Research Article

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Rapid and efficient microwave-assisted extraction of *Caesalpinia sappan* Linn. heartwood and subsequent synthesis of gold nanoparticles

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Abstract: Since microwave (MW)-assisted synthesis of gold nanoparticles (AuNPs) using *Caesalpinia sappan* (CS) extract as both a reducing and stabilizing agent is currently unavailable, a MW-based synthesis protocol was investigated and presented for the first time in this work. In addition, to rapidly prepare the reactant for this purpose, the MW-assisted extraction of CS heartwood was studied. From the optimization experiments, it was found that the extraction using the MW irradiation at 300 W for 3 min produced the extract with high and reproducible brazilin content which could be readily used for the synthesis of AuNPs. Under the optimal synthesis conditions, roughly spherical CS-AuNPs with an average diameter size of 49.6 nm and acceptable 28-day stability were obtained within only 1 min. The resulting CS-AuNPs were capable of selective binding to Fe^{2+}, Fe^{3+}, and Al^{3+}, leading to particle aggregation as well as noticeable change of color and shift of UV-Vis absorption maxima. From these results, CS-AuNPs could be fabricated via this fast, green, and efficient route. Furthermore, their potential application for colorimetric sensing of certain metal ions was preliminarily explored and proposed in this work.

Keywords: microwave, extraction, synthesis, gold nanoparticles, *Caesalpinia sappan* Linn.

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1 Introduction

Currently, green and sustainable routes to the fabrication of gold nanoparticles (AuNPs), versatile materials with a wide range of applications have become the focus of interest [1,2]. In terms of energy efficiency, microwave (MW) irradiation provides more rapid and uniform heating, compared to conventional heating which relies on conduction/convection, due to internal heating arising from dipolar polarization and ionic conduction. This phenomenon usually results in shorter reaction time, lower energy consumption as well as better yield, properties, homogeneity, and reproducibility of AuNP products [3]. In the aspect related to the reagents used, several plant extracts can act as an excellent green reducing agent and stabilizing/capping agent for the synthesis of AuNPs as it is easily obtainable, safe, and inexpensive, thereby precluding the use of hazardous chemicals [4]. For these reasons, a large number of works on the MW-assisted synthesis of AuNPs via the use of plant extracts have been published in recent years [5–8].

Caesalpinia sappan Linn. (CS), commonly known as Brazil or Sappan wood, is a plant distributed in Southeast Asia. This dried heartwood has been used as a dye for textile, ingredient in food and beverage as well as a medicinal agent with various pharmacological activities, e.g., antioxidant, anti-inflammatory, and antibacterial activities [9]. Among the chemical constituents naturally occurring in CS heartwood, brazilin is the major colored compound containing heterotetracyclic structure. It can act as a reducing agent since it has a tendency to be oxidized to brazilein, a compound with one fewer hydroxyl group because it is replaced by the carbonyl functional group. From the review, the use of CS extract as a reducing and stabilizing

agent for the synthesis of AuNPs is still limited. Until now there is the only work by Chartarrayawadee et al. in which conventional heating was used for both the extraction of CS heartwood and the subsequent synthesis of AuNPs using the resulting extract [10]. Despite the successful fabrication of AuNPs, the overall process in that work was relatively time-consuming and required elaborate procedure such as lyophilization of the extract and overnight incubation of the synthesis reaction. In addition, the possible application of the CS-AuNPs obtained has not yet been studied or proposed.

To fill these research gaps, the aim of this work was to investigate a MW-assisted procedure for the synthesis of AuNPs using CS heartwood extract. Apart from this, since the reports about the MW-accelerated extraction of CS heartwood without the use of organic solvents are still limited, a green and rapid MW-based protocol for the extraction of CS heartwood using only water as a solvent was also investigated. In many studies, it has been reported that MW irradiation causes disruption of plant cells, thereby facilitating the mass transfer of solvent into the plant material and the release of the plant constituents [11]. Therefore, it is more efficient extraction method than conventional heating in terms of the requirement of less solvent and energy consumption, higher yield, and faster operation [12]. After the synthesis, CS-AuNPs were characterized and the metal-binding capability was primarily examined. Therefore, this is the first report that presented rapid and efficient routes to the extraction of CS heartwood and subsequent synthesis of AuNPs using MW irradiation. Additionally, the potential application of the resulting CS-AuNPs for colorimetric sensing of certain metals was preliminarily explored and proposed.

2 Materials and methods

2.1 Materials and reagents

Tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$), $FeSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$, K_2SO_4, $CaSO_4 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$, and $Al_2(SO_4)_3 \cdot 18H_2O$ were obtained from Sigma-Aldrich (Missouri, USA). CS heartwood powder was purchased from a local market in Thailand. A brazilin standard with a purity of >99% was purchased from Chengdu Biopurify Phytochemical Ltd, China. All other chemicals were of analytical grade. Distilled water was used throughout the work. Prior to use for the synthesis of CS-AuNPs, all glassware was cleaned using freshly prepared aqua regia solution ($HCl:HNO_3$, 3:1 by volume) and then rinsed thoroughly with water.

2.2 Extraction of CS heartwood

2.2.1 MW-assisted extraction

One gram of CS heartwood powder was added to 20 mL of water in a 50 mL-MW vessel. The samples were irradiated with MW (100, 200, or 300 W) using a MWe synthesizer (Discover SP, CEM Corporation, USA) set in a fixed power mode. After 1, 2, and 3 min, heating was stopped. The samples were collected by immediately immersing the tubes in ice and then subjected to centrifugation at 10,000 rpm and filtration through a 0.45 μm membrane filter. The clear extract was stored at 4°C until further use.

2.2.2 Extraction using conventional heating

One gram of CS heartwood powder was added to 20 mL of boiling water while stirring in a 50 mL vessel, which was immersed in a water bath set at 100°C. At 5, 15, 30, and 60 min, the samples were collected in the same manner as that of MW-assisted extraction. The clear extract was stored at 4°C until further use.

2.3 Determination of brazilin content in CS extract

The contents of brazilin in the CS extracts were determined by high-performance liquid chromatography (HPLC) (Agilent 1200 Infinity, Agilent Technologies, USA) following the method described by Warinhomhaun et al. [13]. The chromatographic separation was achieved on a C_{18} column (150 mm × 4.6 mm, 5 μm) by gradient elution with methanol and 2.5% v/v acetic acid as the gradient mixtures. The injection volume was 20 μL. The flow rate was 1 mL·min^{-1}, the detection wavelength was 280 nm and the column temperature was 35°C. The retention time of brazilin was 10.2 min. From the analysis results, the extraction method which gave the highest brazilin content in the extract was used for the preparation of the CS extract employed for the synthesis of AuNPs.

2.4 MW-assisted synthesis of CS-AuNPs

CS-AuNPs were synthesized by the reduction of $HAuCl_4$ using the CS extract as a reducing agent and stabilizing agent. For this purpose, a MW synthesizer (Discover SP, CEM Corporation, USA) set in a dynamic control mode was used. To establish the optimized synthesis protocol, 5 mL of $HAuCl_4$ solution of different concentrations (0.5, 1.5, and 2.5 mM) was mixed with the CS extract of different volumes (200, 400, and 600 µL). The synthesis was carried out at 50°C, 60°C, 70°C with the reaction time of 0.5, 1, 2 min. After the MW irradiation, the solutions were rapidly cooled by immersing the reaction vessels in the ice bath to stop the reaction. Finally, the CS-AuNPs were stored at 4°C in a refrigerator until further use.

2.5 Characterization of CS-AuNPs

The synthesized CS-AuNPs were characterized in several aspects. The physical appearance, i.e., color and clarity were examined visually and the colloidal property was confirmed by Tyndall effect using a laser beam. The ultraviolet-visible (UV-Vis) absorption spectra were recorded on a multimode plate reader (Victor Nivo, PerkinElmer, USA). To study the chemical functionality, the solution of CS-AuNPs was centrifuged at 14,000 rpm for 15 min and the precipitate was collected and then dried at 70°C overnight. The dried CS-AuNPs were analyzed by Fourier transformed infrared (FTIR) spectrometry (Thermo Nicolet Nexus 4700 FTIR spectrometer, Thermo Fisher Scientific, USA) using the KBr disc method. The FTIR spectra of CS-AuNPs and freeze-dried CS extract were compared. The mean hydrodynamic diameter and zeta potential of AuNPs were recorded by Zetasizer Nano-ZS (Malvern Instruments, UK). The morphology and particle size of CS-AuNPs were also studied by transmission electron microscopy (TEM) (Philips® Model TECNAI 20) with 80 kV accelerating voltage.

2.6 Stability study of CS-AuNPs

The solutions of CS-AuNPs prepared by using MW irradiation and conventional heating were kept at 4°C. After 28 days, the particle size as measured by zetasizer was compared to that of Day 0.

2.7 Preliminary study of metal binding property of CS-AuNPs

Since brazilein is capable of binding to certain metals, the study was conducted to preliminarily investigate whether fabricated CS-AuNPs could bind to metals and result in the aggregation and color change or not. The binding experiment was done by mixing 100 µL of CS-AuNPs solution with 100 µL 20 mM Fe^{2+}, Fe^{3+}, Al^{3+}, K^{+}, Ca^{2+}, Zn^{2+}, and Mg^{2+} solution. The reaction was left at room temperature for 10 min. Then, the UV-Vis absorption spectra were recorded to observe a shift of maximum wavelength.

3 Results and discussion

3.1 Optimal condition for MW-assisted extraction of CS heartwood

While organic solvents [14] or mixtures of organic solvents and water such as ethanol/water [13,15] were employed for the extraction of brazilin from CS heartwood in most previous studies, in this work only water was used as an extractant so that the resulting extract could be subsequently used for the synthesis of AuNPs without removal of organic solvents. Since MW-assisted extraction is superior to conventional methods for extraction of plant materials in terms of fast and deep internal heating, it was chosen in this work. To establish the optimal condition for the MW-assisted extraction of CS heartwood, the effects of MW power level and irradiation time on the yield of brazilin in the extract were evaluated. As shown in Figure 1a, more brazilin was extracted when higher power and longer irradiation time were used. However, since the MW synthesizer used in this study would terminate the irradiation after 3 min to avoid the over-the-limit of the generated pressure when the maximum power of 300 W was used, the irradiation using 300 W for 3 min was chosen for the extraction. Under this condition, the yield of brazilin was 13.1 ± 0.4% w/w.

In comparison to the extraction carried out by heating the plant samples in a water bath at 100°C, MW-assisted extraction was found to be more efficient since the conventional method gave a significantly lower brazilin yield (9.72 ± 1.56% w/w) and it required a much longer extraction time (30 min) (Figure 1b). In several works, it was evidenced that MW irradiation caused disruption of plant

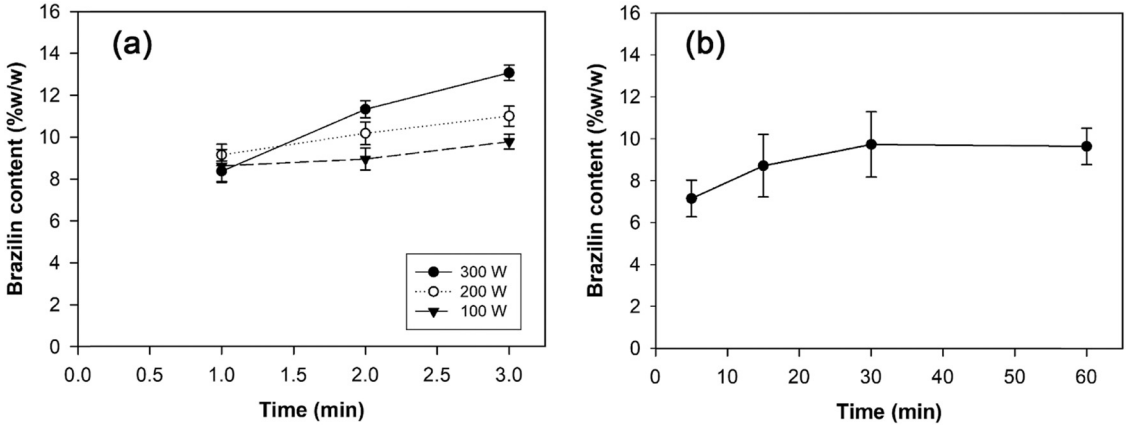

Figure 1: Brazilin contents in the CS-heartwood extract obtained from MW-assisted extraction (a) and extraction using conventional heating at 100°C (b) (n = 3).

cells and facilitated the mass transfer of solvent into the plant material, thereby promoting the efficient release of the plant constituents [11]. Additionally, the shorter heating time might help avoid the degradation and oxidation of brazilin to brazilein [9], allowing the better retention and availability of brazilin in the extract. Because of the more reliable heating processes which were efficiently controlled by a MW synthesizer, the better batch-to-batch reproducibility of the brazilin content was accomplished, as seen by the lower standard deviation values (bars) than those of the extraction using conventional heating. Therefore, it can be concluded that MW-assisted extraction was a faster as well as more efficient and energy-saving means of preparing the CS extract used as the reactant for the synthesis of AuNPs. Apart from that, since CS extract is known to have pharmacological activities, the MW-based protocol established in this study may be applied to the preparation of a high-brazilin aqueous CS extract for medicinal uses or studies.

3.2 Optimal condition for MW-assisted synthesis and characteristics of CS-AuNPs

The as-prepared CS extract from the optimal MW-assisted extraction was used for the MW-mediated synthesis of AuNPs. Since the temperature plays an important role in the synthesis of AuNPs, several studies reported that a MW system with precise temperature control function produced AuNPs with a highly reproducible diameter as well as the narrow size distribution [16,17]. The synthesis in this work was carried out by using a dynamic control mode of the MW synthesizer. In this mode, the temperature could be set and maintained at the desired level by constantly adjusting the MW power during the course of the reaction. To optimize the synthesis reaction, the varied volumes of the extract and concentrations of $HAuCl_4$ solution were initially tested while keeping the heating constant at 60°C for 1 min. The results showed that different compositions of the reactants produced the AuNP solutions with apparently different colors (Figure 2). The use of too low concentration of $HAuCl_4$ solution reactant (0.5 mM) gave the low yields of AuNPs, as indicated by pale pink or yellow color of the product solutions. On the other hand, at a high concentration of $HAuCl_4$ solution (2.5 mM) used together with the large volumes of the CS extract (400 and 600 µL), the solutions of CS-AuNPs appeared in dark color. It was probable that the large excess of functional groups of the CS extract at high concentrations might cause side reactions and/or affect the reduction process of gold ions, therefore producing the AuNPs with undesirable properties [10]. Of the reactants tested, those composed of 5 mL of 1.5 mM $HAuCl_4$ solution and 400 µL of the CS extract gave a red-wine colored solution and contained the smallest AuNPs. Based on this finding, the parameters related to MW irradiation were further optimized. While temperature and time of MW heating significantly affected the formation and the characteristics of AuNPs prepared by using different reducing agents in several studies [17–20], these two parameters had less pronounced effects on the resulting AuNPs, compared to the reactant composition, since they all gave similar red solutions (Figure 3). However, as determined by the zetasizer, the smallest particle size of CS-AuNPs with a narrow size distribution was obtained

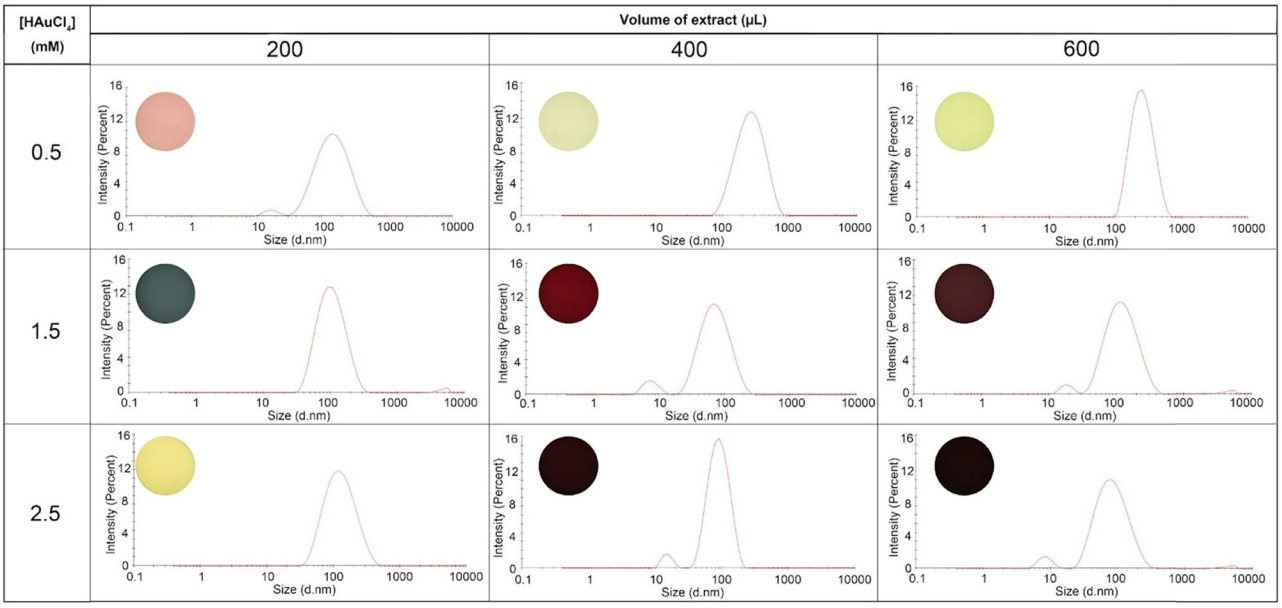

Figure 2: Color of solutions (shown in circle) and particle size distribution of CS-AuNPs obtained from the MW-assisted synthesis using different volumes of the extract and concentrations of $HAuCl_4$ solution. The reactions were conducted by MW irradiation at 60°C for 1 min.

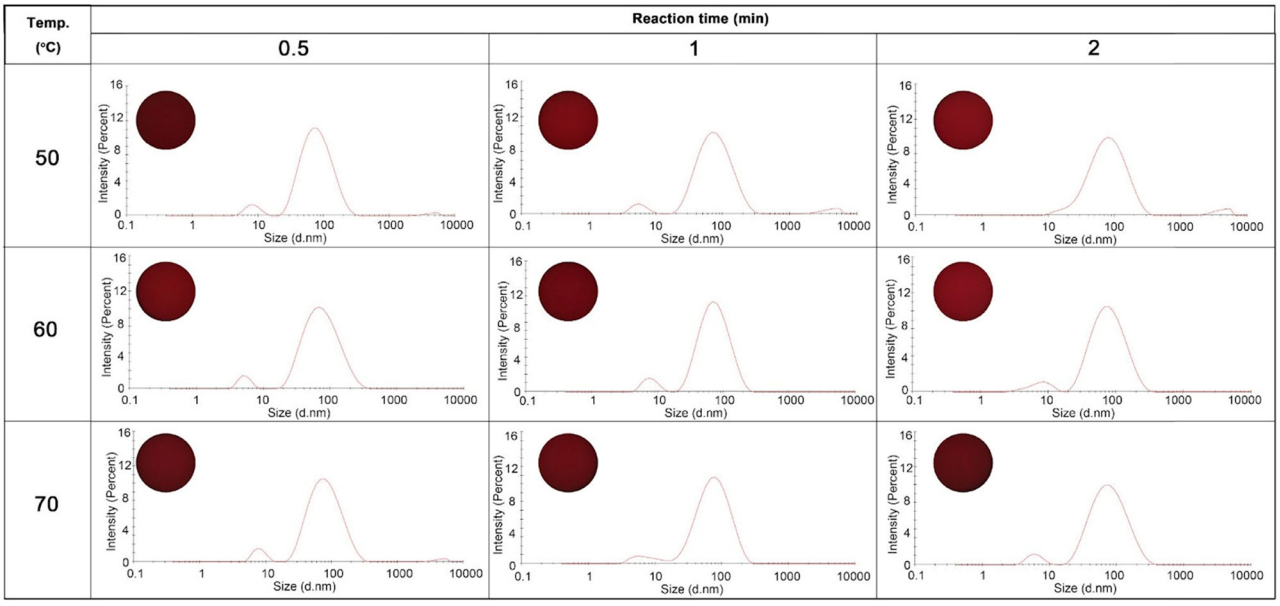

Figure 3: Color of solutions (shown in circle) and particle size distribution of CS-AuNPs obtained from the MW-assisted synthesis using different heating temperatures and reaction times. The reactions were conducted using the reactants composed of 5 mL of 1.5 mM $HAuCl_4$ solution and 400 μL of the CS extract.

from a 1 min MW irradiation in which the temperature was kept constant at 60°C.

The solution of CS-AuNPs prepared under this condition had a red-wine color (Figure 4a) with a UV-Vis absorption peak at 544 nm (Figure 4b). Light scattering was observed when a laser beam passed through the solution (Figure 4a), indicating the colloidal characteristic of AuNPs. From the TEM photograph, CS-AuNPs had a roughly spherical shape (Figure 5) with an average diameter of 17.7 ± 4.4 nm. As determined by the zetasizer, the hydrodynamic size of CS-AuNPs was 49.6 ± 0.4 nm. The zeta potential of CS-AuNPs was about −13.6 mV,

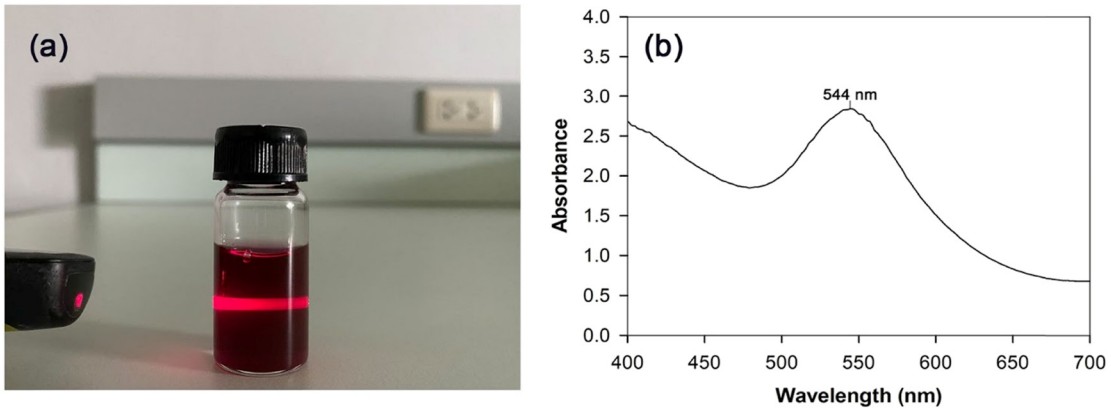

Figure 4: Color and Tyndall effect (a) and UV-Vis spectrum of CS-AuNP solution (b).

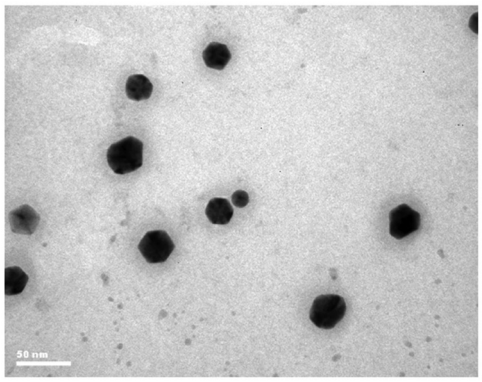

Figure 5: TEM image of CS-AuNPs.

probably due to the negatively charge of brazilein and other phytochemicals, e.g., phenolic compounds, flavonoids, and tannins in the CS extract which capped on the AuNPs [14].

FTIR spectrum of CS-AuNPs (Figure 6) was recorded in order to confirm the synthesis reaction and study the presence of functional groups. By comparing the FTIR spectrum of CS-AuNPs with that of CS extract, both of them showed a broadband peak at 3,400 cm^{-1} due to stretching vibrations of the hydroxyl group overlapping with the C–H stretching vibrations peak at 2,923 cm^{-1}. In the low-wavenumber region, the two spectra also had similar peaks. However, the spectrum of CS-AuNPs showed a peak at 1,507 nm^{-1}, representing C═O stretching,

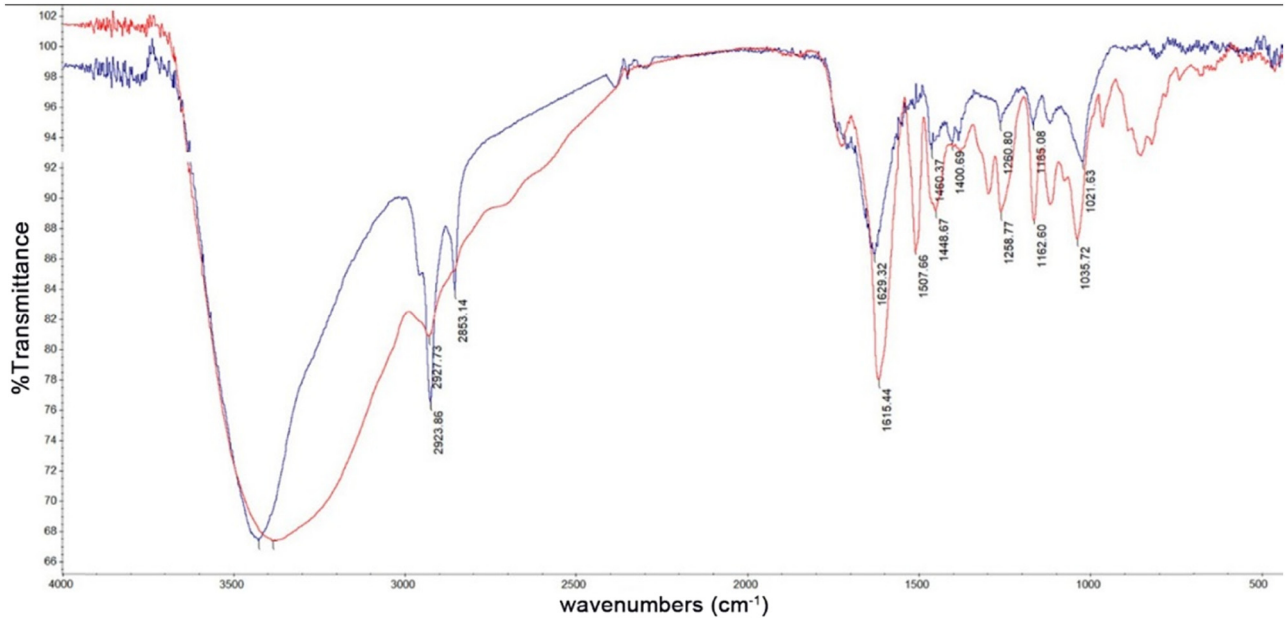

Figure 6: FTIR spectra of CS-AuNPs (red line) and CS extracts (blue line).

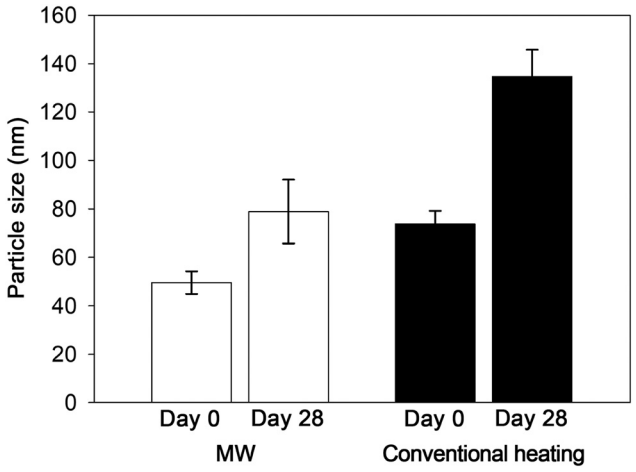

Figure 7: The average hydrodynamic particle size of CS-AuNPs prepared by MW irradiation (MW) and conventional heating after storage at 4°C for 28 days (n = 3).

which was absent in the spectrum of CS extract due to the formation of the carbonyl group in brazilein after the hydroxyl group of brazilin underwent the oxidation reaction [21]. The results confirmed that the main capping compound on the CS-AuNPs surface was brazilein.

Chartarrayawadee et al. reported the synthesis of CS-AuNPs using conventional heating [10]. In that work, the CS extract which was lyophilized after the extraction by conventional heating at 80°C for 3 h was reconstituted into the solution and then mixed with $HAuCl_4$ solution. Subsequently, the mixture was stirred overnight (12 h) at room temperature followed by heating the solution at 90°C for 1 h, resulting in quasi-spherical and short-length earthworm-like shaped CS-AuNPs with the average hydrodynamic size of 49 nm, which was comparable to those obtained from MW-assisted synthesis in this study (50 nm). However, since the use of MW irradiation significantly shortened the time required for both extraction of

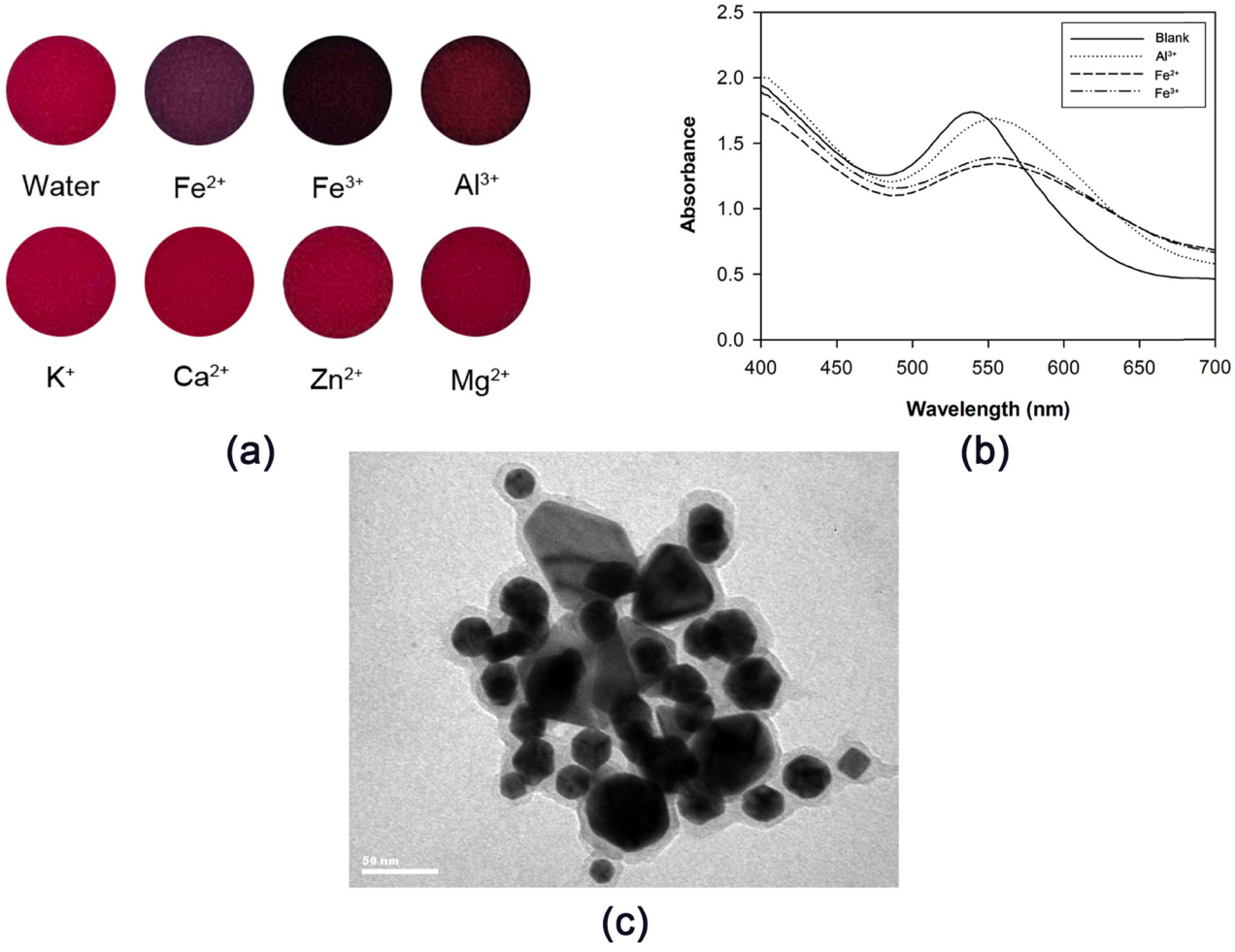

Figure 8: Color of the reaction solutions after the addition of metal ions to the CS-AuNPs solution (water was used as a blank) (a), shift of absorption bands due to Fe^{2+}, Fe^{3+}, or Al^{3+} (b), and TEM photograph of the CS-AuNPs aggregation induced by Fe^{2+} (c).

CS heartwood to 3 min and the synthesis of CS-AuNPs to only 1 min. Furthermore, the CS extract after the removal of plant debris could be readily used for the synthesis of CS-AuNPs without lyophilization. Thus, the MW-based procedure was more rapid and feasible.

3.3 Stability of CS-AuNPs

After the storage of CS-AuNPs solutions at 4°C for 28 days, the particle size of CS-AuNPs obtained from the MW-assisted synthesis increased from 49.6 ± 4.7 to 78.9 ± 13.2 nm (Figure 7). Nevertheless, it was smaller than 100 nm, which still met the definition of nanoparticles. In contrast, CS-AuNPs which were prepared by the conventional method, using the same composition of reactant, temperature, and time as those used in the MW-assisted synthesis, became larger from 73.7 ± 5.4 to 134.7 ± 11.1 nm. This finding demonstrated that even though the synthesis was conducted under the same conditions, the CS-AuNPs obtained from different means of heating possessed different characteristics. It was probable that MW irradiation provided the synthesis reaction with MW effects, i.e., those resulting from the material and wave interactions and the dipolar polarization, in addition to the effects of efficient heating [18–20]. It can be concluded that CS extract was efficient in stabilizing AuNPs over a period of time and CS-AuNPs obtained from the MW-based method were more stable than those synthesized by using conventional, convective heating.

3.4 Metal ion binding property of CS-AuNPs

Since brazilein forms stable coordination complexes with certain metal ions [22,23], the metal binding ability of CS-AuNPs in which brazilein was present on the surface as the capping agent was preliminarily investigated. After the addition of Fe^{2+}, Fe^{3+}, or Al^{3+} to the CS-AuNPs solution, the color change from red to purple was clearly observed whereas negligible alteration was seen with other ions (Figure 8a). In addition, the presence of Fe^{2+}, Fe^{3+}, or Al^{3+} shifted the absorption bands of CS-AuNPs solution from 544 to 555 nm (Figure 8b). The TEM photograph revealed that, Fe^{2+}, Fe^{3+}, and Al^{3+} were capable of binding to CS-AuNPs and inducing the aggregation of particles (Figure 8c), leading to the chromogenic and spectral changes. These results implied that CS-AuNPs had the potential for use as a colorimetric sensor for selective detection of these ions, e.g., aluminum hydroxide in antacid, ferrous sulfate in supplements, and contaminating ions in water or pharmaceutical preparations. For this purpose, the optimization of the reaction conditions, e.g., pH and quantity of AuNPs used in the assay, and the validation of the developed analytical method must be further investigated.

4 Conclusions

The MW-based methods for the extraction of CS heartwood and subsequent synthesis of AuNPs using the extract as both a reducing and stabilizing agent were reported. By the MW irradiation, the extraction was accomplished in 3 min, yielding the extract containing high and reproducible brazilin content which could be readily used for the synthesis of AuNPs. Under the optimized synthesis conditions, i.e., the reactant composition, MW heating time, and reaction temperature, spherical CS-AuNPs with small size and acceptable stability were obtained within 1 min. Furthermore, the resulting CS-AuNPs were capable of selective binding to Fe^{2+}, Fe^{3+}, and Al^{3+} and inducing particle aggregation. This phenomenon led to the noticeable change of color and shift of UV-Vis absorption maxima, implying their potential for colorimetric sensing of these metal ions.

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