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Original Article

FORMULATION AND EVALUATION OF HERBAL CAPSULE CONTAINING *CURCUMA MANGGA* VALETON and ZIJP. EXTRACT AND ITS IMMUNOSTIMULATORY EFFECTS

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ABSTRACT

Objective: The present study was carried out to formulate and evaluate *C. mangga* extract as granule mass in a capsule dosage form as well as investigate its immunostimulatory effects.

Methods: The granule mass was evaluated for its bulk density, tapped density, Carr's index, Hausner ratio, angle of repose, and flow ability. Meanwhile, capsule weight and disintegration time were determined to evaluate the *C. mangga* capsule. The immunostimulatory effects of extract in capsule dosage form were investigated by measuring delayed-type hypersensitivity (DTH) response and IgG production. There were six formula prepared (F1-F6). Formula F1-F6 were vary according to the component of adsorbent (Corn starch/polyvinyl pyrrolidone) and filler (maltodextrin/Avicel/lactose) to obtain the optimum formula.

Results: The granule mass of all formula had a good flow ability and free-flowing properties according to the Carr's index (10.26±3.38 to 17.54±1.60) and flowing time (1.05±0.05 to 1.60±0.08 seconds). Of all the capsule formula, F2 capsule containing dry extract of *C. mangga* with Avicel as the filler met the standard requirement in terms of disintegration time (2.15±0.76 min) and weight uniformity (the deviation was below than 7.5%). The capsule of *C. mangga* extract enhanced the paw thickness as compared to those of the negative control ($P<0.05$). The level of Immunoglobulin G (IgG) was also increased after treatment with the capsule of *C. mangga* extract.

Conclusion: The results suggest that the capsule of *C. mangga* extract has immunostimulatory effects, emphasizing its potential to be used for the treatment of infectious and immunodeficiency diseases.

Keywords: *Curcuma mangga*, Formulation, Capsule, Immunomodulator

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INTRODUCTION

Multiple cells and molecules that can identify and eliminate different pathogens and undesirable substances are involved in human defense [1, 2]. Phagocytes are crucial to innate immunity, while lymphocytes primarily support adaptive immunity. T lymphocytes can be classified into two main lineages based on whether they express the CD4+ or CD8+proteins on their surface. Stimulation of CD4+T cell initiates delayed-type hypersensitivity (DTH) response [3].

Th-1, Th-2, Th-17, and CD4 T regulatory (Treg) cells are the four subpopulations of CD4 T cells that carry out the functions of CD4 T cells [4]. B cells become plasma cells, which are capable of producing antibodies with the aid of th cells. A particular relationship between an antibody molecule (the Fab component) and an antigen allows each antibody to attach to only one antigen. Other antibody components communicate with phagocytes and other immune system components [5]. Immunoglobulin A (IgA), IgD, IgE, IgG, and IgM are the five types of antibodies found in humans. IgG is the immunoglobulin that predominates among them in serum. IgM first appears in the blood during the initial response to a new antigen, followed by IgG. When the production of IgG rises, IgM production falls [6].

Pathological conditions can be driven on by immune system abnormalities or dysfunction. In order to manage and treat illnesses caused on by immune system defects or dysfunction, the immune response system must be modified [7]. Immunomodulators are used to boost or reduce host defense responses in the treatment of disorders where compromised immune responses have a significant impact on the course of the disease. Immunostimulant medications are preferred for the treatment of immunodeficiency and infectious diseases, whereas immunosuppressive medications are frequently utilized to treat inflammation, allergy disorders, and organ rejection [8].

Since ancient times, the use of traditional medicines has increased. One of the medicinal plants utilized as alternative medicine is *Curcuma mangga* rhizome, which has been used to treat cancer, fever, and gastrointestinal disorders [9]. Numerous active secondary metabolites, including flavonoids, saponins, glycosides, terpenoids, and steroids, were discovered to be present [10]. Our prior studies showed that *C. mangga* has immunomodulatory effects on phagocytosis and antibody titer by in *in vivo* experiments [10, 11]. The toxic effect of *C. mangga* rhizomes was also assessed and it was shown that its LD50 was greater than 5000 mg/kg bw, indicating that it was not harmful [12]. It has also been shown that the methanol extract of *C. mangga* exhibits nitric oxide inhibitory activity *in vitro* [13]. The findings highlight *C. mangga* might be developed as an immunostimulatory agent. *C. mangga* extract need to be processed into a dosage form in order to produce a human immunostimulatory agent that is safe and efficient.

One of the most simple prepared dosage forms is capsule. This dosage form requires less method preparation compared to the other dosage form. The crucial factors that need to be considered in capsule preparation are the formulation and evaluation of the granule mass. The efficiency of capsule production, especially in hard capsule filling, is affected by the characteristics of granule mass, which related to the particle properties [14, 15]. The current study was performed to formulate the capsule containing dry extract of *C. mangga* and evaluate its immunostimulatory effects on humoral and cellular-mediated immunity.

MATERIALS AND METHODS

Materials

Chemicals and reagents

The chemicals used in this study were ethanol (SmartLab, Indonesia) and PBS (Sigma, USA). Maltodextine, lactose, corn starch, talc, magnesium stearate, methylparaben, and propylparaben polyvinyl pyrolidon (PVP) were purchased from Bratachem (Indonesia). ELISA kit of IgG was obtained from Komabiotech (Korea).

Plant materials

The plant collection was carried out in North Sumatera, Indonesia. A scientist from Herbarium Medanense (MEDA), Universitas Sumatera Utara, Indonesia, recognized the plant sample with the identity number of 5669/MEDA/2021.

Extraction procedure

The rhizomes were dried, crushed, and then extracted using ethanol as the solvent by maceration. In brief, 500 g of the rhizomes of *C. mangga* were macerated in ethanol (1:10). The solvent was then eliminated with the help of a rotary evaporator to produce an ethanol extract of the *C. mangga* rhizomes [10].

Curcuma mangga dried extract (CMDE) preparation

There were two adsorbents used to prepare the *C. mangga* dried extract (CMDE), polyvinyl pyrolidon (PVP) and corn starch. The ratio between PVP and corn starch with *C. mangga* ethanol extract was 1:2 and 1:1.5, respectively. The *C. mangga* extract was mixed with each of the adsorbents and sieved to homogenize the size. Then the mixture was dried in the drying board. Since the flowing properties of extract-corn starch powder was not good, co-processing of the dried extract (extract with corn starch) in to a granule form was conducted. The co-process was done using 10% cassava starch mucilage with wet granulation method. The term CMDE-PVP and CMDE-CS were used to represent *C. mangga* dried extract with PVP as adsorbent and *C. mangga* dried extract with corn starch as adsorbent, respectively.

The dried extracts were evaluated for their LOD value and flowing properties, including flowing time, tapped density, bulk density, Carr's index, Hausner ratio and angle of repose [15]. The flow ability was examined by measuring the length time for the bulk to flow from a funnel sing. The bulk density was determined by recording the volume (Vo) of a known weight (M) of the granules mixture and after taping until constant volume (Vt) in a measuring cylinder. The bulk density (Pb) and tapped density (Pt) were calculated as M/Vo and M/Vt, respectively. The Carr index (CI%) was determined from Pb and Pt as $\mathrm{Cl} = \frac{\mathrm{Pt}-\mathrm{Pb}}{\mathrm{Pt}}$

The Hausner ratio (HR) was determined using the formula of $\mathrm{HR} = \frac{\mathrm{Pt}}{\mathrm{Pb}}$

The angle of repose (α) was determined by measuring the height (h) and the diameter (d) when a conical pie formed from flowing over a funnel and then calculated the tangent as follow:

$tan\ \alpha = \frac{h}{r}$; α was then deduced from its tangent [15].

Granule mass preparation

The dried extracts were mixed with the excipients to prepare the granule mass for the capsule. The formulation of the granule mass was differentiated into 6 formulas with 3 different fillers, namely lactose, Avicel® and maltodextrin. Capsule shell size number 3 was chosen to prepare the capsule. The mass for each capsule was determined by subtracting the total weight of a filled capsule with the capsule shell. The capsule capacity obtained was 170 mg. The formula of the granule mass for capsule preparation can be seen in table 1.

Table 1: The formula of granule mass of *Curcuma mangga* extract capsule

Ingredients	Weight (mg)					
	F1	F2	F3	F4	F5	F6
CMDE-CS	125	125	125	-	-	-
CMDE-PVP	-	-	-	150	150	150
Corn starch	8.5	8.5	8.5	8.5	8.5	8.5
Talc	1.7	1.7	1.7	1.7	1.7	1.7
Mg stearat	1.7	1.7	1.7	1.7	1.7	1.7
Methyl paraben	0.306	0.306	0.306	0.306	0.306	0.306
Propyl paraben	0.034	0.034	0.034	0.034	0.034	0.034
Lactose	32.76	-	-	7.76	-	-
Avicel	-	32.76	-	-	7.76	-
Maltodextrin	-	-	32.76	-	-	7.76

CMDE-CS: *Curcuma mangga* dried extract-corn starch; CMDE-PVP: *Curcuma mangga* dried extract-poly vinyl pyrrolidone

Granule mass evaluation

Each granule mass formula was evaluated in term of water content, flow ability, tapped density, bulk density, Carr's index, Hausner ratio, angle of repose according to the method described in Carbinatto *et al.* [15].

Curcuma mangga capsule preparation and evaluation

Capsules were prepared with semi-automatic capsule filler. Capsule formula that met requirement of Indonesian Pharmacopoeia and showed the best-flowing properties was chosen to perform the *in vivo* study. Prepared capsules were evaluated for their weight uniformity and disintegration time according to the procedure explained in Indonesian Pharmacopoeia. Weight uniformity was determined by calculating the weight % deviations of 20 capsules [16].

Antigen preparation

Nutrient broth agar (NB) was used to grow *Staphylococcus aureus*, which was then incubated for 24 h. A 1 ml aliquot was mixed with 9 ml of NB, and the cell concentration was determined using the spectrophotometry method until 1 x 10^8 cells/ml was reached. It was also centrifuged for 10 min at 250 °C at 10.000 rpm. The supernatant was discarded. Thereafter, 1 ml of PBS was used to resuspend the cells.

Animals

Wistar male rats weighing 150–200 g were used. Animal Research Ethics Committees (AREC), University of Sumatera Utara (0135/KEPH-FMIPA/2021), evaluated all procedures.

Delayed-type hypersensitivity (DTH) response

DTH response assay was performed to determine the effect of *C. mangga* capsule on cellular immunity. The experiment was carried out by measuring paw volume following a modified previous study [17]. Animals were given capsules containing *C. mangga* extract (100, 200, or 400 mg/kg BW) orally 72 h before being exposed to *S. aureus* (1 x 10^8 cells/ml) by intraperitoneal injection. This treatment was continued for 14 d. The negative control group, meanwhile, received avicel as a component of the basic formula. A positive control was Stimuno® (50 mg/kg BW). On day 14, the paw volume

was measured, then, all the rats were challenged by same amount of *S. aureus* in hind footpad.

Immunomodulatory assay on antibody production

A modified method of an earlier study was used to assess the impact of the extract on Immunoglobulin G (IgG) production [17]. The animals were given capsule containing *C. mangga* extract at dosages of 100, 200, and 400 mg/kg bw 72 h before getting sensitized to *S. aureus* (1 x 10^8 cells/ml) by intraperitoneal injections. The treatment continued for 14 d. The negative control group received avicel as a component of the basic formula. A positive control was Stimuno® (50 mg/kg bw). After the experiment, blood was drawn, and the serum was collected to measure the IgG levels.

Statistical analysis

The data were presented as mean±SD and analyzed using Kruskal-Wallis and Post hoc Mann-Whitney. P<0.05 was indicated as a significant difference.

RESULTS

Characteristic of *Curcuma mangga* dried extract (CMDE)

The dried extract of *C. mangga* showed an orange-brown colour. The colour of the dried extract is different according to the type of plant used in extraction. This result was supported by a previous study which reported that the dried extract of *Picria fel-terrae* leaves was greenish brown [18]. The part of the plant used in the extraction and the content of secondary metabolites in the plant part also interfered to the colour of the extract. The adsorbents used in the drying process of *C. mangga* ethanol extract were PVP and corn starch. These two adsorbents gave different characteristics of the dried extract. The extract that dried with PVP had granule form and the extract dried with corn starch had fine powder form with smaller particle size (fig. 1). The extract that dried with corn starch (CMDE-CS) showed bad flowing property due to the very fine particle size. However, CMDE-CS had lower water content compared to the CMDE-PVP. Therefore, CMDE-CS was co-processed in to granule form to gain better-flowing property. The characteristics of CMDE-PVP and CMDE-CS are presented in table 2.

Characteristic of capsule granule mass

The addition of excipients in granule mass for capsule preparation was aimed to increase the properties of the dried extract and to fill the space in the capsule shell. The mixture of all of the ingredients was shown in fig. 2. Generally, each formula of granule mass gave shorter flowing time and higher Carr's index compared to the dried extract alone. Particularly, in the granule mass formula using CMDE-CS showed slightly higher bulk and tapped densities compared with the CMDE-CS alone. However, formula with CMDE-PVP demonstrated a significant increase of bulk and tapped densities value compared to the CMDE-PVP alone. The influence of fillers addition also exhibited differences between the formula. The most significant effect of the fillers was noticed in the Carr's index parameter. The smallest Carr's index was generated by maltodextrin as seen in F3 (with CMDE-CS) and F6 (with CMDE-PVP). The characteristic results of capsule granule mass can be seen in table 3.

Table 2: Characteristic results of *Curcuma mangga* dried extract

Parameters	CMDE-PVP	CMDE-CS
Tapped density (g/ml)	0.57±0.04	0.31±0.02
Bulk density (g/ml)	0.50±0.03	0.29±0.02
Carr's index (%)	9.82±2.26	7.28±3.44
Hausnerr ratio	1.11±0.03	1.08±0.04
Angle of repose (°)	21.79±1.48	27.12±2.75
Flowing time (sec)	3.28±0.70	3.07±0.61
LOD (%)	8.69±1.34	4.69±1.08

CMDE-CS: *Curcuma mangga* dried extract-corn starch; CMDE-PVP: *Curcuma mangga* dried extract-poly vinyl pyrrolidone; n: 3 Data=mean±SD.

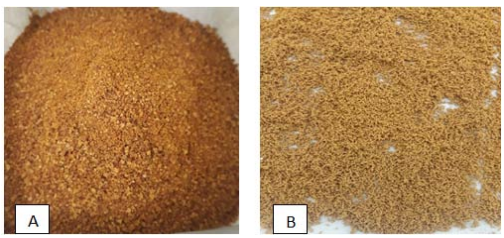

Fig. 1: The dried extract of *Curcuma mangga* with PVP (A) and corn starch (B) as the adsorbents

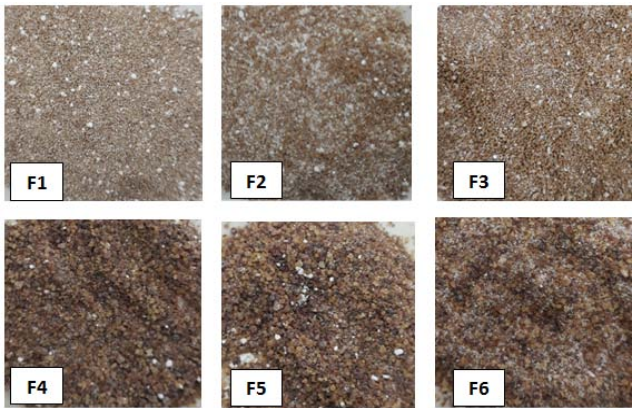

Fig. 2: The mixture of capsule granule mass, F1= mixture of CMDE-CS with lactose as filler, F2= mixture of CMDE-CS with Avicel as filler, F3 = mixture of CMDE-CS with maltodextrin as filler, F4 = mixture of CMDE-PVP with lactose as filler, F5 = mixture of CMDE-PVP with Avicel as filler, F6= mixture of CMDE-PVP with maltodextrin as filler

Table 3: Characteristic results of granule mass for *Curcuma mangga* capsule

Parameters	F1	F2	F3	F4	F5	F6
Tapped density (g/ml)	0.42±0.00	0.38±0.01	0.42±0.01	0.73±0.01	0.67±0.02	0.73±0.00
Bulk density (g/ml)	0.35±0.01	0.31±0.01	0.37±0.00	0.61±0.00	0.58±0.01	0.64±0.00
Carr's index (%)	16.40±2.33	17.54±1.60	10.26±3.38	16.34±1.20	14.32±1.97	12.26±0.09
Hausnerr ratio	1.19±0.03	1.19±0.03	1.11±0.05	1.19±0.02	1.16±0.03	1.13±0.01
Angle of repose (°)	22.13±0.28	22.61±1.23	23.58±1.21	22.62±1.13	20.29±1.80	21.05±1.76
Flowing time (sec)	1.60±0.03	1.60±0.08	1.45±0.02	1.05±0.05	1.19±0.10	1.24±0.09

n: 3, Data=mean±SD

Capsule evaluation

Weight uniformity

Capsule weight uniformity was conducted to ensure that each capsule contained a consistent dose of CMDE. Indonesian Pharmacopoeia stated that in 20 capsules, there should not more than 2 capsules has a deviation more than 7.5% and none of the capsule exceed 15% deviation [16]. As shown in table 4, F1 had one capsule with deviation value more than 15%; F3 had 7 capsules that exceed 7.5%; F4, F5 and F6 also showed capsules that did not meet the requirement of Indonesian Pharmacopoeia. There was only F2 capsule formula that had fulfilled the Indonesian Pharmacopoeia requirement.

Disintegration time

The disintegration time of formula prepared was 3.20±0.67, 2.15±0.76; 3.20±1.11; 10.16±5.56, 3.46±1.37, and 4.13±0.84 for F1-F6, respectively. All of the formula met the requirement of Indonesian Pharmacopoeia disintegration time, which is not more than 15 min. The fastest formula to disintegrate was shown by F2 with 2.15±0.76 min. According to the capsule evaluation, it was obtained that F2 met all the requirements of Indonesian Pharmacopoeia in terms of weight uniformity and disintegration time. This formula was then chosen as the formula to prepare capsule dosage form for animal studies.

Delayed-type hypersensitivity (DTH) response

The administration of *C. mangga* capsule of formula F2 enhanced the footpad thickness of rats as compared to those of negative control (P<0.05). Of all samples tested, *C. mangga* capsule at dose of 400 mg/kg bw demonstrated the highest stimulation on DTH response (fig. 3). Suprisingly, the stimulation was higher than those of positive control with paw volume of 3.14±0.19; 2.14±0.21, for *C. mangga* capsule and positive control, respectively.

Table 4: Weight uniformity result of *Curcuma mangga* capsules

Capsule No.	Deviation (%)					
	F1	F2	F3	F4	F5	F6
1	2.70	3.90	2.30	14.04	7.25	6.34
2	2.70	2.10	8.05	3.15	1.45	4.68
3	2.70	3.90	9.20	2,58	4.35	6.34
4	2.70	3.90	3.45	2.58	7.25	0.83
5	8.11	2.10	8.05	2.58	7.25	0.83
6	18.92	2.10	345	2.58	1.45	6.34
7	2.70	2.10	9.20	2.58	10.14	0.83
8	2.70	3.90	2.30	8.88	7.25	4.68
9	2.70	3.90	9.20	2.58	1.45	4.68
10	2.70	2.10	2.30	2.58	1.45	0.83
11	2.70	3.90	3.45	3.15	7.25	6.34
12	13.51	3.90	3.45	2.58	1.45	0.83
13	8.11	2.10	3.45	14.04	15.94	4.68
14	2.70	2.10	2.30	3.15	7.25	0.83
15	2.70	3.90	9.20	3.15	15.94	0.83
16	2.70	3.90	8.05	8.88	7.25	4.68
17	2.70	2.10	9.20	3.15	1.45	0.83
18	2.70	2.10	8.05	3.15	7.25	11.85
19	2.70	2.10	13.79	8.31	1.45	11.85
20	2.70	2.10	13.79	8.88	1.45	0.83

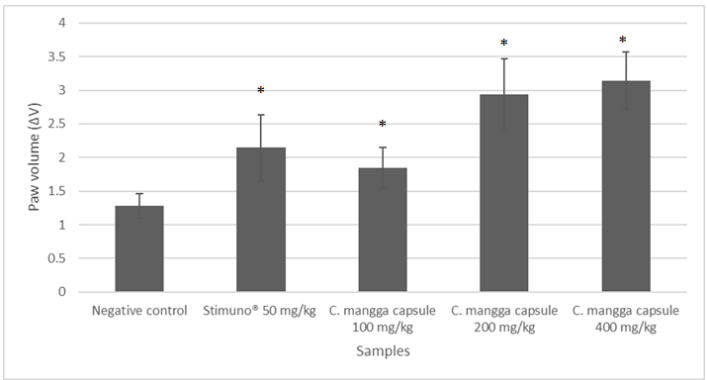

Fig. 3: Effect of capsule containing dry ethanol extract of *C. mangga* on delayed type hypersensitivity response of (Data: mean±**SD, n: 5; *P<0.05 significant to respective control)**

Immunomodulatory assay on antibody production

Fig. 4 shows that *C. mangga* capsule of formula F2 increased the production of immunoglobulin G from rats after infected by *S. aureus,* indicating the enhancement of humoral immunity. The enhancement of IgG production of *C. mangga* capsule was in a dose-dependent manner. The result was in agreement with DTH response, of all the samples, the capsule containing dry ethanol extract of *C. mangga* at the dose of 400 mg/kg bw demonstrated the strongest stimulation.

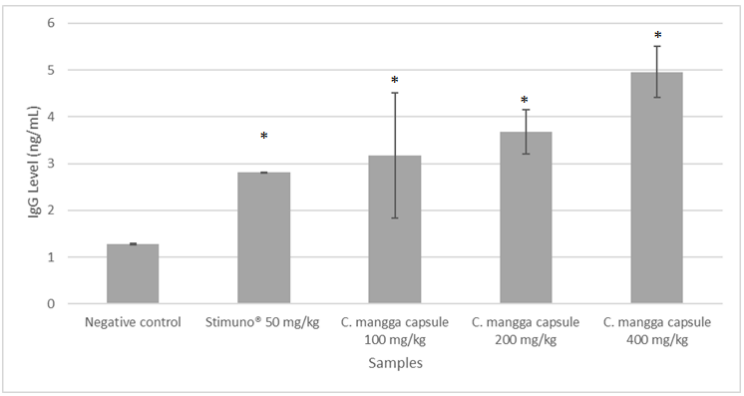

Fig. 4: Effect of capsule containing dry ethanol extract *C. mangga* extract on immnunoglobulin G production (Data: mean±SD, n: 5; *P<0.05 significant to respective control)

DISCUSSION

The bulk densities of the formula containing CMDE-PVP was higher than 0.5 g/ml, indicating that *C. mangga* granules mass had denser characteristic as compared to the those of formula containing CMDE-CS. Usually denser particles experience great limitation to flow due to the particles contact to each other. The bulk density value for each formula was lower than the tapped density value, indicating that there were empty spaces between the particles. However, the difference between tapped and bulk densities of formula containing CMDE-PVP showed greater compared to the tapped and bulk densities difference of formula containing CMDE-CS. The difference between bulk and tapped densities is related to the inter-particle porosity. Greater the difference between tapped and bulk densities resulting higher value of inter-particle porosity. Hence, there are many of pores and empty space between the particles in the formula.

Short flowing time below than 10 seconds indicates that the formula has good flowability. All of the formula showed flowing time between 1.05±0.05 to 1.60±0.08 seconds. It indicated that all of the formula had excellent flowing property. This indicator was supported by the indirect flow-related properties, these include angle of repose, Carr's index and Hausner ratio. All the formula showed the Hausner ratio below than 1.25. The result suggest that the *C. mangga* granule mixture showed good flowing characteristic. The Carr's index below than 16% showed free-flowing property [15]. The Carr's index of all of the formula demonstrated value in the range of 10.26±3.38 to 17.54±1.60. According to Carr category on Carr's index and Hausner ratio [19], F3 and F6 revealed excellent flow properties; meanwhile, F1, F2, F4 and F5 demonstrated good flow property. Of all the formula, F2 showed the highest Carr's index, indicating that F2 had less free-flowing but still in good flowing category [19]. All the formula showed the angle of repose in the range of 20.29±1.80 to 23.58±1.21, which were still below than 30 °indicating good flow ability [20]. Capsule evaluation in terms of weight uniformity and disintegrating time are required to ensure that the capsules produced are consistent and homogeny. The weight uniformity is one parameter to calculate the weight of each capsule content whereby indicating the consistency of the dose distribution in each capsule. In the capsule evaluation result, it was showed that only F2 had fulfilled the Indonesian Pharmacopoeia requirement for weight uniformity. These phenomena could be influenced by several factors, such as the homogeneity of the granule mass and the accuracy of the filling process. Therefore, only the formula that met the Indonesian Pharmacopoeia capsule evaluation should be used for the animal study to evaluate the effectiveness of *C. mangga* extract as immunostimulatory in capsule dosage form. The *C. mangga* capsule of F2 formula was proceeded to animal study.

An *in vivo* investigation utilizing Wistar rats infected with *S. aureus* was conducted to assess the immunomodulatory properties of capsules containing dried ethanol extract of *C. mangga* rhizomes. To learn more about the immunomodulatory effects of *C. mangga* extract on DTH response and antibody generation, *in vivo* investigations on immune response were carried out. *S. aureus* was injected into every animal used in this study. Rats with *S. aureus* infections had their paw volume increased by the *C. mangga* capsule. The increase in paw edema is a sign that the rat immune system's DTH response has been stimulated. The result aligned with our previous studies, which showed that *C. mangga* extract stimulated phagocytosis activity [10]. The examination into the development of antibodies also showed that *C. mangga* capsule boosted IgG production in rats. When *C. mangga* capsule was administered at a dose of 400 mg/kg bw, it showed the greatest activation of the DTH response and IgG production among all the samples.

CONCLUSION

The granule mass of *C. mangga* dried extract shows good flow ability property, as well as the capsule formulation of F2 met the requirement of Indonesian Pharmacopoeia. Furthermore, the capsule containing dried extract of *C. mangga* was able to stimulate the immune response, especially on the DTH response and antibody production. The results indicate that *C. mangga* capsule has the potential to be developed into a marketed immunomodulating product. However, further studies are required to evaluate the effect in humans by clinical study.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that they have no conflicting interest.

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