

# Immunomodulatory Effect of Water Extract of *Stachytarpheta jamaicensis* (L.) Vahl

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## ABSTRACT

*Stachytarpheta jamaicensis* (L.) Vahl traditionally is used to treat several diseases, such as urinary tract infections, rheumatic, and pharyngitis. The objective of this study was to test immunomodulatory effect of water extract of *S. jamaicensis* (L.) Vahl. herbs. Immunomodulatory assay of water extract of *S. jamaicensis* herbs have been examined in male Balb/c mice using carbon clearance assay. The doses of the extract were 62.5, 125, and 250 mg/kg bw. Parameter of immunomodulatory effect was phagocytic indexes after seven days administration of the extract. The result showed that water extract of *S. jamaicensis* at doses of 62.5 and 250 mg/kg bw had lower phagocytic index compared to Zymosan A, but higher phagocytic indexes compared to Methylprednisolone. The water extract of *S. jamaicensis* may be considered as an immunosuppressant agent.

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## INTRODUCTION

Immunomodulators are substances that help to correct immune systems, and it divides into immunostimulant and immunosuppressant. Immunosuppressants are the agents which suppress the immune system and are used for the control of pathological immune response in autoimmune disease, graft rejection etc. Immunostimulants are the agent which enhance body's resistance against infections; they enhance the basal levels of immune response, and in individuals with impairment of immune response as immunotherapeutic agent (Patil, 2012).

Several medicinal plants had been proved for their immunomodulatory effect, such as *Allium sativum*, *Aloe vera*, *Andrographis paniculata*, *Azadirachta indica*, *Curcuma longa*, *Carica papaya*, *Plantago major*, *Tinospora cordifolia*, etc (Mukherjee, 2014). Another plant that had been reported to have immunostimulatory action was *Stachytarpheta cayennensis* C. Rich. The study indicated that methanol extract of

*S. cayennensis* possesses immunostimulatory action with significant synergistic effects with artesunate, and can therefore, offer immune boosting activities in disorders of immune suppression (Okoye, 2014). Other alternative of *Stachytarpheta* family that had anti-inflammatory effect was *Stachytarpheta jamaicensis* (L.) Vahl (Sulaiman, 2007).

*S. jamaicensis* is an annual herb, traditionally used to treat urinary tract infections, rheumatic, pharyngitis, blood purifier, irregular menstruation, vaginal discharge, hepatitis A in Indonesia. It also uses as antilithiasis and diuretic. The parts of *S. jamaicensis* that can be used are leaves, flowers, and roots (Dalimartha, 2000). The objective of this study is to test immunomodulatory effect of water extract of *Stachytarpheta jamaicensis* (L.) Vahl. herbs.

## MATERIAL AND METHODS

### Plant collection and determination

The herbs of *S. jamaicensis* were collected from Lembang, West Java, Indonesia and identification of the plant was done by School of Life Science and Technology, Institut Teknologi Bandung.

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### Phytochemical analysis

Phytochemical analysis of the herbs and water extract of *S. jamaicensis* was done to evaluate the presence of alkaloids, tannins, saponins, flavonoids, steroid, and terpenoid.

### Animal and Immunomodulatory Assay

Immunomodulatory assay of water extract of *S. jamaicensis* herbs have been examined in male Balb/c mice using carbon clearance assay (Wagner, 1991). All animal experiments were conducted under institutional ethical guidelines.

The animals (male Balb/c mice) were obtained from Biosciences and biotechnology research center, Institut Teknologi Bandung, Bandung, Indonesia and housed under standard laboratory conditions. The animals fed with standard mice feed and water *ad libitum*.

The animals were divided into six groups (four in each).

Group I : received aquadest orally at a dose of 50 ml/kg bw.

Group II : received Methylprednisolone intra peritoneally at dose of 1.4 mg/kg bw i.p.

Group III : received Zymosan A intra peritoneally at dose of 10 mg/kg bw i.p

Group IV : received ethanol extract of *S. jamaicensis* orally at a dose of 62.5 mg/kg bw.

Group V : received ethanol extract of *S. jamaicensis* orally at a dose of 125 mg/kg bw.

Group VI : received ethanol extract of *S. jamaicensis* orally at a dose of 250 mg/kg bw.

The animals were given the substance for 7 days. In the 8th day, prior the experiment, the animals were fasted for 4 hours except water was given *ad libitum*. Then blood samples (20  $\mu$ L) were taken and the hematology profile were evaluated using hematology analyzer (Melet Schloessing®). After 2 hours, each mouse receives an intravenous injection of 0.2 mL per 20 g of an Chinese ink (Yamura 233, black, Indonesia) dispersion (1.6 mL ink diluted in 8.4 mL 1% gelatin-salt solution). Blood samples (20  $\mu$ L) were taken at intervals of approximately 3, 6, 9, 12, and 15 minutes from the retro-orbital plexus and was soluted in 4 mL 1% acetic acid.

The absorbance of each solution was measured spectrophotometrically at 650 nm wavelength. Density reading, plotted against time give the regression line. Clearance value (Cv) is obtained as the ratio of the slope regression line of sample to the slope regression line of control. Carbon clearance assays correlated with phagocytic indexes (PI) (Wagner, 1991)

$$Cv = PI = \frac{\text{Slope of the regression line of sample}}{\text{Slope of regression line of control}}$$

At the end of the experiment, all animals were sacrificed and indexes of spleen were calculated. To assess histological profile,

the lymph of each group were paraffin embedded and prepared using microtome. They were double stained with hematoxylin and eosin and were observed by light microscope.

### Statistical analysis

All the values expressed are Mean  $\pm$  S.E.M. Statistical evaluations were performed by Student's t-test at the 95% confidence level using an SPSS program for Windows 17.0. Values of  $p < 0.05$  were considered significant.

## RESULT AND DISCUSSION

### Phytochemical analysis

Phytochemical analysis of herbs and the water extract *S. jamaicensis* showed alkaloids, flavonoids, saponin, triterpene, monoterpene and sesquiterpene as shown in Table 1.

**Table 1:** Phytochemical analysis of herbs and water extract of *S. jamaicensis*.

Parameter	Result	
	Herbs	Water extract
Alkaloid	+	+
Tannin	-	-
Saponins	+	+
Flavonoids	+	+
Steroid	+	+
Triterpene	+	+
Monoterpene and sesquiterpene	+	+

(+) = present; (-) = absent

### Immunomodulatory Assay

Carbon clearance assay based on the rate of removal of injected colloidal carbon particles from the bloodstream. When colloidal carbon is injected intravenously, the carbon is removed by the sessile intravascular phagocytes in the liver and spleen. It would describe reticuloendothelial phagocytic activity. The Kupffer cells of the liver take up approximately 90% and the splenic macrophages 10% (Wagner, 1991).

In this study, we used two comparator, zymosan and methylprednisolone, to evaluate immunomodulatory effect of water extract *S. jamaicensis*. Zymosan is composed of beta-glucans, mannans, chitins, and it acts as immunostimulant by activate several macrophages receptors, including TLR2, dectin-1, the mannose receptor, and CD11b/CD18 (complement receptor 3). Zymosan also synergistically activates inflammatory pathways leading to TNF alfa production and an oxidative burst (Du *et al.*, 2006). Methylprednisolone in smaller doses has effect as immunosuppressive and anti-inflammatory by limiting cytokine production. Glucocorticoids suppress the cell-mediated immunity. They act by inhibiting genes that code for the cytokines IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8 and TNF- $\gamma$ , the most important of which is the IL-2. Smaller cytokine production reduces the T cell proliferation. Glucocorticoids also suppress the humoral immunity, causing B cells to express smaller amounts of IL-2 and of IL-2 receptors. This diminishes both B cell clone expansion and antibody synthesis (Rathee, 2012).

### Growth pattern

Water extract of *S. jamaicensis* did not cause changes on growth pattern of the assay animals. Figure 1 showed that body weight of all dose was not significantly different compared to control group ( $p>0.05$ ).

### Phagocytosis indexes

As stated in the above, clearance value (Cv) was obtained as the ratio of the slope regression line of sample to the slope regression line of control and it correlated with phagocytic indexes (PI) (Wagner, 1991) Density reading of each group is shown in Table 2 and phagocytic indexes is shown in Figure 2.

Water extract of *S. jamaicensis* at dose of 62.5 and 250 mg/kg bw had lower phagocytic index compared to Zymosan A as immunostimulant, but higher phagocytic indexes compared to Methylprednisolone as immunosuppressant. It showed that water extract of *S. jamaicensis* had probability as immunosuppressant.

### White blood cell differentiant's profile

The effect of water extract of *S. jamaicensis* on white blood cell's differentiation profile could be seen in Table 3. WBC

differentiate that can be count were lymphocytes, mid-sized (including monocytes, basophils, eosinophils), and granulocyte. The result showed that water extract of *S. jamaicensis* could reduce total amount of midsized cell, and granulocyte, eventhough it was not significantly different compared to control group ( $p>0.05$ ). Water extract of *S. jamaicensis* increased amount of lymphocyte and was not significantly different compared to control group ( $p>0.05$ ). After the animal received water extract of *S. jamaicensis* for seven days, it showed that water extract could reduce total amount of midsized cell (including monocytes, basophils, eosinophils), and granulocyte, but increased the amount of lymphocyte. Basophil play an important part in allergic inflammation. Bashophil is also one of the major players in the IgG-but not IgE-mediated systemic anaphylaxis. The depletion of basophils protects experimental animal from death due to anaphylactic shock (Mukai, 2009).

### Spleen indexes

The effect of water extract of *S. jamaicensis* on spleen indexes could be seen in Figure 3. The result showed that water extract of *S. jamaicensis* had no effect on indexes of the spleen ( $p>0.05$ ).

**Table 2:** Effect of water extract of *S. jamaicensis* on carbon clearance rate.

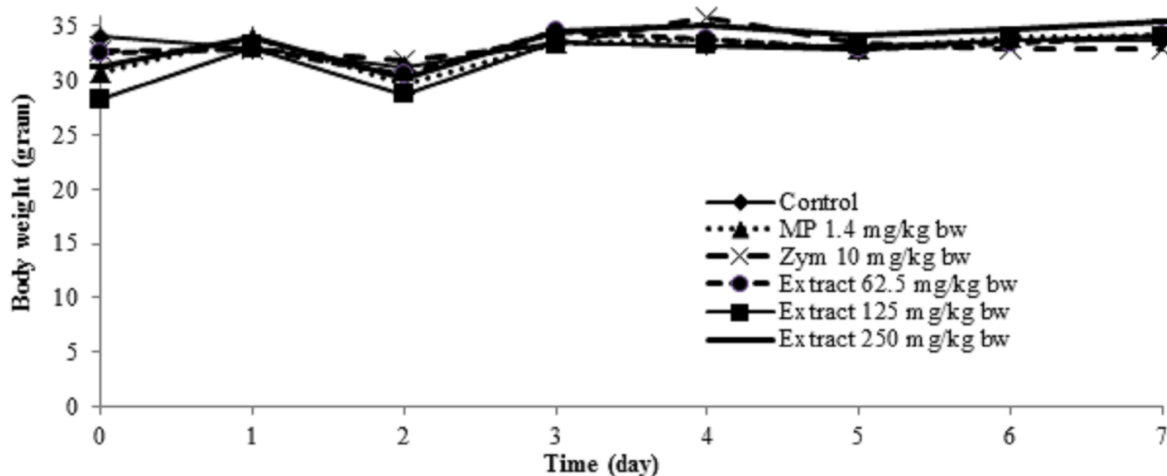
Group	Amount of carbon in the blood (%Transmittance) after time post injectin (minutes)					Slope regression line
	3	6	9	12	15	
Control	44.52±13.14	38.09±2.52	38.82±2.52	37.17±4.25	36.76±5.02	-0.2876
Methylprednisolone 1.4 mg/kg bw	40.12±7.87	42.71±7.18	42.76±9.24	41.45±9.65	44.18±8.41	-0.1310
Zymosan 10 mg/kg bw	36.14±3.96	33.20±5.03	37.51±1.42	34.53±3.29	31.81±5.41	-0.3234
Water extract of <i>S. jamaicensis</i> 62.5 mg/kg bw	35.71±9.12	41.39±9.67	38.13±8.04	39.81±9.88	36.50±5.58	-0.2172
Water extract of <i>S. jamaicensis</i> 125 mg/kg bw	40.49±13.21	41.24±12.37	41.76±10.32	44.15±11.53	39.12±7.25	-0.1068
Water extract of <i>S. jamaicensis</i> 250 mg/kg bw	40.31±13.37	43.19±10.61	42.68±10.32	42.86±9.14	38.35±7.40	-0.1984

n=4; \* $p<0.05$ , compared to control group using t-test

**Table 3:** The effect of water extract of *S. jamaicensis* on haematological profile.

Group	Parameter of haematology		
	Lymphocyte	Mid Sized Cell	Granulocyte
Control	11.15±1.77	4.11±1.43	3.68±3.66
Methylprednisolone 1.4 mg/kg bw	10.76±3.07	3.08±1.57	2.13±1.37
Zymosan 10 mg/kg bw	12.28±4.22	3.65±1.31	1.27±0.56
Water extract of <i>S. jamaicensis</i> 62.5 mg/kg bw	13.89±1.49	3.82±0.98	1.28±0.85
Water extract of <i>S. jamaicensis</i> 125 mg/kg bw	13.25±4.05	3.28±1.32	1.67±1.39
Water extract of <i>S. jamaicensis</i> 250 mg/kg bw	13.71±2.88	2.88±0.58	0.97±0.18

n=4; \* $p<0.05$ , compared to control group using t-test



**Fig. 1:** The effect of water extract of *S. jamaicensis* on growth pattern.

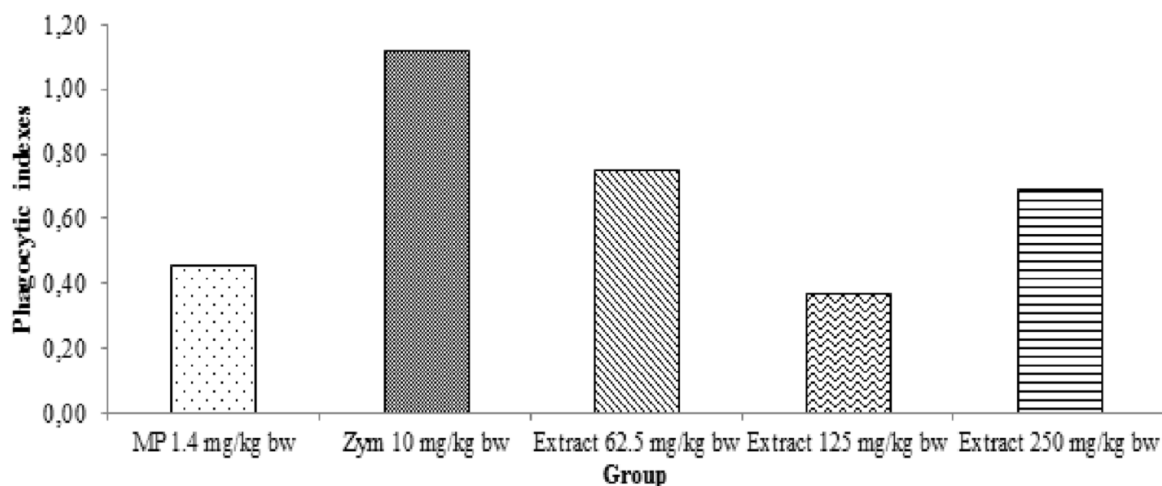


Fig. 2: Effect of water extract of *S. jamaicensis* phagocytic activity.

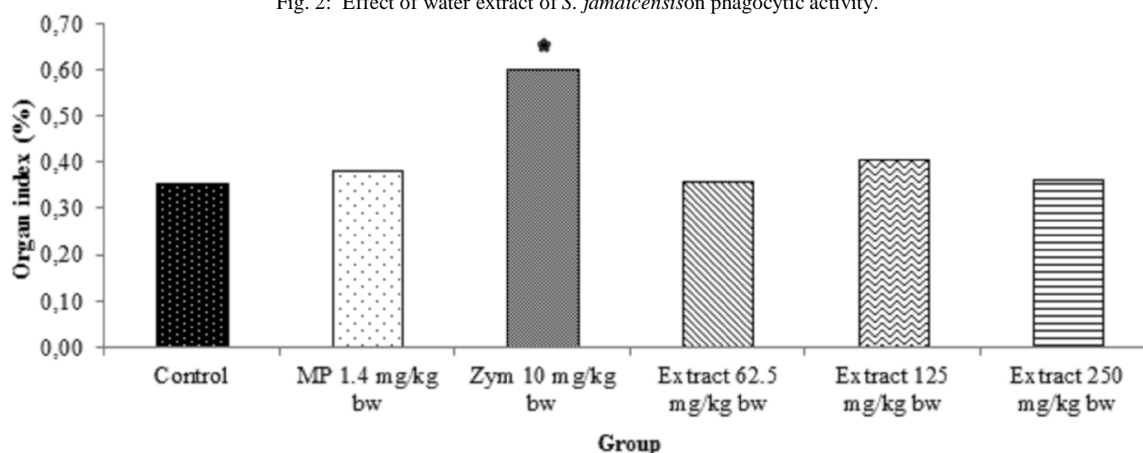


Fig. 3: Effect of water extract of *S. jamaicensis* indexes of the spleen. n=4; \*p<0.05, compared to control group using t-test

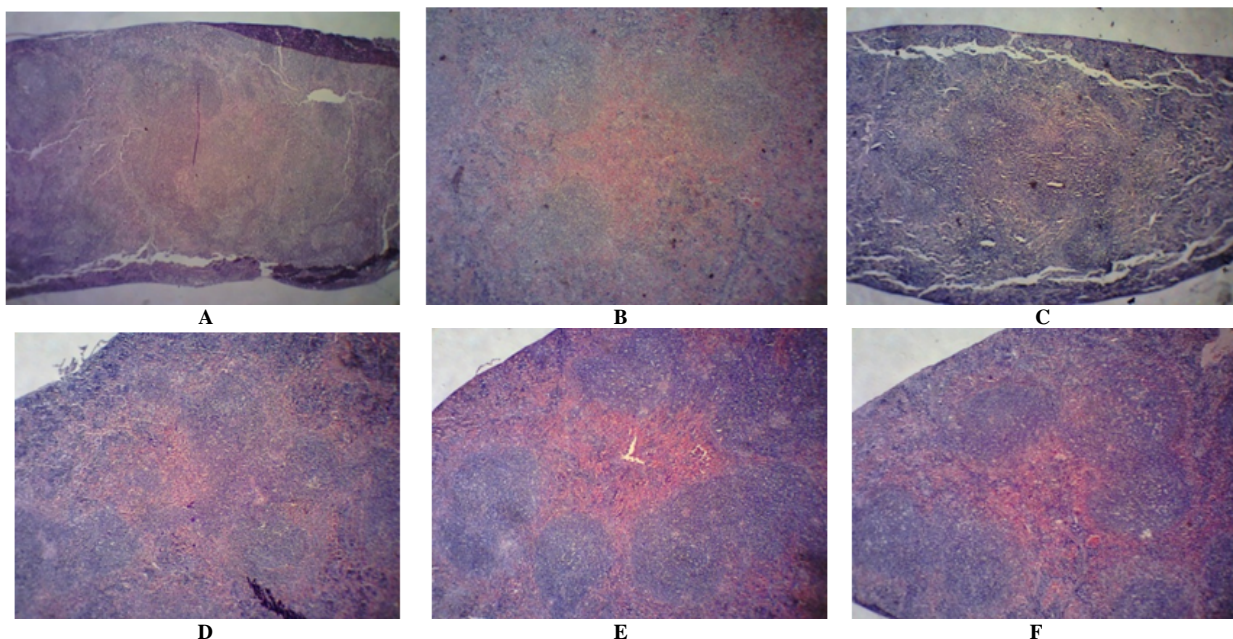
### Histology of the spleen

The effect of water extract of *S. jamaicensis* on histology of the spleen could be seen in Figure 4. Histology spleen of the animal whom received water extract of *S. jamaicensis* showed that it had near similarity with methylprednisolone rather than Zymosan.

The result of carbon clearance assay showed that water extract of *S. jamaicensis* had probability as immunosuppressant. Phagocytosis indexes of water extract of *S. jamaicensis* had similarity with methylprednisolone rather than Zymosan. It was also supported by spleen indexes and histology of the spleen. Histology spleen of the animal whom received water extract of *S. jamaicensis* showed that it had near similarity with methylprednisolone rather than Zymosan. Immunosuppressant effect of ethanol extract of *S. jamaicensis* is shown by its anti-inflammatory effect. It suggests that the extract can inhibit arachidonate COX and had ability to reduce proliferation of macrophages, neutrophils, fibroblast and multiplication of small blood vessels in both acute and chronic models of inflammation (Sulaiman, 2009). Idu *et al* (2006) had been evaluate acute hypotensive effect of the aqueous extract of the leaves of *S. jamaicensis* which it may be partly due to the negative chronotropic effect or to a direct effect on vascular smooth muscle

(Idu, 2006). Besides, anti-inflammatory/immunosuppressive cytokines could inhibit endothelial dysfunction because it protect against the impairment of endothelial function by counteracting the effects of pro-inflammatory cytokines, such as interleukin (IL)-35, and suppressing oxidative stress (Shao, 2014).

As described above, water extract of *S. jamaicensis* had alkaloids, flavonoids, saponnin, triterpene, monoterpene and sesquiterpene. *Stachytarpheta jamaicensis* (L.) Vahl leaf extract due to the flavonoid content had the ability to reduce the level of TNF- $\alpha$  as inflammation agent by inhibit the metabolism of prostaglandins in the cyclooxygenase pathway (Kharisma, 2012) Effect of antiinflammatory from *Stachytarpheta jamaicensis* (L.) Vahl could be because it has lanostane triterpenoids and steroidal glycoside (Okwu, 2009; Okwu, 2010). Lanostane triterpenoids is a potent drug with the ability to block the release of the enzymes responsible for pains and inflammation (Okwu, 2009), while steroid glycosides also had oxytocic and antioxidant functions and could be use in the treatment of rheumatoid arthritis (Okwu, 2010). The *in vitro* effects of ethyl acetate extracts of *S. jamaicensis* leaves showed that the extract significantly inhibited the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the respiratory burst of rat peritoneal macrophages (Alvarez, 2004).



**Fig. 4:** Histology of the spleen (a) Control (b) Methylprednisolone 1,4 mg/kg bw (c) Zymosan 10 mg/kg bw (d) Water extract of *S. jamaicensis* 62.5 mg/kg bw (e) Water extract of *S. jamaicensis* 125 mg/kg bw (f) Water extract of *S. jamaicensis* 250 mg/kg bw

## CONCLUSION

The study showed that water extract of *S. jamaicensis* had ability as immunosuppressant, but the mechanism should be determine further.

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