Original Article

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Antioxidant activity and potential of *Caesalpinia sappan* aqueous extract on synthesis of silver nanoparticles

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Summary **The aim of this study was to investigate the antioxidant activity of *Caesalpinia sappan* aqueous extract (CE) and its potential on synthesis of silver nanoparticles (AgNPs). The antioxidant activity of CE was investigated using ferric reducing antioxidant power (FRAP) assay and two radical scavenging methods using 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) as free radicals. Silver nitrate ($AgNO_3$) was used as precursor for the synthesis of AgNPs. Effects of $AgNO_3$ concentration, reaction temperature, and duration of reaction were investigated. The obtained AgNPs was characterized using UV-Vis and photon correlation spectrophotometers. The antimicrobial activity of AgNPs was studied by means of diffusion method. The results from FRAP demonstrated that CE had high reducing property of 78.7 ± 2.4 mM Fe^{2+}/mg. The trolox equivalent antioxidant capacity of CE determined by ABTS was 64.8 ± 4.2 µM/mg. The concentration of CE that can inhibit 50% of DPPH radicals (IC_{50}) was 51.2 ± 3.2 µM. These results indicated that CE possesses strong antioxidant and reducing activities. The present study also showed that CE can act as reducing agent to produce AgNPs. The concentration of $AgNO_3$, reaction temperature, and reaction time play an important role on the particles size and zeta potential of the obtained AgNPs. The antimicrobial activity of the AgNPs against *Escherichia coli*, *Candida albicans*, and *Streptococcus mutants* was stronger than against *Staphylococcus aureus*.**

Keywords: Caesalpinia sappan, silver nanoparticles, reducing activity, antimicrobial activity

1. Introduction

Nowadays, silver nanoparticles (AgNPs) are of high interest due to their particular properties and wide applications. AgNPs are used to inhibit many pathogenic including bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, and *Pseudomonas aeruginosa* (*1-3*) and fungi like *Aspergillus* and *Candida* spp. (*4,5*). AgNPs can be synthesized by redox reaction of silver salt as a precursor and a reducing agent from synthetic chemicals. Recently, the eco-friendly process of AgNPs production was developed using reducing agents from natural sources like plants (*5*), algae (*6*), and microorganisms (*7*).

Caesalpinia sappan is a plant belongs to familily Leguminosae. It is wildly distributed and cultivated in Southeast Asia, Africa and the America (*8*). The wood of *C. sappan* contains several phytochemicals in alkaloids, phenolics, flavonoids, and glycosides (*9*). The major active compound of *C. sappan* is brazilin and brazilein, an oxidized from of brazilin (*10,11*). Many biological activities from different parts of *C. sappan* have been reported such as antioxidant activity from heart woods (*12*), antihelmintic property from leaves (*13*), and antimicrobial activity from barks (*14*).

The aim of this study was to synthesize AgNPs

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by using *C. Sappan* extract as a reducing agent. The plant extracts that can be used as reducing agents in AgNPs should dissolve well in water and its aqueous solution should have antioxidant or reducing property. However, all extracts of *C. Sappan* previously reported to have antioxidant activity were extracted from organic solvents and cannot dissolve well in water. Therefore, it is an essential to clarify whether the aqueous extract of *C. Sappan* has an antioxidant and reducing activities. In the present study, the reducing property of *C. sappan* aqueous extract was determined using ferric reducing antioxidant power (FRAP). The antioxidant activity of this extract was investigated by means of two standard radical scavenging methods using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) as free radicals. The AgNPs were synthesized using silver nitrate ($AgNO_3$) as a precursor in the redox reaction. Effects of $AgNO_3$ concentrations, reaction temperature, and duration of reaction were studied. The obtained AgNPs were confirmed by UV-Vis absorption. The size, size distribution, and zeta potential of the obtained AgNPs were determined by photon correlation spectrophotometer (PCS).

2. Materials and Methods

2.1. *Materials*

All chemical reagent were analytical grade and purchased from commercial source without further purification. Two chemicals used as free radicals; DPPH and ABTS as well as 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma Adrich, Inc (St. Louis, MO, USA). $AgNO_3$ and sodium hydroxide (NaOH) 97% were supplied by RCI Lab-scan Co., Ltd. (Bangkok, Thailand). Hydrochloric acid (HCl) 37% was purchased from Carlo erba reagents (Rodano, Metropolitan City of Milan, Italy). Ferrous sulphate heptahydrate ($FeSO_4•7H_2O$) was purchased from RFCL limited (New Delhi, Delhi, India). Iron (III) chloride hexahydrate ($FeCl_3$) was purchased from Honeywell Riedel-de-Haën™ (Seelze GmbH Manufacturing Facility, Seelze, Hanover, Germany). Tryptic soy agar (TSA) and broth (TSB) were supplied by Difco™ (Balti-more, Maryland, USA). Brain heart infusion agar (BHI-A) and broth (BHI-B) were purchased from Becton, Dickinson and Company (Franklin Lakes, New Jersey, USA). Sabouraud dextrose agar (SDA) and broth (SDB) were purchased from BBL™ (Baltimore, Maryland, USA). All other chemicals and solvents were of analytical reagent grade or the highest grade available.

2.2. *Microbial strains*

Two aerobic bacterial strains, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 (*E. coli*) which represent for Gram-positive and Gram-negative bacteria, respectively, and two strains facultative Gram-positive bacteria, *Streptococcus mutans* DMST 9567 and *S. mutans* DMST 41283 were used as pathogenic bacteria. *Candida albicans* ATCC 10231 was used as pathogenic fungi for the investigation of antimicrobial activity of the synthesized AgNPs.

2.3. *Collection of plant*

The fresh plant of *C. sappan* was collected from the local area in Chiang Mai province, Thailand. The heart wood of *C. sappan* was washed thoroughly using distilled water several times to remove dust and cut into small pieces before drying in the hot air oven at 50°C. The dried plant materials were ground into coarse powder.

2.4. *Preparation of plant extract*

Exact weight of 2 g of dried plant powder was macerated in 100 mL deionized water with constant stirring at room temperature for 24 h. The plant extract was filtered through Whatman No.1 filter paper and filtrate was subjected to a Freeze dry (Virtis®, Warminster, Pennsylvania, USA) to obtain an extract as a lyophilized form of *C. sappan* aqueous extract (CE). CE was stored in the refrigerator at 4°C for further studies.

2.5. *Antioxidant activity of CE*

2.5.1. *FRAP assay*

The FRAP assay was done according to the method previously described (*15*) with some modification. Briefly, the FRAP reagent was freshly prepared by mixing 2.5 mL of 10 mM TPTZ solution in 40 mM HCl with 2.5 mL of 20 mM FeCl3 and 25 mL of 0.3 M acetate buffer pH 3.6. An exact amount of 20 µL of aqueous CE was mixed with 120 µL of FRAP reagent in 96 well plate. Blank samples were prepared by mixing acetate buffer and CE. The samples and blank were incubated for 10 min at room temperature and the absorbance of the samples was determined at 595 nm using microplate reader (Bio-Rad, Model 680, USA). The reducing power of the samples was evaluated by calculating the amount of Fe^{2+} produced by CE aqueous solution using the calibration curve of $FeSO_4$. The experiment was done in triplicate.

2.5.2. *ABTS assay*

This method was done by using ABTS free radical decolorization assay developed by Re *et al*. (*16*) with some modification. Briefly, the ABTS radicals was generated by reacting ABTS solution (7 mM) with

2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain the absorbance of 0.7 ± 0.2 at 750 nm. CE aqueous solution was separately diluted with ethanol to reach a concentration of 0.1 mg/mL. An aliquot of 20 µL ethanolic test solution of CE was added to 180 µL ABTS free radical solution. The sample was incubated for 5 min and measured at 750 nm using a microtiter plate reader. All measurements were performed in triplicate. The free radical scavenging activity of each sample was expressed as trolox equivalent antioxidant capacity (TEAC), which was obtained by comparing the absorbance change at 750 nm in a reaction mixture containing CE with that containing trolox. This index is defined as the millimolar concentration of a trolox solution which antioxidant capacity is equivalent to 1.0 mg of CE.

2.5.3. *DPPH*

The scavenging activity on DPPH radical of CE was determined by modifying the methods of Gamez *et al*. (*17*). An aqueous solution of CE was diluted with ethanol to prepare an ethanolic test solution of different concentrations. DPPH was dissolved in ethanol and mixed with certain amount of the ethanolic CE solution. The solution was adjusted to a final DPPH concentration of 100 µM and the CE final concentrations of 0.1-1.0 mg/mL. The mixture was shaken vigorously and left to stand for 30 min in the dark place at room temperature (28°C). The amount of DPPH remaining in each period of standard was determined spectrophotometrically at 540 nm using a microtiter plate reader. All measurements were performed in triplicate. The radical scavenging activity was calculated as % inhibition.

2.6. *Synthesis of AgNPs*

In the synthesis of AgNPs, an aqueous solution of 1mg/mL CE was stirred and the pH was adjusted to 6 by HCl or NaOH. Then an aqueous solution of $AgNO_3$ was added dropwise to the CE solution until the volume ratio of CE to $AGNO_3$ was 100:1 and the final concentration of $AgNO_3$ was 0.01, 0.05, 0.1, 0.5, and 1 mM. The effect of reaction temperature was studied at 28, 50, 75, and 90°C and the reaction time studied was 15-300 min. The reaction mixture was kept under continuous stirring for all conditions.

2.7. *Characterization of AgNPs*

2.7.1. *UV-Vis absorption*

Characterization of the AgNPs was performed using UV–Vis spectrophotometer (Shimadzu UV 2450 double-beam spectrophotometer, Shimadzu-2450, Kyoto, Japan). The obtained AgNPs was diluted to 100 folds with deionized water before subjecting to this investigation. The optical property of the AgNPs solution was observed in the wavelength range of 300-800 nm. The UV-Vis absorbance spectra were recorded at room temperature.

2.7.2. *PCS*

The obtained AgNPs from different conditions were investigated for particles size, size distribution, and zeta potential by PCS (Malvern Zeta sizer Nano-Zs, Malvern Instruments, Worcestershire, UK). Each sample was diluted to 100 fold with deionized water before measurement. The size average of particles, size distribution expressed as polydispersity index (PdI), and zeta potential were recorded from three measurements taken of three independent AgNPs batches.

2.8. *Antimicrobial activity test of AgNPs*

The antimicrobial activity of the obtained AgNPs against the pathogenic microorganism; *S. aureus*, *E. coli*, *S. mutans*, and *C. albicans* was tested by means of Kirby-Bauer method (*18*). The aerobic and facultative bacteria were grown in TSA and BHI-A, respectively at 37°C for 24 h. Then they were diluted with TSB and BHI-B, respectively to a final density of 1.5×10^6 colony forming unit (CFU)/mL. *C. albicans* were cultured in SDA at 37°C for 36-48 h. The fungal suspension was diluted with SDB to a final concentration of $1\text{-}2 \times 10^5$ CFU/mL. The suspensions of the test microorganisms were swabbed on the surface of their corresponding agars. The wells of 6 mm were made on agar plates and each AgNPs sample of 40 µL was filled in the wells, whereas CE and $AgNO_3$ solutions were used as the negative controls. Ampicillin 100 µg/mL and Amphotericin B 100 µg/mL were used as the positive controls against bacterial and fungal strains, respectively. The plates were incubated at 37°C for 24 h and 48 h for bacterial and fungal strains, respectively. The antimicrobial activity of AgNPs was evaluated by determining the diameter of clear zone of inhibition (mm). All samples were done in triplicate for each pathogen.

2.9. *Statistical analysis*

Descriptive statistics for continuous variables were calculated and reported as a mean ± standard deviation. Data were analyzed using a One-way analysis of variance and Duncan's multiple range test using Statistic a software version 17 (SPSS Inc., Chicago, Illinois, USA). *P*-value less than 0.05 was considered as significant difference.

3. Results

3.1. *Antioxidant and reducing activities of CE*

The color of CE powder was dark orange-red and could dissolve well in water. The antioxidant and reducing activities of CE were performed when CE was in aqueous solution. The results from all tests demonstrated that CE aqueous solution possessed strong antioxidant and reducing capacities. The reducing activity of CE solution from FRAP was 78.7 ± 2.4 mM/mg whereas the free radical scavenging property of CE determined by ABTS showed the TEAC value of 64.8 ± 4.2 µM/mg. The minimum concentration to scavenge 50% of free radicals (IC_{50}) obtained from DPPH was 51.2 ± 3.2 µM

3.2. *Synthesis and antimicrobial activity of AgNPs*

3.2.1. *Effect of $AgNO_3$ concentration*

In this experiment, the reaction temperature was fixed at 75°C and the reaction time was fixed at 60 min. The concentration of $AgNO_3$ was varied from 0.025 to 10 mM. The color of CE solution is orange whereas $AgNO_3$ solution is colorless. In the synthesis process, the color of mixture between CE and $AgNO_3$ was slowly changed from orange turned to brown-gray color which indicating the formation of AgNPs. The high concentration of $AgNO_3$ showed rapid change in color whereas the color change in low concentration of $AgNO_3$ was slow. Figure 1 show particles size and zeta potential value of obtained AgNPs obtained from different concentration of $AgNO_3$. It was found that the particles size of AgNPs that synthesized from 0.025, 0.5, 1, 5, and 10 mM $AgNO_3$ were 411.8 ± 20.4, 245.3 ± 34.2, 183.7 ± 15.8, 160.8 ± 4.0, and 233.3 ± 42.4 nm, respectively with the PdI values 0.28 ± 0.1, 0.24 ± 0.1, 0.23 ± 0.1, 0.18 ± 0.1, and 0.22 ± 0.1, respectively. The zeta potential of the AgNPs obtained from these concentrations were –24.6 ± 6.8, –27.4 ± 2.6, –28.0 ± 3.2, –32.6 ± 4.1, and –20.1 ± 8.1 mV, respectively.

For antimicrobial activity, it was found that AgNPs obtained from 5 mM $AgNO_3$ showed the highest activity against all microbial strains as they demonstrated the significantly widest inhibition zones. The inhibition zones of the AgNPs of this condition against *S. aureus* and *E. coli* were 13.4 ± 0.3 and 16.7 ± 0.7 mm, respectively whereas that against *S. mutans* DMST 9567 and *S. mutans* DMST 41283 were 14.1 ± 0.7 and 13.7 ± 0.3 mm, respectively. The inhibition zone of AgNPs against *C. albicans* was 14.3 ± 0.7 mm as shown in Table 1.

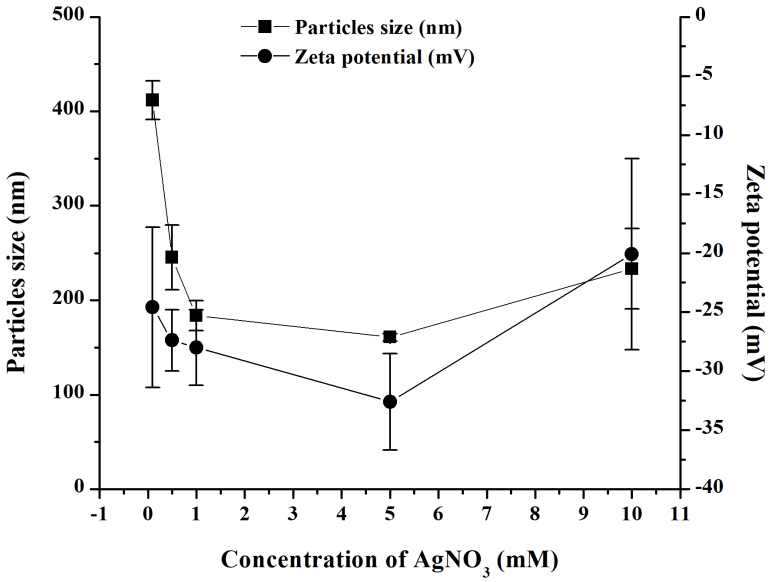

Figure 1. Effect of concentration of AgNO3 on particles size and zeta potential of AgNPs.

Table 1. Effect of concentration of $AgNO_3$ on antimicrobial activity of AgNPs

Concentration of $AgNO_3$ (mM)	Diameter of inhibition zone (mm)				
	S. aureus ATCC 25923	*E. coli* ATCC 25922	*S. mutans* DMST 9567	*S. mutans* DMST 41283	*C. albicans* ATCC 10231
0.1	NZ	NZ	NZ	NZ	NZ
0.5	9.2 ± 0.7	13.8 ± 0.7	9.0 ± 0.7	9.4 ± 0.8	9.2 ± 0.7
1	11.5 ± 0.7	15.2 ± 0.7	10.7 ± 0.3	11.2 ± 0.7	12.4 ± 0.6
5	13.4 ± 0.3	16.7 ± 0.7	14.1 ± 0.7	13.7 ± 0.3	14.3 ± 0.7
10	12.6 ± 0.5	14.8 ± 0.3	13.6 ± 0.7	12.8 ± 0.8	13.1 ± 0.7

NZ: No inhibition zone

3.2.2. *Effect of reaction temperature*

In the study of effect of temperature on the obtained AgNPs, the concentration of $AgNO_3$ was fixed at 5 mM and the reaction time was fixed at 60 min. The reaction temperature studied were at 28, 50, 75, and 90°C. The size of the AgNPs obtained from these conditions were 1310.1 ± 178.7, 222.2 ± 130.8, 137.5 ± 23.2, and 219.1 ± 81.2 nm, respectively with the PdI values of 0.48 ± 0.1, 0.32 ± 0.1, 0.18 ±0.1, 0.22 ± 0.1, respectively. The zeta potential of the AgNPs obtained from these concentrations were –5.1 ± 2.9, –19.6 ± 1.3, –29.3 ± 1.5, and –28.4 ± 2.6 mV, respectively as shown in Figure 2.

For antimicrobial activity, it was found that AgNPs obtained from the reaction temperature was 75°C showed the highest activity against all microbial strains as they demonstrated the significantly widest inhibition zones. The inhibition zones of the AgNPs of this condition against *S. aureus*, *E. coli*, *S. mutans* DMST 9567, *S. mutans* DMST 9567, and *C. albicans* were 12.4 ± 0.4, 16.8 ±0.6, 13.4 ± 0.7, 13.2 ± 0.3, and 14.5 ± 1.6 nm, respectively whereas AgNPs synthesized at 28°C showed no inhibition zone against all strains. The results show in Table 2.

3.2.3. *Effect of reaction time*

In the study of effect of reaction time, the concentration of $AgNO_3$ was fixed at 5 mM and the reaction temperature was fixed at 75°C. The reaction period studied was in the range of 15-300 min. The results of UV-Vis spectra showed that increase in reaction time caused significant increase in UV-Vis absorption. However, it was noted that the absorption of AgNPs obtained from the reaction tome of 60 and 90 min showed no significant difference. Moreover, the absorption intensity was decrease with the reaction time at 120, 180, and 300 min, respectively. The highest intensity of the prepared AgNPs spectra was obtained (data not shown) when the following condition was use; $AgNO_3$ at 5 mM, reaction temperature at 75°C and the reaction time of 60 min. The effect of duration of reaction on particles size and zeta potential was shown in Figure 3. The particles size of AgNPs obtained from the reaction times of 30, 60, 180, and 300 min was 128.7 ± 1.85, 120.9 ± 0.8, 235.3 ± 3.87, and 929.23 ± 68.9 nm, respectively whereas the PdI values of Effect of duration reaction of synthesis AgNPs at 30, 60, 180, and 300 min PDI 0.20 ± 0.1, 0.18 ±0.1, 0.24 ± 0.1, and 0.45 ± 0.1, respectively. The zeta potential of the AgNPs obtained from these concentrations were –31.1 ± 1.2, –30.6 ± 0.8, –24.2 ± 1.4, and –14.7 ± 3.2 mV, respectively.

For antimicrobial activity, it was found that AgNPs obtained from the reaction time of 60 min showed the highest activity against all microbial strains as they

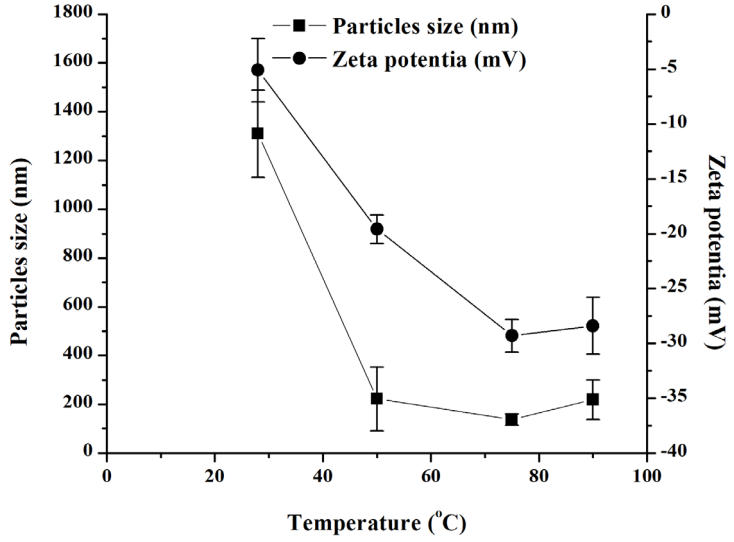

Figure 2. Effect of reaction temperature on particles size and zeta potential of AgNPs.

Table 2. Effect of temperature on antimicrobial activity of AgNPs

Temperature (°C)	Diameter of inhibition zone (mm)				
	S. aureus ATCC 25923	*E. coli* ATCC 25922	*S. mutans* DMST 9567	*S. mutans* DMST 41283	*C. albicans* ATCC 10231
28	NZ	NZ	NZ	NZ	NZ
50	9.5 ± 0.8	12.1 ± 0.7	10.1 ± 0.3	10.4 ± 0.7	11.6 ± 1.2
75	12.4 ± 0.4	16.8 ± 0.6	13.4 ± 0.7	13.2 ± 0.3	14.5 ± 1.6
90	12.4 ± 0.6	14.8 ± 0.4	11.6 ± 0.8	10.8 ± 0.7	10.5 ± 0.4

NZ: No inhibition zone

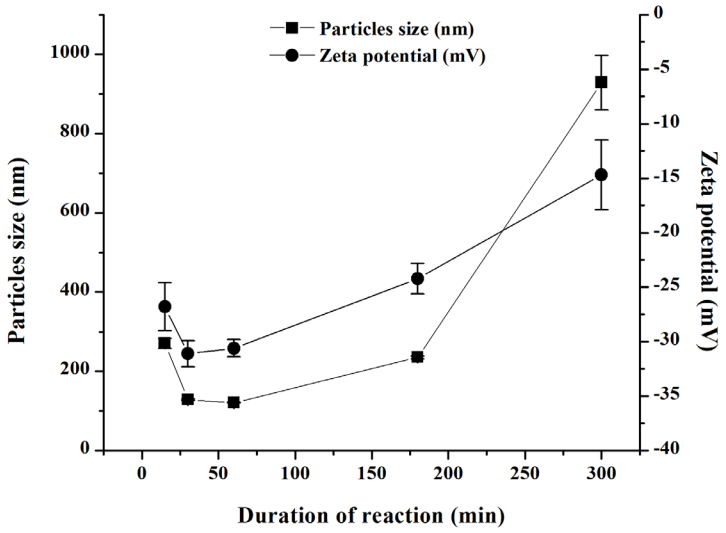

Figure 3. Effect of reaction time on particles size and zeta potential of AgNPs.

Table 3. Effect of duration of reaction on antimicrobial activity of AgNPs

Duration of reaction (min)	Diameter of inhibition zone (mm)				
	S. aureus ATCC 25923	*E. coli* ATCC 25922	*S. mutans* DMST 9567	*S. mutans* DMST 41283	*C. albicans* ATCC 10231
15	8.0 ± 0.5	9.0 ± 0.5	NZ	NZ	NZ
30	11.2 ± 0.5	12.0 ± 0.25	11.3 ± 0.3	10.5 ± 0.7	11.4 ± 0.7
60	12.0 ± 0.5	16.5 ± 0.5	13.6 ± 0.7	13.4 ± 0.7	14.5 ± 0.7
180	10.25 ± 0.5	11.5 ± 0.25	12.8 ± 0.7	12.4 ± 0.3	13.5 ± 0.7
300	NZ	NZ	NZ	NZ	NZ

NZ: No inhibition zone

demonstrated the significantly widest inhibition zones when compared with those obtained from the reaction time of 15, 30, 180 min, whereas those obtained from the reaction time of 300 min showed no inhibition zone against all microbial strain. The inhibition zones of the AgNPs obtained from the reaction time of 60 min against *S. aureus*, *E. coli*, *S. mutans* DMST 9567, *S. mutans* DMST 9567, and *C. albicans* were 12.0 ± 0.5, 16.5 ± 0.5, 13.6 ± 0.7, 13.4 ± 0.7, and 14.5 ± 0.7 mm, respectively as shown in Table 3.

4. Discussion

Previously, antioxidant property of *C.sappan* was investigated and reported that the activity was according to the phenolic compounds (*19*) such as alkaloid, tannins, steroids, and flavonoids (*14,20*), which most of flavonoid compounds in the extracts are flavone and flavonol (*21*). Most of these compounds are not soluble in water. The synthesis reaction of AgNPs needs to be in aqueous system. Therefore, it is essential to investigate whether there are some water soluble active compounds of this plant. The results in the present study reveal that the aqueous extract of *C. sappan* still possesses antioxidant and reducing properties. These results indicated that there are some bioactives in *C. sappan* that are water soluble and have antioxidant and reducing properties.

In the synthesis process, it has been reported that may factors including pH (*22*), reaction temperature (*23*), concentration of a precursor (*24*), and reaction time (*24*) that might affect the obtained AgNPs. Therefore, in the present study that the aqueous solution of *C. sappan* extract was used as a reducing agent, we investigated all of these factors and the results are in agreement with the previous reports that the concentration of the precursor ($AgNO_3$), the reaction temperature, and the reaction time play an important role particularly on the size and antimicrobial activity of the obtained AgNPs. The results in the present study show that the smallest size of AgNPs can be obtained from the suitable condition using 5 mM $AgNO_3$.

The obtained AgNPs synthesized from temperature 75°C showed smallest particle (137.5 ± 23.2 nm) and highly negative charge (–29.3 ± 1.5 mV). It was found that higher temperature gave smaller size of AgNPs. However, the temperature higher than 75°C was found to be not suitable condition for AgNPs preparation. Our results are in good agreement with the other research groups who have reported the AgNPs synthesized by using tea leaf extract that the particles size of AgNPs increased with the increased temperature

(*25*). The reaction time also affect the obtained AgNPs synthesized by CE. The obtained AgNPs synthesized at 60 min showed the smallest particles size, highest negative zeta potential, and low PdI value. The antimicrobial activity was according to the size of AgNPs. It was found that the smaller particle size gave the higher antimicrobial activity. It is considered that smaller size AgNPs having the large surface area which available for interaction microorganism cell than the larger size AgNPs (*26*). The mechanism of antimicrobial activity of AgNPs has been proposed that it is due to the adhesion of AgNPs on the surface of the microorganisms and change the properties of their membranes (*27*). Moreover, the effective of AgNPs on microbial is dependent on the microorganisms genus, species, strain and also isolates (*28*). The AgNPs obtained in the present study show less effective against *C. albicans* than bacteria. This is considered that *C. albicans* as a eukaryotic cell has thicker cell wall. The cell wall of *C. albicans* is composed of β1, 3-glucan that can form cross-linkages with other compounds and increase the strength and integrity of the cell wall. Therefore, it is more difficult for AgNPs to penetrate into fungal cell in comparison with the bacterial cells.

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References

1. Ajitha B, Ashok Kumar Reddy Y, Sreedhara Reddy P. Biosynthesis of silver nanoparticles using *Plectranthus amboinicus* leaf extract and its antimicrobial activity. Spectrochim Acta - Part A Mol Biomol Spectrosc. 2014; 128:257-262.
2. Ranganathan Nithya RR. Synthesis of silver nanoparticles using a probiotic microbe and its antibacterial effect against multidrug resistant bacteria. African J Biotechnol. 2012; 11:11013-11021.
3. Velusamy P, Das J, Pachaiappan R. Greener approach for synthesis of antibacterial silver nanoparticles using aqueous solution of neem gum (*Azadirachta indica* L.). Ind Crop Prod. 2015; 66:103-109.
4. Sagar G, Ashok B. Green synthesis of silver nanoparticles using *Aspergillus niger* and its efficacy against human pathogens. Eur J Exp Biol. 2012; 2:1654-1658.
5. Ashour AA, Raafat D, El-Gowelli HM, El-Kamel AH. Green synthesis of silver nanoparticles using cranberry powder aqueous extract: Characterization and antimicrobial properties. Int J Nanomedicine. 2015; 10:7207-7221.
6. Abdel-Raouf N, Al-Enazi NM, Ibraheem IBM, Alharbi RM, Alkhulaifi MM. Biosynthesis of silver nanoparticles by using of the marine brown alga *Padina pavonia* and their characterization. Saudi J Biol Sci. 2018. 1-9
7. Saeb ATM, Alshammari AS, Al-Brahim H, Al-Rubeaan KA. Production of silver nanoparticles with strong and stable antimicrobial activity against highly pathogenic and multidrug resistant bacteria. Sci World J. 2014. 1-9
8. Wu Z, Bao H, Zhou F, Liu J, Meng F, Feng L, Lu J, Zhang Q, Ye Y, Lin L. *Cytotoxic cassane* diterpenoids from the seeds of *Caesalpinia sappan*. Chinese Chem Lett. 2017; 28:1711-1715.
9. Kim KJ, Yu HH, Jeong SI, Cha JD, Kim SM, You YO. Inhibitory effects of *Caesalpinia sappan* on growth and invasion of methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol. 2004; 91:81-87.
10. Ye M, Xie W, Lei F, Meng Z, Zhao YN, Su H, Du LJ. Brazilein, an important immunosuppressive component from *Caesalpinia sappan* L. Int Immunopharmacol. 2006; 6:426-432.
11. Nirmal NP, Rajput MS, Prasad RGSV, Ahmad M. Brazilin from *Caesalpinia sappan* heartwood and its pharmacological activities: A review. Asian Pac J Trop Med. 2015; 8:421-430.
12. Nirmal NP, Panichayupakaranant P. Antioxidant, antibacterial, and anti-inflammatory activities of standardized brazilin-rich *Caesalpinia sappan* extract. Pharm Biol. 2015; 53:1339-1343.
13. Harjit K, Amini MH, Suttee A. Evaluation of antioxidant and anthelmintic properties of *Caesalpinia sappan* L. leaves. Int J Pharmacogn Phytochem Res. 2016; 8:362-368.
14. Mohan G, Anand SP, Doss A. Efficacy of Aqueous and Methanol extracts of *Caesalpinia sappan* L. and *Mimosa pudica* L. for their potential antimicrobial activity. South As JBiolSci. 2011; 1:48-57.
15. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem. 1996; 239:70-76.
16. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999; 26:1231-1237.
17. Gamez EJC, Luyengi L, Sang Kook Lee, Zhu LF, Zhou BN, Fong HHS, Pezzuto JM, Kinghorn AD. Antioxidant flavonoid glycosides from *Daphniphyllum calycinum*. J Nat Prod. 1998; 61:706-708.
18. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45:493-496.
19. Tuekaew J, Siriwatanametanon N, Wongkrajang Y, Temsiririrkkul R, Jantan I. Evaluation of the antioxidant activities of Ya-hom intajak, A thai herbal formulation, and its component plants. Trop J Pharm Res. 2014; 13:1477-1485.
20. Gan R, Xu X, Song F, Kuang L, Li H. Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. J Med Plants Res. 2010; 4:2438-2444.
21. Rusita YD, Suhartono. Flavonoids content in extracts secang (*Caesalpinia Sappan* L.) maceration method infundation analysis and visible ultraviolet spectrophotometer. IJMRHS. 2016; 5:176-181.
22. Mock JJ, Barbic M, Smith DR, Schultz DA, Schultz S. Shape effects in plasmon resonance of individual

colloidal silver nanoparticles. J Chem Phys. 2002; 116:6755-6759.
23. Nayak D, Ashe S, Rauta PR, Kumari M, Nayak B. Bark extract mediated green synthesis of silver nanoparticles: Evaluation of antimicrobial activity and antiproliferative response against osteosarcoma. Mater Sci Eng C. 2016; 58:44-52.
24. Dong C, Cao C, Zhang X, Zhan Y, Wang X, Yang X, Zhou K, Xiao X, Yuan B. Wolfberry fruit (*Lycium barbarum*) extract mediated novel route for the green synthesis of silver nanoparticles. Optik. 2017; 130:162-170.
25. Sun Q, Cai X, Li J, Zheng M, Chen Z, Yu CP. Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity. Colloids Surfaces A Physicochem Eng Asp. 2014; 444:226-231.
26. Mahendran G, Ranjitha Kumari BD. Biological activities of silver nanoparticles from *Nothapodytes nimmoniana* (Graham) Mabb. fruit extracts. Food Sci Hum Wellness. 2016; 5:207-218.
27. Tripathi DK, Tripathi A, Shweta, Singh S, Singh Y, Vishwakarma K, Yadav G, Sharma S, Singh VK, Mishra RK, Upadhyay RG, Dubey NK, Lee Y, Chauhan DK. Uptake, accumulation and toxicity of silver nanoparticle in autotrophic plants, and heterotrophic microbes: A concentric review. Front Microbiol. 2017; 8:1-16.
28. Pietrzak K, Gutarowska B. Influence of the silver nanoparticles on microbial community in different environments. Acta Biochim Pol. 2015; 62:721-724.

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