

# Imunomodulator Properties of White Turmeric (Curcuma Mangga Val.) in Vivo

Dwiyati Pujimulyani

dwiyati@mercubuana-yogya.ac.id

Mercu Buana University of Yogyakarta

Wisnu Adi Yulianto

Mercu Buana University of Yogyakarta

Tri Indarto

Mercu Buana University of Yogyakarta

Sulkhan Windrayahya

Indonesia International Institute for Life Sciences

#### Research Article

Keywords: White turmeric, immunomodulatory, antioxidant

Posted Date: February 26th, 2024

**DOI:** https://doi.org/10.21203/rs.3.rs-3949963/v1

License: © (1) This work is licensed under a Creative Commons Attribution 4.0 International License.

Read Full License

**Additional Declarations:** No competing interests reported.

## **Abstract**

White turmeric contains bioactive compounds that have potential as immunomodulators. This research aims to assess the antioxidant properties and quantify the total phenolic content of white turmeric in vitro and test immunomodulatory properties in vivo. The study utilized a Completely Randomized Block Design (CRBD) with 2 treatment factors, namely the variation in parts of white turmeric rhizomes (main and tiller) and the variation in blanching time using the steam blanching method (0; 2.5; 5; 7.5; and 10 min). The analyses carried out were antioxidant activity of DPPH method (2,2 diphenyl-1-pickrylhidrazyl), and total phenolic content of white turmeric. Selected white turmeric powder based on in vitro test was analysed for immunomodulator (SOD (Superoxide Dismutase), IL-1 (Interleukin-1), IL-6 (Interleukin-6), IL-8 (Interleukin-8), IgE (Immunoglobulin E), IgG (Immunoglobulin G), dan IgM (Immunoglobulin M)). Selected white turmeric is the main rhizome with 5 min steam blanching. The analysis showed antioxidant activity of 81.46% RSA and total phenolic content of 6.08 mg EAG/g db. The results of in vivo studies showed that rats given white turmeric had an SOD value of 72.92% significantly better than rats given Na-CMC (Sodium Carboxymethyl Cellulose) and not significantly different from commercial supplements (stimuno). The IL-1 value of 0.40 pg/ml and IL-8 value of 48.53 pg/ml of rats given white kunir were significantly better than rats given Na-CMC and stimuno. The antibody values of IgE 74.02 ng/ml, IgG 18.20 ng/ml and IgM 2.97 ng/ml of rats treated with white turmeric were significantly better than those treated with Na-CMC and stimuno. The antibody values indicates that white turmeric has immunomodulatory effects.

# 1. Introduction

At the end of the year 2019, an outbreak of a disease known as Covid-19 emerged, caused by the coronavirus SARS-CoV-2 [1]. Herbal plants are a sought-after commodity by the community due to the existence of the coronavirus to boost immune system, one of which is white turmeric. White turmeric rhizome (Curcuma mangga Val.) is a type of empon-empon widely found in Indonesia. The content of bioactive compounds of empon-empon is stated to be useful as an immunomodulator [2], [3]. Immunomodulators are substances or agents that influence the immune system by stimulating, strengthening, or regulating immune responses [4]. Immunomodulators can work in various ways, including enhancing immune cell activity, modulating immune protein production, or regulating inflammatory responses [5], [6]. White turmeric rhizome also effective as an antiaging agent [7] and can increase the body's immunity [8]. The content of herbal plants that can be used as immunomodulators are flavonoids, quercetine, curcumin, catechin, epicatechin-gallate, which work as potential inhibitors of various infections [9], [10]. White turmeric has main components that function as secondary metabolites, namely phenols, tannins, curcumin and flavonoids [11]. Secondary metabolism involves a series of chemical reactions that are not directly involved in the growth and development of plants but contribute to various other functions, including responses to the environment and defense against pathogenic organisms. Secondary metabolism involves a series of chemical reactions that are not directly involved in the growth and development of plants but contribute to various other functions, including responses to the environment and defense against pathogenic organisms [12], [13].

Blanching is a pre-treatment in food processing [14], [15]. The blanching process is divided into two methods, namely steam blanching and hot water blanching [16]. Steam blanching minimises the loss of water-soluble components such as vitamins, minerals and sugars [17]. Blanching can reduce the antioxidant activity of some fruits [18], but increased antioxidant activity in carrots, [16], oil extracts [19] and seedless grapes [20]. The increase in antioxidant activity during the blanching process is due to the change of inactive to active compounds [21]. The antioxidant activity is in accordance with the results of [22], that heating tannins showed an increase in antioxidant activity compared to without heating.

Previous research related to white turmeric includes anticholesterol [23], antidiabet [24], and antiaging [25]. The potential of white turmeric due to steam blanching on immunomodulatory properties has not been previously investigated. Based on this, the study's primary goal is to assess the antioxidant potential of white turmeric in vitro and immunomodulatory properties in vivo.

## 2. Materials and methods

#### 2.1 Materials

White turmeric rhizomes aged 9–10 months were obtained from CV Windra Mekar. The analysis used various chemicals including distilled water, pure ethanol (Merck), BHT (*Butylated Hydroxytoluene*, Sigma), DPPH solution (2,2-*diphenyl*-1-*picrylhydrazyl*), Sigma-Aldric) 0,1 mM, pure Folin-cilocalteu (Merck),  $Na_2CO_3$  (Merck, 20%), saturated  $Na_2CO_3$ , Na-CMC (*Sodium Carboxymethyl Cellulose*) suspension stimuno dan *S. aureus* 1,0x10<sup>8</sup> sel/mL.

#### 2.2 Equipment

The chemical equipment used include measuring cups (*pyrex Iwaki*), beaker glass (*pyrex Iwaki*), test tubes (*pyrex Iwaki*), measuring pipettes (*pyrex*), volumetric flasks (*pyrex Iwaki*), dropper pipettes, stirring rods, micro pipettes (Acura 825 autoclavable), analytical scales (Ohaus Pioneer PA214), filter paper (Whatman no 42), vortex (Maxi Mix II type 37600) and UV-Vis spectrophotometer (*Shimadzu UV mini 1240*).

#### 2.3 Measurement Procedure

Various phases in this research involve the conversion of white turmeric into powder with variations in the parts of the white turmeric rhizome (main rhizome and tiller) and the experiment includes altering the duration of steam blanching within a range of times (0, 2.5, 5, 7.5, and 10 min). and analysis of white turmeric powder in vitro and in vivo.

#### 2.4 Preparation of White Turmeric Powder

White turmeric is sorted to separate the main rhizome and tillers. After sorting, white turmeric is peeled and washed. The washed white turmeric undergoes steam blanching treatment with durations of 0; 2.5; 5; 7.5; and 10 minutes. The blanched white turmeric is drained, sliced to a thickness of 1–2 mm, and dried using a modern cabinet dryer at 55 °C for 11 hours. The dried white turmeric is finely blended and sieved through a 60-mesh sieve.

#### 2.5 Method of antioxidant activity using DPPH (2,2-diphenyl-1-pikrilhidrazil) method [26]

The assessment of antioxidant activity utilizing the DPPH method was conducted by taking 0.2 ml of sample and then adding 0.1 mM DPPH solution as much as 3.8 ml, then vortexed for 1 minute and kept in the dark at room temperature for 30 minutes during the incubation period which is 28°C. Blank was made using ethanol. The spectrophotometer was utilized to gauge the absorbance at  $\lambda$  517 nm. The activity of free radical scavenging was indicated as % Radical Scavenging Activity.

$$%RSA = 1 - \frac{absorbance sample}{absorbance control} \times 100\%$$
(1)

### 2.6 Total Phenolic Content (TPC) Folin-Ciocalteu [27]

The extract's total phenolic content was quantified using the Folin-Ciocalteu reagent with minor modification. 50  $\mu$ l of the sample were combined with 250  $\mu$ l of Folin-Ciocalteu solution, left undisturbed for 1 minute, followed by the addition of 750  $\mu$ l of 20% Na<sub>2</sub>CO<sub>3</sub>. After vortexing, distilled water was introduced to achieve a total volume of 5 ml. Subsequently, the incubation was conducted at ambient temperature for 2 hours, then measured with a spectrophotometer at  $\lambda$  760 nm.

TPC (mg GAE/g wb) = 
$$\frac{\text{concentration ppm} \times 100}{\text{weight of sample (g)}}$$
...

(2)

TPC (mg GAE/g db) =  $\frac{100}{(100 - \text{moisture content})} \times \text{TPC wb}$ .....(3)

#### 2.7 Imonomodulator Assay in vivo

The measurement of SOD, IL-1, IL-6, IL-8, IgE, IgG, and IgM was applied to the blood of experimental rats. The categorization involves 24 male Wistar rats, segregated into 4 groups. Group 1 serves as the control with a standard feed, group 2 is a 0.5% Na-CMC suspension for negative control, group 3 is a suspension of stimuno 0.9 mg / 200 g b / b, group 4 is white kunir 16.2 mg / 200 g b / b. On the 8th day, blood was drawn using a blood glucose meter. On the 8th day, blood was drawn using a micropipette. IL-1, IL-6, and IL-8 were determined using the spectrophotometric method according to the kit employed. All samples from the treatment were adapted for 1 week, then given treatment according to the standard feed of each group. The treatment was conducted for 16 days, and on the 14th day, an injection of *S. aureus* at a

concentration of 1.0x10<sup>8</sup> cells/mL was administered, with a volume of 0.1 mL intraperitoneally (directly injected into the peritoneal cavity).

SOD determination was carried out using 0.6 mL hepatic supernatant reacted with 2.70 mL of 50 mM  $Na_2CO_3$  buffer containing 0.01 mM EDTA (pH 10), 0.06 mM, 10 mM of xanthine, 0.5% BSA 0.03 mM and 2.5 mM NBT as much as 0.03 mM. Subsequently, xanthine oxidase was introduced at a concentration of 0.04 units and left to stand. After 30 min, it was measured at  $\lambda$  560 nm. Calculation of SOD levels as follows [28].

Activity SOD (%) = 
$$\left(1 - \frac{A}{B}\right) \times 100\%$$
 .....(4)

Description:

A = Absorbance of sample

B = Control absorbance

Determination of immunomodulatory effects on antibody values (IgE, IgG and IgM) in male rats is measured by the antibody titer method by agglutination [29]. Determination of the immunomodulatory effect observed on day 17th, blood samples of each rat were taken through a vein in the tail and tissue was taken.

#### 2.8 Statistical Analysis

The research design employed is a Completely Randomized Block Design (CRBD) with 2 treatment factors, namely variation of white turmeric rhizome parts (main and tiller) and variation of blanching time using the steam blanching method (0; 2.5; 5; 7.5 and 10 min).

# 3. Result and discussion

The results of the antioxidant properties analysis of white turmeric powder are presented in Table 1.

Table 1 Analytical results of white turmeric powder

Rhizome Part	Time Blanching	Antioxidant Activity (% RSA)	TPC (mg GAE/g db)	
Main	0	76,26 ± 1,40 <sup>ab</sup>	3,67 ± 0,14 <sup>c</sup>	
Main	2.5	78,64 ± 0,42 <sup>de</sup>	4,87 ± 0,13 <sup>f</sup>	
Main	5	81,46 ± 0,63 <sup>g</sup>	6,08 ± 0,11 <sup>h</sup>	
Main	7.5	79,95 ± 0,49 <sup>ef</sup>	5,55 ± 0,29 <sup>g</sup>	
Main	10	78,93 ± 0,14 <sup>de</sup>	4,33 ± 0,14 <sup>de</sup>	
Tiller	0	74,88 ± 0,14 <sup>a</sup>	2,73 ± 0,16 <sup>a</sup>	
Tiller	2.5	77,10 ± 0,21 <sup>bc</sup>	3,23 ± 0,22 <sup>b</sup>	
Tiller	5	80,83 ± 0,62 <sup>fg</sup>	5,63 ± 0,11 <sup>g</sup>	
Tiller	7.5	78,19 ± 0,62 <sup>cd</sup>	4,60 ± 0,06 <sup>ef</sup>	
Tiller	10	75,87 ± 0,13 <sup>ab</sup>	4,04 ± 0,08 <sup>d</sup>	

Description: Different letters in columns indicate significant difference with 95% significance level.

## 3.1 DPPH Antioxidant Activity (% RSA)

The DPPH antioxidant activity (% RSA) of white turmeric powder is presented in Fig. 1.

The findings presented in Table 1 indicate a substantial impact of rhizome variations and the duration of steam blanching on the antioxidant activity of the resulting white turmeric powder. Antioxidants are substances that donate electrons or act as reducing agents [30]. These substances possess a low molecular weight but can hinder the progression of oxidation reactions by impeding the generation of radicals [31], [32]. In Table 1, it is show that the antioxidant activity values increase with the steam blanching treatment. However, upon reaching the 7.5-minute mark, the antioxidant activity values decrease. It is suspected that at the 5th minute the increase in antioxidant activity has reached its maximum value. The research is substantiated by the findings by [33] that the antioxidant activity of white turmeric after blanching showed higher results than white turmeric rhizomes without blanching. Blanching treatment on black turmeric (*Curcuma aeruginosa* Roxb.) using distilled water and citric acid as the media enhances its antioxidant activity values [34]. The elevation in antioxidant activity observed during blanching is believed to result from transforming inactive compounds into active ones. This is by the results of [35] that tannin heating showed an increase in antioxidant activity compared to without heating. The increase in antioxidant activity with heat treatment also occurs in sorghum seeds, with an

increase in antioxidants by 40.9% and 59.1% [36]. The main rhizomes and tiller treated with 5 min blanching were not significantly different.

3.2 TPC (mg GAE/g)

TPC (mg GAE/g) of white turmeric powder is presented in Fig. 2.

The results of analysis of variance in Table 1, showed that rhizome variation and blanching time had a significant effect on TPC in the white kunir powder produced. The highest TPC were found in the main rhizome and tiller white turmeric powder with 5 min blanching time treatment. White turmeric powder has the highest TPC of 6.08 mg GAE/g in the main rhizome with a blanching time of 5 min, which means that each gram of powder is comparable to 6.08 mg of gallic acid. The phenolic content of a substance correlates with its antioxidant activity; the higher the antioxidant activity, the greater the phenolic content, and vice versa [37]. The amount of TPC in white turmeric is thought to be due to the contribution of curcuminoid compounds in white turmeric. This compound is known for its potent antioxidant properties. Therefore, the phenolic content of white turmeric, including curcuminoids, can majorly contribute to the antioxidant activity of white turmeric rhizomes [38], [39]. Blanching white turmeric powder has a higher content than fresh white turmeric. This is following the research of [33] which states that blanching treatment can increase the TPC. White turmeric subjected to pressurized blanching also produces high phenolic content. This is attributed to the degradation of glycosides into aglycones and the breakdown of phenolic complexes into simpler phenolic compounds [23].

#### 3.3 Immunomodulator Analysis Results

The best result of white turmeric powder from antioxidant activity analysis was powder from the main rhizome which was steam blanching for 5 min. The best powder was carried out in vivo immunomodulatory analysis including SOD, IL-1, IL-6, IL-8, IgE, IgG and IgM.

# 3.3.1 SOD

SOD is an intracellular antioxidant whose role is to protect cells from oxidant interference and oxidative stress that causes several diseases and degeneration processes such as aging and carcinogenesis [40]. SOD stands as a key antioxidant enzyme that serves as a crucial defense mechanism against free radicals. It operates as an internal cellular defense system, converting oxygen (O<sub>2</sub>) into hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and oxygen, which are further detoxified by catalase (CAT) and glutathion peroxidase (GPx) [41]. Analysis was conducted to determine the Superoxide Dismutase (SOD) levels in Wistar rats that had been injected with 0.1 mL of *Staphylococcus aureus* 10<sup>8</sup> and treated with white turmeric powder, Na-CMC, and Stimuno. The results of SOD levels are presented in Table 2.

Table 2 SOD values in rats

Sample	SOD (%)
Normal	82.03 ± 2.92 <sup>c</sup>
Na-CMC	23.44 ± 4.07 <sup>a</sup>
Stimuno	70.84 ± 4.38 <sup>b</sup>
White turmeric	72.65 ± 2.92 <sup>b</sup>

Table 2 shows that rats injected with Na-CMC have low blood SOD levels. Whereas rats treated with white turmeric and stimuno have higher SOD levels close to normal rats. Rats treated with white turmeric as much as 16.2 mg / 200 g bw had blood SOD levels of 72.65%, not significantly different derived from rats subjected to stimuno treatment 0.9 mg / 200 g bw had SOD levels of 70.84%. High levels of SOD in the blood of normal mice indicate a healthy condition [42]. High levels of SOD in the blood in normal rats indicate a healthy state. Stimuno is a product containing antioxidant *Phyllanthus niruri* from meniran. The phytochemistry of plants belonging to the genus *Phyllanthus* can enhance or suppress immune responses [43]. White turmeric is a rhizome containing curcumin, phenols, and flavonoids that are immunomodulators. White turmeric contains curcumin, phenols, and flavonoids. This proves that the content of bioactive compounds in white turmeric and stimuno can increase SOD levels in mice injected with *Staphylococcus aureus* bacteria. The higher the SOD levels in mice, the healthier the mice are.

In previous research showed that the in vivo study of SOD of wistar rats fed with white turmeric that had been blanched with different methods had a higher value than the negative control [23], stated that SOD levels in rats given white turmeric extract affected SOD values, this is because white turmeric extract contains curcuminoids. Giving antioxidants in curcumin can neutralise free radicals that can cause oxidative stress [44].

# 3.3.2 IL-1, IL-6, IL-8

IL-1, IL-6, IL-8 values are presented in Table 3.

Table 3
Results of IL-1, IL-6 and IL-8

Sample	IL-1 (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)
Normal	0.27 ± 0.01 <sup>a</sup>	65.11 ± 1.57 <sup>a</sup>	43.09 ± 3.17 <sup>a</sup>
Na-CMC	1.46 ± 0.03 <sup>d</sup>	126.04 ± 1.81°	115.36 ± 4.56 <sup>d</sup>
Stimuno	0.63 ± 0.01°	78.22 ± 0.96 <sup>b</sup>	79.34 ± 3.05 <sup>c</sup>
White turmeric	0.51 ± 0.03 <sup>b</sup>	75.76 ± 1.82 <sup>b</sup>	55.23 ± 6.73 <sup>b</sup>

Description: Different letters in columns indicate significant difference with 95% significance level

IL-1 is a group of cytokine polypeptides, namely IL-1α, IL-1β, and IL-1Ra, that hold significant significance in governing the immune system and inflammatory responses [45]. Based on Table 3, it can be seen that normal rats, rats given Na-CMC, stimuno and white turmeric have significantly different IL-1 levels. IL-1 and IL-8 levels of rats given white turmeric are significantly lower than supplements from the market (stimuno). Low levels of IL-1 may indicate a lack of inflammatory activity, suggesting a healthy condition in mice [46]. Rats treated with white turmeric as much as 16.2 mg / 200 g bw had blood IL-1 levels of 0.51 pg / ml, rats treated with stimuno 0.9 mg / 200 g bw had blood IL-1 levels of 0.63 pg / ml and rats treated with Na-CMC as much as 1.46 pg / ml.. Normal rats have an IL-1 value of 0.27 pg/ml. The data shows that mice given white turmeric have IL-1 values close to normal mice because the curcumin compound in white turmeric can normalise the IL-1 value of rats injected with *Staphylococcus aureus* bacteria. Research by [47] explains that curcumin compounds can inhibit the release of body compounds that cause inflammation or pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α. Has better anti-inflammatory than commercial on the market (stimuno). Curcumin can affect several molecular targets and biochemical pathways involved in inflammation [48]. This allows curcumin to operate at various levels to alleviate inflammation.

IL-6 is a protein cytokine that functions as a messenger that activates the immune system against foreign invasion and can help fight the spread of cancer [49]. IL-6 values in rats treated with white turmeric were significantly different from rats treated with Na-CMC, but not significantly different from those treated with commercial supplements (stimuno). This is because white turmeric contains curcumin compounds that can help reduce IL-6 values. Curcumin can regulate the expression of genes involved in immune responses, including genes related to the production of IL-6, leading to a reduction in the production of IL-6 by immune cells [50]. Research by[51], states that diabetic rats treated with white turmeric powder as much as 1.5 g and 4.5 g showed a significant decrease in IL-6, TNF-α, and IL-8 levels from week 1 to week 4. The indicates that the phenolic compounds and flavonoids present in white turmeric contribute to the reduction of pro-inflammatory cytokines [52].

Table 3 shows that mice given white turmeric have a low IL-8 value close to the IL-8 value of normal mice. White turmeric contains curcumin compounds that can help normalise IL-8 values. Curcumin has

antioxidant properties that can help reduce oxidative stress, which may contribute to the production of IL-8. By diminishing oxidative stress, curcumin can positively influence the levels of IL-8. IL-8 is a chemokine that contributes directly to macrophage infiltration and activation in adipose tissue (Pujimulyani et al., 2022). Research by [51] stated that white turmeric powder given to rats for one month can reduce IL-6, TNF- $\alpha$ , and IL-8 levels in diabetic rats. Research by [53] also mentioned that curcumin can regulate (NF)- $\alpha$  through IKKb by reducing the expression of IL-8, TNF- $\alpha$ , and IFN- $\alpha$ .

Based on the values of IL-1, IL-6, and IL-8, the mice treated with white turmeric significantly differ from those with only Na-CMC as the negative control, and the results are close to those of normal mice. IL-1 and IL-8 values of white turmeric rats significantly differed from those given commercial supplements (stimuno). This indicates that the administration of white turmeric is better at normalising IL-1 and IL-8 values compared to commercial supplements (stimuno) and Na-CMC.

# 3.3.3 IgE, IgG, dan IgM

IgE, IgG dan IgM values are presented in Table 4.

Table 4 IgE. IgG and IgM

Sampel	IgE (ng/ml)	IgG (ng/ml)	IgM (ng/ml)	
Normal	63.72 ± 1.65 <sup>a</sup>	16.53 ± 1.11 <sup>a</sup>	2.05 ± 0.14 <sup>a</sup>	
Na-CMC	125.39 ± 3.61 <sup>d</sup>	92.56 ± 3.31 <sup>d</sup>	32.56 ± 0.38 <sup>d</sup>	
Stimuno	79.53 ± 0.81 <sup>c</sup>	25.00 ± 2.02 <sup>c</sup>	4.79 ± 0.19 <sup>c</sup>	
White turmeric	76.36 ± 1.05 <sup>b</sup>	20.17 ± 0.89 <sup>b</sup>	3.80 ± 0.37 <sup>b</sup>	

Description: Different letters in columns indicate significant difference with 95% significance level

IgE antibodies are generally found in the blood in small amounts. However, IgE antibodies will increase when the body experiences inflammatory reactions due to allergies [54]. IgE antibody tests are performed to detect allergic diseases and parasitic infections. The results of IgE, IgG and IgM measurements of mice in each treatment group are presented in Table 4.

Based on Table 4, the IgE value of mice given white turmeric was 76.36 ng/ml. The IgE value in rats given white turmeric is close to normal rats 63.72 ng/ml. Mice given white turmeric have better IgE values than those given commercial supplements (stimuno) and mice given Na-CMC. This is suspected because white turmeric contains phytochemical compounds, including curcumin that may reduce IgE. According to [55] and [56], the administration of curcumin significantly prevents the increase of IgE, IL-4, nitrous

oxide (NO) and eosinophil peroxidase in the respiratory tract. So, inflammatory responses in rhinitis such as sneezing, nasal congestion, nasal itching and eye lacrimation are reduced.

IgG stands as the predominant antibody type present in the bloodstream and various other bodily fluids. When an antigen enters the body, white blood cells will form IgE antibodies to fight it. Based on Table 4, the IgG value in mice treated with white turmeric at 20.17 ng/ml was better than those treated with Na-CMC and stimuno. Research by [57] and [58], states that white turmeric can increase the production of antibodies in the body. These antibodies include immunoglobulin G (IgG) and natural killer (NK) cells. The reason for the IgG levels in mice treated with white turmeric powder approaching those of normal mice may involve the anti-inflammatory and immunomodulatory properties of compounds present in white turmeric, such as curcumin, which contains antioxidants that regulate immune and inflammatory responses. Previous research has stated that curcumin in rhizomes can enhance the growth of young carp, reduce FCR (Feed Conversion Ratio), improve disease resistance, innate immunity, and antioxidant capacity in fish, as well as attenuate inflammatory responses [59].

Table 4 shows that the IgM value in mice is significantly different. IgM is a type of immunoglobulin produced by the immune system in response to the invasion of pathogens or foreign substances [60]. High IgM levels can indicate an ongoing or past infection. White turmeric treatment rats had better IgM values than those treated with commercial supplements (stimuno) and Na-CMC. IgM values in negative control mice had high values as a sign of active infection. The higher the IgM levels, the higher the infection levels in mice, and vice versa. The impact of curcumin found in white turmeric significantly influences both the innate and adaptive immune systems, particularly in pathological circumstances. Curcumin efficiently regulates the activities of T cells, B cells, dendritic cells, monocytes, macrophages, and neutrophils [61], [62].

#### 3.4 Body Weight of Rats

The results of the study of the administration of Na-CMC, stimuno and white turmeric on the body weight of rats can be seen in Table 4.

Table 4
Body weight data of Wistar rats (g)

Sampel	BB After Adaptation	BB on 7th days	BB on 14th days	BB before injection	BB after injection
Normal	182.00 ± 3.46 <sup>a</sup>	189.17 ± 2.86 <sup>a</sup>	195.33 ± 3.39 <sup>a</sup>	201.33 ± 2.16 <sup>a</sup>	206.83 ± 2.93 <sup>b</sup>
Na-CMC	179.50 ± 4.60 <sup>a</sup>	186.17 ± 4.36 <sup>a</sup>	192.00 ± 4.29 <sup>a</sup>	198.33 ± 4.08 <sup>a</sup>	199.50 ± 4.04 <sup>a</sup>
Stimuno	181.00 ± 3.35 <sup>a</sup>	188.50 ± 3.01 <sup>a</sup>	194.67 ± 3.72 <sup>a</sup>	200.67 ± 3.27 <sup>a</sup>	206.00 ± 4.00 <sup>b</sup>
White urmeric	178.16 ± 3.31 <sup>a</sup>	185.33 ± 3.72 <sup>a</sup>	191.50 ± 3.83 <sup>a</sup>	197.83 ± 3.87 <sup>a</sup>	202.83 ± 4.07 <sup>ab</sup>

Description: Different letters in columns indicate significant difference with 95% significance level

Table 4 indicates that the body weight of rats administered with white turmeric and stimuno does not exhibit a significant difference compared to normal rats. However, rats administered with Na-CMC show a significant difference in body weight compared to normal rats and a decrease in the body weight of mice. Each treatment showed a significant difference after the rats were injected with *S. auerus*. Rats suspended in 0.5% Na-CMC had the lowest body weight compared to other rats. This indicates that *S. auerus* can inhibit body weight development in rats.

In rats given white turmeric had a body weight that was not significantly different from normal rats and those given commercial supplements (stimuno). Giving white turmeric can maintain the body weight of rats close to normal rats. This is because white turmeric contains flavonoids and phenols that can inhibit the growth of *Staphylococcus auerus* in the body. Flavonoids and phenolics have a tendency to bind to bacterial proteins, thus inhibiting enzyme activity which will interfere with the metabolic process of bacteria [63]. Flavonoid and phenol compounds can affect the permeability of bacterial cell membranes, causing damage to cell structures, and inhibiting vital functions [64]

# 4. CONCLUSION

The best white turmeric powder is the main rhizome with a steam blanching time of 5 min which has antioxidant and immunomodulatory properties. Variations of rhizomes and length of steam blanching time have an antioxidant activity analysis value of the DPPH method which is 81.46% RSA and TPC of 6.08 mg EAG/g bk. The results of in vivo studies, rats given white turmeric powder had SOD values of 72.92%, IL-1 0.40 pg/ml, IL-6 68.66 pg/ml, IL-8 48.53 pg/ml, IgE antibodies 74.02 ng/ml, IgG 18.20 ng/ml and IgM 2.97 ng/ml close to normal rats as controls compared to rats given Na-CMC 0.5% and commercial supplements (stimuno). This indicates that white turmeric has immunomodulatory effects.

## **Declarations**

#### **ACKNOWLEDGEMENT**

The author would like to thank University of Mercu Buana Yogyakarta for providing research fund through the Umbrella Research Programme with grant number 177/B.01/H1/III/2023.

#### APPROVAL LETTER FOR ETHICAL SUITABILITY

The Research Ethics Commission of Alma Ata University affirms that the research has adhered to ethical principles in accordance with the Declaration of Helsinki 2008 with Number: KE/AA/II/101053/EC/2023.

#### **COMPETING INTERESTS**

The authors declare that they have no conficts of interest.

#### **DATA AVAILABILITY**

Datasets from the current study are available from the corresponding author upon request.

# **Author Contribution**

DP= contributed to the research and drafted the paperWAY= contribute to editing the manuscriptTI= contributing to research and drafting the paperSW= contributed to manuscript editing

## References

- 1. V. G. da Costa, M. L. Moreli, and M. V. Saivish, 'The emergence of SARS, MERS and novel SARS-2 coronaviruses in the 21st century', *Archives of Virology*, vol. 165, no. 7, pp. 1517–1526, Jul. 2020, doi: 10.1007/s00705-020-04628-0.
- 2. L. Hakim, *Rempah dan herba kebun-pekarangan rumah masyarakat: keragaman, sumber fitofarmaka dan wisata kesehatan-kebugaran.* Yogyakarta: Diandra Creative, 2015.
- 3. S. Sukmawati, I. Musfiroh, Muchtaridi, and A. Fristiohady, 'Qust Al Hindi (*Saussurea Lappa*): A Narrative Review of Its Phytochemistry and Pharmacological Potential Against Covid-19', *Int J App Pharm*, pp. 1–7, Dec. 2022, doi: 10.22159/ijap.2022.v14s5.17.
- 4. T. Behl *et al.*, 'Exploring the multifocal role of phytochemicals as immunomodulators', *Biomedicine & Pharmacotherapy*, vol. 133, p. 110959, Jan. 2021, doi: 10.1016/j.biopha.2020.110959.
- 5. W. Jiang and J. Xu, 'Immune modulation by mesenchymal stem cells', *Cell Proliferation*, vol. 53, no. 1, p. e12712, Jan. 2020, doi: 10.1111/cpr.12712.
- 6. A. A. Rabaan et al., 'Role of Inflammatory Cytokines in COVID-19 Patients: A Review on Molecular Mechanisms, Immune Functions, Immunopathology and Immunomodulatory Drugs to Counter Cytokine Storm', Vaccines, vol. 9, no. 5, 2021, doi: 10.3390/vaccines9050436.

- 7. D. Pujimulyani, Temu Mangga: Lalapan Berkhasiat sebagai Antiaging, in *Pangan Fungsional Indonesia*, 1st ed., in Buah dan Sayuran. , Bogor: IPB Press, 2023, pp. 237–244.
- 8. Yuandani, I. Jantan, A. S. Rohani, and I. B. Sumantri, 'Immunomodulatory effects and mechanisms of curcuma species and their bioactive compounds: a review', *Front. Pharmacol.*, vol. 12, p. 643119, Apr. 2021, doi: 10.3389/fphar.2021.643119.
- 9. H. A. Alhazmi *et al.*, 'Medicinal Plants and Isolated Molecules Demonstrating Immunomodulation Activity as Potential Alternative Therapies for Viral Diseases Including COVID-19', *Frontiers in Immunology*, vol. 12, 2021, [Online]. Available: https://www.frontiersin.org/articles/10.3389/fimmu.2021.637553
- 10. L. J. Valencia-Hernández, J. A. Ascacio-Valdés, J. E. Wong-Paz, H. Khan, and C. N. Aguilar, 'Immune Booster Property of Epigallocatechin-3-Gallate and Catechin', in *Nutraceuticals and Functional Foods in Immunomodulators*, R. K. Kesharwani, R. K. Keservani, and A. K. Sharma, Eds., Singapore: Springer Nature Singapore, 2022, pp. 291–312. doi: 10.1007/978-981-19-2507-8\_12.
- 11. D. Pujimulyani, W. A. Yulianto, A. Setyowati, P. Prastyo, S. Windrayahya, and A. Maruf, 'White saffron (*Curcuma mangga* Val.) attenuates diabetes and improves pancreatic β-cell regeneration in streptozotocin-induced diabetic rats', *Toxicology Reports*, vol. 9, pp. 1213–1221, 2022, doi: 10.1016/j.toxrep.2022.05.014.
- 12. S. Khare *et al.*, 'Plant secondary metabolites synthesis and their regulations under biotic and abiotic constraints', *Journal of Plant Biology*, vol. 63, no. 3, pp. 203–216, Jun. 2020, doi: 10.1007/s12374-020-09245-7.
- 13. H. Aguirre-Becerra *et al.*, 'Role of Stress and Defense in Plant Secondary Metabolites Production', in *Bioactive Natural Products for Pharmaceutical Applications*, D. Pal and A. K. Nayak, Eds., Cham: Springer International Publishing, 2021, pp. 151–195. doi: 10.1007/978-3-030-54027-2\_5.
- 14. A. Ciurzyńska *et al.*, 'The Effect of Pre-Treatment (Blanching, Ultrasound and Freezing) on Quality of Freeze-Dried Red Beets', *Foods*, vol. 10, no. 1, 2021, doi: 10.3390/foods10010132.
- 15. A. Sarkar, S. Rahman, M. Roy, M. Alam, M. A. Hossain, and T. Ahmed, 'Impact of blanching pretreatment on physicochemical properties, and drying characteristics of cabbage (*Brassica oleracea*)', *Food Res.*, vol. 5, no. 2, pp. 393–400, Apr. 2021, doi: 10.26656/fr.2017.5(2).556.
- 16. H. Wang *et al.*, 'Effects of vacuum-steam pulsed blanching on drying kinetics, colour, phytochemical contents, antioxidant capacity of carrot and the mechanism of carrot quality changes revealed by texture, microstructure and ultrastructure', *Food Chemistry*, vol. 338, p. 127799, Feb. 2021, doi: 10.1016/j.foodchem.2020.127799.
- 17. B. Liu, X. Fan, C. Shu, W. Zhang, and W. Jiang, 'Comparison of non-contact blanching and traditional blanching pretreatment in improving the product quality, bioactive compounds, and antioxidant capacity of vacuum-dehydrated apricot', *J Food Process Preserv*, vol. 43, no. 3, p. e13890, Mar. 2019, doi: 10.1111/jfpp.13890.
- 18. R. F. Dibanda, E. Panyoo Akdowa, A. Rani P., Q. Metsatedem Tongwa, and C. M. Mbofung F., 'Effect of microwave blanching on antioxidant activity, phenolic compounds and browning behaviour of some

- fruit peelings', *Food Chemistry*, vol. 302, p. 125308, Jan. 2020, doi: 10.1016/j.foodchem.2019.125308.
- 19. T. Kaseke, U. L. Opara, and O. A. Fawole, 'Effect of blanching pomegranate seeds on physicochemical attributes, bioactive compounds and antioxidant activity of extracted oil', *Molecules*, vol. 25, no. 11, p. 2554, May 2020, doi: 10.3390/molecules25112554.
- 20. J. Wang, H.-W. Xiao, X.-M. Fang, A. S. Mujumdar, S. K. Vidyarthi, and L. Xie, 'Effect of high-humidity hot air impingement blanching and pulsed vacuum drying on phytochemicals content, antioxidant capacity, rehydration kinetics and ultrastructure of Thompson seedless grape', *Drying Technology*, vol. 40, no. 5, pp. 1013–1026, Apr. 2022, doi: 10.1080/07373937.2020.1845721.
- 21. J. Nie, 'Effects of various blanching methods on fucoxanthin degradation kinetics, antioxidant activity, pigment composition, and sensory quality of Sargassum fusiforme', *LWT-Food Science and Technology*, vol. 143, p. 111179, 2021, doi: https://doi.org/10.1016/j.lwt.2021.111179.
- 22. K. Wuttikul and M. Sainakham, 'In vitro bioactivities and preparation of nanoemulsion from coconut oil loaded *Curcuma aromatica* extracts for cosmeceutical delivery systems', *Saudi Journal of Biological Sciences*, vol. 29, no. 12, p. 103435, Dec. 2022, doi: 10.1016/j.sjbs.2022.103435.
- 23. D. Pujimulyani, U. Santoso, S. Luwihana, and A. Maruf, 'Orally administered pressure-blanched white saffron (*Curcuma mangga* Val.) improves antioxidative properties and lipid profiles in vivo', *Heliyon*, vol. 6, no. e04219, pp. 1–8, 2020, doi: https://doi.org/10.1016/j.heliyon.2020.e04219.
- 24. D. Pujimulyani *et al.*, 'Hypoglycemic Activity of *Curcuma mangga* Val. Extract via Modulation of GLUT4 and PPAR-γ mRNA Expression in 3T3-L1 Adipocytes', *Journal of Experimental Pharmacology*, vol. 12, pp. 363–369, 2020, doi: https://doi.org/10.2147/JEP.S267912.
- 25. D. Pujimulyani *et al.*, 'Elastase, Hyaluronidase and Tyrosinase inhibitor activities antiaging of Curcuma mangga Val. extract and its fractions', *IOP Conf. Ser.: Earth Environ. Sci.*, vol. 379, no. 1, p. 012004, Nov. 2019, doi: 10.1088/1755-1315/379/1/012004.
- 26. B. J. Xu and S. K. C. Chang, 'A comparative study on phenolic profiles and antioxidant of legumes affected by extraction', *Journal of Food Science*, vol. 72, pp. 59–66, 2007.
- 27. D. Pujimulyani, S. Raharjo, Y. Marsono, and U. Santoso, 'Aktivitas Antioksidan dan Kadar Senyawa Fenolik Pada Kunir Putih (*Curcuma mangga* Val.) Segar dan Setelah Blanching', *AGRITECH*, vol. 30, no. 2, pp. 68–74, 2010.
- 28. A. Yunarsa and I. P. G. Adiatmika, 'Kadar antioksidan superoksida dismutase (SOD) hati tikus pada aktivitas fisik berat', *E-Jurnal Medika Udayana*; *Vol 7 No 4 (2018): E-Jurnal Medika Udayana*, 2018, [Online]. Available: https://ojs.unud.ac.id/index.php/eum/article/view/38730
- 29. L. B. Sebayang and A. S. Hasibuan, 'Uji Efek Imunumodulator Vco (Virgin Coconut Oil) Pada Tikus Jantan', *JBL*, vol. 11, no. 2, p. 139, Sep. 2021, doi: 10.35799/jbl.v11i2.35663.
- 30. M. Parcheta, R. Świsłocka, S. Orzechowska, M. Akimowicz, R. Choińska, and W. Lewandowski, 'Recent Developments in Effective Antioxidants: The Structure and Antioxidant Properties', *Materials*, vol. 14, no. 8, 2021, doi: 10.3390/ma14081984.

- 31. J. C. Kurniawan, E. Suryanto, and A. Yudistira, 'Analisis Fitokimia dan Uji Aktivitas Antioksidan dari Getah Kulit Buah Pisang Goroho (*Musa acuminate* (L.))', vol. 2, no. 03, 2013.
- 32. J. Dumanović, E. Nepovimova, M. Natić, K. Kuča, and V. Jaćević, 'The Significance of Reactive Oxygen Species and Antioxidant Defense System in Plants: A Concise Overview', *Frontiers in Plant Science*, vol. 11, 2021, doi: 10.3389/fpls.2020.552969.
- 33. D. Pujimulyani, S. Raharjo, Y. Marsono, and U. Santoso, 'Pengaruh Blanching Terhadap Aktivitas Antioksidan, Kadar Fenol, Flavonooid, dan Tanin Terkondensasi Kunir Putih (*Curcuma mangga* Val.)', vol. 30, no. 3, 2010.
- 34. D. Pujimulyani, S. Windrayahya, and Irnawati, 'The Effects of Media and Blanching Time on the Antioxidative Properties of *Curcuma aeruginosa* Roxb', *Indonesian Journal of Pharmacy*, vol. 32, no. 2, pp. 244–250, 2022.
- 35. T. Kim, J. Silva, M. Kim, and Y.-S. Jung, 'Enhanced antioxidant capacity and antimicrobial activity of tannic acid by thermal processing', *Food Chemistry*, vol. 118, pp. 740–746, Feb. 2010, doi: 10.1016/j.foodchem.2009.05.060.
- 36. S. A. Almaiman, N. A. Albadr, S. Alsulaim, H. F. Alhuthayli, M. A. Osman, and A. B. Hassan, 'Effects of microwave heat treatment on fungal growth, functional properties, total phenolic content, and antioxidant activity of sorghum (*Sorghum bicolor* L.) grain', *Food Chemistry*, vol. 348, p. 128979, Jun. 2021, doi: 10.1016/j.foodchem.2020.128979.
- 37. A. A. Feregrino-Pérez *et al.*, 'Polyphenolic Compounds and Antioxidant Capacity in Native Maize of the Sierra Gorda of Querétaro', *Agronomy*, vol. 14, no. 1, 2024, doi: 10.3390/agronomy14010142.
- 38. K. Pal *et al.*, 'Analysis of rhizome colour content, bioactive compound profiling and ex-situ conservation of turmeric genotypes (*Curcuma longa* L.) from sub-Himalayan terai region of India', *Industrial Crops and Products*, vol. 150, p. 112401, Aug. 2020, doi: 10.1016/j.indcrop.2020.112401.
- 39. D. U. C. Rahayu, D. A. Setyani, H. Dianhar, and S. Purwantiningsih, 'Phenolic compounds from indonesian white turmeric (Curcuma zedoaria) rhizomes', *Asian J Pharm Clin Res*, pp. 194–198, Jun. 2020, doi: 10.22159/ajpcr.2020.v13i7.38249.
- 40. P. Saxena, K. Selvaraj, S. K. Khare, and N. Chaudhary, 'Superoxide dismutase as multipotent therapeutic antioxidant enzyme: Role in human diseases', *Biotechnology Letters*, vol. 44, no. 1, pp. 1–22, Jan. 2022, doi: 10.1007/s10529-021-03200-3.
- 41. M. U. Sheilaadji, M. Y. Listiawan, and E. Ervianti, 'Hubungan Kadar Antioksidan Superoxide Dismutase (SOD) dengan Indeks Bakterial (IB) pada Pasien Kusta Baru Tipe Multibasiler (MB) tanpa Reaksi', vol. 31, no. 3, 2019.
- 42. M. N. Islam *et al.*, 'Superoxide dismutase: an updated review on its health benefits and industrial applications', *Critical Reviews in Food Science and Nutrition*, vol. 62, no. 26, pp. 7282–7300, Sep. 2022, doi: 10.1080/10408398.2021.1913400.
- 43. J. M. Dahanayake, P. K. Perera, P. Galappaththy, and M. Arawwawala, 'A mini review on therapeutic potentials of *Phyllanthus niruri* L.', *Trends in Phytochemical Research*, vol. 4, no. 3, pp. 101–108, 2020.

- 44. K. Jakubczyk, A. Drużga, J. Katarzyna, and K. Skonieczna-Żydecka, 'Antioxidant Potential of Curcumin—A Meta-Analysis of Randomized Clinical Trials', *Antioxidants*, vol. 9, no. 11, 2020, doi: 10.3390/antiox9111092.
- 45. P. Anton-Pampols *et al.*, 'The Role of Inflammasomes in Glomerulonephritis', *International Journal of Molecular Sciences*, vol. 23, no. 8, 2022, doi: 10.3390/ijms23084208.
- 46. K. Pyrillou, L. C. Burzynski, and M. C. H. Clarke, 'Alternative pathways of IL-1 activation, and its role in health and disease', *Frontiers in Immunology*, vol. 11, 2020, [Online]. Available: https://www.frontiersin.org/articles/10.3389/fimmu.2020.613170
- 47. P. P. Sordilo and L. Helson, 'Curcumin Suppression of Cytokine Release and Cytokine Storm. A Potential Therapy for Patients with Ebola and Other Severe Viral Infections', *In Vivo*, vol. 29, no. 1, p. 1, Jan. 2015, [Online]. Available: http://iv.iiarjournals.org/content/29/1/1.abstract
- 48. P. Liczbiński, J. Michałowicz, and B. Bukowska, 'Molecular mechanism of curcumin action in signaling pathways: Review of the latest research', *Phytotherapy Research*, vol. 34, no. 8, pp. 1992–2005, Aug. 2020, doi: 10.1002/ptr.6663.
- 49. N. Kumar, A. Vyas, S. K. Agnihotri, N. Chattopadhyay, and M. Sachdev, 'Small secretory proteins of immune cells can modulate gynecological cancers', *Seminars in Cancer Biology*, vol. 86, pp. 513–531, Nov. 2022, doi: 10.1016/j.semcancer.2022.02.008.
- 50. S. Makuch, K. Więcek, and M. Woźniak, 'The Immunomodulatory and Anti-Inflammatory Effect of Curcumin on Immune Cell Populations, Cytokines, and In Vivo Models of Rheumatoid Arthritis', *Pharmaceuticals*, vol. 14, no. 4, 2021, doi: 10.3390/ph14040309.
- 51. D. Pujimulyani, W. A. Yulianto, A. Setyowati, P. Prastyo, S. Windrayahya, and A. Maruf, 'White saffron (Curcuma mangga Val.) attenuates diabetes and improves pancreatic β-cell regeneration in streptozotocin-induced diabetic rats', *Toxicology Reports*, vol. 9, pp. 1213–1221, Jan. 2022, doi: 10.1016/j.toxrep.2022.05.014.
- 52. D. Pujimulyani, W. A. Yulianto, A. Setyowati, S. Arumwardana, and R. Rizal, 'Antidiabetic and antioxidant potential of *Curcuma mangga* Val extract and fractions', *Asian Journal of Agriculture and Biology*, vol. 6, no. 2, pp. 162–168, 2018, [Online]. Available: https://www.asianjab.com/antidiabetic-and-antioxidant-potential-of-curcuma-mangga-val-extract-and-fractions/
- 53. I. M. Zuhri, L. Mas'udah, A. Isyfi Faizati, and R. Muti'ah, 'Fitokimia Dan Farmakologi Tanaman Empon-Empon Sebagai Imunomodulator Pada Penyakit Saluran Pernapasan: Systematic Review', *J. Food Pharm. Sci*, pp. 555–569, Mar. 2022, doi: 10.22146/jfps.3378.
- 54. P. Satitsuksanoa, M. Daanje, M. Akdis, S. D. Boyd, and W. van de Veen, 'Biology and dynamics of B cells in the context of IgE-mediated food allergy', *Allergy*, vol. 76, no. 6, pp. 1707–1717, Jun. 2021, doi: 10.1111/all.14684.
- 55. V. N. Thakare, M. M. Osama, and S. R. Naik, 'Therapeutic potential of curcumin in experimentally induced allergic rhinitis in guinea pigs', *International Immunopharmacology*, vol. 17, no. 1, pp. 18–25, Sep. 2013, doi: 10.1016/j.intimp.2013.04.025.

- 56. M. H. Boskabady, F. Shakeri, and F. Naghdi, 'Chapter 7 The effects of *Curcuma Longa* L. and its constituents in respiratory disorders and molecular mechanisms of their action', in *Studies in Natural Products Chemistry*, vol. 65, Atta-ur-Rahman, Ed., Elsevier, 2020, pp. 239–269. doi: 10.1016/B978-0-12-817905-5.00007-X.
- 57. S. Khalid *et al.*, 'Therapeutic potential of Curcumin in *Curcuma longa*: A Review', vol. 5, pp. 32–41, Nov. 2020, doi: 10.5281/zenodo.4058892.
- 58. J. Mishra, A. Bhardwaj, and K. Misra, 'Chapter 8 Curcuma sp.: The Nature's Souvenir for High-Altitude Illness', in *Management of High Altitude Pathophysiology*, K. Misra, P. Sharma, and A. Bhardwaj, Eds., Academic Press, 2018, pp. 153–169. doi: 10.1016/B978-0-12-813999-8.00008-2.
- 59. J. Ming *et al.*, 'Optimal dietary curcumin improved growth performance, and modulated innate immunity, antioxidant capacity and related genes expression of NF-κB and Nrf2 signaling pathways in grass carp (*Ctenopharyngodon idella*) after infection with Aeromonas hydrophila', *Fish & Shellfish Immunology*, vol. 97, pp. 540–553, Feb. 2020, doi: 10.1016/j.fsi.2019.12.074.
- 60. K. B. Megha and P. V. Mohanan, 'Role of immunoglobulin and antibodies in disease management', International Journal of Biological Macromolecules, vol. 169, pp. 28–38, Feb. 2021, doi: 10.1016/j.ijbiomac.2020.12.073.
- 61. R. M. Srivastava, S. Singh, S. K. Dubey, K. Misra, and A. Khar, 'Immunomodulatory and therapeutic activity of curcumin', *International Immunopharmacology*, vol. 11, no. 3, pp. 331–341, Mar. 2011, doi: 10.1016/j.intimp.2010.08.014.
- 62. L. Lu *et al.*, 'Targeted immunomodulation of inflammatory monocytes across the blood-brain barrier by curcumin-loaded nanoparticles delays the progression of experimental autoimmune encephalomyelitis', *Biomaterials*, vol. 245, p. 119987, Jul. 2020, doi: 10.1016/j.biomaterials.2020.119987.
- 63. Z. Zulkarnain, C. Muthiadin, F. Nur, and St. A. Sijid, 'Potensi Kandungan Senyawa Ekstraksi Daun Patikan Kebo (*Euphorbia hirta* L.) Sebagai Kandidat Antibiotik Alami', *TEKNOSAINS*, vol. 15, no. 2, p. 190, Aug. 2021, doi: 10.24252/teknosains.v15i2.19545.
- 64. G. Donadio *et al.*, 'Interactions with Microbial Proteins Driving the Antibacterial Activity of Flavonoids', *Pharmaceutics*, vol. 13, no. 5, 2021, doi: 10.3390/pharmaceutics13050660.

# **Figures**

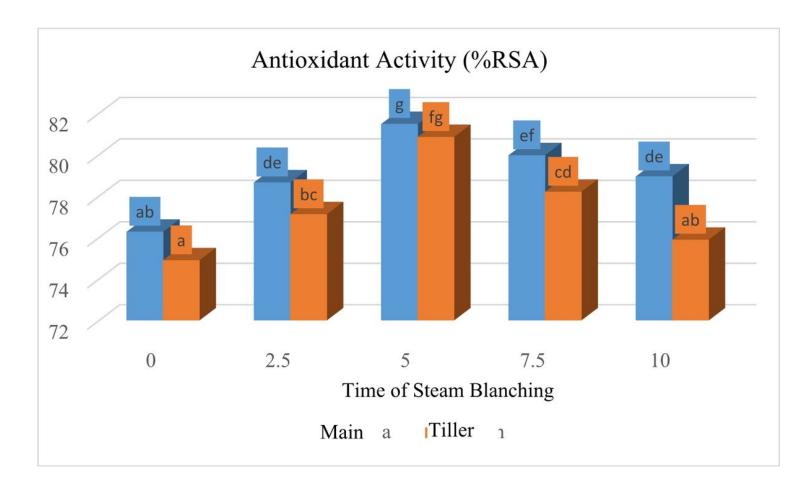


Figure 1

Antioxidant activity DPPH (%RSA)

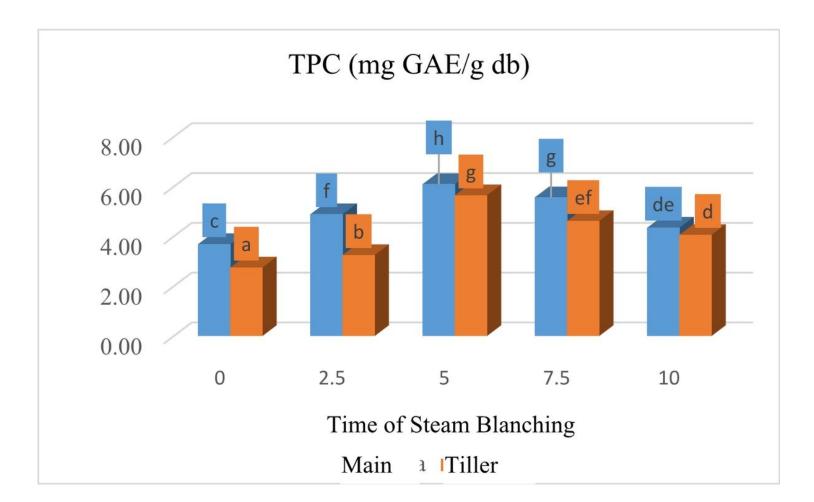


Figure 2

TPC (mg GAE/g db)