



# The Role of Herbal Medicine in Suppressing the Incidence of Typhoid Fever in Indonesia

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**Abstract.** Typhoid fever is one of the common diseases in developing countries. This is attributable to *Salmonella typhi* bacteria. The water from the unripe *Manila sapodilla* was often to be given as a drink on typhoid fever patients. Indonesia is a country that is rich in natural ingredients that can be used as medicine. Currently we are still faced with the problem of typhoid fever that never goes away in Indonesia. Many people in some areas have turned to herbal treatment using available natural ingredients to treat typhoid fever, one of which is to consume sapodilla manila fruit which is strong and effective enough to cure typhoid fever quickly. The method used in this study was to extract the manila sapodilla fruit with the maceration method which was then tested *in vitro* and *in vivo* to determine the effectiveness of the manila sapodilla fruit in reducing salmonella typhi colonies that cause typhoid fever. This study was an murine-based *in vivo* approach where our mice were divided into four groups: two treatment groups, and negative and positive references. *Manila sapodilla* fruit extract was used to reduce *Salmonella typhi* colonies that cause typhoid fever. Our results showed that there was a significant decrease in *Salmonella typhi* bacteria that was treated with *Manila sapodilla* extract at either dose of 510 mg and 750 mg. This suggested that sapodilla manila extract play a role in weakening *Salmonella typhi* colonies. Hence, this was deemed as a potential anti typhoid fever as a substitute for the use of antibiotics.

**Keywords:** manila fruit · typhoid fever · TNF- $\alpha$

## 1 Introduction

Tropical infectious disease, namely typhoid fever or better known as typhus disease in Indonesian, is a disease caused by the *Salmonella typhi* bacteria, the *Salmonella typhi*

bacteria enters through food contaminated by flies' feet, cockroaches' feet, rat's feet where the animal's feet have been infected. Carrying the typhoid fever bacteria and the animal is called an effector or carrier of bacteria the bacteria that were carried earlier and contaminated by food will then be eaten by the patient where some of the bacteria will die in the stomach and some will get to the intestines and in this intestine these bacteria will then multiply and cause intestinal infections [1, 2]. After the bacteria have reproduced, it will cause inflammation in the intestines which will lead to the implementation of fever in patients who we know as typhoid fever [3, 4].

Typhoid fever caused by *Salmonella typhi* bacteria is almost evenly distributed in all developing countries in the world where the incidence continues to increase, especially during the transition season, it is also still being investigated why this happened for the incident in Indonesia itself, this typhoid fever is very complained of in the world. Some provinces that are still known for their water sanitation are not clean. Because the spread of typhoid fever is quite significant in Indonesia, research is needed to be able to break the chain of transmission and spread of this typhoid fever so that in the future people can be more careful and not be infected by this *Salmonella typhi* bacteria [5–7]. Until now, the incidence of typhoid fever cases is still quite high, found in both men and women in hospitals and primary clinics. Typhoid fever still requires antibiotic therapy while we know that giving antibiotics causes a lot of resistance and also allergies in patients [3, 8, 9].

This research is one of the studies that will reveal how much herbal treatment is needed, one of which is Manila sapodilla fruit to replace the use of antibiotics in dealing with typhoid fever which is quite high, the case is mainly in Indonesia where the Manila sapodilla fruit used is the thick Manila sapodilla fruit, namely which is about three to four months old and this fruit is a whole fruit that is not deformed and also not affected by pests where the size of the selected fruit is a fruit that has a diameter of 3.5 cm and the fruit is light brown and has no defects on the outer skin of the fruit so that fruit allows for the same size and also homogenized with other fruits which we will then proceed to the process of making extracts [10–12]. The petals are usually arranged in two circles; single leaves, located alternately, often clumping at the ends of twigs. Flat-edged, slightly hairy, shiny dark green, oval to slightly lanceolate,  $1.5\text{--}7 \times 3.5\text{--}15$  cm, base and tip wedge-shaped, stem 1–3.5 cm, leaf bone protruding on the underside. Low branched, sapodilla trunk with rough skin blackish gray to dark brown [13–15]. All parts contain latex, a thick milky white sap. The benefits of sapodilla sapodilla fruit have been widely known empirically in the community, namely the raw fruit is used for the treatment of typhoid fever by washing/cleaning the raw fruit, then the fruit is shredded and the results of the grated are squeezed using fine leaves and the filter results are drunk on typhoid fever sufferers [16]. Ripe fruit can be used as an ingredient in syrup or if it is fermented it can be made into wine or vinegar. This tree of sapodilla manila can be used as a protected tree in front of the yard and its fruit so that its leaves can be used for treatment.

The tree itself can be an ornamental plant or medicinal plant that can be used by the community. Manila sapodilla has long been used by the people of Indonesia as an alternative treatment for typhoid fever, by grating the fruit and squeezing the water contained therein using a soft cloth. This treatment has been shown to be effective in curing typhoid fever patients [17].

## 2 Materials and Methods

### 2.1 Animal Preparation and Acclimatization

This study was a pure experimental study (True-Experimental Design) using a completely randomized design (CRD) conducted in the laboratory. The design of this study used a randomized design with Matching Pretest design - Posttest Comparison Group Design. The subjects were 10–14 weeks-male Balb/c mice with weights ranged from 30–40 g. All conducts were adhered to the standardized guideline by the WHO. The mice were acclimatized in a light-dark circle per 12 h for a week.

### 2.2 Manila Sapodilla Extract

The fruit of Manila sapodilla were macerated then submerged into 96% ethanol for 24 h. The ethanol was then pulled out of the fruit through vacuum filtration. Typically, 15 kg Manilla sapodilla produced 150 thick dark brown extract referred to as Rizqan. We treated our mice with 510 mg and 750 mg of the rizqan to two separate groups.

### 2.3 Manila Sapodilla Extract

The PCR was carried out by doubling DNA at 94°C for 3 min, the cycle was repeated 38 times at 54°C (30 s). Detecting the GAPDH gene using a forward/sense primer: AGAGGGAAATCGTGCGTGAC. The PCR protocol was performed by doubling DNA at 94°C for 10 min, the cycle was repeated 32 times at 54°C (30 s). And the primary reverse/antisense: CAATAGTGATGACCTGGCCGT according to Tomomi Yajima's protocol. QRT-PCR using the Green QRT-PCR master mix kit, one stage. This protocol is optimized for Mx4000 instruments. Passive dye reference was included in the reaction, diluted 1:500. Solutions containing dyes are kept away from light. Dilute 2 x SYBR Green QRT-PCR master mix and store on ice. Following the initial defrosting of the master mix, the unused portions were stored at 4°C with caution, avoiding repeated freeze-thaw cycles. The experimental reaction was prepared by making cocktail reagent constituted by 25 µl (including trial RNA), 12.5 µl of 2 x SYBR Green QRT-PCR master mix plus x µl of starting primer (concentration optimized) plus more Nuclease –free PCR – H2 level x µl of final primer (optimized concentration) as well as 0.375 µl of the reference dye solution from step 1 (optional) and 1.0 µl of the RT/Rnase enzyme blocking mixture to 50 µl of the total reaction volume can also be used. The reaction was mixed slowly so no bubbles formed (not rotated), then the mixture was distributed to test tubes by adding x µl of experimental RNA to each test tube. The mix prior to running on the Realtime PCR machine (CFX Connect system, Biorad Laboratories, Real Time PCR 96 well 0.1 ml, USA).

## 2.4 ELISA Method for TNF- $\alpha$

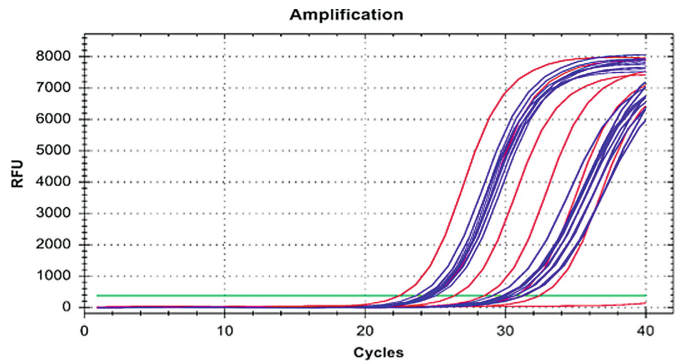
Serum samples were prepared along with all reagents according to the TNF- $\alpha$  kit used. In the first stage, 100  $\mu$ l of standard liquid, blank or sample was added to each well then incubated for 1 h at 37°C. Properly aspirate fluid from each well. Next, 100  $\mu$ l of Biotinylated Detection Antibody was added to each well and again was incubated for 1 h at 37°C. The plate was washed 3 times. As much as 100  $\mu$ l of Streptavidin-HRP Complex was added to each well then incubated. After 3x wash, the substrate (TMB) was dripped as much as 90  $\mu$ l per well. The color development was stopped using 50  $\mu$ l mild acid. The data was quantified using ELISA Reader 270 (Biomérieux, France) at 450 nm of wavelength.

## 3 Results

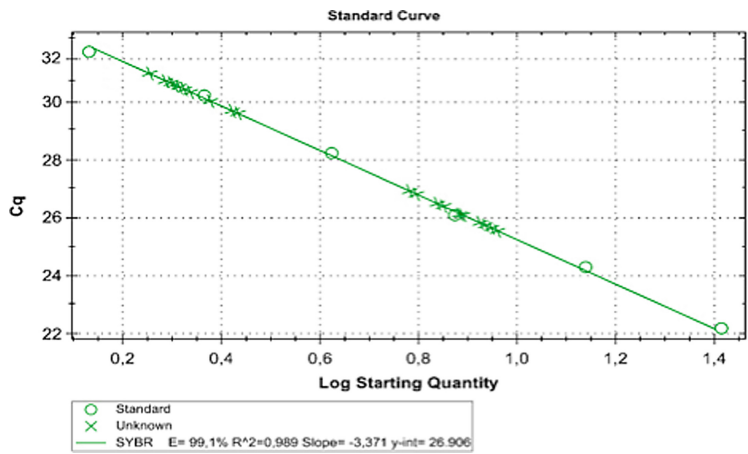
Observation (H-4) to (H-30) found a significant decrease in *HMGB1* mRNA expression in the four groups, namely EBSM (sapodilla fruit extract) 510 mg/Kg BW ( $p = 0.011$ ,  $p < 0.05$ ), EBSM (sapodilla fruit extract) 750 mg/Kg BW ( $p = 0.000$ ,  $p < 0.05$ ), Levofloxacin 98 mg/Kg BW ( $p = 0.000$ ,  $p < 0.05$ ) and distilled water ( $p = 0.033$ ,  $p < 0.05$ ). The same thing was performed in the observation (H-10) to (H-30) found a significant decrease in *HMGB1* mRNA expression in the four groups, namely EBSM (sapodilla fruit extract) 510 mg/KgBW ( $p = 0.011$ ,  $p < 0.05$ ), EBSM (sapodilla fruit extract) 750 mg/KgBW ( $p = 0.000$ ,  $p < 0.05$ ), Levofloxacin 98 mg/KgBW ( $p = 0.000$ ,  $p < 0.05$ ) and distilled water ( $p = 0.021$ ,  $p < 0.05$ ).

There was a significant decrease in TNF- $\alpha$  concentration ( $p = 0.024$ ,  $p < 0.05$ ) in the group given EBSM (sapodilla fruit extract) 510 mg/KgBW on observations (H-4) to (H-10) to (H-30). The same thing was also found in the group given EBSM (sapodilla fruit extract) 750 mg/KgBW found a significant decrease in TNF- $\alpha$  concentration ( $p = 0.018$ ,  $p < 0.05$ ); the group given Levofloxacin 98 mg/KgBW also experienced a significant decrease in TNF- $\alpha$  concentration ( $p = 0.001$ ,  $p < 0.05$ ). The control group showed a significant decrease in the concentration of TNF- $\alpha$ , namely 0.012. Four experimental design treatments decreased the concentration of TNF- $\alpha$ .

Independent t-test was carried out to see whether the data of the tested animals from the four groups (group 1 sapodilla fruit extract 510 mg/KgBB, group 2 sapodilla fruit extract 750 mg/KgBB, group 3 Levofloxacin 98 mg/KgBB, and group 4 aquades) homogeneous or not. Then, the paired t-test and anova test was performed to see whether there is a change that occurs between one group and another in one observation. Furthermore, the LSD test was carried out to see the differences in changes that occurred between groups. The repeated ANOVA test was carried out for changes that occur in observations made more than twice. The test results showed significant difference ( $p < 0.05$ ) which can be seen in Table 1 and Figs. 1 and 2.



**Fig. 1.** Amplification of *HMGB1* gene expression at the first dilution



**Fig. 2.** Amplification of *HMGB1* gene expression at the second dilution

**Table 1.** The value of the TNF-a concentration on the ELISA examination with a concentration of 10 times the dilution

NO	SAMPLE NUMBER	CONCENTRATION (PG/ML)	CONCENTRATION 10X
1	A01	11,435	114,348
2	A02	7,395	73,949
3	A03	8,742	87,415
4	A04	12,950	129,497
5	A05	6,385	63,850
6	A06	9,583	95,832
7	A07	12,108	121,081

(continued)

**Table 1.** (continued)

NO	SAMPLE NUMBER	CONCENTRATION (PG/ML)	CONCENTRATION 10X
8	A08	10,593	105,931
9	A09	14,296	142,963
10	A10	8,068	80,682
11	B01	17,663	176,629
12	B02	12,445	124,447
13	B03	13,623	136,230
14	B04	19,009	190,095
15	B05	15,980	159,796
16	B06	13,960	139,597
17	B07	18,336	183,362
18	B08	12,781	127,814
19	B09	16,485	164,846
20	B10	11,098	110,981
21	C01	18,841	188,411
22	C02	17,326	173,262
23	C03	14,970	149,696
24	C04	19,514	195,145
25	C05	18,673	186,728
26	C06	16,990	169,896
27	C07	20,019	200,194
28	C08	21,198	211,977
29	C09	17,831	178,312
30	C10	22,881	228,810
31	D01	19,851	198,511
32	D02	19,178	191,778
33	D03	16,821	168,212
34	D04	20,188	201,878
35	D05	19,346	193,461
36	D06	18,504	185,045
37	D07	22,208	222,077
38	D08	23,554	235,543
39	D09	20,693	206,927
40	D10	23,891	238,910

## 4 Discussion

High Motility Group Box 1 (*HMGB1*) is a cytokine expressed in almost all cells that experience inflammation. *HMGB1* will be released once an injury that afflicts a cell for the purpose of regulating the inflammation occurs.. *HMGB1* is found in almost all damaged and inflamed cells. *HMGB1* is transported into the nucleus by a nuclear import complex, which then retains it in the preformed protein. *Manila sapodilla* fruit is easily available. *Manila sapodilla* fruit can also be an alternative medicine because it is natural and safe for consumption.

Pathogenic microbial infections causing diarrhea are usually caused by *Bacillus cereus*, *Bacillus anthracis*, *Campilobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherechia coli*, *Listeria monocytogenes*, *Pseudomonas cocovenenans*, *Salmonela sp*, *Shigella sp*, *Staphilococcus aureus*, and *Vibers*. *Salmonella typhi* is a Gram-negative, rod, non-spore-forming, motile, encapsulated and flagellated bacteria (move with vibrating hairs). These bacteria can live at a pH of 6–8 at a temperature of 15–410 C (optimal temperature 37 C). These bacteria can die by heating at 54.4 C for one hour, treatment at a temperature of 60 C for 15–20 min, pasteurization, boiling and chlorination. The occurrence of *S. typhi* transmission to humans is by the faecal-oral route. Most of it is due to contamination of contaminated food or drinks. Research on the antibacterial activity test of sapodilla fruit extract (*Achras zapota L*) against *Salmonella typhi* using the *in vitro* method showed a significant decrease in the administration of sapodilla extract 510 mg/kg with a value of  $p = 0.009$  ( $p < 0.05$ ), a significant decrease when giving manila sapodilla fruit extract 750 mg/kg BW with a value of  $p = 0.007$  ( $p < 0.05$ ), a significant decrease in the provision of levofloxacin 98 mg/kg BW with a value of  $p = 0.009$  ( $p < 0.05$ ), and a significant reduction for giving distilled water 0.11 cc/Kg BW with a value of  $p = 0.004$  ( $p < 0.05$ ).

The results of our research proved that the intervention of sapodilla fruit extract can reduce the expression of the *HMGB1* mRNA gene at a dose of 510 mg/Kg BW and 750 mg/KgBW for observation at days 4 to 30. This was due to the content of compounds contained in sapodilla fruit which can suppress bacterial growth thereby reducing the number of bacteria. This process resulted in the reduced number of damaged host cells and *HMGB1* levels which will stick to TLR4 to anticipate the inflammatory process. The same thing was also found in the positive control group levofloxacin 98 mg/KgBW and the aquades group. The group of mice that received 750 mg/kgBW of sapodilla fruit extract after *Salmonella typhi* infection intraperitoneal showed a decrease in expression of the *HMGB1* mRNA gene was higher by 4.19 ng/mL compared to the dose of 510 mg/KgBW only 1.90 ng/ml on day-observation 4th to the 30th day. Whereas in the positive control group given levofloxacin 98 mg/KgBW, a decrease of 5.97 ng/ml was almost the same when compared to the decrease in *HMGB1* mRNA gene expression in the group given sapodilla sapodilla fruit extract 750 mg/kgBW. The same thing was also found in the negative control group who also experienced a decrease in expression of the *HMGB1* mRNA gene, but only 1.78 ng/ml which was the lowest of the three other groups.

Our results were in line with research conducted by Bhutia, et al. (2011) which reported that sapodilla fruit extract has significant antibacterial activity with *n*-hexane and 70% ethanol. Furthermore, another study conducted by Arsyad, et al. (2016) also

reported that *in vitro* manila sapodilla fruit extract had the ability to inhibit the growth of *Escherichia coli*. This is because the sapodilla fruit extract contains tannin and flavonoid compounds which can inhibit the growth of negative bacteria. Tumor necrosis factor alpha (TNF- $\alpha$ ) is a pleiotropic cytokine that plays a role in the inflammatory process, initiates polymorphonuclear (PMN) and activates it so that PMN can reach the site of infection. The main sources of TNF- $\alpha$  are mononuclear phagocytes and antigen-activated T cells, NK cells, and mast cells. Lipopolysaccharide is a potent stimulator of macrophages to secrete TNF- $\alpha$ .

Table 4 shows that after giving sapodilla fruit extract at a dose of 510 mg/Kg BW ( $p = 0.011$ ,  $p < 0.05$ ) and 750 mg/Kg BW ( $p = 0.004$ ,  $p < 0.05$ ), there was a decrease in the concentration of TNF- $\alpha$  at mice that were infected with *Salmonella typhi* intraperitoneal. The same thing was found in the positive control for Levofloxacin 98 mg/KgBW ( $p = 0.023$ ,  $p < 0.05$ ). This was because the administration of sapodilla fruit extract can reduce the amount of *Salmonella typhi* bacteria so that it can suppress the infection process in the host directly causing reduced release of cytokines by macrophages. Research conducted by Kema, et al. (2016) also reported that sapodilla fruit extract was effective as an antibacterial at ten Gram positive and twelve Gram negative bacteria. This was due to the content of compounds contained in sapodilla fruit extract, namely tannins, flavonoids and triterpenoids which could inhibit bacterial growth causing bacterial lysis.

## 5 Conclusion

From the results of the study above, it can be concluded that the sapodilla manila fruit has the potential to reduce *Salmonella typhi* colonies that cause typhoid fever, where this fruit has long been used by the people of Indonesia. The extract obtained from the sapodilla manila fruit can be considered as a substitute for antibiotics that have been used as a medicine for patients suffering from typhoid fever. So that in the future we can reduce the incidence of resistance and allergies to antibiotics.

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