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Green Synthesis of Silver Nanoparticles using Aqueous Fruit Peel Extract of *Citrus aurantifolia:* Optimization, Its Characterization and Stability Test

(Sintesis Hijau Nanozarah Perak menggunakan Ekstrak Kulit Buah Berair *Citrus aurantifolia*: Pengoptimuman, Pencirian dan Ujian Kestabilannya)

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Abstract

In this present study, silver nanoparticles were synthesized by green biological synthesis method using plant extract from fruit peel of *Citrus aurantifolia* as reducing agents. All the parameters in the synthesis of silver nanoparticles (AgNPs) were optimized to achieve a better yield, controlled size and stability of the particles. The biosynthesis of silver nanoparticles was monitored via UV-vis spectrophotometer and stability test was done. The resulting UV-Vis spectra of synthesized AgNPs from *C. aurantifolia* fruit peel extract (CAFPE) showed standard surface plasmon resonance band at 420 nm which indicated the presence of AgNPs. The optimum result was obtained with an optimum concentration at 4 mM $AgNO_3$, leaving in a dark room temperature for 24 h and using a concentration 1:3 ratio (extract: silver nitrate). Moreover, the stability of the CAFPE-AgNPs was also observed after 30 days of synthesis and even up to 10 months, indicating optimization plays major role towards the stability fate of nanoparticles. The FTIR analysis showed possible functional groups of biomolecules that play roles in the bioreduction and capping of silver nanoparticles. In addition, it is believed that these parameters are highly suitable for bulk production of single spherical AgNPs with diameter 29.6- 45.2 nm confirmed via FESEM. Thus, the obtained results clearly suggest that optimization of silver nanoparticles may have important role in attaining a better yield and stability of metal nanoparticles, refraining back to its original structure or particles.

Keywords: *Citrus aurantifolia*; green synthesis; optimization; plant extract; silver nanoparticles

ABSTRAK

Dalam kajian ini, nanozarah perak telah disintesis melalui kaedah sintesis biologi hijau menggunakan ekstrak tumbuhan daripada kulit buah *Citrus aurantifolia* sebagai agen penurunan. Semua parameter dalam sintesis nanozarah perak (AgNPs) telah dioptimumkan untuk mencapai hasil yang lebih baik, saiz terkawal dan kestabilan zarah. Biosintesis nanozarah perak diuji melalui spektrofotometer UV-vis dan ujian kestabilan juga telah dilakukan. Spektrum UV-Vis terhasil bagi AgNPs tersintesis daripada ekstrak kulit buah *C. aurantifolia* (CAFPE) menunjukkan jalur resonans plasmon permukaan piawai pada 420 nm yang menunjukkan keberhasilan pembentukan AgNPs. Keputusan optimum diperoleh dengan kepekatan optimum pada 4 mM $AgNO_3$, dibiarkan dalam suhu bilik gelap selama 24 jam dan menggunakan nisbah kepekatan 1:3 (ekstrak: perak nitrat). Selain itu, kestabilan CAFPE-AgNPs juga diperhatikan selepas 30 hari sintesis dan malah sehingga 10 bulan, menunjukkan pengoptimuman memainkan peranan utama ke arah nasib kestabilan nanozarah. Analisis FTIR menunjukkan kebarangkalian kumpulan biomolekul yang memainkan peranan dalam bioreduksi dan agen penutup nanozarah perak. Selain itu, adalah dipercayai bahawa parameter ini sangat sesuai untuk pengeluaran pukal AgNPs sfera tunggal dengan diameter 29.6 - 45.2 nm yang turut disahkan melalui FESEM. Oleh itu, keputusan yang diperoleh dengan jelas menunjukkan bahawa pengoptimuman nanozarah perak mungkin mempunyai peranan penting dalam mencapai hasil yang lebih baik dan ke arah kestabilan nanozarah logam, selain daripada menahan kembalinya AgNPs kepada struktur atau zarah asalnya.

Kata kunci: *Citrus aurantifolia*; ekstrak pokok; nanozarah perak; pengoptimuman; sintesis hijau

Introduction

Nanotechnology has recently shed light as a promising method in developing nanomaterials suitable for all biomedical applications. 'Nano-' or Nanobiotechnology intends to synthesis, improve, and utilize nanomaterials in the current field of nanotechnology (Barik Tapan 2021). In fact, 'nano' was the top trending search globally in the interdisciplinary field such as biology, medicine, chemistry, and physics. Nanoparticle was coined by Norino Taniguchi referring to all technological developments at nanoscale sizing ranges between 1 and 100 nm (Taniguchi 1974). The potential of nanotechnologies in revolutionizing a wide array of applications such as drug delivery, diagnostics, bioanalysis, biosensors, gene delivery, artificial implants, tissue engineering, and pest management (Elechiguerra et al. 2005).

Common metal nanoparticles widely used in the field of research are silver, gold, platinum, copper, iron, palladium with their own different applications based on their physical properties. According to reports, silver nanoparticles (AgNPs) are non-toxic, safer towards the environment, cost-effective, and consume less energy (Ahmed et al. 2016; Qu et al. 2014; Sun et al. 2014). Nanoparticle synthesis is usually carried out by physical and chemical methods. However, these methods suffer from high energy demand, high pressure, high temperature, complex instruments and harm usage of toxic chemicals. Likewise, the toxic chemicals may imply an environmental risk, provide low product yield and even expensive. Thus, there is a need to develop an eco-friendly alternative.

Another option in synthesizing metal nanoparticles is through biological method, an eco-friendly and green technique. In this methods, the synthesis is mediated either by plants, fungi, or microorganisms. It is evident from earlier reports that plants are better options for synthesis of nanoparticles. Nanoparticles produced from plant parts either the leave, root, peel contributes to a more stable colloid nanoparticle and rate of synthesis is much more faster (Iravani 2011).

Since the benefits of green synthesis method of nanoparticles outweigh the chemical synthesis, such that, it is cost-effective, good stability in the nanoparticles formation, minimal time, non-toxic by-product, produces less pollution, as well as increases environmental and human health safety (Malhotra & Alghuthaymi 2022; Ying et al. 2022). Hence, they are suitable for a large-scale biosynthesis of silver nanoparticles.

Although the mechanisms of the biological reaction with nanoparticle remains unclear, it is theorize that plant metabolites play a role as a reducing agent responsible for the reduction of silver ions (Ag^{+} to Ag^{0}), leading to the formation of AgNPs (Ahmed et al. 2016). Numerous studies have been conducted on the biosynthesis of AgNPs from plant parts or plant extracts, including the leaves of *Coleus amboinicus*, the seeds of *Medicago sativa*, the peel of *Citrus sinensis*, the roots of *Panax ginseng*, the seeds of *Weissella oryza*, the stems of *Terminalia arjuna*, and the stems of *Cordia dichotoma* (Arya et al. 2018).

Interestingly, all plant parts will eventually play role in the process of synthesis AgNPs, however, it depends on which functional metabolites want to act. Reports also suggest that different mechanisms for synthesizing nanoparticles lies in different plant species or parts (Baker et al. 2013). For instance, Eugenol, the main terpenoid of *Cinnamomum zeylanisum*, was found to play main role in the synthesis of gold and silver nanoparticles (Makarov et al. 2014). Notably, dicot plants contain many secondary metabolites that may be suitable for nanoparticle synthesis (Singh et al. 2016).

In the present study, fruit peel extract of *C. aurantifolia* was used as reducing and capping agent to produce AgNPs. *C. aurantifolia* (family: Rutaceae) or popularly known as 'key lime' is cultivated worldwide, especially in tropical and subtropical regions of Asia and Southeast Asia (Sunday et al. 2015). Though the peel of the fruit is commonly used as a waste material, this *Citrus* spp peel extracts have been known to have its capability to synthesize AgNPs and even tested for their biological effect for examples antimicrobial, antioxidant and cytotoxic properties (Alkhulaifi et al. 2020; Jalani et al. 2018; Narang & Jiraungkoorskul 2016; Niluxsshun, Masilamani & Mathiventhan 2021; Reena et al. 2017).

C. aurantifolia fruit peels have abundance of secondary metabolites such as flavonoids and saponins (Herawati, Ekawati & Yusmiati 2020). This bioactive phytoconstituents activates its property as an antioxidant, such that, strong evidence were shown from the peel of *C. aurantifolia* extract (Boshtam et al. 2011). Therefore, the use of peel fruit waste was proven for its reducing chemical properties considering it as an economic benefits for the study (Balavijayalakshmi & Ramalakshmi 2017; Ibrahim 2015; Kaderides & Goula 2017).

In present work, AgNPs were synthesized via biological method in the presence of *C. aurantifolia* fruit peel extract as a reducing and capping agent, with optimized parameters. Optimum size of AgNPs plays an important role in the effectiveness and validity of the

studies confirming its nanoparticle characteristics as an active ingredients. An optimized parameters of $AgNO_3$ will result in an optimum size of AgNPs as well as the efficacy of the study. Herein, the biosynthesis of AgNPs was done under very mild reaction conditions. Parameters influencing the synthesis of AgNPs such as reaction time, $AgNO_3$ concentration, effect extract to $AgNO_3$ ratio, and stability test after several months also were explored.

Materials and Methods

Preparation of *Citrus aurantifolia* aqueous fruit peel extract

The fruit peels of *C. aurantifolia* was purchased from Chemical Engineering Pilot Plant (CEPP), Universiti Teknologi Malaysia, Malaysia, in the form of extract powder. 1 g of fruit peels powder were dissolved with 100 mL of ultra-pure water. The mixture was boiled for 40 min. The suspension thus obtained was cooled, filtered through Whatman filter paper No. 1 (25 µm), and kept under refrigeration for further used.

Biosynthesis of silver nanoparticles

Silver nanoparticles used in this study were synthesized based on preliminary studies conducted by Jalani et al. (2018) with slight modification. The concentration of 4 mM aqueous solution of $AgNO_3$ were prepared using ultra-pure water and used for the synthesis of silver nanoparticles with the help of CAFPE as a reducing agent. 10 mL of this extract was mixed with 30 mL of 4 mM of $AgNO_3$ solution, and kept in water bath (70 °C; 2 h). The formation of AgNPs then was confirmed as the colour changes into reddish brown colour. Then, AgNPs were centrifuged at 40,000 rpm for 15 min and washed to discard clear supernatant solution (to remove unwanted metabolites or so-called unbound plant extract residue). The pellet was then obtained and dried using a Freeze Dryer (Labconco, USA).

Characterization of silver nanoparticles

Synthesized silver nanoparticles were confirmed using UV-vis absorption spectra (first confirmatory test for nanoparticle characterization), against ultra-pure water as blank. A small aliquot of sample (0.5 mL) was diluted with 4 mL of ultra-pure water prior the measurement. The absorbance of solution was recorded at the wavelength of 320-800 nm in Beckman–DU 720 spectrophotometer to confirm the silver ions reduction.

The sample in the lyopholized powder form were used for the characterization of Fourier Transform Infrared Spectroscopy (FTIR) and Field Emission Scanning Electron Microscope with Energy Dispersive X-Ray Spectroscopy (FESEM- EDX) analysis. This is to further confirm the state of the sample in the nanoparticle property. The sample slide was created by simply dropping a very small amount of the sample's powder of silver nanoparticles onto a copper grid coated with carbon, and then wiping away any surplus solution using blotting paper. FESEM instrument (FEI Nova NanoSEM 23, United States) was well- equipped with a Thermo energy dispersive x-ray spectroscopy (EDX) attachment. The plausible mechanisms between Ag-NPs and the functional groups present in the CAFPE, were predicted using the FTIR spectrometer using the potassium bromide (KBr) pellet technique. The sample was run through a spectrum, scanning from 650 to 4000 cm^{-1} at 4 cm^{-1} resolution.

Optimization of nanoparticles synthesis time

The time of this reaction was optimized by using different time intervals. The reaction time was monitored at 2 h and 4 h in a water bath at 70 °C and 24 h in a room condition without heat treatment (constant: concentration at 4 mM of $AgNO_3$ and 1:3 extract to silver ratio). The absorbance of the resulting solutions was measured spectrophotometrically.

Concentration of silver nitrates solution

In this reaction, the concentration of $AgNO_3$ was optimized using different concentrations of 1, 2, 3, 4, and 5 mM, respectively (constant: 2 h reaction time; 1:3 extract to silver ratio). The absorbance of the resulting solution was measured spectrophotometrically.

Effects of $AgNO_3$ amount to the formation of AgNPs

Assuming the pyramid diagram points, three concentrations ratio of leaf extract and $AgNO_3$ was optimized with constant concentration of 4 mM $AgNO_3$ solution (1:1, 1:3, 1:9). The absorbance of the resulting solutions was measured spectrophotometrically.

Stability study

The optimized solution of silver nanoparticles were kept in dark at room temperature. The stability of the

synthesized particles was monitored up to 30 days and 10 months after optimization by using UV–vis spectral analysis.

Results and Discussion

Present study illustrated an eco-friendly way to synthesis silver nanoparticles using CAFPE as reducing agent. The $AgNO_3$ is used as a metal precursor without the assistance of any other toxic reagents. The formation of AgNPs were monitored by UV–vis spectrum and compared within 30 min of exposure.

Optimization of different parameters

Different parameters were optimized for the synthesis of silver nanoparticles, which includes the reaction time, concentration of $AgNO_3$ and concentration ratio of leaf extract to $AgNO_3$. The formation of AgNPs was confirmed when the colour changes from light yellow to reddish brown colour (Figure 1). This indicates that the reduction has occurred (Ag^+ to Ag^0), and forming the AgNPs.

All metal nanoparticles have specific absorption spectra. In other words, different metal nanoparticles have different maximum absorption peak, such that band of 530 nm is the transverse surface plasmon absorption, typical for gold nanoparticles, whereas standard absorption band at 362 nm for zinc oxide nanoparticles (Song et al. 2011; Zhang et al. 2006). In this study with the use of silver nanoparticles, previous literatures shows that the standard absorption band for the formation of AgNPs are indicated by the band ranges from 350 nm to 450 nm (depending on the size, shape, and crystallinity of the particle) (Arya et al. 2018). On the surface of the nanoparticles, incident light creates oscillations in conduction electrons, hence electromagnetic radiation is absorbed. This phenomenon has caused the metal nanoparticles to reflect a very strong optical absorption of radiation in the visible and ultraviolet light due to the excitation of surface plasmons on the surface (Wolny-Koładka & Malina 2017).

Effect of substrate concentration

One of the important optimization parameter for the maximum synthesis of AgNPs is on the concentration of $AgNO_3$. Maximum reduction results in the stabilization performance. It was reported that the yield of AgNPs increased when the concentration was increased (Jalani et al. 2018). In this study, the reaction showed that there was a gradual increased in the formation of AgNPs by 4 mM $AgNO_3$ concentration compared to the other concentrations (1 mM to 3 mM). The optimum concentration can be observed at this 4 mM of $AgNO_3$ (Figure 2). However, as the concentration further increased to 5 mM, the peak intensity decreases, thus indicating non-uniformity in particle size. This confirms with the previous study whereby at certain point of concentration, the peak declines as the concentrations were too high (Mohapatra, Kuriakose & Mohapatra 2015). This occurrence arise was assumed due to the less availability of functional groups in the peel extract that can act as reducing agent. Even though there are plenty of silver ions available for aggregation, however the unlimited availability of these functional group (reducing agent) in extract has eventually prevents the formation of AgNPs (Alafandi et al. 2021; Jalani et al. 2018). Despite all the studies in synthesizing method of AgNPs using various plant parts. It is to note that

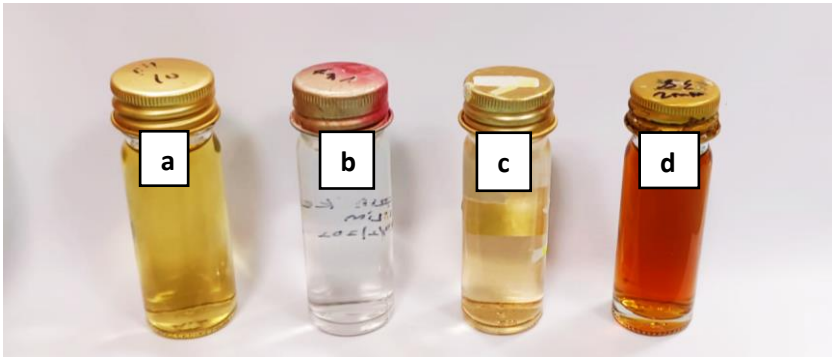

FIGURE 1. The colour of aqueous solution (a) peel extract alone, (b) silver nitrate alone and (c) after adding the silver nitrate into peel extract before the synthesis (d) formation of AgNPs synthesized using extract after the synthesis

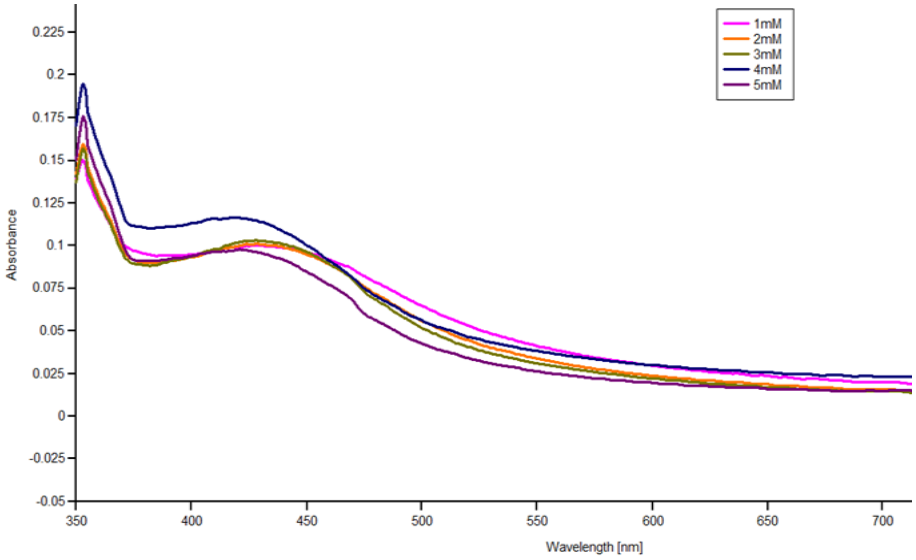

FIGURE 2. UV–vis spectra of AgNPs at different concentration of silver nitrate (Constant parameter: 1:3 – extract to silver nitrate ratio, 70 ºC; 2 h)

different plant species, tissues or parts have different concentration and composition of secondary metabolites (functional group). This may be explained that various morphological diversity were found in previous literature includes triangles, hexagons, pentagons, cubes, spheres, and ellipsoids. The secondary metabolites present in plants are hypothetically said to play role in the reduction process of the $AgNO_3$, hence forming AgNPs. Thus, herein, the concentration at 4 mM of $AgNO_3$ is enough and optimum for the availability presence of secondary metabolites in this specific fruit peel extract of *C. aurantifolia* plant, hence to the formation of AgNPs.

EFFECT OF TIME SYNTHESIS REACTION

As generally accepted in the previous published literatures, reaction time is one of the key factor in the synthesis of metal nanoparticles, such that it controls the size of the nanoparticles. Also, it is a part in reaching the yields point of every reaction. Hence, reaction time is needed in determining the optimum and stability fate of nanoparticles formation. The higher the reaction time during nanoparticle synthesis, the larger the size growth and crystallinity of metal NPs, but also the higher the density of samples per volume, which consequently resulted in an increase in absorption peak of UV-Vis (Karaagac & Köçkar 2020; Ozel, Kockar & Karaagac 2015; Yazdani et al. 2021). This means that the reaction times are more effective on the size growth and crystallinity of the nanoparticles formation.

From this research, the Surface Plasmon Resonance (SPR) band characteristics of Ag-NPs were detected at 420 nm (Figure 3), which corresponds to the standard SPR of silver nanoparticles established previously (Krishnaraj et al. 2012). Interestingly, this SPR appeared at 420 nm at the start of the reaction and remained stable at this same wavelength even after the reaction was finished, specifically for room condition. However, lengthening the period of reaction to 4 h were of no advantage for this study, such that the peak declines and become flattened. One of the study also reported similar results whereby increasing the reaction time has resulted in the decrease of particle size of the synthesized silver (Pourmortazavi et al. 2015). However, most of the literature stated otherwise, such that increasing reaction time results in more formation of silver nanoparticles (Bhuyar et al. 2020; Devi & Joshi 2015). This is largely due to a significant amount of Ag^+ being converted to Ag^0, thus resulting in excellent SPR band. Thus, an ideal time period is necessary.

One of the characteristics of generated $AgNO_3$ is that it is unstable and tends agglomerate easily after the synthesis, resulting in bigger particle sizes. Thus, optimum duration is needed. The optimum of required to complete the reaction in our investigation was not

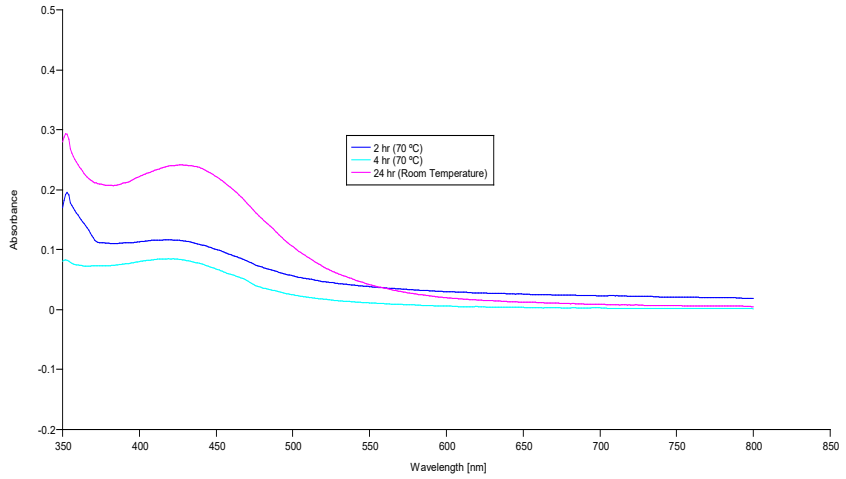

FIGURE 3. UV–vis spectra of AgNPs at different reaction time (Constant parameter: 4 mM of $AgNO_3$, 1:3 ratio (extract: silver nitrate))

using a heat treatment, but just at room temperature left for 24 h in a dark room to allows etching. The synthesis will stop when they reach thermodynamic and kinetic stability. Plus, previously stated in the literature that spherical AgNPs are known to have a characteristic UV-Vis absorbance peak around 400 nm based on the electron oscillations (surface plasmons) of such materials at the nanoscale (Bélteky et al. 2021; Yusof et al. 2018).

EFFECT OF $AgNO_3$ AMOUNT TO THE FORMATION OF AgNPs

The concentration ratio is among crucial parameters in the synthesis of metal NPs, especially when using plant extract. The concentration ratio may vary from different plants or plant parts. Results in Figure 4 indicates that the highest peak of AgNPs synthesized using extract were obtained when using extract-to-$AgNO_3$ molar ratio of 1:3. The highest peak at particular molar ratio (peel extract : $AgNO_3$) showing those amounts are adequate to produce more yield of AgNPs rather than other molar ratios. Much more lower and higher molar ratio (i.e., 1:1 and 1:9) for extract-to-$AgNO_3$ may produce lower AgNPs due to either limited or excessive amount of $AgNO_3$, hence hinder the stabilizer to form complex with Ag^+ (Alafandi et al. 2021). As a result, in generally accepted that under sufficient Ag^+ precursor, the SPR intensity can be observed via UV-vis analysis. Moreover, the increase in absorbance peak might be due to the OH groups transferred to carbonyl groups through air oxidation in a reaction (Ahmad, Ali & Bashir 2013; Guo et al. 2014; Jalani et al. 2018). As a result, silver ions reduction take place. Earlier reports illustrates that peak intensity variations and shifts in wavelength can be signs of aggregation and surface interactions (Bélteky et al. 2021). Surface plasmon resonance is so crucial in determining the optimization of AgNPs, and even more has also has led to new analytical approaches for biomolecules.

STABILITY TEST

The stability of silver nanoparticles formed after getting the optimized parameters were compared at 24 h after reaction, 30 days and up to 10 months after reaction may be determined by UV-Vis analysis study. Result in Figure 5 showed that no alteration in the peak at the wavelength of 420 nm even after a month period at dark room temperature. It is summarized that the suspension has a high level of stability despite extended storage and ageing. Interestingly, the SPR peak intensity eventually has increased double triple towards its peak, showing a high peak at 10 months of reaction compared to a month and 24 h after reaction. The SPR peak intensity 24 h reaction showed a broad peak, which indicates that the particles are polydispersed (Bhuyar et al. 2020). With the time period aspect, this totally proved that there was an increased in the formation of the synthesized AgNPs as the times passes. In addition, this has clearly indicates a strong stability biosynthesis of silver nanoparticles. Therefore, this evident has proved us that optimization

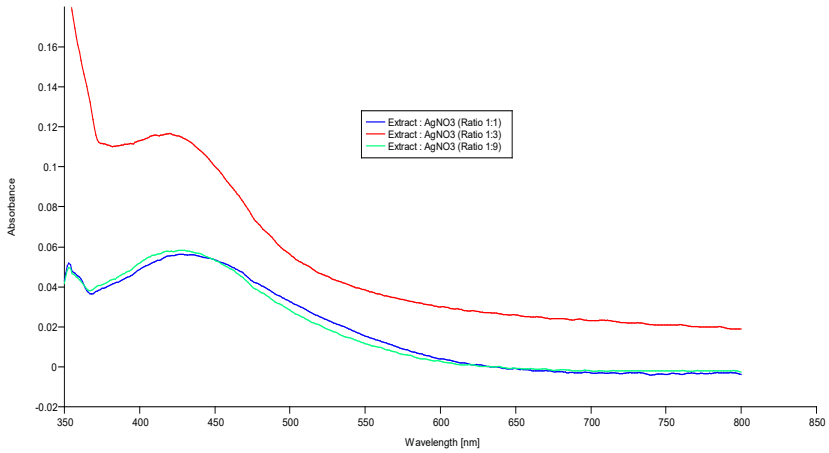

FIGURE 4. UV–vis spectra of AgNPs at different concentration ratio of CAFPE synthesized AgNPs with 4 mM of $AgNO_3$, (Constant parameter: 10 ml peel extract; 70 °C; 2 h)

step in the synthesis of AgNPs was crucial to the stability and aggregation of the particle. Hence, preventing the nanoparticles to go back towards its original properties or structure (size growth, and product aggregation). It was also confirmed by previous study that a better yield, controlled size, and stability were all achieved by optimizing the reaction medium used in the synthesis process (Krishnaraj et al. 2012). Another report stated that the stability was also observed at even at a longer period of time, up to 8 months after the synthesis (Arya et al. 2018). The length of the wave depends on how easily the electrons are excited; the easier the electrons being excited, the longer the wavelength of light it can absorb (Dada et al. 2018). Further important nanoparticle characterization signatures for ought to be carried out which are very imperative to this study.

CHARACTERIZATION USING FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Fourier transform infrared (FTIR) spectrum analysis were used to identify the possible biomolecules that contributes in the reduction and capping of the bioreduced silver nanoparticles. The FTIR spectrum of the silver nanoparticles synthesized using CAFPE showed 12 distinct absorption bands that appeared in various regions, indicating the presence several compounds in the plant extract (Figure 6). The resulting FTIR spectra includes: 3813.12, 3744.98, 3298.31, 2931.91, 1734.65, 1641.43, 1534.41, 1374.49, 1223.53, 1070.95, 809.45, and 699.37 cm^{-1}, respectively. Broad peaks appearing at 3813.12 cm^{-1} indicates the presence of hydrocarbon compound, resulting in the bending and stretching vibrations of the O-H. The bands at 3744.98 cm^{-1} is due to O-H stretch referring to the presence of polyphenols. The peak at 3298.31 cm^{-1} can be assigned to N-H stretching from amino groups. The peak at 2931.91 cm^{-1} corresponds to stretch vibrations of aliphatic C-H bonds. The peak at 1734.64 cm^{-1} corresponds to carbonyl ester group. The peak at 1641.41 cm^{-1} is related to the stretching of amide I linkages due to the carbonyl vibrational in proteins, while peak at 1534.41 cm^{-1} indicates the stretching of N–H bending of amide I. The C–N stretching of aromatic amines was confirmed by the 1374.49 cm^{-1}. The peak at 1223.53 cm^{-1} band is assigned to C-O vibrations. The peak at 1070.95 cm^{-1} associated to the C–N stretching (amine groups). The peak at 809.45 cm^{-1} pointed out on the stretching mode of Ag–O, indicating presence of AgO in monoclinic phase. The peak at 699.37 cm^{-1} is related to C=C of bend alkyne.

As can been from Figure 6, there is a major difference in the reduction or decrease of that particular functional group from CAFPE towards the formation of AgNPs- CAFPE. In other words, those functional group were completely being used for the formation of AgNPs. Thus, they are the main functional group that plays role in the process of bio-reduction. In agreement with this statement, study by Perumal et al. (2023) also claims that the absence or reduce of FTIR peaks intensity (functional group) after synthesis were responsible for the production of AgNPs. In context of this study, we

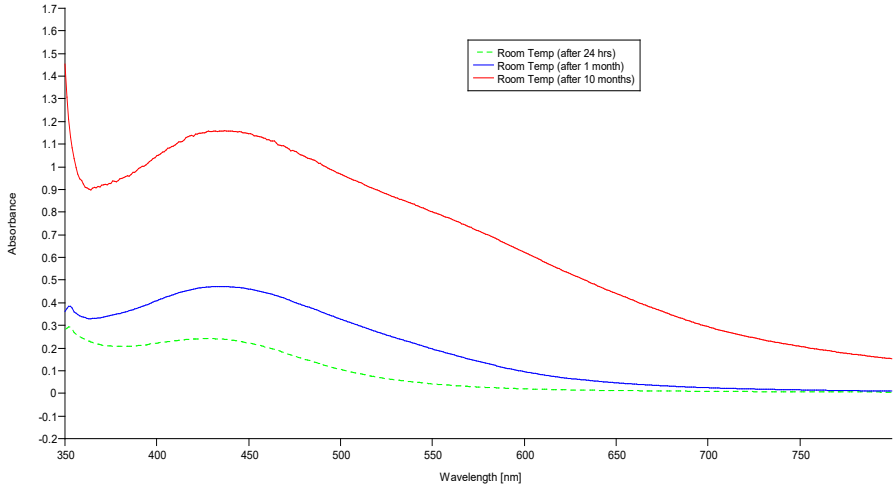

FIGURE 5. UV-vis spectra of the formation of an optimized AgNPs from CAFPE for the stability test at 24 h after synthesis reaction in comparison with 30 days and 10 months reaction

discovered that the main functional group includes, carbonyl group (protein), COO^-, O-H (phenol), C-N, C-H and C-C bond, had actively participated in the formation of silver nanoparticles using fruit peel extract of *Citrus aurantifolia.*

The exact mechanisms between AgNPs formation with the functional group present in CAFPE remain to be elucidated. However, it has been proposed that proteins, amino acids as well as secondary metabolites, such as flavonoids, tannins, alkaloids, polyphenols, and terpenoids in CAFPE have significant roles in metal salt reduction and, furthermore, act as capping and stabilizing agents for synthesized nanoparticles. This is in agreement with the study by Mustapha et al. (2023), mentioning CAFPE contains flavonoid, tannins, terpenoids, phenols, alkaloids, and saponins. For instance, this O-H group (phenol) in CAFPE form AgNPs by reacting with the Ag^+ ion in the metal salt, as reported by Rakhman et al. (2022). Plus, the hydroxyl groups present in the B and C rings of kaempferol (flavonoid group) may participate in the metal NPs formation, likewise the flavonoid presence in CAFPE (Raghavan et al. 2015). In other investigation by Raja et al. (2014), tannins were involved in reduction of silver ions to silver nanoparticles, mainly through the hydroxyl groups. Thus, this might answer the role of tannin in CAFPE. Moreover, study by El-Kassas and El-Sheekh (2014) showed that the carbonyl group from proteins and the hydroxyl functional group from polyphenols of *Corallina officinalis* extract could assist in forming and stabilizing gold nanoparticles.

Some researchers also discovered that phenolic compounds from plants, such as flavonoids may serve as the capping agents. It is well aware that the presence of these functional groups on the surfaces of noble metal nanoparticles may act as a protective coating and hence, providing the stability to the nanoparticles. Functional groups with strong affinity for metal nanoparticles includes amino (-NH), carboxylic acid (COOH), mercapto (SH), and cyano (CN) (Corbierre et al. 2001; Mandal, Fleming & Walt 2002; Shan et al. 2003; Teranishi, Kiyokawa & Miyake 1998).

In addition, reports also claim that different mechanisms for nanoparticle synthesis exist in different plant species or parts (Baker et al. 2013). Plus, these phytochemicals potentially play the same role as a reducing agent, which reduces the metal ions on their surface. For instance, when comparing to a same plant with different plant parts, amides, carboxyl groups, hydroxyl group, carbonyl groups and anhydride groups of *C. aurantifolia*- juice extract, was found to play main role in the synthesis of metal NPs (Adebayo-Tayo, Akinsete & Odeniyi 2016). In the case of *C. aurantifolia* leave extract, the aliphatic amines, amide, aliphatic methyl group was believed to play role in the bio-reduction process of metal NPs.

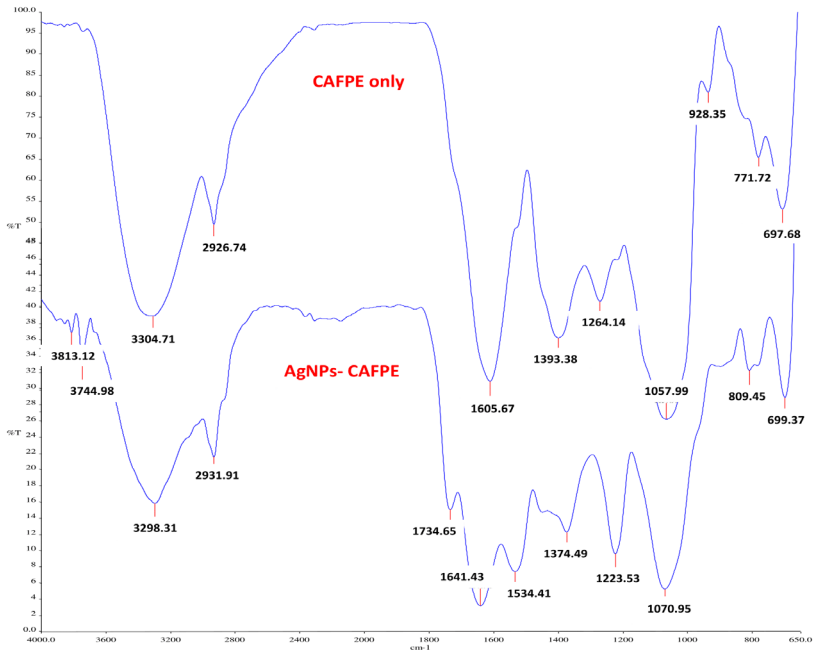

FIGURE 6. The Fourier-transform infrared spectra of CAFPE only and silver nanoparticles synthesized using CAFPE (AgNPs-CAFPE)

Additionally, when the $AgNO_3$ comes into touch with compex biological fluids (plant metabolites), they selectively adsorbed on the *nanoparticle* surface due to their strong binding affinity, hence creating a corona that interacts with biological systems. These corona layers serve as additional efficacy compared to a bare biological nanoparticles (Monopoli et al. 2020). Thus, biological nanoparticles are more effective due to the attachment of biological functional groups on the surface of synthesized nanoparticles from the biological sources, such as CAFPE. Especially in medicinal plants, there are abundant metabolites with pharmacological activity that are hypothesized to attach to the synthesized nanoparticles, hence, enhancing more the effectiveness of the nanoparticles formation.

ANALYSIS OF SILVER NANOPARTICLE USING FESEM - EDX

A field emission scanning electron microscope (FESEM) is a common technique to characterize topography of the surface morphology of synthesized silver nanoparticles. In any nanomaterials, it is recommended from the previous studies to use FESEM as compared to SEM as they tend to produce high quality image. Figure 7 shows the micrograph of Ag nanoparticles produced by the reaction of $AgNO_3$ solution with CAFPE after 24 h in room temperature. Silver nanoparticles were single (29.6- 45.2 nm), spherical in shape without any significant agglomeration at different magnifications of x 20,000, x 100,000 and x 160,000 (Figure 7(A)-7(C)).

Since the FESEM was well equipped with an EDX detector, the results were recorded (Figure 8). Silver was identified as the sample's most commonly found and abundant element by the EDX spectrum's results, which showed a distinct peak at 3.0 keV, with a percentage of 55.69% overall. Therefore, this result of silver abundancy confirms the sample's element that synthesized using $AgNO_3$ to form AgNPs. Other elements such as carbon (20.89%), oxygen (19.04%), chlorine (3.52 %), phosphorus (0.48 %), and sulphur (0.39 %) were also recorded at low concentrations (Table 1).

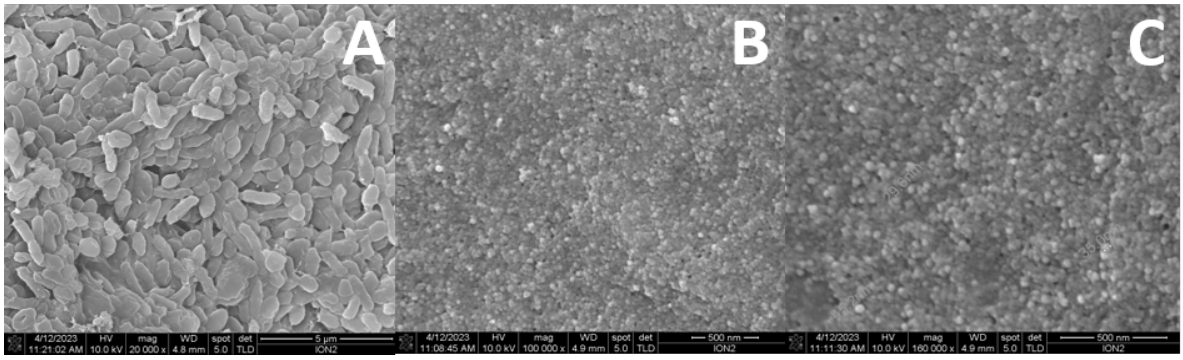

FIGURE 7. FESEM micrographs showing smooth surfaces and spherically shaped silver nanoparticles at magnifications of x 20,000 (A), x 100,000 (B), x 160,000 (C)

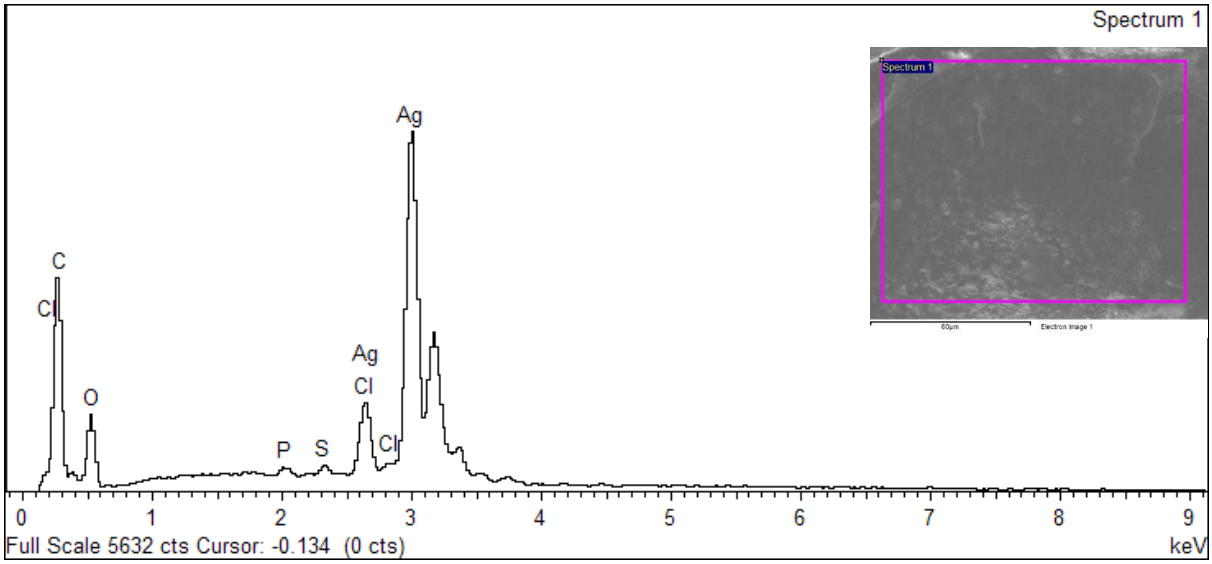

FIGURE 8. EDX spectra of synthesized AgNPs and FESEM micrograph showing EDX analysis regions

TABLE 1. The percentage of elemental compositions of CAFPE synthesized silver nanoparticles

Elements	Ag	C	O	Cl	P	S	Total
Weights percentage (%)	55.69	20.89	19.04	3.52	0.48	0.39	100.00

Conclusion

The work represents that silver nanoparticles with greater stability were synthesized using simple, affordable, and eco-friendly green biological synthesis approach. The single step process is ideal for mass production because it is quick and does not require the complex steps compared to other protocols. This documented study used a green mediated AgNPs synthesis and optimized with different parameters in the reaction medium to develop a high yield of AgNPs. The optimized conditions for high yield of AgNPs synthesis are 4 mM $AgNO_3$, leaving for eching in a dark room for 24 h, with concentration 1:3 ratio (extract: silver nitrate) and it is believed that these parameters are highly suitable for bulk production of single spherical AgNPs with diameter 29.6 - 45.2 nm confirmed via FESEM. In context of this study, we also discovered that the main functional group includes, carbonyl group (protein), COO^-, O-H (phenol), C-N, C-H, and C-C bond, have plays major role in the formation of silver nanoparticles using fruit peel extract of *Citrus aurantifolia*. Interestingly, the optimized AgNPs exhibited a stable formation of nanoparticles after a month period of time and even up to 10 months in the stability test, assuming the particles have gain a better yield, controlled size, and more stable after optimizing the reaction medium used in the synthesis process. Thus, this has conclude that optimization parameters are necessary for the fate of metal nanoparticles.

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