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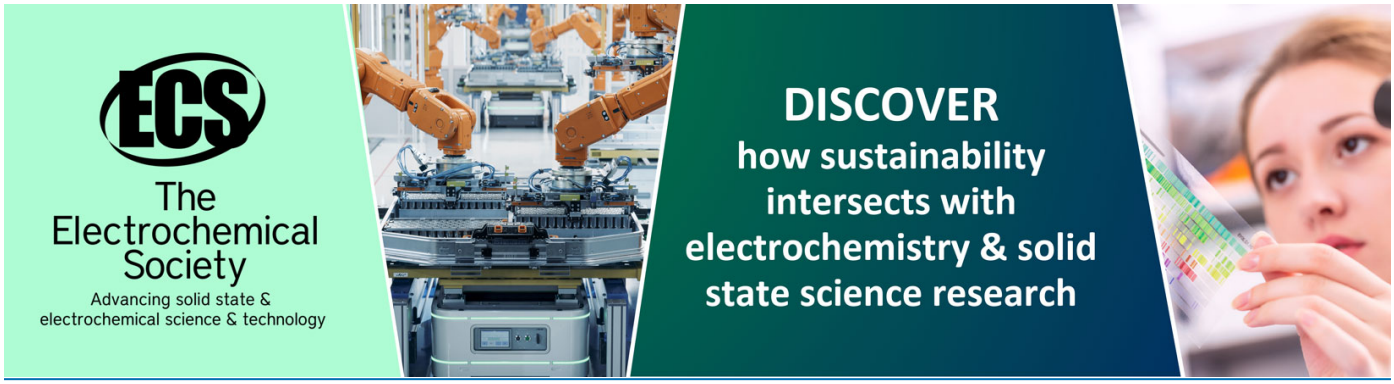

Antibacterial Mangosteen (*Garcinia mangostana* Linn.) peel extract encapsulated in Chitosan

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Abstract. Mangosteen peel extract contains xanthone compound having phenol basic structure. Because xanthone is insoluble in water, therefore mangosteen peel extract encapsulated by polymer substance through encapsulation technology is necessity. Mangosteen peel were powdered to 3 sizes. The nanomangosteen peel ethanol extract was encapsulated in various of concrentration chitosan. The particle size of encapsulated products were determined by particle size analyzer (PSA) and also analyzed of antibacterial activities . The nanomangosteen peel extract showed antibacterial activity stronger impact to 3 tested bacterial: *S. aureus*, *B. cereus*, and *S. flexinery* than 40 mesh and 20 mesh size. Encapsulated of nanomagosteen peel extract with chitosan showed that the particle size of PN, AN, and BN were 308.30, 342.42 ,and 421.26 nm respectively with polydispersity index (PI) of 0.14, 0.31, and 0.11 respectively, however antimicrobial activities of encapsulated products still have not satisfied yet.

1. Introduction

Nowadays, new trendsetter in science technology is nano technology that is material technology engineering by size accuracy until nanometer scale. Nanoparticle has been used as one of physical approach to change and increase pharmacokinetic and pharmacodynamics from several of drug molecules. Recently, nanoparticle from polymer substance whether natural or synthetic polymer is used as potential drug delivery system, for spreading ability in body organs during certain time, and ability to deliver drug in the right target. Nanoparticle from biodegradable and compatible polymer subtance is one of good development to deliver drug as this speciment form was thought to be able to be adsorbed completely in digestive system after entering body [1]. One of natural polymers developed was chitosan. From former research of Sugita *et al*. [2] , the best of three chitosan formulas have encapsulated ketoprofen. and the products have efficiency of encapsulated of 87.5% with average of size particle aroud 222,1 nm. Hardi *et al.* [3] reported that ketoprofen activity with concentration of 5.95 mg/L in 500 mg microcapsule is able to inhibit COX-2 enzyme work until 90,86%. This value is higher than ketoprofen in conventional form, 88%, and even is higher than ketoprofen adsorbed in β-cyclodextrin complex [4]

Now, modern countries are vying to take lead in this nanotechnology, such as US, West European countries (England, Germany, France, etc), Asia (Japan, South Korea, Taiwan, China, Israel, etc).

Indonesia also developed this technology in some areas like nanocomposite, nanoparticle, nanostructure material, nanotubes, nanocatalyst, and nanofilter, but has not yet penetrated into herbal nanoparticle technology. While in China, herbal nanoparticle production attracted a lot of attentions especially for China traditional herbal [5]. Indonesia has plant biodiversity, in which more than thousand million plants grow in Indonesia land, and one of them is mangosteen(*Garcinia mangostana* L).

Mangosteen is Indonesian tropical plant which traditionally has been used as anticancer herbal [6,7,8], antimalarial herbal [9,10], immunomodulator herbal[8], heart treatment, atherosclerosis (plaque in blood vessels), hypertension, and thrombosis [11]. Claim of various properties is thought to be related with secondary metabolites content in mangosteen peel which is rich in xanthone compound. Xanthone content in mangosteen peel reaches 40% and was thought to be responsible for pharmacology activity as anti-oxidant, anti-proliferative, anti-inflammation, and antimicrobial [11]. It was generally known that xanthones is polyphenol cyclic ketone compound ($C_{13}H_{18}O_2$). Almost all of xanthone derivative molecules have phenol functional group, so xanthones are often called as polyphenol. Because of astringent taste from its peel, xanthone insolubility in water, and requirement to maintain that compound stability in order to make the biological activity remains vibrant, so mangosteen peel extract encapsulation by polymer substance through encapsulation technology is required. Therefore, herbal based nanoparticle with mangosteen peel extract and the assay to antimicrobial activity are developed through this research. Nanoparticle gives a lot of advantages such as increases solubility, decrease medical dosage, and increase herbal drug absorption [12]. It is wished from this research, especially through nanoparticle technology innovation, mangosteen peel extract strength as antimicrobial could be increased.

2. Experimental procedures

2.1 General Experimental Procedures

Antibacterial activity was conducted using disc diffusion methods. Three bacterias, i.e. *Staphylococcus aureus, Bacillus cereus,* and *Shigella flexinery* from Departement of Biology IPB were used for antibacterial activity assays. Tetracycline was selected as positive control while dimethyl sulfoxide (DMSO) was used as negative control. Inhibition index was measured with the following equation (Equation 1).

$$Inhibition\ index = \frac{Inhibition\ zone\ of\ sample}{Paper\ disc\ diameter} \qquad (1)$$

2.2 Mangosteen Peel Crude Extract Preparation

Mangosteen peel simplicia was smoothed to 3 particle size that is 841 μm, 420 μm, and 213,6 nm. As much as 500 gram from every particle size were exhaustively extracted three times with ethanol (1:3) at room temperature and while stirred occasionally. After filtering and evaporating the solvent, 23.53g, 20.83g and 25.68g crude extract, respectively were yielded.

2.3 Mangosteen Peel Crude Extract – Chitosan Encapsulated Particle Production and Characterization

Chitosan formula used refers to Sugita *et al.* [2] research result which is shown in Table 1. Every formula was mixed with 0,2 mg/mL mangosteen peel crude extract. As much as 200 mL chitosan solution was added by 80 mL tripolyphospate (TPP) solution, then the mixture was added by mangosteen peel crude extract and oleic acid solution while stirred in room temperature. After that, the mixture was sonicated for ± 3 hours in frequency of 20 kHz and amplitude of 20 %. Then, solution was centrifuged by 15.000 rpm for 30 minutes. The obtained supernatant was changed into powder by using spray drier. Nanoparticles granules were measured by PSA (particle size analyzer).

Table 1 Combination of chitosan concentration, TPP, and oleic acid

Formula	Chitosan % (b/v)	TPP (mg/mL)	Oleic Acid (mg/mL)
P	3.0	0.84	1.5
B	2.5	0.84	0.8
A	2.5	0.84	0.1

Source: *Sugita et al.* [2]

2.4 Antimicrobial Activity Test of Mangosteen Peel Crude Extract With and Without Chitosan Encapsulation

The used method was micro-dilution. Extract is dissolved in DMSO and made in various concentration at concentration range of 15,6 - 2000 ppm. Medium NB sample and inoculant bacteria were placed into 96 sterile microplate and incubated for 24 hours. Bacteria used for this research were *S. aureus* and *B. cereus*. The determined parameters were minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC).

3. Results and Discussion

Water content of three samples (841 μm, 420 μm, and 213,6 nm) are similar, that is around 11 %, but yield of crude extract were different from 3 different size of mangosteen peel sample. Yield of nano size mangosteen peel is the highest among the other size. Because as the particle size become smaller, sample contact area increases. Hence, it enlarges contact between samples and eases solvent to penetrate into sample when extraction process takes place. The impact of downsizing was significantly seen in extract yield, but was not seen in water content.

Table 2 shows inhibitory diameter measurement result from various size mangosteen peel extract. From 3 tested bacteria, 2 bacteria are classified into gram positive bacteria (*S. aureus* and *B. cereus*) and 1 bacteria is a gram negative (*S. flexineri*). They showed inhibition indicated by inhibitory area at test agar plate. Inhibitory diameter average of *S. aureus* for 3 extract sizes is the highest among the other bacteria. Yet, measured inhibitory diameter (mm) is still lower than kanamycin (positive control) inhibitory diameter. From those 3 extract sizes, inhibitory diameter average of nano mangosteen peel extract is the highest among the other extract sizes.

Table 2. Bacteria inhibitory diameter average of various sizes mangosteen peel extract

Bacteria	Extract size	Inhibitory diameter average (mm) from 3 replicate in test concentration (mg/mL)						
		Kanamycin (0,05)	0,12	0,10	0,08	0,06	0,04	0,02
S. aureus	Nano (213,6 nm)	16.3	7.8	7.5	6.7	6.7	6.5	6.3
	420 μm	19	8.2	7.5	7	7	6.3	5.8
	841 μm	18.3	7.7	7.2	6.5	6.8	6.8	6.2
B. cereus	Nano (213,6 nm)	9.3	7.3	7	6.8	6.7	6.5	6.3
	420 μm	9.2	7.3	7	6.8	6.3	6.2	6.2
	841 μm	9	6.7	6.5	6.2	6	6.2	6
S. flexineri	Nano (213,6 nm)	17	7.2	6.7	6.7	6.3	6.1	6
	420 μm	13	6.2	6.1	6.2	5.8	5.9	6.3
	841 μm	14.7	6.2	6.6	6	5.8	5.7	5.5

Data in Table 2 showed that generally, as the extract concentration increases, the measured inhibitory diameter also increases. The nano mangosteen peel crude extract showed slightly higher inhibition than the other two having bigger size (841 and 420 μm). With the smaller size, this is become possible, since contact area become larger, so more components contained in the sample are extracted and synergized to inhibit bacteria activity. Besides, probably as the size become smaller, the components undergo bacteria cell wall easier to influence that bacteria activity. It is as stated by Prusty *et al*. [12], nanoparticle gives many advantages such as increases herbal drug absorption and solubility compared with drug preparation roughly.

Poeloengan and Praptiwi [13] stated that gram positive bacteria cell wall has single layer containing 1-4% lipid, while gram negative bacteria cell wall has three layers consisting of lipoprotein, phospholipid outer membrane, and lipopolysaccharide. Analysis data of *S. flexineri* shows inhibition indicated by transparent zone existence, while that zone is not exist for *E.coli*. Whereas those bacteria are classified into gram negative bacteria. It may be caused by cell wall thickness difference of those both bacteria. Gram negative bacteria has thick cell wall that is peptidoglycan (2-7 nm), existing between inner and outer membrane. The outer membrane (7-8 nm thick) consists of lipid, protein, and lipopolysaccharide. Peptidoglycan (murein) is bacteria cell wall main component which is rigid. It is also responsible to maintain cell integrity and determine its form. Gram negative bacteria (like *E. coli*) has double membrane system in which plasma membrane is covered by permeable outer membrane.

A compound involving as antibacterial, generally do activity through inhibition in cell wall, in membrane function, in protein and nucleic synthesis, protein molecule change, and also enzyme inhibition [14]. Based on Romas *et al.* [15] xanthone, saponin, terpenoid, tannin, and flavonoid compounds in mangosteen peel have antimicrobial activity. Xanthone can retard cell replication. Saponin can increase surface tension of bacteria cell wall so it will be strained very strongly and causes cell membrane destruction since the most important components such as protein, nucleic acid, and nucleotide serve as bacteria survival, out of the cell. The lipophilic terpenoid can destroy cell membrane, and then tannin can inactivate bacteria cell adhesion (attached molecule at host cell) existing in cell surface which is able to inhibit protein transport enzyme through cell membrane, while flavonoid has very active property to slow down bacteria and fungi growth. Ardananurdin *et al.* [16] stated that polyphenol inhibits microorganism enzyme work through oxidized compound structure, probably through reaction with sulfidril functional group or through nonspecific interaction with microorganism protein. Besides, polyphenol is also able to denaturate bacteria protein. Based on this data, mangosteen peel extract nano is continued to encapsulation stage with A, B, and P chitosan formula.

Mangosteen peel crude extract encapsulation process was conducted by chitosan through ionic gelation. Nanomangosteen peel crude extract is encapsulated by 3 kinds formula used by Sugita *et al.* [2]. Those 3 chitosan-TPP formula gave better nanoparticle size and adsorption efficiency for ketoprofen drug, that is 222 nm and 87% respectively. It is wished that by using Chitosan-TPP cross linked as coating is able to control its release. Soppimath *et al.* [17] stated that drug delivery system in nanoparticle form more facilitate the active compound to enter or diffuse through biological membrane toward blood stream system than conventional drug form (Figure 1). TPP and oleic acid in chitosan formula serve as crosslinking agent and surfactant respectively. TPP enhances chitosan membrane and oleic acid existence will stabilize emulsion in every formula mixture, since 3 formula used are *o/w* (oil in water) emulsion type. Oleic acid addition in the mixture serves in decreasing inters surface tension [18]. Chitosan formula used are A, B, and P formula (Table 1) and the encapsulated result is then called as AN, BN, and PN formula.

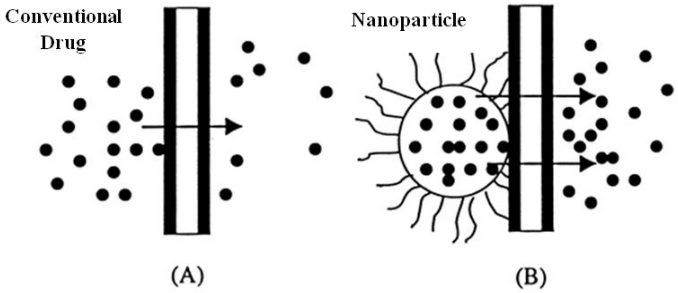

Figure 1 Diffusion process of conventional drug (A) and nanoparticle coated-drug (B) through biological membrane [17]

Biological membrane passed by active compounds (drug) is blood-brain barrier (BBB) having tight endothelial cell path so it will limit drug ability to pass and go toward central nervous system (CNS). By using nanoparticle, it will bind to endothelial core layer so drug will pass biological membrane easier [17]. The releasing of curcuma extract from encapsulation system was reported by Herdini *et al.* [19]. It stated that curcuma extract released from chitosan-alginate membrane is controlled by diffusion mechanism. That diffusion process takes place at 37ºC (human body temperature), in which membrane will expand and in the other hand, body liquid existence will cause membrane become swollen so the active compounds will diffuse out of the coating. Controlled diffusion process from those active compounds, active compounds releasing will pass biological membrane with more controlled than conventional active compound form which is very passive.

Chitosan nanoparticle formation mechanism is by ionic interaction between chitosan polycationic (positive functional group because of protonated amine group, $-NH_3^+$) and TPP polyanionic (negative functional group because of phosphate ion existence, $-PO_4^-$). This interaction creates stable matrix which make extract easier to be trapped, and release again from coating matrix [20]. The used parameters to predict chitosan degradation into smaller molecules is through turbidity value measurement. The mixture is sonicated to observe ultrasonic wave acoustic property propagated through medium. Sani *et al.* [21] stated that sonication method will cause cavitation or micro bubbles formation, and when those bubbles reach certain volume in which they are not able to absorb energy anymore, they will be unstable and finally rupture. This change is characterized through turbidity value. The measured turbidity for AN, BN, and PN formula are 32.6, 37, and 58.5 NTU respectively. The process is continued to centrifugation and the obtained supernatant turbidity is measured. Centrifugation process decrease turbidity value of those 3 formula (AN, BN, and PN) to 23.6, 19, and 54 NTU respectively. Turbidity value decrement indicates particle size downsizing. Analysis result of 3 formula by PSA shows that the three formula gives particle size less than 450 nm with polydispersity index (PI) $\leq$ 0,3. PI value visualizes particle size distribution. As the PI value become lower, the uniformity degree become better [22] and also shows stability of nanoparticle system. Data in Table 3 showed that PI value for all formula $\leq$ 0,3. It means the formed system is mono dispersion or particle size distribution which is tend to be narrow and the particle is not tend to form aggregate [23]. PSA analysis result for 3 formula is shown in Table 3. The antimicrobial activity advance test is only applied to PN formula as its size is the smallest among of all samples, and the chosen microbe is *S aureus*.

PN sample antimicrobial activity test was conducted by micro dilution method using 1 bacterium that is *S. aureus* with tetracycline antibiotic as positive control. PN sample is able to inhibit *S. aureus* growth by minimum inhibition cencentration (MIC) value at concentration of 2000 ppm. This value is still not satisfactory since extract without encapsulation has MIC value at concentration of 500 ppm. Inhibitory activity from those 2 samples are also still high than tetracycline concentration as positive control that is 62,5 ppm. From minimum lethal concentration (MLC) observation result, mangosteen peel extract without encapsulation can kill *S. aureus* at concentration of 2000 ppm, while PN sample still does not show lethal activity. It is probably caused by active compounds which are still not released perfectly since chitosan matrix expansion is not maximum, so active compound diffusion is still not released or encapsulated extract concentration is not maximum. Nata *et al.* [24] reported that drug diffusion behavior

through chitosan membrane is begun with swelling process followed by membrane pores opening so drug releases from matrix. Roihanah *et al.* [25] stated that one of factors influencing antibacterial compound to inhibit or kill bacteria is depend on concentration. Mangosteen peel extract concentration used is 0,2 mg/mL.

Table 3 Mangosteen peel extract-chitosan formula encapsulated size and polydispersity index (PI) value

Sample	Concentration		Particle size (nm)	Polydispersity index (PI)
	Chitosan (%b/v)	Oleic acid (mg/mL)		
AN	2,5	0,8	342,42	0,305
BN	2,5	0,1	421,26	0,114
PN	3,0	1,5	308,30	0,140

This chitosan formula had ever been used to encapsulate methanol extract of *C. fistula* leaves and *D. repens* fruit by using concentration of 0,01% (b/v) with particle size in the range of 80-475 nm, and PI value of 0.9 - 4.2. Wijaya *et al.* [26] reported that both particles are able to inhibit *P. falcifarum* growth. IC_{50} of chitosan formula - *C. fistula* leaves methanol extract and chitosan - *D. repens* fruit methanol extract are 0.004 and 0.08 μg/ml respectively. Saha *et al.* [27] reported that ampicillin drug coated by chitosan-TPP nanoparticle showed higher inhibitory diameter (14 mm) than conventional ampicillin (12 mm) after 48 hours. Hardi *et al.* [3] used that chitosan formula to encapsulate ketoprofen drug and *in vitro* anti-inflammation assay to COX-2 enzyme work. Ketoprofen concentration used for anti-inflammation assay is 5.95 mg/L in 500 mg nanoparticle, while conventional ketoprofen concentration giving therapy effect is 15-25 mg/L, so to reach that therapy effect around1.5 g nanoparticle is required. Ketoprofen-chitosan coated is able to inhibit COX-2 enzyme work until 90.86%, higher than conventional form (88.73%). Ultra sonication process does not destruct the compounds and still gives high activity even though in the manufacturing process, specimens were exposed with high heat.

4. Conclusion

The nanomangosteen peel extract showed antibacterial activity stronger impact to 3 tested bacterial: *S. aureus*, *B. cereus*, and *S. flexinery* than 40 mesh and 20 mesh size, however its encapsulated form has not shown satisfactory antibacterial activity. The PN has particle size of 308.30 nm, lower than AN and BN, respectively with polydispersity index (PI) of 0.14.

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