Ultrasound-assisted green synthesis of gold nanoparticles using citrus peel extract and their enhanced anti-inflammatory activity

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\textbf{ABSTRACT}

Ultrasound and plant extract are two green approaches that have been used to synthesize gold nanoparticles (AuNPs); however, how the combination of ultrasound and citrus peel extract (CPE) affects the structure characteristics and the bioactivity of AuNPs remains unknown. Here we investigated the effects of ultrasound conditions on the particle size, stability, yield, phenolic encapsulation efficacy, and the anti-inflammatory activity of AuNPs. The results showed that temperature was positively correlated to the particle size and the anti-inflammatory activity of synthesized AuNPs. Increasing the power intensity significantly decreased the particle size, while increased the change of total phenolic content (ΔTPC) in the reaction mixture. The increase of ΔTPC caused the enhanced anti-inflammatory activity of AuNPs. The AuNPs synthesized with or without ultrasound treatment were characterized using UV–Vis, DLS, SEM, TEM, EDS, XRD, and FT-IR. The result verified the formation of negatively charged, spherical, stable, and monodispersed AuNPs. AuNPs synthesized with ultrasound (AuNPs-U) has smaller particle size (13.65 nm vs 16.80 nm), greater yield and anti-inflammatory activity (IC\textsubscript{50} 82.91 vs 157.71 μg/mL) than its non-ultrasound counterpart (AuNPs-NU). HPLC analysis showed that hesperidin was the key reductant for the synthesis of AuNPs. AuNPs-U also inhibited the mRNA and protein expression of iNOS and COX-2 in the LPS-induced Raw 264.7 cells. Our research elucidates the relationship between the reaction conditions and the structure characteristics and the anti-inflammatory activity of AuNPs synthesized using CPE with the help of ultrasound, thereafter, provides a feasible and economic way to synthesize AuNPs that can be used to ameliorate inflammation.

\textbf{1. Introduction}

Gold nanoparticles (AuNPs) is one of the most attractive metal nanoparticles (NPs) due to their unique physical and chemical properties, good biocompatibility, high dispersibility, high stability, and low toxicity [1–3]. They have been used as the biomedicine (anti-cancer drug or drug carrier, cancer detection), biosensors, catalyst, and anti-microbial agents [1–3]. Different physical and chemical approaches have been used to synthesize metal NPs, however, these methods are either not cost-effective, or need toxic chemical reducing agents which are harmful to environment. In recent years, green synthesis of metal NPs has received tremendous attention due to the advantages of energy efficiency, safety, less toxicity, and environmentally friendly [4]. Ultrasound is one of the attractive green approaches that has been used in metal NPs synthesis because it is capable to produce smaller and narrower size distribution NPs, shorten reaction time, synthesize without reducing agent under ambient temperature [4]. Sonochemical synthesis of AuNPs has been extensively studied by different researchers who mainly focus on understanding the effects of ultrasound frequency [5,6], power [7], organic additives [8–10], time[11], type of air [8],

\begin{itemize}
  \item **Abbreviations:** AuNPs, gold nanoparticles; AuNPs-U, gold nanoparticles synthesized by citrus peel extracts under ultrasonic conditions; AuNPs-NU, gold nanoparticles synthesized by citrus peel extracts without ultrasound treatment; COX-2, cyclooxygenase-2; CPE, citrus peel extract; DLS, dynamic light scattering; EDS, energy dispersive spectrometer; FE-TEM, field emission transmission electron microscopy; FT-IR, Fourier Transform-Infrared spectra; HRP, horseradish peroxidase; IFN-γ, interferon γ; IL-1β, interleukin 1β; IL-6, interleukin 6; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MT, million tons; NF-kB, nuclear factor κB; NO, nitric oxide; NPs, nanoparticles; PGE-2, prostaglandin E2; PVDF, Polyvinylidene difluoride; SEM, scanning electron microscope; TNF-α, tumor necrosis factor; TPC, total phenolic content; XRD, X-ray diffractometer.

\end{itemize}

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concentration of reducing agents [11], stabilizer [12], on the rate of sonochemical reduction of Au(III) to AuNPs, shape and size of AuNPs. The change in the properties of anti-inflammatory activity is crucially, related to the surface of AuNPs and to the stabilizers present in the suspension. However, how the change of different ultrasound conditions affects this property directly is unfounded and whether there is a relationship between the particle size and anti-inflammatory activity remain unknown.

Except for ultrasound, plant extracts have also been widely used in the green synthesis of metal NPs because they are rich in proteins, carbohydrates, ketones, aldehydes, polyphenols, flavonoids, phenolic acids, and alkaloids, which can serve as reducing, capping, and stabilizing agents, thereby reducing the use of toxic chemicals. The extracts of different plant parts, including leaf, root, bean, seed, fruit, bark, flower, and juice have been used to synthesize AuNPs, however, the use of citrus peel extract (CPE) to synthesize AuNPs is scarce [13]. The reported particle size of AuNPs ranges from 2 – 600 nm and the shapes are spherical, triangular, rod, decahedral, icosahedral, pentagon, hexagon, and cubic depending on the varied chemical composition of plant extract, pH, temperature, time, the mass ratio of plant extract, and metal ion [1].

According to the Statistical Bulletin of 2020, the total global production of citrus fruits is estimated to be 143.67 million tons (MT), among which 76.29 MT are orange [14]. Citrus peel, which accounts for about 40–50% of the total citrus fruit, is mainly discarded in the land field with only a small proportion is used in animal feed, thus causing severe environmental problem. However, citrus peel is rich in organic acid, ascorbic acid and malic acid), phenolic acids (caffeic acid, ferulic acid, p-coumaric acid, gallic acid, and chlorogenic acid) and flavonoids (hesperidin, hesperetin, rutin, naringenin, narinutein, and tangeritin), and the flavonoids are more abundant in the peels than in the juice [15]. These phytochemicals are good reducing agents and stabilizers which also possess anti-inflammatory, antioxidant, antibacterial, antiallergic and anticancer functions [15,16]. CPE has been used to synthesize ZnO [17], silver [18], copper [19], and Fe2O3 [22] NPs. Most of the research used CPE to green synthesize silver NPs for antibacterial purpose. There is very limited research studied on the green synthesis of AuNPs using CPE. Yuan et al. synthesized AuNPs using Citrus maxima peel extract and studied its catalytic and antibacterial capacity [19]. The impacts of HAuCl4 concentration, temperature, time, and pH on the synthesis of AuNPs were investigated. However, only UV–Vis spectrum was used to show the change of nanoparticle reaction system under the above four reaction conditions. Yang et al. studied the impact of different pH on the yield, morphology and size of AuNPs using Citrus Sinensis peel extract [20]. However, the research comprehensively studied how the green synthesis conditions in combination of CPE and ultrasound affect the particle size, stability, the changes of phenolic compounds, and the anti-inflammatory activity of AuNPs are scarce. Therefore, synthesis of AuNPs using CPE in assistant with ultrasound is a promising green synthesis approach which will not only combine the advantages of CPE and ultrasound, but also provide an alternative way to produce value-added products from citrus peels.

Inflammation is the natural response of the body to the biological, chemical, or physical stimuli [23]. Numerous researches showed that chronic inflammation is closely associated with various diseases such as cancer, diabetes, cardiovascular diseases, atherosclerosis, arthritis, inflammatory bowl diseases, and autoimmune diseases [23–25]. AuNPs have been reported to possess anti-inflammatory activity. Elbagory et al. showed that AuNPs synthesized by Hypoxis hemerocallidea aqueous extract and its major bioactive hypoxoside reduced the production of interleukin – 1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) in THP-1 cells and interferon γ (IFN-γ) in NK92 cells [26]. AuNPs synthesized using Panax ginseng leaf extract can inactivate NF-κB signalling pathway, thereby inhibiting prosta glandin E2 (PGE-2), cyclooxygenase-2 (COX-2), nitric oxide (NO), inducible nitric oxide synthase (iNOS), IL-6, and TNF-α in lipopolysaccharide (LPS)-induced Raw 264.7 cells [27]. However, the research comprehensively studied how the green synthesis conditions in combination of CPE and ultrasound affect the particle size, stability, the changes of phenolic compounds, and the anti-inflammatory activity of AuNPs is scarce. It is well known that different plants contain different bioactive reducing agents, therefore, it is also important to elucidate the contribution of phytochemicals in the plant extract in the synthesis of AuNPs and the anti-inflammatory mechanism of the synthesized AuNPs herein.

In the present study, we synthesized AuNPs using CPE under ultrasound treatment. The impact of ultrasonic temperature, mass ratio of citric peel to chloroauric acid, pH, ultrasonic time, and ultrasonic power on the particle size, change of total phenolic content (ΔTPC), and the NO inhibitory activity were investigated and the interrelationship between the synthesis conditions and the above attributes were assessed. AuNPs synthesized with or without ultrasound treatment were characterized and compared using multi-spectroscopy and microscopy methods. The changes of phytochemicals in the CPE before and after synthesis were analyzed using HPLC to elucidate the contribution of different phytochemicals to the biosynthesis of AuNPs. Finally, the anti-inflammatory activity of AuNPs-U was assessed by measuring the NO, iNOS, and COX-2 in LPS-induced Raw 264.7 cells.

2. Materials and methods

2.1. Materials

Chloroauric acid (HAuCl4·3H2O) was purchased from Shanghai (RHAWN Co. Ltd., Shanghai, China). HPLC grade (>98%) phytochemical standards were purchased from Chengdu Must Bio-Technology Co. Ltd. (Chengdu, China). Dulbecco’s Modified Eagle’s Medium (DMEM), antibiotic mixture (100×), fetal bovine serum (FBS), and LPS were purchased from Gibco (Grand Island, NY, USA). RNA easy fast cell kit, fastKing gDNA dispensing RT SuperMix, and RealUniversal Color PreMix were purchased from Tiangen Biotech Co. Ltd. (Beijing, China). Primers, including iNOS (5′ forward, CTT CAA CCC CAA GGT TGT CTG CAT and 3′ reverse, ATG TCA TGA GCA AAG GCC CAG AAC), COX-2 (5′ forward, TTG TTG AGT CAT TCA GAC GAC AGA T and 3′ reverse, CAG TAT TGA GGA GAA CAG ATG GGA TT), and β-actin (5′ forward: AGG GCC GAG CGG GAA ATG CTG 3′ reverse: CTT CTT GCT GAT CCA CAT CT) were synthesized by Sangon Biotech (Shanghai, China). iNOS, COX-2, and β-actin primary antibodies and horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG were purchased from Cell Signaling Technology (Massachusetts, USA). Bicinchoninic acid (BCA) protein assay reagent and BeyoECL star substrate were purchased from Beyotime Biotechnology (Shanghai, China). Polyvinylidene difluoride (PVDF) membrane was purchased from Merck Millipore (Temecula, USA). Raw 264.7 cells were purchased from Type Culture Collection Cell Bank of Chinese Academy of Sciences (Shanghai, China). All other reagents are analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Zhenjiang, China).

2.2. Preparation of citrus peel extract (CPE)

Oranges (Citrus sinensis var. Valencia) were purchased from the local market in Zhenjiang on July 2020. The peels were separated from the fruits and then dried in a hot air oven at 60°C for 72 h. The dried citrus peels were powdered and passed through a 100-mesh sieve. The obtained citrus peel powders (2 g) were extracted twice with 20 mL, 80% aqueous methanol under sonication for 20 min. The supernatants were combined and the methanol was evaporated under reduced pressure, followed by reconstituted with ddH2O to a final volume of 25 mL and then stored in a refrigerator for further use.

2.3. Synthesis of AuNPs

HAuCl4·3H2O stock solution (1%, w/v; 7.85 mL) was mixed with
different volume of CPE and the final volume was made up to 200 mL using ddH₂O. The reaction mixture was sonicated in a self-designed and manufactured multi-frequency power ultrasonic device from Dr. Haile Ma’s group (Jiangsu University) under different reaction conditions [28]. After sonication, the mixture was centrifuged at 11000 \( \times \) g for 1 h at 4 °C. The supernatant was collected for TPC and phytochemical analysis. The precipitates were collected and washed with ddH₂O for three times followed by re-dispersed in 10 mL ddH₂O. An aliquot of gold nanoparticle (AuNPs) was stored in a refrigerator for cell culture and the rest of the AuNPs were freeze-dried for particle characterization. The ultrasound conditions included: ultrasonic temperature (50, 57.5, 65, 72.5, 80 °C), mass ratio of citrus peel to HAuCl₄·3H₂O (15 : 1, 20 : 1, 25 : 1, 30 : 1, 35 : 1), pH (6, 7, 8, 9, 10), ultrasonic time (10, 15, 20, 25, 30 min), and ultrasonic power (60, 90, 120, 150, 180 W). After obtaining the optimal conditions, AuNPs were also synthesized without ultrasound, namely, AuNPs-U and AuNPs-NL, respectively. Ultrasonic frequency was set at 40 kHz. All the experiments were repeated three times. The yield of the obtained AuNPs is calculated as follows:

\[
\text{Yield} \left( \% \right) = \frac{\text{weight of gold nanoparticles}}{\text{weight of Au in chloroauric acid}} \times 100 
\]

(1)

2.4. Determination of total free polyphenol content (TPC)

TPC in the supernatant of reaction mixture after reaction and the CPE before NPs synthesis were determined using the Folins-Ciocalteu colorimetric method described in Chen et al. [29]. The percentage change of TPC before and after AuNPs synthesis were calculated using Equation (2).

\[
\Delta \text{TPC} \left( \% \right) = \frac{\text{TPC of CPE before ultrasonication} - \text{TPC of CPE after ultrasonication}}{\text{TPC of CPE before ultrasonication}} \times 100
\]

(2)

2.5. Determination of phytochemicals in the CPE using HPLC

The Shimadzu LC system (LC-20AD) equipped with a Phenomenex Kinetex C18 (100 × 4.8 mm, 5 μm) column (Phenomenex, Torrance, CA, USA) and a diode array detector was used to analyze the phytochemicals. The appropriates diluted CPE (20 μL) were separated with mobile phases that include solvent A (0.1% trifluoroacetic acid) and solvent B (acetonitrile) with the following gradient: 0 min, 26%-27% B; 25 min, 26%-27% B; 25–28 min, 27 %-30% B; 28–33 min, 30%-40% B; 33–40 min, 40%-65% B; 40–45 min, 65%-15% B; 45–50 min, 15% B. Hesperetin, naringenin, hesperidin, and narirutin were detected at 280 nm and caffeic acid, p-coumaric acid, ferulic acid, chlorogenic acid, tangeretin, and nobiletin were detected at 330 nm. The column temperature was set at 25 °C and the flow rate was 1.0 mL/min. The concentration of phytochemicals is expressed as μg/mL in CPE.

2.6. Characterization of AuNPs

The AuNPs were characterized using multi-spectroscopy and microscopy methods. UV–Vis spectra (200 – 800 nm) of diluted AuNPs were recorded by a UV–Vis spectrophotometer (Agilent Technologies Inc., USA). A laser particle size analyzer (Litesizer 500, Anton Paar, Shanghai, China) was used to analyzed the particle size distribution and Zeta potential of AuNPs. The Fourier Transform-Infrared (FT-IR) spectra (500 – 4000 cm⁻¹) were obtained using (Nicolet Nexus 470 spectrometer, USA). The morphology of AuNPs was observed by a scanning electron microscope (SEM) (JSM-7800F, JEOL, Japan) and a field emission transmission electron microscopy (FE-TEM) (JEM-2100, JEOL Ltd., Japan) equipped with an energy dispersive spectrometer (EDS) to evaluate the elements present in AuNPs. For SEM measurement, freeze-dried AuNPs were added to the conductive adhesives and the excess powders were blown away. For TEM measurement, a drop of the synthesized AuNPs was deposited on a carbon coated copper grid and evaporated at 50°C for two hours. The crystallinity of AuNPs was determined using a X-ray diffractometer (Bruker AXS Ltd., Germany).

2.7. Anti-inflammatory activity of AuNPs

2.7.1. Analysis of nitric oxide (NO) inhibitory activity

The NO inhibitory activity of AuNPs was evaluated according to the method described by Chen et al. [30]. Raw 264.7 cells were grown in the complete DMEM which contains 100 U/mL penicillin, 100 g/mL streptomycin, and 10% FBS in a 37°C cell culture oven containing 5% CO₂. The medium was changed every other day. Raw 264.7 cells were seeded in 96-well plates at a density of 10⁵ cells / well and incubated overnight, thereafter, the cells were pre-incubated with AuNPs for 1 h followed by stimulation with 0.1 μg/mL LPS and incubation for another 24 h. Thereafter, 100 μL cell culture supernatant was reacted with 100 μL Griess reagent, and the absorbance was observed at 575 nm with a microplate reader. The standard curve of sodium nitrite was used to calculate the concentration of NO. The half-maximal inhibition concentration (IC₅₀) of each sample was calculated to estimate the anti-inflammatory capacity of AuNPs. Cell viability was measured by MTT assay [30] and only the viability of the cells > 90% were used.

2.7.2. Real-Time quantitative reverse transcription PCR (qRT-PCR)

Raw 264.7 cells (1 × 10⁵ cells /well) were seeded in 6-well plates and cultured overnight. After pre-treating with different concentrations (20, 40, 80 μg/mL) of AuNPs-U for 1 h, Raw 264.7 cells were incubated with 0.1 μg/mL of LPS for 24 h. Total RNA of the cells was extracted using RNA Easy Fast Cell Kit according to the manufacturer’s instructions. The RNA with a 260/280 > 1.8 was reverse transcribed into cDNA using FastKing gDNA Dispelling RT SuperMix kit, and then proceed quantitative PCR using RealUniversal Color Premix (SYBR Green) kit. The qPCR program was: pre-denaturation at 95 °C for 15 min; denaturation at 95 °C for 10 s, annealing at 55 °C for 20 s, extension at 72 °C for 30 s, 40 cycles. The sequences of the primers, including iNOS, COX-2, and β-actin were synthesized based on the sequences described by Chen & Kitts [31].

2.7.3. Western blot

The expressions of iNOS and COX-2 proteins were determined using the method described by Chen & Kitts [31]. Raw 264.7 cells in 6-well plates were lysed with 200 μL of RIPA cell lystate on ice for 30 min. After centrifugation at 14000 × g for 15 min under 4 °C, the supernatant was collected and the protein concentration was measured by BCA assay. Proteins were separated on an 8% SDS-PAGE gel followed by transferred to PVDF membrane. After blocking with 5% skimmed milk in the blocking buffer for 1 h at room temperature, the membrane was incubated with 1:1000 diluted primary antibody (iNOS, COX-2, β-actin) at 4 °C overnight followed by incubated with HRP-labeled goat anti-rabbit IgG for 1 h. The blot was exposed to beyoECL star substrate and visualized by an enhanced chemiluminescence system (Tanon 4600, Shanghai, China).
2.8. Statistical analysis

Data were analyzed by one-way ANOVA with Tukey’s post hoc test to compare the significant difference among the treatments pair-wisely using MINITAB software (Version 17, Minitab Inc., State College, PA). A P < 0.05 denotes significantly different.

3. Results and discussion

In the present study, the effects of ultrasound conditions, including temperature, mass ratio of CPE and chloroauric acid, pH, time, and ultrasound power on the particle size, zeta potential, ΔTPC in the CPE solution, and the NO inhibitory activity of the synthesized AuNPs were investigated to discover the relationship between ultrasound conditions and the characteristics and bioactivity of AuNPs. The UV–Vis spectrum in Fig. 1 showed the characteristic peaks in the range of 542–588 nm, indicating the successfully synthesis of AuNPs in all the reaction systems. The negative zeta potential values range from −27.28 to −51.21 mV suggested that the stable AuNPs were formed (Table 1).

3.1. The effect of ultrasonic conditions on the synthesis of AuNPs using CPE

3.1.1. Ultrasonic temperature

Fig. 1A showed the UV–Vis spectra and the color of AuNPs synthesized under different temperature. The width and maximum UV–Vis absorbance wavelength of the characteristic peak reflect the size and properties of the synthesized AuNPs. In general, the wider the peak the larger of the particle size [32]. As the temperature increased, the color of the AuNPs solution gradually changed from pink to violet, and then changed back to dark pink at 72.5 °C. The color of AuNPs is associated with the particle size. As the particle size increases, the color changes from pink to wine red, then to blue-violet, and last to black due to the surface plasmon resonance of AuNPs [33,34]. The mean particle size (MPS) measured by a laser particle size analyzer further confirmed that the particle size of AuNPs increased as the temperature increased from 50 °C to 65 °C and then decreased as the temperature further increased to 72.5 °C (Table 1), which is in consistent with the findings from Gan et al. [35]. This phenomena is due to the Oswald ripening phenomenon, where small AuNPs tend to agglomerate to form larger NPs at higher temperatures [36]. The negative zeta potential value indicates that the negative organic molecules (e.g. OH−) are encapsulated in the metal nanoparticles, which reduces the repulsive force between AuNPs, hinders agglomeration, and improves their stability [37]. This result was in accordance with the change of TPC after ultrasonication (Table 1). The percentage change of TPC (ΔTPC, %) ranged from 27.02% to 30.75%, which was not significantly affected by the reaction temperature. The reduced TPC in the CPE indicated that the phenolic compounds functioned as reducing agent and contributed to the negative zeta potential value due to their rich in hydroxyl group. The ΔTPC of the CPE in the synthesis system treated at 72.5 °C was the maximum, while the IC50 of NO inhibitory activity were smallest (119.33 μg/mL), indicating that the greater encapsulation of phenolic compounds on the AuNPs may contribute to its higher anti-inflammatory activity. Therefore, in the following experiment, the temperature was set at 72.5 °C.

3.1.2. Mass ratios of CPE and chloroauric acid

Fig. 1B showed that with the increase of the mass ratio of CPE to chloroauric acid, the yield of AuNPs increased, while ΔTPC showed a reverse trend due to the increasing phenolic compounds added in the reaction system (Table 1). The mass ratio did not affect the zeta potential; however, it significantly influenced the particle size and the NO inhibitory. The MPS showed an increase trend when the mass ratio enhanced from 15 : 1 to 25 : 1 because that the reducing power increased as more CPE was added, thus the AuNPs were formed and agglomerated instantly, resulting in an increase in the particle size. When the mass ratio reached 30 : 1, the particle size began to decrease, which may be associated with the excessive CPE that caused strong interactions...
between biomolecules on the surface of NPs, thereby preventing the agglomeration of newly formed AuNPs, and thus resulting in a decrease in the particle size [34]. Prathna et al. [38] reported that as the concentration of the lemon juice increased, the electron density increased significantly due to the charged groups in the reducing agent restricting the free electrons of the metal clusters within a small volume. Moreover, the surrounding reducing agent also increased the surface change of NPs, thus reduced the particle size. AuNPs prepared at the mass ratio of 20:1 have the lowest IC₅₀ of 108.71 μg/mL, therefore, in the following experiment, a mass ratio of 20:1 was used.

3.1.3. pH

Fig. 1C showed that the neutral and slightly acidic pH (6 and 7) conditions produced greater quantity of AuNPs compared with the basic pH (8, 9, 10) conditions. However, the absolute values of zeta potential of AuNPs synthesized under pH 6 and 7 were lower than that of basic conditions, indicating that AuNPs synthesized under basic condition were more stable. When pH reached 10, the absorption peak of AuNPs has a tendency of red shift, indicating that the synthesized AuNPs have a larger particle size and have a tendency to agglomerate, which was confirmed herein by evaluating the MPS (Table 1). pH is a critical factor to influence the electrical charges of the biomolecules in the plant extract, thereby affecting their reducing, capping, and stabilizing capacities, and thus the size and shape of NPs [39]. The impacts of pH on the particle size, shape and reaction rate varied in different research depending on the plants and the metal ions. Khalil et al. [39] reported that slightly alkaline condition (pH = 8) increased the reducing and stabilizing power of reducing agents in the olive leaf extract, therefore accelerated the reaction rate of silver nanoparticle synthesis. However, strong acidic (pH ≤ 5) or basic (pH ≥ 10) conditions slowed down the reaction rate. Khalil et al. [39,40] also found that AuNPs synthesized using olive leaf extract under acidic conditions were unstable and precipitated within 12 h. However, in the range of pH from 7.3 to 9.6, the reaction rate, yield, and particle size significantly increased, which followed a decrease when the pH further increased to 10. We found that IC₅₀ of AuNPs synthesized at pH 9 was the lowest, which was associated with the smallest particle size. Therefore, pH 9 was applied in the following experiments.

3.2. Ultrasound time

Fig. 1D showed that ultrasound time did not significantly affect the yield of AuNPs. Mobaraki et al. [41] reported a similar trend that the absorption peak of AuNPs moved to a longer wavelength with the increase of time during the process of reaction. We also found that ΔTPC and zeta potential were significantly decreased as the ultrasound time increased, which was associated with the increased particle size. The possible reason was that as the ultrasound time increased, the loaded phenolic compounds in AuNPs released into the reaction solution again due to the acoustic cavitation effect, thereafter reduced the absolute values of zeta potential, and thus the particles were prone to agglomeration to increase the particle size. The IC₅₀ of AuNPs was gradually increased as the ultrasound time increased to 25 min. This result may be due to the larger particle size and the less encapsulated phenolic compounds in AuNPs. Therefore, the selected optimal synthesis time was 10 min.

### Table 1

The impacts of ultrasonic conditions on the MPS, Zeta potential, ΔTPC, and NO inhibitory activity (IC₅₀) of AuNPs.

| Ultrasonic conditions | Levels | MPS (nm) | Zeta potential (mV) | ΔTPC (%) | IC₅₀ (μg/mL) |
|-----------------------|--------|----------|---------------------|----------|--------------|
| Temperature¹ (°C)     | 50     | 29.77 ± 0.36³ | -40.19 ± 1.03³ | 30.42 ± 2.13³ | 305.21 ± 0.57³ |
| 57.5                  | 67.07 ± 2.06³ | -34.45 ± 2.74³ | 28.97 ± 1.08³ | 139.64 ± 4.62³ |
| 65                    | 76.35 ± 7.14³ | -44.26 ± 0.81³ | 28.10 ± 3.44³ | 165.91 ± 4.86³ |
| 72.5                  | 54.54 ± 0.79³ | -70.66 ± 1.08³ | 30.75 ± 2.20³ | 119.33 ± 5.07³ |
| 80                    | 69.95 ± 2.05³ | -40.93 ± 1.08³ | 27.02 ± 2.69³ | 200.21 ± 10.48³ |
| Mass ratio²           | 15     | 56.76 ± 0.25⁴  | -48.28 ± 2.43³ | 44.18 ± 3.98³ | 127.41 ± 6.55³ |
| 20                    | 67.37 ± 4.57⁴  | -45.91 ± 0.99³ | 34.10 ± 1.51³ | 108.71 ± 1.49³ |
| 25                    | 93.87 ± 4.08⁴  | -51.21 ± 2.59³ | 29.79 ± 0.17³ | 155.01 ± 11.11³ |
| 30                    | 54.54 ± 0.79³  | -47.06 ± 1.08³ | 30.75 ± 2.20³ | 119.33 ± 5.07³ |
| 35                    | 61.88 ± 5.25⁴  | -50.13 ± 0.98⁴  | 19.05 ± 1.31³ | 156.80 ± 9.79³ |
| pH²                   | 6      | 61.02 ± 2.06⁴  | -27.28 ± 0.71³ | 32.33 ± 3.50³ | 116.75 ± 0.60³ |
| 7                    | 55.49 ± 4.55³ | -32.94 ± 1.26³ | 24.78 ± 1.56³ | 112.50 ± 5.53³ |
| 8                    | 67.37 ± 4.57⁴  | -45.91 ± 0.99³ | 34.10 ± 1.51³ | 108.71 ± 1.49³ |
| 9                    | 54.79 ± 5.08⁴  | -46.35 ± 1.39³ | 27.23 ± 1.48³ | 103.69 ± 3.27³ |
| 10                   | 75.41 ± 4.50⁵  | -44.23 ± 1.69³ | 30.18 ± 4.45³ | 130.22 ± 3.99³ |
| Time³ (min)           | 10     | 49.91 ± 2.53³  | -40.86 ± 3.04³ | 43.70 ± 2.32³ | 98.06 ± 6.19³ |
| 15                   | 54.79 ± 5.08³ | -46.35 ± 1.39³ | 27.23 ± 1.48³ | 103.69 ± 3.27³ |
| 20                   | 47.32 ± 1.83³ | -37.64 ± 1.98³ | 16.53 ± 0.78³ | 151.57 ± 9.98³ |
| 25                   | 88.15 ± 4.38³ | -37.07 ± 1.49³ | 17.65 ± 0.49³ | 173.16 ± 4.53³ |
| 30                   | 100.61 ± 0.17³ | -32.89 ± 0.82³ | 17.14 ± 1.14³ | 134.76 ± 9.12³ |
| Power⁴ (W)            | 60     | 125.36 ± 4.40⁴  | -34.41 ± 1.53³ | 36.86 ± 1.21³ | 130.98 ± 7.09³ |
| 90                   | 67.70 ± 1.93³ | -36.85 ± 2.52³ | 41.06 ± 2.61³ | 129.89 ± 8.14³ |
| 120                  | 49.91 ± 2.52³ | -40.86 ± 3.04³ | 43.70 ± 2.32³ | 98.06 ± 6.19³ |
| 150                  | 67.52 ± 4.81³ | -35.22 ± 2.05³ | 47.85 ± 3.57³ | 117.04 ± 2.45³ |
| 180                  | 66.55 ± 4.90³ | -37.37 ± 1.94³ | 52.07 ± 5.82³ | 136.41 ± 6.48³ |

MPS: mean particle size. Data were expressed as mean ± SD; Means do not share letters denote significant difference at P < 0.05.

¹ The ultrasound conditions are as follows: ultrasound frequency = 40 kHz, mass ratio = 30.1, pH = 8, ultrasound time = 15 min, ultrasonic power = 120 W, ultrasonic temperature = 50–80 °C.

² The ultrasound conditions are as follows: ultrasound frequency = 40 kHz, ultrasonic temperature = 72.5 °C, pH = 8, ultrasound time = 15 min, ultrasonic power = 120 W, the mass ratio = 15–35.

³ The ultrasound conditions are as follows: ultrasound frequency = 40 kHz, ultrasonic temperature = 72.5 °C, the mass ratio = 20, ultrasound time = 15 min, ultrasonic power = 120 W, pH = 6–10.

⁴ The ultrasound conditions are as follows: ultrasound frequency = 40 kHz, ultrasonic temperature = 72.5 °C, the mass ratio = 20, pH = 9, ultrasonic power = 120 W, ultrasound time = 10–30 min.

⁵ The ultrasound conditions are as follows: ultrasound frequency = 40 kHz, ultrasonic temperature = 72.5 °C, the mass ratio = 20, pH = 9, ultrasound time = 10 min, ultrasonic power = 60–180 W.
3.3. Ultrasound power

Fig. 1E showed that as the ultrasound power increased, the absorbance peak was blue shift and the intensity was increased, indicating that the yield of AuNPs increased and particle size decreased, which was further confirmed by the MPS results in Table 1. The MPS decreased as the ultrasound power increased from 60 to 120 W and then followed an increase trend when the ultrasound power increased to 180 W, which was similar to the change of IC50. The increase of ultrasound power was associated with the increasing of ΔTPC, indicating that higher ultrasound power helped the encapsulation of phenolic compounds in the AuNPs. Dheyab et al. [37] found that when the ultrasound output power increased from 12 to 36 W, the size of the AuNPs decreased. IC50 of AuNPs prepared under 120 W was the lowest (98.06 μg/mL) with the smallest particle size (49.91 nm), which may be easier uptake by the cells. It is known that the cellular uptake of nanoparticles was affected by the particle size. The nanoparticles with the size of 50 nm have maximum cellular uptake, however, smaller (15–30 nm) or larger (70–240 nm) particles decreased the uptake [42]. Therefore, ultrasound power of 120 W was chosen.

3.4. Correlation analysis of ultrasound conditions and measured attributes of AuNPs

Fig. 2 showed the correlation between the ultrasound conditions and the particle size, ΔTPC and IC50 of AuNPs. The reaction temperature was inversely correlated to the IC50, suggesting that increasing temperature can increase the NO inhibitory activity of AuNPs. The mass ratio was positively correlated with IC50, suggesting that increasing the CPE concentration did not increase the anti-inflammatory activity of AuNPs. The ΔTPC was negatively correlated with ultrasound time, suggesting that the longer ultrasound treatment and the lower ultrasound power, the lesser phenolic compounds were encapsulated into AuNPs. Therefore, ultrasound power of 120 W was chosen.
Fig. 4. Characterization of AuNPs synthesized by CPE. (A) Zeta potential, (B) Particle size distribution, (C) SEM, (D) FT-TEM.
reaction systems, the lower IC caused the decrease of particle size. The greater change of TPC in the encapsulated in the AuNPs. The increase of ultrasound power also caused the decrease of particle size. The greater change of TPC in the AuNPs-NU (81.34 ± 4.41 nm) was significantly smaller than that of AuNPs-NU (16.80 ± 4.41 nm). Both the results of TEM and dynamic light scattering (DLS) measurements showed that the AuNPs-U have smaller particle size compared with AuNPs-NU. TEM measured the metallic diameter of solid sample of NPs, while DLS determined the hydrodynamic diameter of the liquid sample of NPs[44]. Therefore, the force such as van der Waals interaction in the solution tends to increase the particle sizes in the liquid sample analyzed by DLS[38]. Similar result was found by Barros et al.[44] who found that the particle size of silver NPs synthesized by orange peel extract determined by DLS was significantly larger compared with the size measured by TEM. EDS spectra (Fig. 5A) of both nanoparticles showed a characteristic peak of elemental gold at 2.15 keV. XRD spectra (Fig. 5B) of both AuNPs have diffraction peaks at 20 values of 38.3°, 44.2°, 65.0°, and 77.8°, which correspond to the four characteristic lattice planes of Bragg’s reflection at (1 1 1), (2 0 0), (2 2 0), and (3 1 1), respectively[45]. The FT-IR spectrum is used to identify the functional groups and biomolecules involved in the biotransformation process. The comparison of the FT-IR spectra of CPE and AuNPs can infer what bioactivities in CPE were responsible for reducing the metal ions [46,47]. Fig. 5C showed CPE has a strong band at 3375 cm⁻¹, corresponding to the O–H stretching. This peak was smaller and shifted to 3458 cm⁻¹ in AuNPs-NU, while in AuNPs-U, O–H stretching band disappeared. The peaks at 2921, 2935, 2940 cm⁻¹ corresponded to the C–H stretching[48]. CPE and both AuNPs all have a sharp peak at 2344 cm⁻¹, which was stronger in the AuNPs than in the CPE. Peaks at 1621 and 1652 cm⁻¹ correspond to the C = C stretching[49] in CPE and AuNPs-NU, respectively, indicating the presence of aromatic C = C. However, no similar peak was detected in AuNPs-U, instead, a peak at 1718 cm⁻¹ was detected, representing the C = O stretching. The peak at 1417 cm⁻¹ in CPE represents O–H bending, while in AuNPs, peaks at 1526 and 1529 cm⁻¹ indicate the presence of C = C bonds[50]. The peaks observed at 1417 cm⁻¹ and 1621 cm⁻¹ were shifted to 1526 cm⁻¹ and 1718 cm⁻¹ in AuNPs-U and 1528 cm⁻¹ and 1652 cm⁻¹ in AuNPs-NU, respectively. Similar results were observed in the silver NPs synthesized using orange peel extract [44]. The peaks at 1058, 1032, 1049 cm⁻¹ in these three samples

3.5. Characterization of AuNPs prepared with or without ultrasonication under optimal conditions

The AuNPs synthesized under optimal conditions with (AuNPs-U) or without (AuNPs-NU) ultrasound treatment were characterized to compare the different attributes, including the stability, morphology, and particle size of these two NPs. Fig. 3A shows the UV–Vis spectra of CPE, AuNPs-U, and AuNPs-NU. There were two UV absorption peaks at around 280 nm and 330 nm in CPE, which contribute to the characteristic absorption peaks of phenolic acids and flavonoids of citrus [43]. In addition, the results showed that in both reaction systems, a characteristic absorbance peak of AuNPs was found at 551 nm (AuNPs-NU) and 561 nm (AuNPs-U), respectively. AuNPs-U have a narrower peak width and higher peak height compared with AuNPs-NU, indicating that ultrasound treatment decreased the particle size and increased the yield which was 72.7% and 62.5%, respectively in these two synthesis systems. The low recovery of AuNPs is partially due to the loss of NPs during the washing step. The zeta potential values of AuNPs-U and AuNPs-NU were −38.76 ± 4.73 mV and −42.02 ± 3.77 mV, respectively (Fig. 4A), indicating that stable AuNPs were obtained in both reaction systems. After 4-month storage under 4 °C, both NPs have similar UV–Vis spectra as the particles that were just synthesized with a slight decrease at maximum absorption wavelength (Fig. 3B), suggesting that AuNPs prepared in both systems were stable during storage.

The color of AuNPs-U in water was wine red, while a blue-violet color was observed in AuNPs-NU. The reason is that ultrasound can reduce the particle size of AuNPs. This result was confirmed by the particle distribution analyzed by the laser particle analyzer (Fig. 4B) which detected the MPS of AuNPs-U was 52.44 ± 4.93 nm, and less proportion, another predominant group has much larger particle size. SEM image (Fig. 4C) displayed the morphology of AuNPs synthesized under ultrasonic and non-ultrasonic conditions, from which it can be seen that the particle size of AuNPs-U is smaller and better dispersed. TEM image (Fig. 4D) confirmed that the synthesized AuNPs were mostly spherical and the particle size of AuNPs-U (13.65 ± 3.90 nm) was significantly smaller than that of AuNPs-NU (16.80 ± 4.41 nm). Both the results of TEM and dynamic light scattering (DLS) measurements showed that the AuNPs-U have smaller particle size compared with AuNPs-NU. TEM measured the metallic diameter of solid sample of NPs, while DLS determined the hydrodynamic diameter of the liquid sample of NPs[44].

Fig. 5. Characterization of AuNPs synthesized by CPE. (A) EDS, (B) XRD, (C) FT-IR.
represent the C-O stretching [50]. FT-IR results indicated that phenolic compounds in the CPE could be the main reducing agents for synthesis of AuNPs. However, the different wavenumbers in the ultrasound and non-ultrasound treated AuNPs suggested that ultrasound may modify the structures of the functional groups of the bioactives in the CPE.

3.6. Phytochemical changes in the CPE during the synthesis of AuNPs

It is well known that the acoustic cavitation of ultrasound causes the sonolysis of water into OH• and H• radicals which in turn react with organic compounds RH to produce reducing species and H2 that serve as reductants to reduce metal ion into atom [4,51]. Phytochemicals such as caffeic acid, ferulic acid, p-coumaric, hesperidin, narirutin, nobiletin, tangeretin in CPE are antioxidants possessing reducing power [15,16]. In the present study we compared ΔTPC in the CPE before and after AuNPs synthesis in ultrasound and non-ultrasound reaction systems. In the system with ultrasound treatment, ΔTPC was 45.72% which was significantly (P < 0.05) lower than that of 53.53% in the non-ultrasound treatment system. This result is possible because ultrasound produced reducing species also contributed to the reduction of Au (III), thus less

Fig. 6. The HPLC chromatograph of phytochemical standards (A), CPE before synthesis (B) and CPE after synthesis (C) at 280 nm. Compounds: 1, Chlorogenic acid; 2, Caffeic acid; 3, P-coumaric acid; 4, Ferulic acid; 5, Narirutin; 6, Hesperidin; 7, Naringenin; 8, Hesperetin; 9, Nobiletin; 10, Tangeretin.

Table 2
The impacts of ultrasound on the change of phenolic substance on AuNPs.

| Treatment | Chlorogenic acid (μg/mL) | Ferulic acid(μg/mL) | Narirutin (μg/mL) | Hesperidin(μg/mL) | Nobiletin (μg/mL) |
|-----------|-------------------------|---------------------|------------------|------------------|------------------|
| Ultrasonic |                          |                     |                  |                  |                  |
| Before    | 2.10 ± 0.03             | 1.06 ± 0.09         | 41.66 ± 0.17     | 40.66 ± 0.22     | 1.38 ± 0.09      |
| After     | 1.06 ± 0.13             | N.D.                | 28.85 ± 2.41     | 1.18 ± 0.91      | 0.93 ± 0.11      |
| Change %  | 49.52 ± 6.27            | 100.00 ± 0.00       | 27.83 ± 2.79     | 97.91 ± 2.12     | 32.65 ± 5.59     |
| Non-ultrasonic |                      |                     |                  |                  |                  |
| Before    | 1.95 ± 0.04             | 0.93 ± 0.01         | 38.56 ± 0.25     | 39.90 ± 0.64     | 1.22 ± 0.03      |
| After     | 0.86 ± 0.05             | N.D.                | 27.38 ± 4.42     | 0.33 ± 0.19      | 0.71 ± 0.11      |
| Change %  | 55.81 ± 2.56            | 100.00 ± 0.00       | 23.11 ± 5.22     | 99.18 ± 0.47     | 41.16 ± 1.90     |

Data were expressed as mean ± SD. N.D.: not detected.
Fig. 7. Effects of AuNPs synthesized with or without ultrasound treatment on nitric oxide production and percentage inhibition in LPS-induced Raw 264.7 cells. (A) AuNPs-U, (B) AuNPs-NU. Raw264.7 cells were pre-treated with the indicated concentrations of samples for 1 h, and then stimulated with LPS (0.1 μg/mL) for 24 h. Error bars represent the standard deviation (n ≥ 3). ### denotes P < 0.001 when compared positive (+) to negative (−) control. Data do not share same alphabet letter denote significant difference (P < 0.05).

Fig. 8. Effects of AuNPs-U on iNOS and COX-2 expressions in LPS-induced Raw264.7 cells. A, iNOS mRNA; B, COX-2 mRNA; C, iNOS protein; D, COX-2 protein. Raw264.7 cells were pre-treated with the indicated concentrations of samples for 1 h, and then stimulated with LPS (0.1 μg/mL) for 24 h. Error bars represent the standard deviation (n ≥ 3). ### denotes P < 0.001 when compared positive (+) to negative (−) control. Data do not share same alphabet letter denote significant difference (P < 0.05).
phenolic compounds were needed; or ultrasound produced smaller NPs, which have smaller surface area and the smaller space between Au after aggregation, therefore, less phenolic compounds were encapsulated in the AuNPs-U compared with AuNPs-NU [52].

To further elucidate which phenolic compounds in CPE contribute to the reduction of Au (III), we further determined the phytochemical compositions of CPE before and after nanoparticle synthesis. Chlorogenic acid, ferulic acid, narirutin, hesperidin, and nobiletin were detected in CPE, while caffeic acid, p-coumaric, hesperitin, naringenin, and tangeretin were not detectable (Fig. 6). Table 2 showed that narirutin and hesperidin are two major flavonoids in CPE, with a concentration of about 40 μg/mL, which were significantly greater than nobiletin, chlorogenic acid, and ferulic acid. Hesperitin and naringenin were not detectable in the CPE, suggesting that the major citrus flavonoids in the CPE are in glycosidic form. After synthesis, all these five detected phenolic compounds were significantly decreased (P < 0.05). Narirutin was decreased 27.83 ± 2.79% and 23.11 ± 5.22%, respectively in the ultrasound and non-ultrasound systems. However, the degree of decrease of hesperidin was much greater than narirutin, reaching 97.97 ± 2.12 % and 99.18 ± 0.47%, respectively, indicating that hesperidin was a more important contributor to reduce Au (III) compared with narirutin. When compared the changes of these five phenolic compounds in both ultrasound and non-ultrasound synthesis systems, they decreased to a similar extent, indicating that ultrasound didn’t increase the encapsulation efficacy of phenolic compounds in AuNPs. Narirutin and hesperidin both belong to flavanone-7-O-rutinoside, however they are structurally different in the B ring. Hesperidin has a –OH group at the C-3′ of B ring, which does not exist in narirutin. The –OH group at the C-4′ of B ring of narirutin was replaced by –OCH3 group in hesperidin. Majo et al. reported that C-3′ catechol structure significantly increased the antioxidant activity of flavanone glycoside [53]. Therefore, hesperidin has greater antioxidant activity compared with narirutin, which suggested that hesperidin was the most important reducing agent in the CPE. Barros et al. [44] also reported the reducing capacity of hesperidin in orange peel for synthesis of silver NPs. The reducing power of citrus flavonoids hesperidin, hesperetin, rutin, naringenin, quercetin and diosmin for the synthesis AuNPs were compared by Sierra et al. [54] under the alkaline condition at room temperature. They found that hesperetin and diosmin have greater capacity to reduce Au (III). Moreover, citrus fruits are rich in citric acid and ascorbic acids which can be good reducing agent for synthesis AuNPs as well [55]. The phytochemical profile in different species, varieties, and parts of citrus fruits varied, which results in the different key reductants in these samples. Our study showed that in the citrus peel used in present study, hesperidin was the most important flavonoids that contribute to the reduction of Au (III), while the impact of nobiletin, chlorogenic acid, and ferulic acid were negligible.

3.7. Anti-inflammatory effects of AuNPs

In recent years, the medicinal and biological applications of AuNPs have attracted enormous attention due to their various advantages such as biocompatible, low toxicity, and easy absorption [56]. A number of studies showed that AuNPs possessed anti-inflammatory activity. Ahn et al. [27] reported that AuNPs synthesized using the extract of Panax ginseng leaves inhibited inflammation through reducing expression of various inflammatory mediators, including NO, PGE-2, TNF-α, IL-6, iNOS, and COX-2 by blocking NF-κB and MAPK pathways. Similar anti-inflammatory activity of hexagonal AuNPs synthesized by fruit extract of Prunus serrulata was also observed [50]. As shown in Fig. 7, AuNPs-U and AuNPs-NU both dose-dependently inhibited NO production in LPS-induced Raw 264.7 cells, however, the NO inhibitory activity of AuNPs-U (IC50 = 8.29 μg/mL) was significantly (P < 0.05) greater than AuNPs-NU (157.71 μg/mL), indicating that ultrasound was able to increase the anti-inflammatory activity of AuNPs. As shown above, ultrasound did not increase the encapsulation efficiency of phenolic compounds, however, it decreased the particle size of AuNPs. Therefore, our result suggested that the reduced particle size contributed to the greater anti-inflammatory activity of gold nanoparticles. Gao et al. showed that the size of nanoparticles is a key factor in determining the anti-inflammatory efficacy of peptide-GNP hybrids [57]. They reported that the order of anti-inflammatory effect of peptide-GNP followed 20 nm > 13 nm > 5 nm [57]. We found that AuNPs-U (13.65 ± 3.90 nm) has significantly greater anti-inflammatory activity than AuNPs-NU (16.80 ± 4.41 nm). This is possible because that the bioactivity of AuNPs is also influenced by the bioactives encapsulated on the surface of NPs. We further investigated the anti-inflammatory mechanism of AuNPs-U through monitoring the gene and protein expressions of COX-2 and iNOS. Fig. 8A and 8B showed that AuNPs-U dose-dependently inhibited COX-2 and iNOS mRNA expression in LPS-induced Raw 264.7 cells. Moreover, the protein expressions of COX-2 and iNOS were also inhibited by AuNPs-U dose-dependently (Fig. 8C and 8D). Our results suggested that AuNPs-U showed anti-inflammatory activity through inhibition of gene and protein expressions of iNOS and COX-2 as well as reduction of NO production.

4. Conclusion

In the present study, an eco-friendly, easy, and rapid green synthesis approach was used to synthesize AuNPs using CPE under the assistance of ultrasound, which can not only valorization of citrus peels, but also produce bioactive AuNPs that can be used to ameliorate inflammation. The particle size, stability, yield, phenolic encapsulation efficacy, and anti-inflammatory activity of AuNPs were affected by the ultrasonic conditions, including ultrasonic temperature, time, power, mass ratio of orange peel to chloroauric acid, and pH. The anti-inflammatory activity of AuNPs was positively correlated with the ultrasound temperature, the mass ratio of citrus peel to chloroauric acid, and negatively correlated with the change of TPC. In general, the increase of temperature and the decrease of ultrasound power caused the decrease in particle size. We also found that AuNPs with the intermediate particle size of about 50 nm analyzed by DLS have high anti-inflammatory activity. Under the optimal reaction condition that includes temperature of 72.5 ℃, mass ratio of 20 : 1, pH of 9, ultrasound time of 10 min, and ultrasound power of 120 W, the synthesized AuNPs possessed the greatest anti-inflammatory activity. AuNPs synthesized under this optimal condition possess smaller particle size, better dispersibility, greater yield, and higher anti-inflammatory activity compared with the non-ultrasound treated counterparts; however, the phenolic encapsulation efficacy of AuNPs-U was not increased. We also elucidated that hesperidin was the most important flavonoid in the CPE functioning as the reductant for the biosynthesis of AuNPs. Moreover, AuNPs-U showed anti-inflammatory activity through inhibition of iNOS and COX-2. Therefore, our study comprehensively demonstrates the relationship between the different ultrasound conditions and the yield, size, stability, phenolic encapsulation capacity and the anti-inflammatory activity of AuNPs synthesized using CPE. We provide a green route for the synthesis of AuNPs in combination of the advantages of ultrasound and citrus peels and the potential of AuNPs in controlling inflammatory diseases.

CRediT authorship contribution statement

Ling Gao: Experimental design, Investigation, Methodology, Data analysis, Writing – original draft. Suhuan Mei: Investigation, Methodology. Haile Ma: Resources. Xiumin Chen: Conceptualization, Experimental design, Supervision, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence
the work reported in this paper.

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Appendix A. Supplementary data

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