Short Communication:
Stimulatory effect of *Curcuma mangga* on immune response against *Staphylococcus aureus*

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Abstract. *Yuandani, Nugraha SE, Laila L, Silaban SD, Ramadhani F. 2020. Short Communication: Stimulatory effect of Curcuma mangga on immune response against Staphylococcus aureus. Nusantara Bioscience 12: 109-113.* Recently, the ethanol extract of *Curcuma mangga* Val. rhizomes were found to have immunomodulatory activity by enhancing phagocytic ability. This study was carried out to evaluate the effect of *C. mangga* rhizomes on antibody titer and delayed-type hypersensitivity response in normal and doxorubicin-induced rats against *Staphylococcus aureus*. The extract was administered orally at doses of 100, 200, and 400 mg/kg body weight (bw) to rats for 72 hours prior to sensitization of *Staphylococcus aureus*, then continued for 14 days. Doxorubicin (4.67 mg/kg bw) was administered on days 8 and 11 to suppress the immune system. The antibody titer of normal and immune-suppressed rats after the treatment with *C. mangga* ethanolic extract was significantly higher than the rats with negative control (P<0.05). Ethanol extract of *C. mangga* also demonstrated stimulation on delayed-type hypersensitivity response which was indicated by the increase of paw volume. The extract at dose of 400 mg/kg bw showed higher immunostimulatory activity than Levamisole as a positive control. The results indicated that the ethanol extract of *C. mangga* shows immunostimulatory activity, confirming its potential to be developed as a therapeutic agent.

Keywords: *Curcuma mangga*, *Staphylococcus aureus*, doxorubicin, antibody titer, delayed-type hypersensitivity response

INTRODUCTION

Human body has developed a remarkably sophisticated defense system consisting of various organs, tissues, and cells that are distributed throughout most of the body. It is used to repeal invasive pathogens including bacteria, viruses, fungi, and parasites such as protozoa and worms that may harm the body. Human immune response is divided into two main categories such as innate immunity and adaptive immunity (Beutler 2004). Innate immune system is our first line of defense while adaptive immune system acts as the second line of defense and provides protection against re-exposure to the same pathogen. Innate immunity is the most universal and acts the most rapidly, mostly depends on polymorphonuclear neutrophils (PMNs), peripheral blood mononuclear, and macrophage cells, whereas adaptive immune response depends upon lymphocytes, which provide long term immunity (Beutler 2004; Saroj et al. 2012). In response to invasive bacterial infection, phagocytes (such as macrophages) perform a number of phagocytic activities, including migration to the site of inflammation and adherence towards vascular endothelial cells. Then these pathogens are killed once they are recognized by the intracellular destruction (Beutler 2004; Kobayashi et al. 2005). Macrophages also initiate the adaptive immune response to most pathogens by presenting antigen to CD4+ T cells via class II major histocompatibility complex (MHC) antigen (Chapel et al. 2006).

Normally the immune system recognizes the body own tissues as 'self' without any reaction against them, but reacts only against foreign antigens. However, immune responses are not always beneficial (Descotes 2011). Defects or malfunctions in immune system can cause disorders either to the innate or adaptive immune response which can lead to illness or disease. Such disorders are generally caused by one of the following three cases such as inappropriate reaction to self-antigen (known as autoimmunity), overactive immune response (known as hypersensitivity reaction), or ineffective immune response (known as immunodeficiency) (Zhernakova et al. 2009; Warrington et al. 2011). Thus, immunomodulators are required to treat those disorders.

Immunomodulators are substances used to modulate the components of immune system, including both innate and adaptive arms of immune response, and keep them in a highly prepared state for any threat they may encounter (Patil et al. 2012). Immunomodulator substance is necessary for the treatment of various diseases or to encounter adverse events of some drugs, such as doxorubicin. Doxorubicin inhibits cell division, hence its

usage in chemotherapeutic treatment of malignant diseases. However, this drug suppresses immune response (Carvalho et al. 2009). Thus, immune-enhancing agent is required to protect the body against infectious agents. Staphylococcus aureus is a gram positive bacteria that can cause some health problems, such as pneumonia, skin infections, and endocarditis Myles et al. 2012).

There are several chemical immunomodulators available in the market. Unfortunately, some of these commercial drugs have side effects. Therefore, safer drugs are required as alternatives. Natural products can become one of the important sources of new alternative and safe immunomodulatory agents (Elgert 2009). Since ancient times, focus on the use of traditional herbs as alternative treatment has been revived all over the world. Nowadays, people also use some of the crude extracts of certain herbs as daily food supplements (Yeap et al. 2007). Thus, the research on natural products continues to explore a variety of lead structures, which may be used as templates for the development of new drugs by the pharmaceutical industry (Lahlou 2013). Along this path, several plant extracts and their isolates have been reported for their immunomodulatory activity. Previous studies reported immunomodulatory effects from *Camellia sinensis, Coriolus versicolor, Phyllanthus niruri, Rosa canina, Chromolaena odorata, Curcuma domestica, Curcuma xanthorrhiza, Garcinia mangostana, Piper nigrum, Labisia pumila*, and *Zingiber cassumunar* (Yeap et al. 2007; Chairul et al. 2009; Jantan et al. 2011; Hidanah et al. 2018; Rahayu et al. 2018; Pramudya et al. 2019).

Curcuma mangga Val rhizomes are one of the medicinal plants that has been used widely as folk medicine. *C. mangga* belongs to Zingiberaceae (Hong 2016). It is an origin of Indo-Malaysia and distributed widely in various countries, such as China, Taiwan, Thailand, and Australia. In Malaysia, it is known as temu pauh, while in Thailand it is called kha min khao. *C. mangga* rhizome has a specific smell like mangoes. This plant has been used as the empirical treatment of various diseases like cancer, fever, and stomach disorders (Malek et al. 2011). The plant has been found to have anti-cancer, anti-inflammatory, and antifungal activities (Jantan et al. 2003; Ruangsang et al. 2010; Karsono et al. 2014). The biological activities of *C. mangga* might be due to the presence of various active compounds which include flavonoids, terpenoids and steroids (Abas et al. 2005; Malek et al. 2011). Previous studies have also reported the pharmacological activities of *C. manga* as immunomodulating agent by enhancing phagocytosis ability in mice (Yuandani et al. 2019). In the present study, the activity of *C. mangga* rhizome to stimulate the immune response against *S. aureus* both in normal rats and doxorubicin-induced rats was investigated.

MATERIALS AND METHODS

Reagents and instruments

Various chemicals were used in this study, such as phosphate buffer saline (PBS) and natrium carboxymethylcellulose (Na CMC) purchased from Sigma (USA), levamisole (Soho, Indonesia), and Ethanol (SmartLab, Indonesia). Some instruments used in this study were plethysmometer (Ugo Basile, Italy) and Rotary evaporator (Heidolph, Germany).

Plant materials

Rhizomes of *C. mangga* were collected from North Sumatra, Indonesia. The plant sample was identified and authenticated by a biologist in Herbarium Medanense, Universitas Sumatera Utara, Indonesia (4679/MEDA/2019).

Extraction procedure

Curcuma mangga rhizomes were dried and ground, then it was macerated by ethanol 96% with a ratio of 1:10. Thereafter, the filtrate was combined and the solvent was evaporated by using a rotary evaporator to get ethanol extract of *C. mangga*.

Antigen preparation

Staphylococcus aureus was cultivated in nutrient broth agar (NBA) and incubated for 24 h. 1 ml Aliquot was added with 9 mL NBA. The concentration of the cell was calculated by using spectrophotometry method to obtain 1×10^8 cells/mL concentration. Next, the mixture was centrifuged at 10,000 rpm for 10 minutes at 25^0C. The supernatant was discarded. Then, the cells were resuspended with 1 ml of PBS.

Delayed-Type Hypersensitivity (DTH) response

Delayed-type hypersensitivity (DTH) response activity of the extract was investigated by the increase or decrease in paw volume based on the modified method by Ahirwal et al. (2015). Wistar rats were divided into several groups such as normal group and doxorubicin-induced group. Each group was further divided into negative control group (administered with Na CMC 0.5%), positive control (orally administered with levamisole 25 mg/kg BB), and treatment group (orally administered with *C. mangga* extract at various doses of 100, 200, and 400 mg/kg bw). *Curcuma mangga* extract or Na CMC 0.5% or levamisole were administered to the animals for 72 hours prior to sensitization of *S. aureus* (1×10^8 cells/mL) by intraperitoneal injection and then continued until 14 days. Doxorubicin (4.67 mg/kg bw) was used to suppress the immune response. Doxorubicin was administered on days 8 and 11 in immune-suppressed groups. On day 14, the rat's paw volume was measured by using plestimometer. Then, all of the test animals were injected with the same amount of *S. aureus* in the hind footpad. After 24 hours, the paw volume was measured again. This study was approved by the Animal Research Ethics Committees of Universitas Sumatera Utara (approval number 0260/KEPH-FMIPA/20I9).

Antibody titer assay

The effect of *C. mangga* on antibody titer was investigated by using agglutination method (Ahirwal et al. 2015). On day 14, before the animal was injected by *S. aureus* to evaluate the DTH response, blood samples were collected from each animal through the vein tail. The serum

was obtained by centrifuging the blood samples. The titer was determined by titrating the *S. aureus* antigen-diluted serum**.** Agglutination was observed visually. Mean log of antibody titer [2(log titer) + 1] was determined to compare the results.

Statistical analysis

The data were presented as mean ± SD and analyzed with SPSS software using a one-way analysis of variance (ANOVA) and followed by post hoc Tukey.

RESULTS AND DISCUSSION

Delayed-Type Hypersensitivity (DTH) response

Microorganisms attack in various different forms, thus various immune responses are required to encounter each type of infection (Kobayashi et al. 2005). This function is mediated by various cells and molecules which are able to recognize and destroy various pathogens and undesirable materials. Cell-mediated defenses and humoral-mediated defenses are different systems but their mechanisms are overlapping and these help to fight invading organisms (Saroj et al. 2012). The cellular response to *S. aureus* was evaluated by determining delayed-type hypersensitivity (DTH) response. Delayed-type hypersensitivity is a subtype of Type IV Hypersensitivity facilitated by CD4+ T-cells effector to the environmental or microbial antigens. This response occurs after the re-exposure to the same environmental or microbial antigens. Antigen Processing Cells (APCs) engulf in the microbial or environmental antigens, then degrade them and present their peptides fragment incorporation with MHC class II antigen on the APCs surface. The interactions T cell receptor (TCR) on T helper surface initiate the differentiation of T helper. Th1 subset releases interferon γ that activates macrophage. Subsequently, the activation of macrophage induces inflammation of local tissue (Chapel et al. 2006). In this study, the extract of *C. mangga* rhizome was able to enhance paw volume of normal and doxorubicin-induced rats after the injection of *S. aureus* (Table 1). The increase in paw volume indicated stimulation on DTH response. The stimulatory activity of *C. mangga* extract on cellular response was dependent on the dose given. The extract at dose of 400 mg/kg bw displayed higher stimulation than Levamisole as a positive control in normal group. The result was in agreement with a previous study that reported the ability of nano curcumin to enhance the DTH response (Afolayan et al. 2018).

Antibody titer

The humoral component of acquired immunity mainly is the antibody produced by B cells. After the recognition of a specific antigen, B cells proliferate and differentiate into plasma cells; releasing a large number of receptor molecules in soluble form that can be secreted. This is known as antibodies (Roitt et al. 2001). They can directly recognize protein, polysaccharide, glycolipid, or nucleic acid antigens in their natural state (undigested). Some activated B cells form memory cells, which can remain silent for a long time but can be activated immediately after the re-exposure to antigen.

Table 1. The effect of *Curcuma mangga* extracts on delayed-type hypersensitivity (DTH) response (mean ± SEM)

Groups	Samples	Paw volume (ΔV)
Normal rats	CMC Na 0.5%	0.41±0.13
	Levamisol 25 mg/ kg bw	1.63±0.09*
	C. mangga extract 100 mg /kg bw	0.88±0.03*
	C. mangga extract 200 mg /kg bw	1.41±0.04*
	C. mangga extract 400 mg /kg bw	2.18±0.20*
Doxorubicin induced-rats	CMC Na 0.5%	0.25±0.04
	Levamisole 25 mg/ kg bw	1.62±0.17*
	C. mangga extract 100 mg /kg bw	0.79±0.03*
	C. mangga extract 200 mg /kg bw	1.28±0.04*
	C. mangga extract 400 mg /kg bw	2.02±0.25*

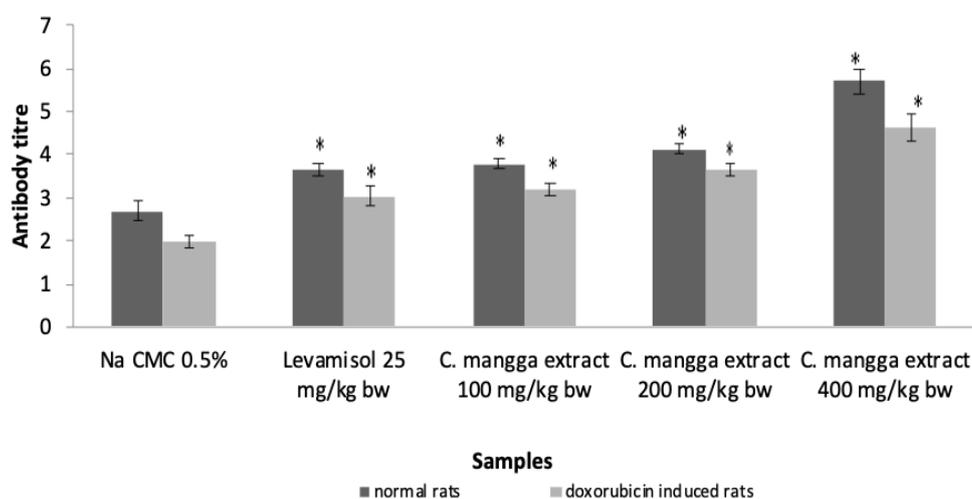Note: *$P<0.05$ significant to respective control

Figure 1. The effect of *Curcuma manga* ethanolic extract on antibody titer against *Staphylococcus aureus*; data is mean ± SEM, *$P<0.05$ significant with respective control

Moreover, B cells have another important function which is to present antigen to helper T cells (Levinson 2012). Adaptive immunity is resulted either by receiving preformed antibody which made it into another host (passive immunity) or from the exposure to environmental or microbial antigens (active immunity) (Saroj et al. 2012). Antibody (Ab), also called immunoglobulins (Ig), can specifically bind to just one antigen by the interaction of an antibody molecule (the Fab portion) against antigen. Other parts of antibody interact with other elements of the immune system such as phagocytes (Chapel et al. 2006). The effect of the extract towards the production of antibody was investigated by agglutination method. Figure 1 shows the effect of *C. mangga* extracts on antibody titer. *Curcuma mangga* rhizomes extract was able to enhance the antibody titer in the response of invading microorganisms against negative control ($P < 0.05$). The stimulatory effect was in a dose-dependent manner, indicating by the enhancement of antibody titer value as the dose increased. Surprisingly, *C. mangga* extract exhibited higher stimulation on antibody titer against *S. aureus* than Levamisole as the positive control. The stimulatory activity of *C. mangga* extract was supported by the enhancement of antibody titer value after doxorubicin was induced (Figure 1). This result aligns with the previous study reported immunostimulatory activity of *C. mangga* to extract on antibody titer against bovine red blood cells (Yuandani et al. 2018).

Various secondary metabolites present in *C. mangga* rhizome might be responsible for its immunostimulatory activity, such as terpenoids, flavonoids, and glycosides. Previous study reported that flavonoids compounds enhance the immune response (Sharma et al. 1996). Hence, it can be concluded that the ethanol extract of *Curcuma mangga* rhizomes depicted stimulatory effect on cellular and humoral mediated immunity. The stimulation was observed in both groups with normal rats and doxorubicin-induced rats, indicating the ability of *C. mangga* to enhance protection against invading microorganisms in normal and impair immune responses. Therefore, *C. mangga* could be developed into an effective immunomodulatory agent. However, further studies are required to confirm their activities on other mechanisms in immune responses.

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