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Antimalarial Drugs Based on Chitosan Nanoparticles of *Cassia fistula* L. and *Duranta repens* L. Fruits Methanol Extract

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ABSTRACT

Cassia fistula L. and Duranta repens L. are medicinal plants which are widely used in treating a variety of diseases, including malaria. The purpose of this study is to synthesize chitosan nanoparticles of Cassia fistula L. leaves and Duranta repens L. fruit methanol extract as antimalarial drug and to determine the in vitro IC_{50} values. Plant chlorophyll and tannin contents of both extracts were eliminated, because they do not have antiplasmodium activity (IC_{50}>100 mg/ml). Synthesis formula A, B, and P falls within the criteria of nanoparticles with particles size range of 80-475 nm. The formula with poloxamer 188 produced better particles sizes and uniformity than the formula with oleic acid. IC_{50} of BCP and BDP formulas are, respectively, 0.004 and 0.08 mg/ml, which indicates the potential as antiplasmodium drugs.

Keywords: *Cassia fistula* L., *Duranta repens* L., Chitosan, Antimalarials

INTRODUCTION

Malaria is a disease caused by blood-parasitic protozoa from genus *Plasmodium* which infecting red blood cells. In 2010, WHO reported 154-289 million malaria cases worldwide, a serious global health issue even until today [1]. It is an endemic disease in more than 90 countries, mostly the developing ones [2]. Indonesia is one of the malaria disease endemic areas with quite high number of cases (especially in Eastern Indonesia) with total reported cases of 1.143.024 in 2009 [3].

Problem with malaria treatment today is the disease's resistance towards commercial antimalarial drugs such as quinine, chloroquine, sulfadoxine, pyrimethamine, kina, amodiaquine, mefloquine, and halofantrine [4]. Thus, the search for the ultimate antimalarial agent is still in motion. One alternative is to utilize medicinal plants such as Trengguli (*Cassia fistula* L.) and Sinyo Nakal (*Duranta repens* L.). The antimalarial potential of these plants have not been much scientifically explored.

Malaria control needs integrated approach which involved prevention (especially vector control) and effective medication. Nanoparticle biomaterial synthesis for antimalarial drug delivery control is one of the strategies developed to search effective precise medication. Chitosan is the biomaterial that can be used for this system. This is due to the intoxic and biocompatible properties of chitosan. In addition, nano sized particles has several advantages as drugs delivery system, such as controlled drug release rate thus lower the frequency of drug administration, deliver drug on target thus reducing systemic side effects, and able to deliver two or more kinds of drug simultaneously for combination therapy to build up synergistic effect and suppress resistance. The aim of this research is to synthesize nanoparticles of *C. fistula* leaves and *D. repens* fruits methanol extracts and determine their *in vivo* IC_{50} as antimalarial agents.

EXPERIMENTAL SECTION

Materials

Analytical instruments used in this study are Perkin Elmer SpectrumOne FTIR, PharmaSpec UV-1700 UV/Vis spectrophotometer, Sorvall RC 5B Plus centrifuge, Delsa Nano C PSA, Col Palmer Probe CV 18 high intensity ultrasonic processor, 2100P Turbidimeter, and common glassware's. This research used *C. fistula* leaves and *D. repens* fruits samples, chitosan from Biotech Surindo, poloxamer 188, sodium tripolyphosphate (STPP), RPHS, sorbitol, hematocrit, blood serum, Giemsa, acetic acid, oleic acid, and organic solvents such as methanol, ethanol, hexane and Dimethyl Sulfoxide (DMSO).

Methods

Chitosan characterization

The characterization of physical and chemical properties of chitosan materials used in this study was water and ash content [5], molecular weight [6], and deacetylation degree determinations [7].

Nanoparticle formula engineering [8]

Herbal extract chitosan nanoparticle formulations were made by mixing chitosan, STPP, herbal extract, and surfactant according to compositions listed in Table 1. For each composition, 0.8 mg/ml STPP solution was used. The first step was mixing 40 ml STPP solution with 100 ml chitosan solution until homogene. After that, 40 ml herbal extract and then 20 ml surfactant were added to the chitosan-STPP solution and stirred at room temperature. Aliquots of 25 ml from each compositions were taken and homogenize by ultrasonic wave at frequency=20 kHz and amplitude=20% for 60 min. The resulting solutions were then centrifuged at 19000 rpm for 2 h to collect particles suspension as supernatant. On every treatment, turbidity measurement was conducted. Particle suspension conversion to powder was done by freeze-drying technique. The sizes of the resulted particles were analyzed by PSA. *In vitro* antimalarial activity test was conducted according to procedure reported elsewhere [9].

Table 1: Herbal extracts chitosan nanoparticle compositions (Sugita et al.)

Group	Formula	Chitosan (% w/v)	*Cassia fistula* (% w/v)	*Duranta repens* (g/ml)	Oleic acid concentration (g/l)	Poloxamer 188 concentration (g/l)
I	ADA	2.5	-	0.044	0.1	-
	BDA	2.5	-	0.044	0.8	-
	PDA	3	-	0.044	1.5	-
II	ADP	2.5	-	0.044	-	0.1
	BDP	2.5	-	0.044	-	0.8
	PDP	3	-	0.044	-	1.5
III	AD	2.5	-	0.011	-	0.1
	BD	2.5	-	0.011	-	0.8
	PD	3	-	0.011	-	1.5
IV	AC	2.5	0.011	-	-	0.1
	BC	2.5	0.011	-	-	0.8
	PC	3	0.011	-	-	1.5

RESULTS AND DISCUSSION

Chitosan characterization results

Table 2 listed the physico-chemical properties of chitosan used in this study. The measured water content is 2 times higher than the specification. This is caused by the hygroscopic nature of chitosan. The measured ash content and DD are relatively close to the specification. The measured molecular weight is half the specification. This is considered due to the different molecular weight determination method and depolimerization or degradation during storage.

Table 2: Chitosan biotech surindo physicochemical properties

Test parameters	Unit	
	Measured	Specification
Water content	8.68%	4.28%[a]
Ash content	0.79%	0.68%[a]
Molecular weight	4.54×10^5 g/mol	9.00×10^5 g/mol[b]
Deacetylation Degree (DD)	91.44%	93.61%[a]

[a]Biotech surindo certificate of analysis; [b]Measurement result by Laboratorium Pusat Penelitian Universitas Sumatera Utara

Surfactant type effect on *D. repens* extract chitosan nanoparticle sizes

In nanoparticle synthesis, surfactants are important component of which type and concentration need to be carefully considered due to their effects on nanoparticle sizes, properties and suspension stability. This research used oleic acid and poloxamer 188 as surfactant. Figure 1 shows turbidity measurement results on group I and II at every treatment steps.

According to Figure 1, sonication and centrifugation proses lower the turbidity value. Bulk particle disintegration into smaller particles with the help of ultrasonic wave which travel through liquid medium has lower the turbidity of group I and II by, respectively, 74 and 58% from their initial step. Meanwhile, centrifugation step has taken turbidity of group I and II further down by 86 and 81% from their sonication step values. This step brings up particle sizes uniformity by redistributing particles position in the container according to their sizes, in other words, this step made bigger heavier particles deposited at the bottom of the container thus separating them from the nanoparticle suspensions. Together, sonication and centrifugation steps have decreased turbidity value of group I and II by 64 and 47% respectively. Thus, turbidity values of formulas that contain poloxamer 188 are much better than those which use oleic acid as surfactant.

Figure 2 also shows similar trend for both IP and average size value. P formula produced particle with better average particle size and IP than B and A formula. This formula has the largest surfactant quantity (1.5 g/l) amongst all. This means larger number of surfactant molecules accumulated on the cavity bubble's gas-liquid interface. This accumulation will lower bubble's surface tension and increase unstable bubble formation rate at cavitation process thus enforcing particle sizes reduction.

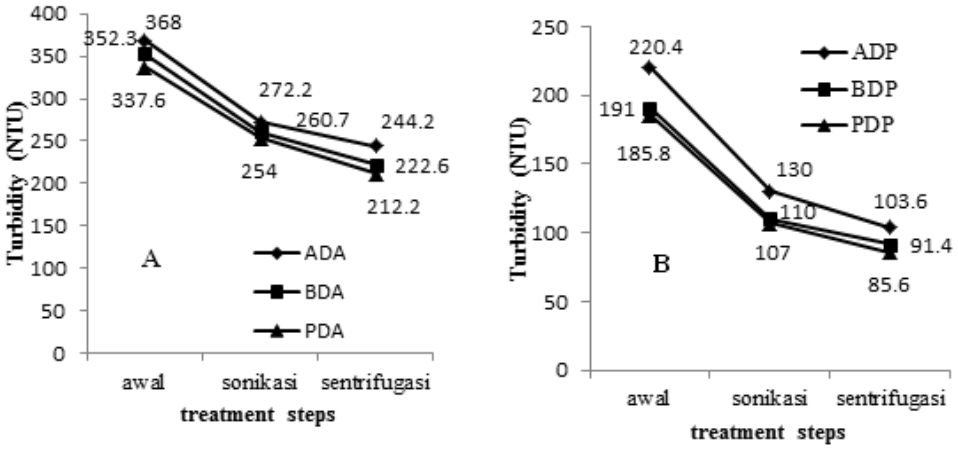

Figure 1: Turbidity measurement results on group I and II at every treatment steps

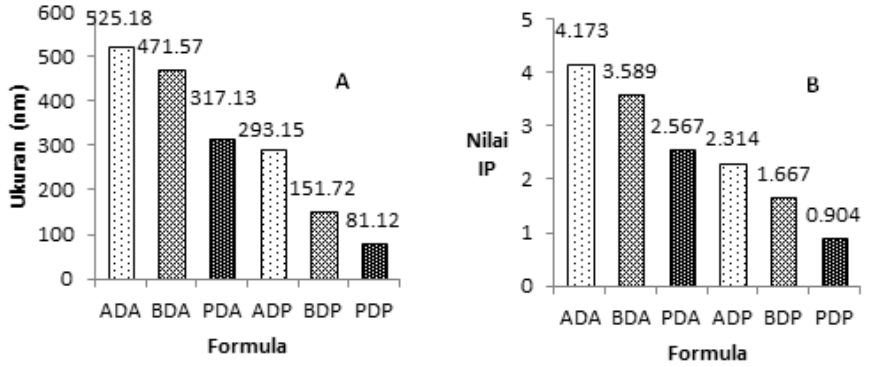

Figure 2: Nanoparticle average size (A) and IP (B) of group I and II

***D. repens* extract concentration effect on nanoparticle sizes**

Particle sizes measurement by PSA method suffers some drawbacks. Double scattering may occur while measuring nanoparticle sizes which can cause the measured size smaller than the actual size. Turbidity measurements of Group II and III at each step are showed in Figure 3. The result indicates that difference in herbal extract concentration (4x) between group III and II does not give any significant effect on turbidity and the value comes down following the same trend.

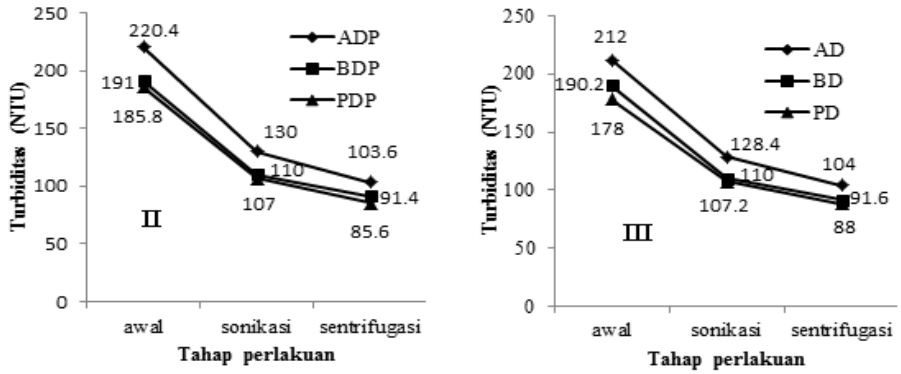

Figure 3: Turbidity measurement results on group II and III at every treatment steps

Figure 4 shows that ADP formula's particle size and IP are better than AD, BDP is better than BD and PDP is better than PD. Chitosan, surfactant, and cross-linker concentration together with nanoparticle formation conditions are the same for every formula. The only difference is

the *D. repens* extract concentrations, group III has 4 times herbal extract concentration of group II. This caused the size and IP of group III formula better. Double scattering phenomenon took place during group III measurement thus lower the measured sizes.

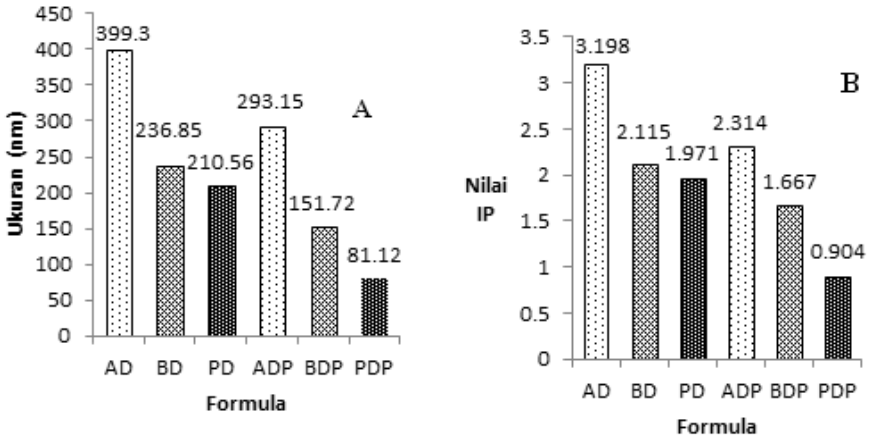

Figure 4: Nanoparticle average sizes (A) and IP (B) of group II and III

Encapsulation result of *C. fistula* extract with chitosan

Effect of surfactant and herbal extract concentration that has been done to *D. repens* extract chitosan nanoparticle synthesis was later adapted to the synthesis of *C. fistula* extract containing chitosan nanoparticle. Turbidity measurement results of group IV on each step is shown by Figure 5. Figure 5 shows the same trend with *D. repens* extract measurement results. Sonication step gave larger effect than centrifugation step. Total turbidity drop is 48%.

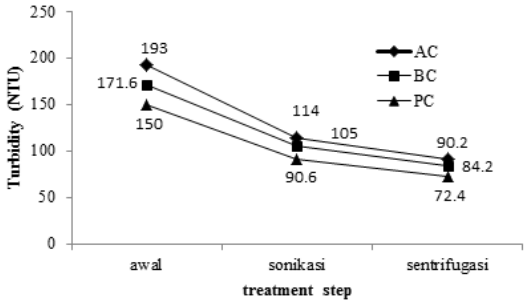

Figure 5: Turbidity measurement results on group IV at every treatment steps

Figure 6 shows that nanoparticle size and IP value of PC formula is less that AC and BC formula. This is due to larger amount of surfactant used in PC formula thus size degradation process at sonication step, namely cavitation, ran more smoothly. Figure 6 also shows that IP value of every formula proportional to particle sizes.

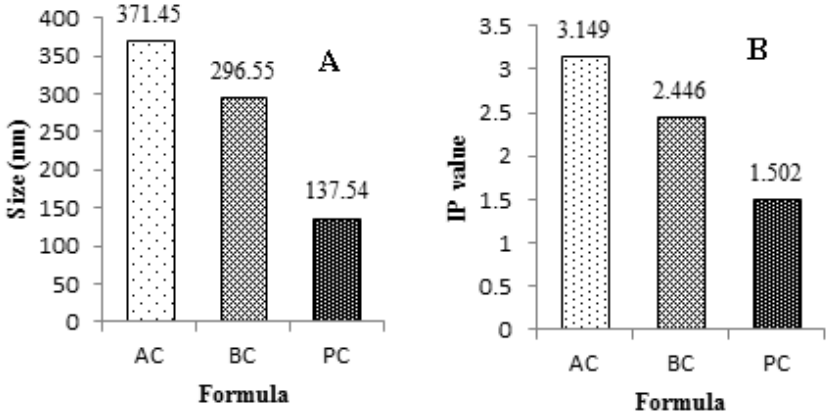

Figure 6: Nanoparticle average sizes (A) and IP (B) on group IV

In vitro activity test result of *C. fistula* leaves and *D. repens* fruit methanol extract chitosan nanoparticle *in vitro* test is initial test that needs to be done to understand this drugs ability to inhibit parasite growth. *P. falciparum* strain 3D7 parasite was used in this research. The results show that parasite growth percentage inversely proportional to the drugs concentration. Figure 7 show that IC_{50} reached by BCP formula at 10^5 × dilution (IC_{50}=0.004 µg/ml) and BDP formula at 10^6 × dilution (IC_{50}=0.08 µg/ml). O'Neil et al. [10] reported that an extract has antiplasmodium activity if its IC_{50} is less than 50 µg/ml and less than 25 µg/ml for fraction. Therefore, BCP and BDP formula has good potential to inhibit *P. falciparum*. This even better than IC_{50} values of *C. fistula* leaves and *D. repens* components. Grace et al. [11] reported IC_{50} values of Trengguli chloroform extract fractions, namely phytol, lutein, and Dilinoleyl Galactopyranosyl Glycerol (DLGG) as 18.9 ± 0.60, 12.5 ± 0.35 and 5.8 ± 0.27 µM respectively. Antimalarial activity of chloroform fraction of *D. repens* fruit methanol extract is able to inhibit *P. berghei* growth with IC_{50}=41.125 mg/kg by wet weight [12].

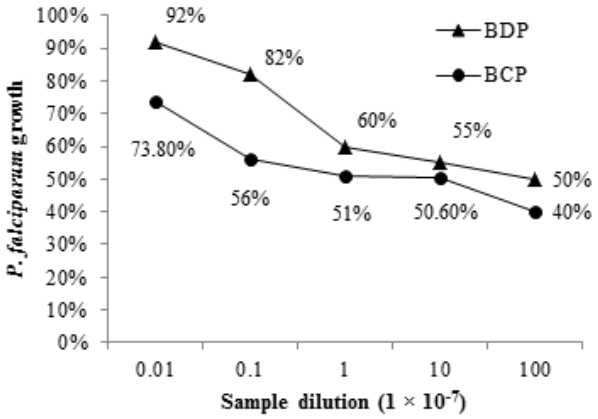

Figure 7: *P. falciparum* parasite growth graphic with BDP (M_0=8 mg/ml) and BCP (M_0=4 mg/ml) formula

Compared to other herbal extracts such as *Calea tenuifolia* methanol extract (19.5 µg/ml), *Momordica charantia* (>50 µg/ml), *Jatropha curcas* L. (>50 µg/ml), *Samanea saman* (10 µg/ml), *Hymenaea courbaril* L. (>50 µg/ml), *Moringa oleifera* Lam. (>50 µg/ml) [13], IC_{50} of BDP and BCP are better against *P. falcifarum*. Madureira et al. [14] reported that *Struchium sparganophorum, Pycnanthus angolensis, Morinda lucia, Tithonia diversifolia,* dan *Cinchona succirubra* also gave less than 10 µg/ml *in vitro* IC_{50} against *P. falcifarum*. Inbaneson et al. [15] reported that *Acalypha indica* and *Jatropha glandulifera* poses good antimalarial activity with IC_{50} value of 43.81 and 49.14 µg/ml, respectively, while *Achyranthes aspera* and *Phyllanthus amarus* show 50 dan 100 µg/ml IC_{50} against *P. falciparum*.

In this study, *P. falciparum* growth inhibitory power of formula B was investigated using two different surfactant namely oleic acid and poloxamer 188 (Figure 8). At 10^5 dilutions, parasite growth treated with BDA is 65% and BDP is 50%. Meanwhile, also at 10^5 dilutions, parasite growth on medium which treated with formulas that contain *C. fistula* extract, BCA and BCP, are 59 and 40%, respectively. Formulas with poloxamer 188 gave better inhibitory power compared to formula which with oleic acid, for both extracts. This is considered due to better ability of poloxamer 188 to form smaller and more uniform nanoparticles thus increasing drug's efficacy.

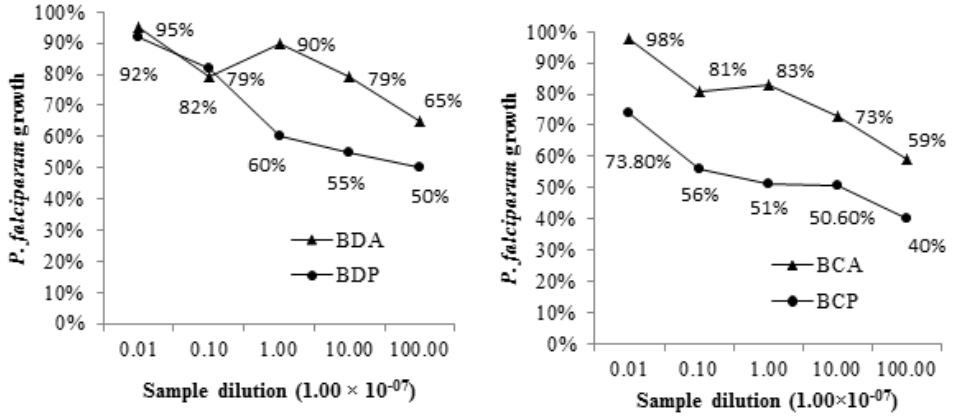

Figure 8: *P. falciparum* parasite growth graphic treated with B formula containing *D. repens* (A) and *C. fistula* (B) with two different surfactants

P. Falciparum growth rate was also compared to fractions from both plants as shown by Table 3. Cf-2, Cf-3, and Cf-4 are fractionation results from *C. fistula* methanol extract by vacuum chromatography using hexane-ethyl acetate with ratio, respectively, 8-2, 7-3, and 6-4 [16]. Dr-35 is *D. repens* tannin free crude extract, while Dr-36 is the fractionation result of Dr-35 by vacuum liquid chromatography using hexane-ethyl acetate (8:2), and Dr-37 is the fractionation result using methanol [17]. Clearly, Table 3 shows that *P. falciparum* can grow better in the medium that has been treated with *C. fistula* or *D. repens* fractions than in medium that has been treated with the chitosan nanoparticle containing the crude plant extracts namely BCP and BDP formula. Thus these formulas, potential as antimalarial new drug is so promising.

Table 3: *P. falciparum* growth rate of *C. fistula* and *D. Repens* extrac fractions

Fraction	Concentration (µg/ml)	*P. falciparum* growth rate
Cf-2	0.0606	58.00%
Cf-3	0.1	60.00%
Cf-4	0.68	40.60%
Dr-35	0.026	64.00%
Dr-36	0.0808	47.00%
Dr-37	0.026	50.00%

CONCLUSION

Synthesis of *C. fistula* leaves and *D. repens* fruits methanol extract chitosan nanoparticle preparations using ionic gelation method have successfully produced particles of which sizes ranging from 80-475 nm. The best IC_{50} against *P. falciparum* reached by BCP and BDP formula: 0.004 and 0.08 µg/ml, respectively. This result shows that encapsulation of *C. fistula* leaves and *D. repens* fruits methanol extract process with chitosan has increased *P. falciparum* inhibitory power of both extracts thus also increasing both plants' antimalarial drugs potential.

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