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High-yield biogenic fabrication and phytochemical screening of silver nanomaterials (AgNMs) from *Kaempferia parviflora* rhizome extract

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ABSTRACT

Nanotechnology is one of the promising scientific advancements that has captured widespread interest across various industries, notably in medicine. The utilization of plants for the synthesis of silver nanomaterials (AgNMs) has emerged as a promising and eco-friendly approach, offering cost-effective solutions for potential biomedical applications. The study aims to optimize the efficiency of biogenic AgNMs fabrication by employing *Kaempferia parviflora* aqueous extraction (KP-AE) as both a reducing and encapsulating agent, thus optimizing the yield of AgNMs. Phytochemical screening was conducted to identify the phytochemical compounds present in KP-AE. Various parameters were optimized, including pH, temperature, and the ratio of KP extract to $AgNO_3$, over different incubation periods. The synthesized AgNMs were analyzed spectroscopically and microscopically using UV-Vis and SEM techniques. At varying concentrations of KP and $AgNO_3$, the KP-AE AgNMs were successfully biogenic fabricated, but the yields varied. As the concentrations of $AgNO_3$ increased, a greater yield of KP-AE AgNMs was achieved. Phytochemical screening KP-AE demonstrated the presence of potential phytochemicals such as alkaloids, saponins, tannins, flavonoids, terpenoids, glycosides, and oils that assisted in the biogenic fabrication of AgNMs. This study established an efficient, affordable, and ecologically sustainable approach for fabricating stable AgNMs using KP-AE AgNMs. Synergistically, reducing and capping potential has been achieved by combining the plant extract in plant-mediated biogenic fabrication, producing stabilized NMs compared to those produced individually. The AgNMs derived from KP-AE exhibit robust antioxidant properties, showcasing promise for further exploration in pharmaceutical applications. Additional research is needed to investigate the biological potential and pharmacological properties of the biogenic fabricated KP-AE AgNMs.

Keywords: *Kaempferia parviflora; biogenic fabrication; silver nanomaterials; optimization and high yield*

INTRODUCTION

Over recent decades, significant advancements have been made in developing a reproducible technique for biogenic production of nanomaterials. Concurrently, there has been a growing market for chemical-free, eco-friendly manufacturing processes. Nanomaterials, owing to their extensive exploration, versatility, and captivating utility, offer myriad solutions across diverse industries such as medicine, nutrition, and energy. The term

"nano" originates from the Greek word "dwarf," denoting "a billionth," highlighting the minute scale of these materials. Nanomaterials can be categorized into distinct groups based on their size, morphological, physical characteristics, and chemical composition.

Nanomaterials consist of lipid-based, metal, ceramic and carbon compositions, each distinguished by their singular physical attributes, typically spanning sizes from 1 nm to 100 nm. Within this spectrum, one-dimensional nanomaterials (1D) such as nanowires, nanorods, and nanotubes exhibit unique structural configurations. Similarly, two-dimensional nanomaterials (2D) like nano coatings, nanolayers, and nanofilms offer distinct properties. Bulk nanomaterials, constituting three-dimensional structures (3D), represent another significant category. Despite their variety, all these nanomaterials adhere to the common constraint of not exceeding 100 nm in size (Ealia et al., 2017).

Metal-based, metal oxide, carbon-based, and quantum dot nanomaterials are the most used forms of nanomaterials (Álvarez-Chima et al., 2023). The shape and morphology of silver nanomaterials (AgNMs) determine their distinct characteristics and properties (Jain et al., 2017). AgNMs can combine, group or agglomerate particles with various morphological characteristics such as sphere-like, cylindrical, and irregular (Zhang et al., 2016). Due to their high surface area and nanoscale size, AgNMs possess unique physical, chemical, and mechanical properties. These distinguishing features enable AgNMs to disperse easily in solution and provide a large surface area to volume ratio, making them ideal for improving the durability of medication and therapeutic administration. These characteristics also make AgNMs suitable for various medical applications.

In the field of nanoscience, synthesizing AgNMs that are both cost-effective and produce high yields has been a significant challenge. However, the biological approach, also known as "green synthesis," has been described as a sustainable, environmentally friendly, and harmless technique for producing AgNMs. This method involves using organisms such as plants, microbes, or biological components. Both plants and microbes are effective biological materials for synthesizing AgNMs. Plant-based extracts are particularly intriguing due to their potential for high yields, sustainability, ease of use, and low toxicity (Singh et al., 2018) (Vanlalveni et al., 2022). Recent research studies have shown that plant extracts can be used to synthesize greener nanomaterials, including cobalt, copper, silver, gold, palladium, platinum, zinc oxide, and iron. Among the biological substitutes, plants and plant extracts appear to be the most advantageous due to their accessibility, low cost, excellent yield, minimal toxicity, and low energy consumption making them an ideal candidate for biogenic fabrication of nanomaterials (Parveen et al., 2016).

Phytochemicals found in plants, particularly phenols and flavonoids, play a vital role in binding nanomaterials and reducing Ag+ to Ag0 ions. Antioxidants are chemicals that counteract cell oxidation-related damage, slowing down or prolonging the oxidative degradation process. Free radicals, which are reactive elements generated by oxidation in the body's cells, can cause cell damage. Plants contain phytochemicals that act as natural stabilizers and reducing agents during the production of AgNMs, helping to prevent this damage (Goodarzi et al., 2018).

Various active phytochemical substances are found in plant extracts, such as polyphenols, reducing sugars, polysaccharides, and organic acids (Tarmizi et al., 2023). Research on the production of silver nanoparticles (AgNMs) using plant extracts has revealed that flavonoids, specifically certain polyphenol types, possess the ability to reduce silver ions into nanomaterials and effectively stabilize them. Flavonoids facilitate this process by releasing -O-H bonds, thereby providing electrons crucial for the reduction of metal ions to nanomaterials. Interaction between flavonoids and silver ions predominantly occurs through the hydroxyl group of the catechol portion, which exhibits lower breakdown energy compared to the hydroxyl group on the aromatic ring. Consequently, this interaction facilitates the reduction of metal ions to nanomaterials. Moreover, a mechanism for stabilizing nanomaterials involves oxidized flavonoids compensating by attaching to the surface of the nanomaterials, effectively impeding their aggregation and ensuring stability (Zuhrotun et al., 2023).

Kaempferia parviflora, more commonly referred to as Thai Ginseng, is a herbaceous plant species deeply rooted in traditional medicinal practices in Thailand and neighbouring regions. Renowned for its therapeutic properties, this herb has long been employed to alleviate metabolic disorders and promote general well-being. The colour of leaves usually appears green, with a reddish tint visible on its underside. The herb has been cultivated for its therapeutic properties in several regions of Asia. The botanical specimen known as Kaempferia parviflora and its associated rhizome, as depicted in Figure 1, is of interest in this study (Chen, et al., 2018; Singh et al., 2023).

In recent years, numerous polyphenolic flavonoids have been isolated from *Kaempferia parviflora*, showcasing multiples of biological properties. These include anti-inflammatory effects (Takuathung et al., 2021; Tuntiyasawasdikul et al., 2022; Horigome et al., 2014), antibacterial activity (Krongrawa et al., 2022; Sitthichai et al., 2022), antioxidant properties (Kwon et al., 2021; Shen et al., 2022), and anti-thrombotic capabilities (Le et al., 2023; Wang et al., 2020). Notably, the compound 5,7-dimethoxylflavone has been specifically investigated for its anti-inflammatory potential (Shen et al., 2022; Kim et al., 2020), while compounds like 5,7,4′-trimethoxyflavone and 5,7,3′,4′-tetramethoxyflavone exhibit antiplasmodial properties (Yenjai et al., 2004). Additionally, rhizome extracts of *Kaempferia parviflora* have been reported to possess antiproliferative and cytotoxic properties (Hossain et al., 2012). The presence of methoxyflavones, as evidenced in Table 1, renders the rhizomes of this plant a valuable source of phenolic compounds, holding significance both physiologically and economically. Compared to other plants such as Citrus species, *Kaempferia parviflora* stands out as a notable reservoir of methoxyflavones with a broad spectrum of biological activities (Asamenew et al., 2018).

Figure 1

Figures showing Kaempferia parviflora species: (A) Plant, (B) Rhizome, (C) Powder

Notes: (A) Kaempferia parviflora plant, recognized for its medicinal properties, (B) Rhizome, the primary source of bioactive compounds used in extracts and (C) Powdered rhizome, commonly used for phytochemical analysis and nanomaterial synthesis.

Table 1

Phytochemicals constituents of Kaempferia parviflora rhizomes

Compound name
6-Hydroxy-7,4′-Dimethoxyflavone
5,7,3′,4′-Tetramethoxyflavone (tetramethylluteolin)
3,5,7,3′,4′-Pentamethoxyflavone (pentamethaquercetin)
5,7-Dimethoxyflavone
5,7,4′-Trimethoxyflavone (trimethylapigenin)
3,5,7-Trimethoxyflavone
3,5,7,4′-Tetramethoxyflavone (tetramethylkaempferol)
5-Hydroxy-7,3′,4′-trimethoxyflavone
5-Hydroxy-3,7,3′,4′-tetramethoxyflavone (ayanin)
5-Hydroxy-7-methoxyfavone (tectochrysin)
5-Hydroxy-7,4′-dimethoxyfalvone
5-Hydroxy-3,7-dimethoxyflavone
5-Hydroxy-3,7,4′-trimethoxyflavone

Notes: Methoxyflavones were reported to be the major source of phytochemical groups in Kaempferia parviflora rhizomes.

METHODOLOGY

Plant material

The rhizome of *Kaempferia parviflora*, originally sourced from Thailand, was procured from Kelantan, Malaysia. A voucher specimen (KM0071/23) was authenticated at the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM). The stems of the freshly obtained rhizomes underwent meticulous cleaning, peeling, and subsequent freeze-drying to produce *Kaempferia parviflora* rhizome powder. This powder was finely ground using a grinder (Retsch Ultra Centrifugal Mill ZM200, Haan, Germany). The resulting finely ground material was carefully stored in a glass jar, ensuring airtightness, and placed in a chiller maintained at a temperature ranging between 4 and 8 °C for future use. *Kaempferia parviflora* (AgNMs) was fabricated using silver nitrate ($AgNO_3$) and optimized at the Research and Diagnostic Laboratory at Management and Science University, Malaysia.
Preparation of plant material

The aqueous deionized water was used to extract *Kaempferia parviflora* (KP-AE) from the dried rhizomes following a combined method from Numat et al. (2022) and Khalir et al. (2022). The magnet stirrer was used when mixing 50 mL of distilled water with 2.5 grams of rhizome powder. The mixture was heated up for 15 minutes at 60 °C. The extraction solution was then centrifuged for 10 minutes at 9000 rpm, filtered through Whatman filter paper No. 1, and used to reduce Ag^+ ions into biogenic production of KP-AE AgNMs. The extract aliquots were used as a capping and reductive material and stored between 4 and 8 °C, Numan et al. (2022) and Khalir et al., (2022).

Biogenic fabrication of silver nanomaterial

In a titration flask, 90 mL of 2 mM ($AgNO_3$) and 10 mL of KP-AE were mixed. The mixture is then consistently stirred at 400 rpm with a magnetic stirrer until the temperature reaches 60°C. While reducing silver nanomaterial from Ag^+ to Ag^0 ions, aluminium foil was wrapped around the flask to keep the light away. The mixture was left to sit in the dark to complete the biogenic reduction. Control $AgNO_3$ was kept throughout the time without mixing with KP-AE. The initial noticeable reduction of Ag^+ ions to Ag^0 silver nanomaterial was observed from the colour changes from clear to brown. From observing the absorbance peak with UV-visible spectroscopy, KP-AE AgNMs was produced effectively using biogenic manufacturing (Liaqat et al*.,* 2022).

Collection biogenic fabrication of silver nanomaterial

Visual observation of the colour produced was used to determine the reduction of silver nanomaterial Ag^+ to Ag^0. The transformation from a light purple colour to a brownish-dark brown coloration can be visually observed (Iravani et al*.,* 2014). The unconverted Ag^+ ions and KP-AE residue were separated from the biogenic fabrication of KP-AE AgNMs by centrifugation at 6000 rpm for 30 minutes (Sorvall ST 16R, Thermo Scientific, Waltham, MA, USA). The KP-AE AgNMs was retrieved after double rinsing and washing and then redistributed in double-distilled water. Soon after re-dispersion, the KP-AE was freeze-dried using a tabletop lyophilizer (ScanVac Coolsafe 110-4). The KP-AE- AgNMs were obtained and stored at 4°C in a light-protected environment. Analysis of the biogenic fabrication KP-AE AgNMs using a UV-Vis spectrophotometer (Multiskan go, Thermo Scientific, Waltham, MA, USA) revealed a peak at approximately 428 nm, which is associated with the establishment and development of AgNMs.

Stability analysis

The optimized fabricated KP-AE AgNMs solutions were kept in the dark for 30 days, and the stability of synthesized AgNMs was determined using UV-Vis spectral analysis.

Percentage yield

To determine the percent yield accurately (Table 3), the initial step involves determining the anticipated quantity of the product through stoichiometry. This value is termed the theoretical yield, representing the maximum achievable amount of product based on the given reactant quantities. On the other hand, the actual yield signifies the quantity of product obtained through practical execution of the reaction in a laboratory context. The percent yield, expressed as a percentage, is the ratio of the actual yield to the theoretical yield, providing insight into the efficiency of the reaction.

$$Percent\ yield = \frac{actual\ yield}{theoretical\ yield}\ x\ 100\%$$

Qualitative analysis of phytochemicals

Phytochemical analysis for the qualitative detection of biologically important compounds, including alkaloids, saponins, tannins, flavonoids, phenols, terpenoids, glycosides in *Kaempferia parviflora*, were examined according to the method with slight modifications by (Thakor et al*.,* 2023); (Kancherla et al*.,* 2019); (Akullo et al*.,* 2023; Muhammad Abdulrazak et al*.,* 2016). These phytochemicals in *Kaempferia parviflora* are responsible for the biogenic fabrication of Ag^+ to Ag^0 that clings onto nanomaterials and increases their biological characteristics and stability of KP-AE AgNMs.

Alkaloids screening (Hager's test)

2 mL of Hager's reagent will be added into 2 mL of 1% aqueous black ginger extract. The presence of alkaloids will be observed by the formation of yellow precipitates.

Saponin screening

2 mL of 1% aqueous black ginger extract will be added into 2 mL of distilled water. The mixture is then stirred in a test tube for 15 minutes until it is homogenous. The presence of saponin will be observed by the formation of a 1 cm layer of frothing foam.

Tannin screening

A solution containing 2 mL of 1% aqueous black ginger extract will be combined with 2 mL of ferric chloride ($FeCl_3$) solution with a concentration of 5%. The presence of dark shade bluish or greenish black indicates the presence of tannin.

Flavonoids screening

A solution containing 1 mL of 2N NaOH will be combined with 2 mL of black ginger extract with the concentration of 1% in water. The presence of flavonoids will be observed by the appearance of yellow colour.

Terpenoids screening (Salkowski test)

A solution containing 1 mL of black ginger extract with a concentration of 1% in water was combined into 2 mL of chloroform. Subsequently, a layer will be formed by cautiously adding 3 mL of concentrated sulfuric acid. The presence of terpenoid will be observed by a reddish-brown hue on the interface of the solution.

Glycoside screening (Benedict's test)

5 mL of Benedict's reagent will be combined with 8-10 drops of black ginger extract, and the mixture will be subsequently heated for 5 minutes using a magnetic stirrer and a hot plate. The presence of carbohydrates will be confirmed by the formation of a dark crimson precipitate.

Characterization of biogenic silver nanomaterial fabrication

Visual evaluation

Visual inspection will be conducted to observe the colour changes in the reaction medium of KP-AE AgNMs, which will indicate the reduction of Ag^+ to Ag^0.

UV-visible spectroscopy analysis

The successfully synthesized KP-AE AgNMs were observed using a UV-Visible spectrometer (HACH DR6000 UV VIS) to investigate the absorbance spectra at the range of 350 to 800 nm to confirm the yield of different parameters: time incubation (1, 2, 12 and 24) hours, pH value (2,4,7 and 11), temperature (5,30,50 and 75) °C, $AgNO_3$ to KP-AE ratio (5:2; 5:4; 5:8; 5:10) mL and concentration of $AgNO_3$ (0.5, 1.0, 1.5, and 2.0) mM. The calibration procedure was used to determine the concentrations of produced AgNMs. The proportional amount of KP-AE utilized in the reaction fully transformed the dissolved silver ions into metallic nanomaterial.

Scanning electron microscopy analysis

The morphology and size of KP-AE AgNMs were examined by scanning electron microscope (SEM) (Quanta 250 FEG), which was used in high-vacuum mode and supplied with a 15 kV acceleration voltage.

RESULTS

Phytochemical analysis

The phytochemical analysis was conducted to identify the chemical constituents present in KP-AE. The results, summarized in Table 2, indicate the presence of various phytochemicals such as alkaloids, saponins, tannins, flavonoids, terpenoids, glycosides, and oils. These compounds were detected using standard qualitative methods, which confirmed their presence in significant quantities. Each of these phytochemicals is known for its distinct bioactive properties, suggesting that KP-AE may possess a broad spectrum of biological activities.

Table 2

Phytochemical analysis on Kaempferia parviflora extract

Compounds	Test Name	Results	Observation
Alkaloids	Wagner	+	Reddish-brown precipitate
Saponins	Saponins	+	Formation layer of foam
Tannins	$FeCl_3$	+	Greenish-black precipitate
Flavonoids	Flavonoids	+	Formation of red precipitate
Terpenoids	Terpenoids	+	Reddish-brown precipitate
Glycosides	Benedict	+	Dark crimson precipitate

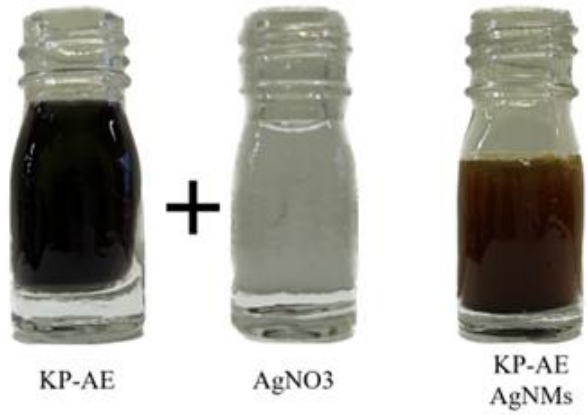
Note: Major phytochemical groups such as alkaloids, saponins, tannins, flavonoids, terpenoids and glycosides are present in the crude extract.

Visual confirmation of KP-AE AgNMs synthesis

As shown in Figure 2, the KP-AE solution, initially purple, turned brownish-red upon the addition of AgNO3. This colour change confirms the successful biogenic synthesis of KP-AE AgNMs, indicating the reduction of Ag+ ions to Ag0.

Figure 2

Observation of colour change during the biogenic synthesis of KP-AE AgNMs



Notes: Oxidation process of active phytochemicals in Kaempferia parviflora aqueous extract (KP-AE) yielding KP-AE silver nanomaterials (KP-AE AgNMs), visualized by colour changes from purple to brownish red.

Characterization of silver nanomaterials

UV-visible spectra analysis

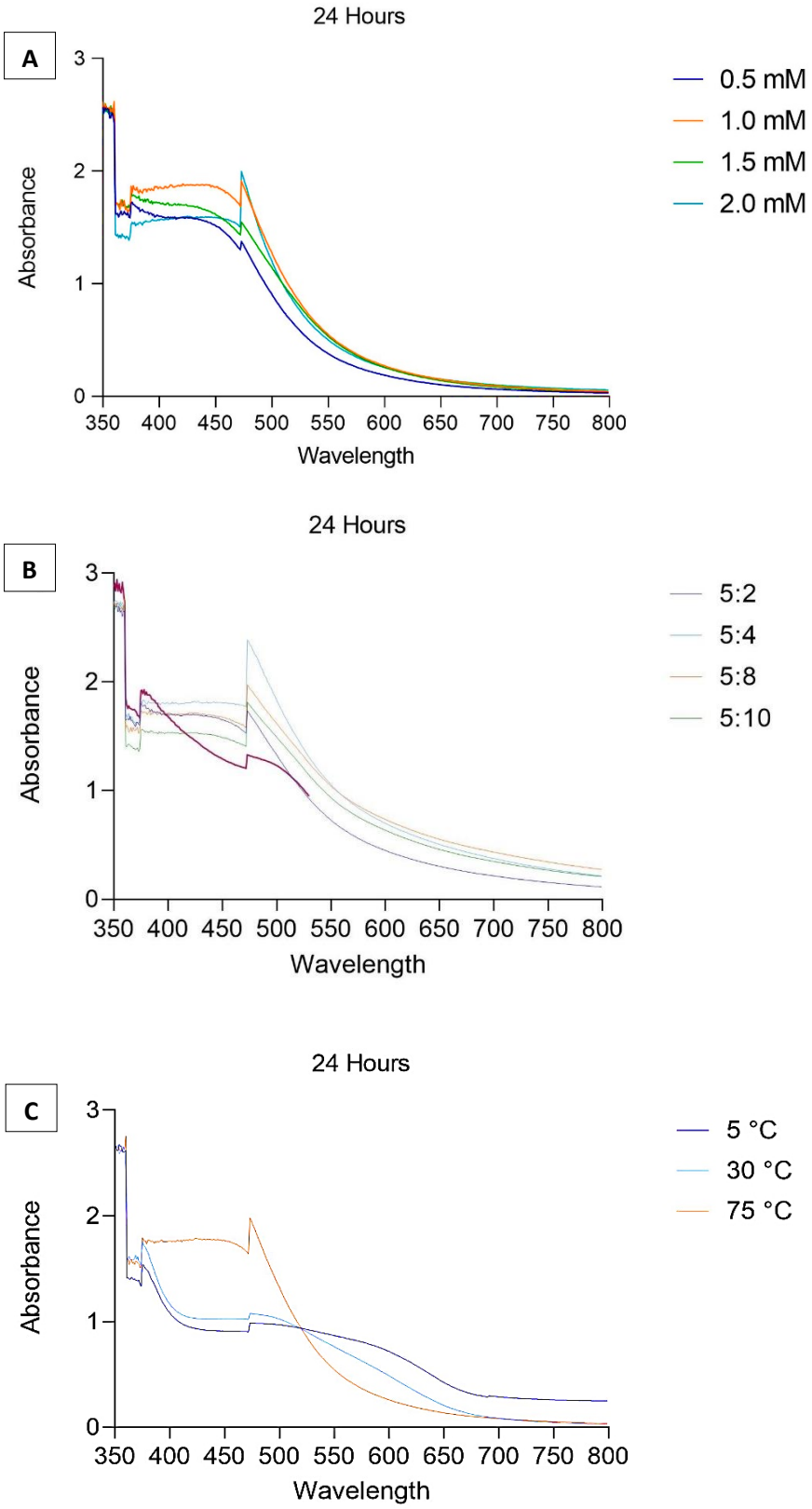
The UV–Vis spectral analysis, illustrated in Figure 3, demonstrated the optimal conditions for the conversion of Ag+ to Ag0 during the synthesis of KP-AE AgNMs. The best parameters were a 5:4 mL ratio of AgNO3 to KP-AE, a 2 mM concentration of AgNO3, a pH of 11, and a temperature of 75 °C, with a 24-hour incubation period. Under these conditions, the SPR band exhibited maximum absorbance peaks at 474 nm, confirming the formation of KP-AE AgNMs. Additionally, the optimization experiments revealed sharp SPR peaks within the 410-480 nm range, indicating successful nanoparticle synthesis.

Field emission scanning electron microscopy (FE-SEM)

The FE-SEM analysis, as depicted in Figure 4, revealed that the KP-AE AgNMs ranged in size from approximately 19 nm to 40 nm. The nanoparticles exhibited a uniform distribution and a predominantly spherical morphology after 24 hours of interaction time. The high-magnification micrographs further demonstrated the surface deposition of the KP-AE AgNMs, confirming that they were well-dispersed with no significant agglomeration observed.

Figure 3

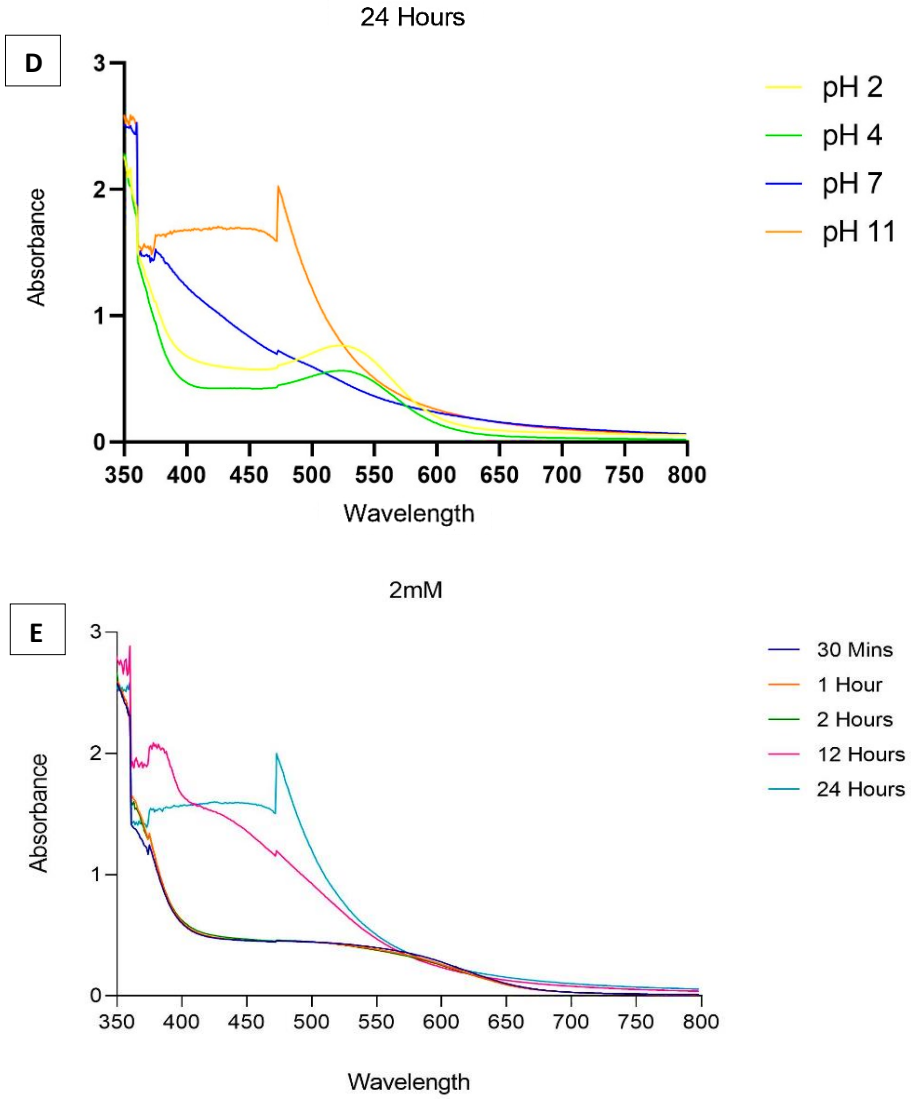
UV-vis spectrum on biogenic fabrication of KP-AE AgNMs



Notes: Effect of varying (A) AgNO3 concentrations; (B) AgNO3 to KP-AE volume ratio and (C) Temperature on the SPR absorption peak during the biogenic fabrication of KP-AE AgNMs

Figure 3 *(continuation)*

UV-vis spectrum on biogenic fabrication of KP-AE AgNMs



Notes: Effect of varying (D) pH; and (E) Incubation period on the SPR absorption peak during the biogenic fabrication of KP-AE AgNMs

Table 3

Yield production on biogenic fabrication of KP-AgNMs

(A)

$AgNO_3$ (mM)	Yield (%)
0.5	12.72
1.0	16.46
1.5	16.31
2.0	17.12

(B)

$AgNO_3$: KP-AE	Yield (%)
5:2	2.55
5:4	8.88
5:8	0.84
5:10	6.42

(C)

Temperature (°C)	Yield (%)
5	3.37
30	6.79
75	10.4

(D)

pH	Yield (%)
2	12.72
4	16.46
7	16.37
11	17.12

(E)

Incubation Period (Hrs)	Yield (%)
1	11.89
2	17.99
12	19.15
24	19.80

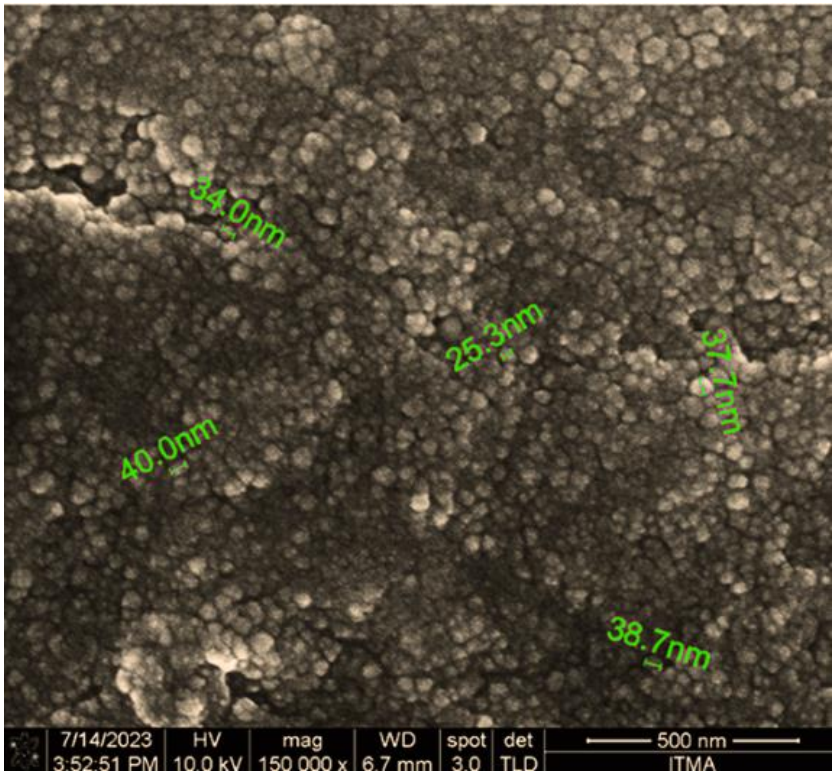
Note: Effect of varying (A) AgNO3 concentrations; (B) AgNO3 to KP-AE volume ratio; (C) Temperature; (D) pH; and (E) Incubation period; on the yield of KP-AE AgNMs during biogenic fabrication.

Field emission scanning electron microscopy (FE-SEM)

The SEM images revealed KP-AE AgNMs ranging from 19 nm to 40 nm, exhibiting uniformity and a spherical morphology after 24 hours of interaction time. Notably, the high-magnification micrographs clearly illustrate the surface deposition of KP-AE AgNMs. As stated by Rose et al. (2019), the presence of bio-organic substances strongly supports our hypothesis that secondary materials derived from KP-AE molecules may serve a critical role as binding agents, effectively coating and encapsulating the nanomaterials.

Figure 4

SEM of biogenic fabrication of KP-AgNMs



Notes: Biogenic fabrication of KP-AE AgNMs at 150x magnification

DISCUSSION

The identification of alkaloids, saponins, tannins, flavonoids, terpenoids, glycosides, and oils in KP-AE underscores the extract's potential as a source of bioactive compounds. Alkaloids and flavonoids, in particular, are well-documented for their antioxidant and anti-inflammatory properties, which may contribute to the therapeutic potential of KP-AE. The presence of these secondary metabolites is not only indicative of the plant's medicinal value but also suggests their possible involvement in the green synthesis of nanomaterials.

According to Varghese et al. (2021), secondary metabolites in plant extracts can facilitate the reduction of metal ions, such as Ag+, to their corresponding nanoparticles (Ag0). In this context, the phytochemicals identified in KP-AE likely play a crucial role in both the reduction process and the stabilization of the synthesized AgNMs. The encapsulation of these nanoparticles by the phytochemicals may also enhance their bioavailability and therapeutic efficacy. Future studies should focus on elucidating the exact mechanisms by which these phytochemicals contribute to the biogenic synthesis of AgNMs and exploring their potential applications in various biomedical fields.

The observed colour change from purple to brownish-red upon the addition of AgNO3 to KP-AE is attributed to surface plasmon resonance (SPR), a phenomenon that occurs when the phytochemical constituents in Kaempferia parviflora facilitate the biogenic reduction and synthesis of Ag+ ions to Ag0. SPR is a characteristic feature of metal nanoparticles, and its occurrence in this study suggests successful synthesis of AgNMs. The factors influencing SPR, including the shape, morphology, size, dielectric environment, and composition of the synthesized nanomaterials, are crucial in determining the optical properties of the nanoparticles.

In this study, the presence of a single SPR peak suggests that the synthesized KP-AE AgNMs likely possess a spherical shape. The role of the phytochemicals in driving this process highlights the potential of KP-AE as a reducing and stabilizing agent in the green synthesis of nanomaterials. Further characterization studies are necessary to confirm the shape and size distribution of these AgNMs and to explore their potential applications.

The UV-Vis analysis confirms that the synthesized KP-AE AgNMs exhibit characteristic surface plasmon resonance (SPR) bands, particularly with maximum absorbance at 474 nm. These results suggest that the parameters used in this study are effective for achieving high yields of AgNMs, aligning with findings from previous research (Melkamu et al., 2021; Saxena et al., 2016; Revathi et al., 2022; Liaqat et al., 2022). The optimal conditions—specifically the 2 mM AgNO3 concentration and a pH of 11—contributed significantly to the stability and size uniformity of the synthesized AgNMs, as evidenced by the sharp SPR peaks.

Further analysis shows that increasing AgNO3 concentration, pH levels, temperature, and incubation time progressively improved the yield and stability of the AgNMs (Table 3). The observed increase in SPR intensity at 75 °C and under alkaline conditions indicates that these parameters are critical for efficient nanoparticle synthesis. These findings corroborate previous studies (Aramwit et al., 2014; Balakumaran et al., 2015; Singh et al., 2020), which have also reported that higher temperatures and alkaline pH values favor the formation of stable and uniform silver nanoparticles.

Moreover, the gradual color change from purple to dark brown over time serves as a visual confirmation of the efficient biogenic reduction process. The progressive increase in SPR intensity with extended reaction time further suggests that prolonged incubation enhances nanoparticle formation and stability (Bhatt et al., 2018; Yusuf et al., 2020). The optimal absorbance observed at 419 nm after 24 hours of incubation signifies that the reaction conditions employed in this study are suitable for producing stable and well-formed KP-AE AgNMs.

The SEM analysis confirms that the KP-AE AgNMs exhibit a consistent size distribution and spherical shape, ranging from 19 nm to 40 nm. This uniformity and morphology suggest effective synthesis conditions. The high-magnification images further indicate that the nanoparticles are well-deposited on the surface, which aligns with the hypothesis that bio-organic substances from KP-AE may play a crucial role in binding, coating, and encapsulating the nanomaterials. These findings are consistent with previous research by Rose et al. (2019), which also highlighted the role of plant-derived secondary metabolites in stabilizing and capping silver nanoparticles.

The FE-SEM analysis supports the effectiveness of the biogenic synthesis method used in this study, as evidenced by the uniform size and spherical morphology of the KP-AE AgNMs. The size range observed (19 nm to 40 nm) is consistent with what is typically expected for silver nanoparticles synthesized using plant extracts, which often results in well-defined and stable nanoparticles. The uniformity in size distribution and morphology suggests that the phytochemicals present in KP-AE played a crucial role in controlling the nucleation and growth of the nanoparticles, leading to the formation of consistently sized AgNMs. Additionally, the high-magnification image illustrates the surface deposition of KP-AE AgNMs, which indicates that the bio-organic substances from the KP-AE extract may act as effective stabilizing agents. This finding is in agreement with the hypothesis proposed by Rose et al. (2019), suggesting that the secondary metabolites in KP-AE could serve as capping and stabilizing agents, preventing agglomeration and enhancing the dispersion of the nanoparticles. The lack of significant agglomeration further highlights the effectiveness of KP-AE in producing stable and well-dispersed silver nanomaterials.

CONCLUSION

The phytochemical components of *Kaempferia parviflora* aqueous extracts were used for this study employing a sustainable, green methodology, yielding spherical shapes with a range size of 19 nm to 40 nm. The most successful approach to biogenic manufacturing has been effectively proven by synthesizing at pH (11), temperature (75°C), incubation time (24 hours), and 2 mM $AgNO_3$ with high product yields. The biogenic fabrication of KP-AE AgNMs is rapid, economical, and non-toxic. However, additional studies are required to demonstrate numerous biological features (such as antifungal, antidiabetic, anti-inflammatory, and cytotoxic potential) as a first step toward pharmaceutical implementation of these biogenic fabrics to produce AgNMs.

AUTHOR CONTRIBUTIONS

Alya Khaizura Azman was responsible for conducting the experiments, drafting the manuscript, and analysing experimental data. Mohammad Aidiel was involved in analysing experimental data and drafting the final version of the manuscript. Deborah Anna Van Oosterhout prepared for drafting the manuscript. Maisarah Abdul Mutalib was responsible for the conception and design of the research, drafting the final version of the manuscript, supervising students, research management, and funding acquisition.

ETHICS APPROVAL

Not applicable.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this work

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