred to as coronary cardiovascular disease, is caused by myocardial ischaemia, hypoxia, and necrosis due to stenosis or blocked arteries lumen secondary to artery atherosclerosis. Myocardial infarct is the commonest kind of cardiopathy. Many researchers hypothesize that the immune and inflammatory response is a very important reason for coronary heart condition. The precise mechanism of action of IL-10 in atherosclerotic disorders and plaque formation has not been fully understood. Potentially, this mechanism can involve inflammation down regulation, coagulation and matrix degradation. Additionally, IL-10 has anti-apoptotic properties in various cell types, and it's been suggested that IL-10 can modulate plaque stability, a minimum of partly, through anti-apoptotic mechanisms [3, 9-10]. This study shows that there was a significant increase in IL-10 levels within the ACS model with *S. polyanthum* leaf extract therapy by using ELISA method. Previous study showed that *S. polyanthum* leaves contain steroid compounds, phenolics, saponins, flavonoids and alkaloids [11, 12]. The main compound in *S. polyanthum* leaves is flavonoids. Flavonoids are polyphenol compounds with anti-inflammatory, anti-platelet, anti-cholesterol, anti-diabetes and anti-oxidant properties. Flavonoids in the *S. polyanthum* are quercetin and fluoretin [12, 13]. In the presence of steroids, IL-10 will be regulated positively by macrophages. This shows that flavonoid compounds and steroids in *S. polyanthum* leaves can increase IL-10 levels.

Previous studies on *S. polyanthum* leaf extract have been reported to have other cardiovascular benefits. Previous

studies by Ismail *et al.* [14] reported that water and methanol extracts of *S. polyanthum* leaves significantly lowered blood pressure in normal Wistar-Kyoto (WKY) mice and spontaneous hypertension by administering the extract intravenously. Ismail *et al.* [15] analyzed the effects of oral *S. polyanthum* leaf extract to WKY rats and conscious spontaneous hypertension, which showed that the administration of oral *S. polyanthum* leaf extract significantly reduces blood pressure in rats with spontaneous hypertension, but not in normal mice [15]. Regarding the mechanism of antihypertensive effect, studies have shown the possibility antihypertensive effects of *S. polyanthum* leaf extract by vasodilatation [16] and by inhibiting angiotensin-converting enzyme (ACE) [17].

In the association to the formation of plaque, Prahastuti *et al.* [13] reported that there was no significant difference with simvastatin administration, but not of *S. polyanthum* leaf extract, may reduce blood cholesterol levels in the dyslipidemic rats model. Scchitano *et al.* [18] concluded that various phytochemicals such as carotenoids, flavonoid polyphenols and non-flavonoids, have been reported to have a role in preventing dyslipidemia through various mechanisms, which will ultimately prevent atherosclerosis in their systematic study. *S. polyanthum* leaf extract can decrease in levels of CRP and MPO, which also means lowers the heart inflammation caused by myocardial infarction in mouse models [8].

4. Conclusions

This study shows the potential benefits of administration of bay leaves in patients with ACS. Prior studies of the advantages of giving *S. polyanthum* leaf extract, shows that *S. polyanthum* leaf extract administration has quite extensive benefits in the circulatory system. *S. polyanthum* leaves have broad therapeutic potential, but further research is required to verify further findings, and examine the side effects and toxicity of *S. polyanthum* leaf extracts before they can be used clinically. In conclusion, there was a significant difference of serum IL-10 between Wistar Strain Mice Model of ACS that was given *S. polyanthum* leaf extracts. The groups given *S. polyanthum* leaf extracts have the higher level of the IL-10 serum.

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