OpenNano 21 (2025) 100224

Contents lists available at ScienceDirect

OpenNano

journal homepage: www.elsevier.com/locate/onano

Are *Bentong* ginger (*Zingiber officinale*) biosynthesized silver nanoparticles safe and effective? An optimization, characterization, and toxicity evaluation study

Nor-Azmiraah Abd Jabar [a], Mahmud Ab Rashid Nor-Khaizura [a,b,*], Siti Izera Ismail [a], Yuet Ying Loo [c], Kah Hui Chong [b], Kousalya Padmanabhan [b], Shan Jiang [b]

[a] Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Malaysia
[b] Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[c] Department of Pathology, Hospital Kuala Lumpur, Jalan Pahang 50586, Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur, Malaysia

ARTICLE INFO

Keywords:
Bentong ginger
Characterization
Ginger powder
Green synthesis
Optimization
Silver nanoparticles
Zingiber officinale

ABSTRACT

The biosynthesis of silver nanoparticles from ginger extract is particularly interesting due to the bioactive compounds present in ginger, which have antioxidant, antimicrobial, and anti-inflammatory properties. The study aims to optimize, characterize, and evaluate the toxicity value of the biosynthesized silver nanoparticles using *Bentong* ginger (*Zingiber officinale*) rhizome extract and commercialized ginger powder extract as reducing and capping agents. The synthesis was optimized regarding pH, silver nitrate concentration, and incubation time for better yield and stability. Additionally, biosynthesized silver nanoparticles were characterized using UV–vis spectrophotometer, X-ray diffraction, Fourier-transform Infrared, and Transmission Electron Microscope analysis. Cytotoxicity test was done using brine shrimp lethality test to determine toxicity value. The result for both *Bentong* ginger rhizome extract and commercialized ginger powder extract indicated that the maximum absorption of biosynthesized silver nanoparticles was 450 nm, with the most optimum pH of 11, 1 mM of silver nitrate concentration, and incubation time of 24 h. The nanoparticles were almost spherical, with an average particle size of 15.08 ± 6 nm. The analysis confirms the presence of phytochemicals in the ginger extract that aids in reducing silver ions into silver nanoparticles. Brine shrimp lethality assay showed the LC_{50} for AgNPs was medium toxic at 838.31 μg/mL. Although silver nanoparticles possess antimicrobial ability, the potential toxicity to human health and environmental concerns must be considered before deploying into food industries. This is the first report utilizing *Bentong* ginger in silver nanoparticle synthesis.

1. Introduction

Nanotechnology has been gaining significant attention for a few decades, with nanoparticle biosynthesis being a critical area of

Abbreviations: AgNPs, Silver nanoparticles; $AgNO_3$, Silver nitrate; DMSO, Dimethyl sulfoxide; HCl, Hydrochloric acid; NPs, Nanoparticles; NaOH, Sodium Hydroxide; FTIR, Fourier-Transform InfraRed; TEM, Transmission Electron Microscope; UV–Vis, Ultraviolet -Visible spectrophotometer; XRD, X-ray Diffraction; *Z. officinale*, *Zingiber officinale*.

* Corresponding author.
E-mail address: norkhaizura@upm.edu.my (M.A.R. Nor-Khaizura).

https://doi.org/10.1016/j.onano.2024.100224
Received 11 October 2024; Received in revised form 28 November 2024; Accepted 5 December 2024
Available online 6 December 2024

research [1]. Nanoparticles (NPs) are building blocks of nanotechnology ranging from 1 to 100 nm in diameter. It has been helpful in many areas due to its size, catalytic ability, stability, and antimicrobial behavior [2]. Small size provides a larger surface area relative to the volume, making them suitable for use in diversified fields. Nanomaterials comprise different shapes, such as nanorods, nanoparticles, and nanosheets, characterized by their dimensionality. Nanomaterials with zero dimension are known as nanoparticles [3]. There are five types of intentionally produced nanomaterials: carbon-based, metal oxides-based, dendrimers, nanocrystals, and nanocomposites [4]. Metal-based NPs like zinc, copper, silver, gold, palladium, and platinum have more advantages than others as they have higher surface area and good adsorption ability of small molecules, which are more commonly used as nanotechnology consumer products [5]. Silver nanoparticles (AgNPs) are extensively studied among all metals due to their substantial antimicrobial activities [6]. Besides that, AgNPs are known for their adaptable properties, and improved physical, chemical, and biological properties, which has caused them to be widely used in numerous industries [7].

Due to its versatility, NPs are appealing and used in many industries. As a new approach, NPs are used in cleaning up the environment as they enhance various chemical reactions through catalytic activity [8]. Nowadays, NPs are also extensively used in the medical field for various applications. Due to their smaller size, NPs are encapsulated in anticancer drugs, which can directly deliver to targeted cancer cells without harming healthy cells [9]. Besides that, metal NPs are used as biosensors in medical facilities to aid in the early detection of tumors and to monitor markers [10]. NPs are utilized against multidrug-resistant pathogens as an alternative to antibiotics. Metal NPs have multiple mechanisms to inhibit pathogens, including adhesion to the cell membrane, production of reactive oxygen species (ROS), cell penetration, and cell signaling modulation [11]. Beyond medicinal applications, NPs are also used in electronic technologies. Quantum dots, commonly known as semiconductor particles, are incorporated in solar cells to enhance optical properties in electronics [12]. NPs are also used in semiconductor devices such as lasers and diodes to improve thermal stability [13], as sensors in controllers, protective coating in devices, and as batteries as they are versatile and stable [14,15]. Although NPs are newly introduced in food industries, research is needed to ensure their safety. It is now used as an antimicrobial agent to inhibit food spoilage and extend the shelf life of food [16]. Besides, NPs are incorporated as packaging materials that can monitor and enhance food conditions [17]. They are also utilized as nanosensors to detect even small concentrations of food hazards, including toxins, pathogens, and metals in foods [18]. As an environmental remediation, silver nanoparticles are used to treat wastewater that exhibits significant photocatalytic activity [19] and combined with oxides to purify air up to 98% from secondary pollution of bacteria and fungi [20].

Generally, metal NPs can be synthesized under three categories: physical, chemical, and biological. The biological method is preferred since it is cheaper and more affordable. These categories are made up of either of two approaches: top-down and bottom-up approaches [21]. However, utilizing NPs in physical and chemical methods often involves harsh chemicals, creating waste, hazardous risks to the ecosystem, and environmental effects [22]. Most of the procedures are time-consuming, tedious, and involve expensive materials. As a promising alternative, the biological method, also known as the green synthesis method, was more compatible with synthesizing nanoparticles. In the biological approach, bio-waste, microorganisms, and plant extracts are three types of medium commonly used. Plant extracts are preferable as they produce a faster and safer synthesis process, resulting in non-toxic derivatives [23]. Plant extracts used with AgNPs through biological processes act as reducing agents during synthesis [24]. It is more environmentally friendly and cost-effective than chemical methods [25].

As a part of the analysis, one variable at a time (OVAT) systematic approach is used for optimization analysis. OVAT is used to evaluate the impact of individual tested variables to enhance the clarity and precision of significant factors. The variables include pH, temperature, silver nitrate and plant extract concentration, incubation time, and agitation [26]. In this study, pH, concentration of silver nitrate, and incubation time were studied for optimization analysis, and UV-visible spectroscopy, Fourier-Transform Infrared (FTIR), X-ray Diffraction (XRD), and Transmission Electron Microscope (TEM) techniques were used for characterization determination. This approach was complemented with statistical analysis to avoid overlooking interactions between variables.

Ginger, scientifically known as *Zingiber officinale*, is a popular spice in the culinary and medicinal fields due to its vast bioactive compounds [27]. Gingerols, shogaols, zingiberene, flavonoids, and phenolics have been found to possess antioxidant, antimicrobial, and anticancer properties, which makes them an excellent option to be used in biosynthesizing silver nanoparticles. The pharmacological benefits of ginger are broad, as it has been used widely since ancient times. Ginger has a significant anti-inflammatory effect that benefits in controlling osteoarthritis and rheumatoid arthritis [28]. Ginger has high antioxidant properties that help neutralize free radicals in the body by reducing chronic illness tendencies and oxidative stress [29]. In addition, ginger has been scientifically proven to aid in cancer treatments by inhibiting cancer cell proliferation, inducing apoptosis, and enhancing chemotherapy drugs' effectiveness [30]. *Zingiber officinale* [31], *Zingiber zerumbet* [32], and *Zingiber cassumunar* [33] are some of the ginger species that have been reported to have effective antimicrobial properties. *Zingiber officinale* Roscoe var. *Bentong* is a species of ginger grown explicitly in the *Bentong* area in Malaysia. It is always high in demand compared to ginger grown in other countries due to its better quality [34]. Although many reports have been established utilizing other species of ginger, this is the first report of utilizing *Bentong* ginger in synthesizing, optimizing, characterizing and determining the toxicity level. Thus, this study aims to optimize, characterize, and determine the toxicity level of the silver nanoparticles biosynthesized with commercialized powdered and *Bentong* rhizome ginger extract.

2. Materials and methods

Fresh rhizomes of *Zingiber officinale* were bought from a plantation located in *Bentong*, Malaysia. $AgNO_3$ was purchased from Sigma-Aldrich, Darmstadt, Germany.

2.1. Preparation of rhizome extract of Z. officinale

Fresh *Bentong* ginger rhizomes were collected, cleaned, and shade-dried. Then, the dried rhizomes were powdered using a grinder and sieved before the extract was stored in an airtight container. 25 g of ginger extract was mixed thoroughly with 250 mL of distilled water (1:10 *w/v*). The mixture was then boiled at 35 °C for 30 min using a water bath and cooled at room temperature. The mixture was then filtered with Whatman filter paper. The aqueous ginger extract was stored at 5 °C for future use.

2.2. Preparation of commercialized Z. officinale ginger extract

Commercially prepared 100 g of *Bentong* ginger powder was bought from a local shop at *Bentong*, Pahang. 25 g of the ginger powder was mixed thoroughly with 250 mL of distilled water before boiling at 35 °C for 30 min in a water bath. Then, the mixture was cooled down before filtering using Whatman filter paper.

2.3. Synthesis of silver nanoparticles

25 mL of ginger extract was added with 125 mL of 1 mM $AgNO_3$ solution and incubated at room temperature under static conditions. A similar setup was prepared without ginger extract as a control.

2.4. Optimization of silver nanoparticles

Three varying parameters tests, pH, concentration of silver nitrate solution, and incubation time, were evaluated in the biosynthesis process of AgNPs using ginger extract. Results obtained through UV–vis spectrophotometer were used to identify the response to the mentioned different parameters and thus determine the optimized value.

2.4.1. pH

The pH of AgNPs synthesis was optimized using different pH values of 3, 5, 7, 9, 11, and 12. Each pH was adjusted using 0.1 N HCl and 0.1 N NaOH. The absorbance of the reaction mixture was measured using a UV–vis spectrophotometer.

2.4.2. Concentration of silver nitrate

Six different types of silver nitrate concentration (1 mM, 2 mM, 4 mM, 6 mM, 8 mM, and 10 mM) were used to study the effect of concentration on the synthesis of AgNPs. The optimum pH of the AgNPs synthesis obtained from the previous result was used, while other parameters remained the same.

2.4.3. Incubation time

Three different time intervals (24 h, 48 h, and 72 h) were chosen for the synthesis to study the incubation time effect against AgNPs. The pH and the concentration of silver nitrate solution were set based on the results obtained from previous tests. Absorbance was measured using a UV–vis spectrophotometer with a wavelength ranging from 350 to 800 nm.

2.5. Characterization of AgNPs synthesized under optimum conditions

2.5.1. UV/vis spectrophotometer

The synthesis of silver nitrate with ginger extract was confirmed by color changes from light yellow to dark brown over time. The reaction mixture was sampled at certain time intervals, and the absorbance value was measured using a Genesys 20 UV–vis spectrophotometer at a wavelength ranging from 350 to 800 nm.

2.5.2. XRD analysis

The mixture was centrifuged at 75,000 × *g* for 30 mins. The pellet was washed a few times in deionized water and dried using a freeze-dryer. Dried nanoparticle powder was coated using an XRD grid, and spectra were recorded using a Philips PW 1830 X-ray generator operating at 40 kV voltage and 30 mA current with Cu Kα1 radiation. 2θ angle used was ranged from 20 to 80°.

2.5.3. FTIR analysis

For FTIR spectroscopy analysis, the Alpha Bruker model with a wave number range of 4000–400 cm^{-1} at room temperature was used to analyze ginger extract and AgNPs.

2.5.4. TEM analysis

For TEM microscopic analysis, the 25μ L sample was sputter coated using a copper stub, and nanoparticle images were analyzed using Joel JEM2100F HRTEM.

2.6. Cytotoxicity assay

2.6.1. Preparation of Artemia salina nauplii

The toxicity of nanoparticles was evaluated using brine shrimp lethality assay using the method by Zhu et al. [35] with slight modification. *Artemia salina* nauplii were prepared and stored at 4 °C. For hatching, artificial seawater was created with a concentration of 3 % (*m/V*) by dissolving 30 g sea salt in 1 L distilled water. The solution was stirred thoroughly before filtering through Millipore filters. Encysted *Artemia* nauplii were incubated in the artificial seawater for 24–48 h at a temperature between 27 °C-30 °C with continuous illumination and vigorous aeration.

2.6.2. Acute toxicity testing

The toxicity testing was conducted according to OECD guidelines [36]. *Artemia* nauplii ($n = 30$ per group) were exposed to seven different concentrations of the AgNPs, ranging from 500 μg/ml to 7.81 μg/ml for 24 h A positive control was included, consisting of 5 % potassium dichromate ($K_2Cr_2O_7$) in 1 mL artificial seawater, while Dimethyl sulfoxide (DMSO) was used as a negative control. All exposures were performed in triplicate within the 96-well plate, each well containing 100 μL of artificial seawater. The experiments were conducted in an incubator at a stable temperature of 27 °C-30 °C. The mortality rate was calculated, and the LD_{50} value was determined using Clarkson's Toxicity Index. The mortality rate was calculated as follows:

$$\text{Mortalityrate}(\%) = \frac{\text{No. of dead } \textit{Artemia salina} \text{ nauplii}}{\text{Initial number of live brine nauplii (control)}} \times 100$$

2.7. Statistical analysis

GraphPad Prism 10 was used for all the data analysis. F-value and p-value were obtained from the optimization absorbance and characterization graph result using one-way ANOVA. All the data was given as mean ± SEM.

3. Results and discussion

3.1. Optimization of varying parameters

The current study signifies ideal conditions of silver nanoparticles (AgNPs) formation synthesized using *Bentong* ginger rhizome extract and commercialized ginger extract powder as a reducing agent. Three types of parameters were optimized to synthesize AgNPs, including pH, concentration of silver nitrate, and incubation time. The volume of ginger extract (25 mL) and incubation temperature (room temperature) were kept constant throughout the study. AgNPs formation was observed using a UV–vis spectrophotometer with an absorbance range of 350–800 nm.

Changes in pH is a vital optimization factor in the stability of silver nanoparticles (AgNPs). The study assessed the effect of six

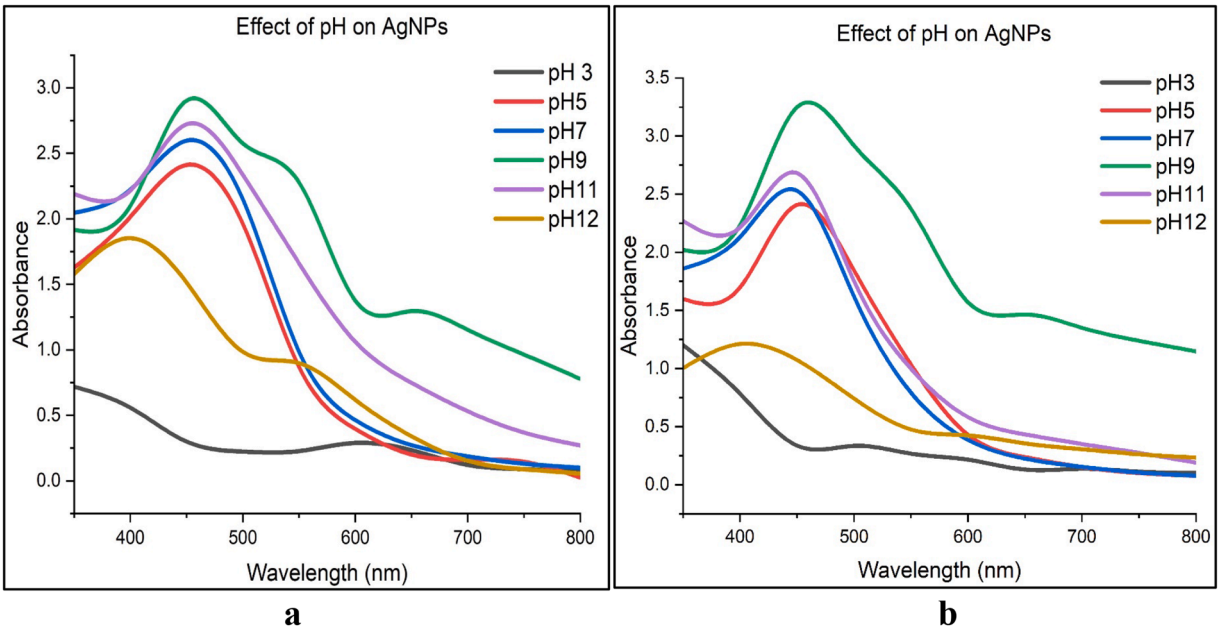

Fig. 1. UV–vis spectra of different pH of biosynthesized silver nanoparticles using rhizome ginger extract (a) and commercialized ginger extract (b).

different pH levels on the synthesis of AgNPs using *Bentong* rhizome extract (a) and commercialized ginger extract (b) (Fig. 1). The concentration of $AgNO_3$ solution (1 mM) and incubation time (24 h) were kept constant. The results indicate that no particles formed at an acidic pH of 3, while a peak was observed around 400 nm, with agglomeration detected at a more alkaline pH of 12. Particles were successfully formed at pH levels 5, 7, 9, and 11, with pH 9 exhibiting the highest absorbance peak, however, agglomeration was also noted at this pH. Statistical analysis further supports these findings. For commercialized ginger extract, the F-value was 4.002 with a p-value of 0.0037, indicating a significant effect of pH on AgNP formation. The low p-value (< 0.05) suggests that the observed differences in absorbance across the pH levels are statistically significant.

Similarly, for *Bentong* rhizome extract, the F-value of 5.359 and a p-value of 0.0005 further demonstrate a highly significant effect of pH on AgNP synthesis. Particles formed within the absorption peak around 420–450 nm in UV–vis spectrophotometer, which corresponds to surface plasmon resonance (SPR) of AgNPs that was established prior [37], further confirming the presence of AgNPs. Previous studies utilizing ginger extract have also proved that alkaline pH favors the formation of nanoparticles [38]. In another study by Tahir et al. (2020), when utilizing a protein called sericin, derived from silkworm to be conjugated with silver nanoparticles, pH 11 showed the highest antimicrobial activity. They concluded that pH is either responsible for changing the solubility of the protein or the protein charge [39]. Thus, pH 11 is considered the best pH as nanoparticles were most stable when synthesized. Another study by Barabadi *et al.* (2021) also proved that at lower pH, the corresponding peaks were polydisperse manners, while at pH higher than 9, they were bell-shaped, which favors this current study [40]. At a higher pH environment, the concentration of H^+ will decrease, causing bacteria to substitute Na^+ with $H^+$for continuous energy production. This substitution will cause increased membrane potential and cell breakdown [41]. Higher pH enhances the antibacterial activity of pathogens, whereas gram-negative bacteria are unable to grow at high pH, which causes membrane hyperpolarization and, eventually, cell lysis [42,43].

Regarding the silver nitrate ($AgNO_3$) concentration effect, pH (11) and incubation time (24 h) were kept constant. The effect of different concentrations on AgNPs synthesized with ginger extract is shown in Fig. 2. For both ginger rhizome extract and commercialized ginger powder extract, 1 mM and 2 mM showed approximately similar absorbance peaks. In contrast, other higher concentrations of $AgNO_3$ showed shifted peaks and agglomeration. The F-value and the p-value for commercialized ginger extract were 3.193 and 0.0135, respectively. On the other hand, for ginger rhizome extract, the F-value was 2.568, and p-value was 0.0372. Both the results have a significant result that shows silver nitrate concentration effects on AgNPs synthesis. This result also aligned with previous studies done by Velmurugan et al. [44] and Mehata [45]. Since a lower concentration yields a similar result,1 mM is the most optimum $AgNO_3$ concentration.

Fig. 3 shows the effect of three different incubation times in synthesizing AgNPs where other parameters were kept constant: pH (11) and $AgNO_3$ concentration (1 mM). The influence of incubation time using ginger rhizome extract and commercialized ginger powder extract was evaluated by measuring absorbance using a UV–vis spectrophotometer, as shown. The absorption band was found to have peaked at 450 nm for all three incubation times for both ginger extracts. Nanoparticles were found to be stable from 24 h to 72 h.

Besides, the observed color changes of the solution from light yellow to dark brown (Fig. 4) also confirmed that silver ion was reduced to silver nanoparticles after 24 h This result is also consistent with Ramzan et al. [46]. The intensity of the color changes increases as incubation time increases. These changes indicate the reduction of silver ions and the formation of AgNPs with three stages: (1) reduction of ions, (2) causing cluster formation, and (3) triggering the nanoparticle formation [47]. This result

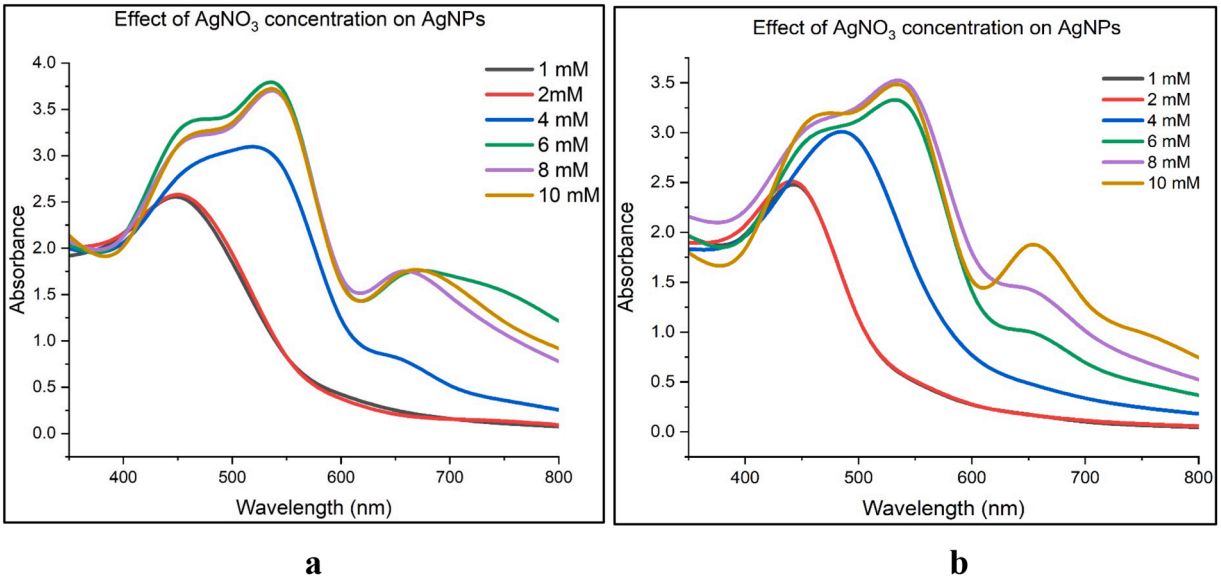

Fig. 2. UV–vis spectra of different concentrations of silver nitrate on biosynthesized silver nanoparticles using rhizome ginger extract (a) and commercialized ginger extract (b).

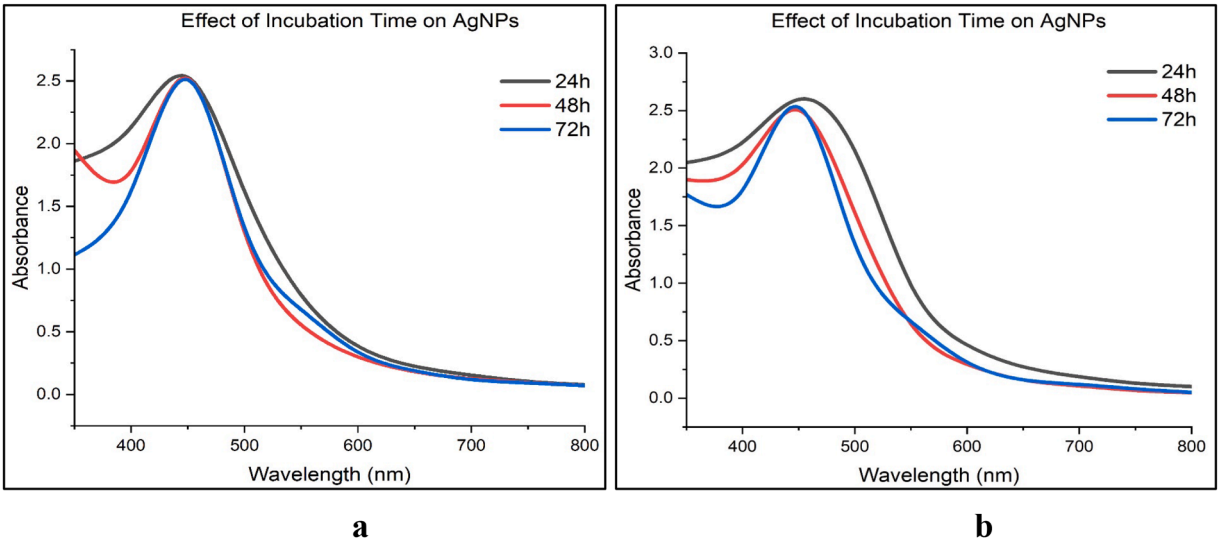

Fig. 3. UV–vis spectra of different incubation time on biosynthesized silver nanoparticles using rhizome ginger extract (a) and commercialized ginger extract (b).

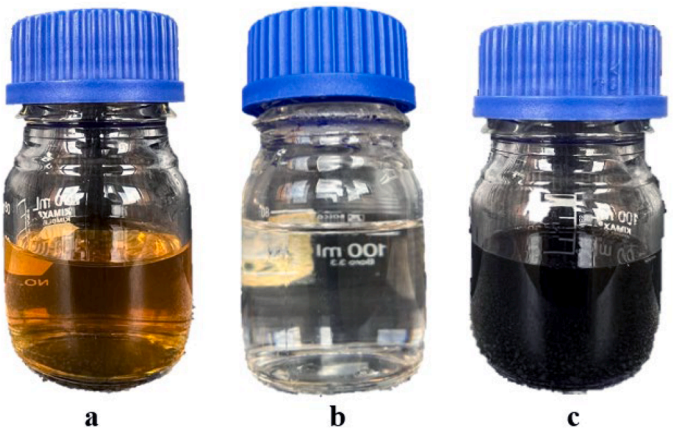

Fig. 4. 1 mM of silver nitrate with *Bentong* ginger rhizome extract; (a) ginger extract alone, (b) silver nitrate solution alone, (c) after synthesizing ginger extract with silver nitrate solution at 24 h

corresponded to previous studies by Krishnaraj et al. (2012), Mehata (2021), and Rajkumar et al. (2021) [45,48,49]. Besides color change, the presence of AgNPs was confirmed with absorbance measurement using a UV–vis spectrophotometer with a ranging wavelength of 350–800 nm. The excitement of silver particles showed an absorbance peak at a wavelength of 450 nm when it was optimized at pH 11 with a silver nitrate concentration of 1 mM and 24 h incubation time. The peak within the range is similar to studies proving the validity of the result [50]. The peak formation showed that bioactive compounds in ginger extract reduce silver ions. The spectrophotometer's surface plasmon resonance (SPR) band also expanded with increasing pH. Since the expansion indicates more production of AgNPs [47], it is seen that acidic and highly alkaline pH causes agglomeration and instability of AgNPs. pH is a significant factor that eventually can change bioactive molecules' size and electrical charge, as well as their ability to interact with molecules and produce AgNPs [49].

The green synthesis production of silver nanoparticles is gaining attention as it is becoming an emerging approach that is eco-friendly, does not utilize harmful chemicals, and is cost-effective [51]. Nanoparticles are small, ranging from 1 to 100 nm, which causes differences in features compared to bulk metals. The small size of nanoparticles is more advantageous as it has an increased surface area-to-volume ratio, which directly increases interaction with other molecules [52]. Thus, the main objective of this study is to optimize synthesized silver nanoparticles (AgNPs) with *Bentong* ginger extract and characterize them.

Plant extracts contain different types and concentrations of phytochemicals that are beneficial as reducing and stabilizing agents [53]. The synthesis of silver nanoparticles using ginger rhizome extract involves a bio-reduction process utilizing the phytochemicals present in ginger. Thus, the production of AgNPs is considered a green synthesis approach. Ginger rhizome has multiple bioactive

compounds, including gingerol, alkaloids, phenols, flavonoids, and terpenoids, each having multiple functions [54]. Gingerol and shogaols aids in reducing silver ions (Ag^{+}) to silver nanoparticles (Ag^{0}) by donating electrons [55]. Curcumene, zingiberene, and zingerone stabilize nanoparticles by forming a protective layer around them upon synthesis [56]. Other phytochemicals and bioactive compounds are also responsible for the reduction process besides acting as capping agent and prevent aggregation of nanoparticles. The size and shape of AgNPs are highly influenced by factors such as concentration of silver nitrate, reaction time, and pH [57], which leads to the objective of this study.

Optimization is the critical preliminary analysis to find the best condition in synthesizing AgNPs. After optimizing, it can be concluded that both ginger rhizome extract and commercialized ginger powder extract showed nearly identical results. However, ginger rhizome extract is preferred for further use in characterization analysis. This is because the extract preparation process of ginger rhizome is known, from harvesting to yielding. On the other hand, during the processing of commercialized ginger powder, the rhizome may go through high-temperature processing such as heating and drying. Bioactive compounds like gingerol can degrade at high temperatures above 90 °C, whereby prolonged exposure to heat could convert gingerol to shogaol, which possesses different biological activity [54]. Besides, the ginger rhizome species were identified and confirmed as *Bentong* ginger, unlike the commercially obtained powder extract, which could be a wild type. Using a well-known extraction methodology and botanical background of plants increases the overall reliability and reproducibility of the study.

3.2. Characterization study

3.2.1. X-Ray diffraction

The crystallinity phase of AgNPs synthesis was examined using the X-ray diffraction (XRD) technique (Fig. 5). Four sharp diffraction peaks were analyzed at 38.17°, 43.94°, 64.55° and 77.45°, which indexed the face-centered cubic (fcc) silver planes of 111, 200, 220 and 311, respectively [58]. The lattice constant calculated from the pattern was $\alpha = 4.0862$ Å. The data obtained matched the Joint Committee on Powder Diffraction Standards (JCPDS) database with card number 00–004–0783. The average nanoparticles' size was calculated using Debye-Scherrer's formula $D = (K\lambda/\beta\cos\theta)$ in which D is the average particle size, K is shaped constant, λ is the wavelength X-ray source (0.1541 nm), β is full width at half maximum (FWHM), and θ is Bragg's angle. The estimated average size of AgNPs was 35 nm. XRD pattern of the analysis was reported using AgNPs powder. Based on the analysis, peaks formed at 111, 200, 220, and 311 showed crystallization occurred on the surface of AgNPs [59]. Besides that, the highest intensity peaks obtained were used to determine the average size of AgNPs. The calculated average size value of 35 nm was higher than the Transmission Electron Microscope (TEM) analysis, which was 15 nm. The difference can be influenced by the slight deviation of particle size used to calculate using Debye-Scherrer's formula and used in TEM analysis [60]. However, researchers have proved that nanoparticles size lesser than 50 nm is favorable for a promising antibacterial activity with minimal cytotoxicity and antioxidant potential [61]. This is due to its smaller size, which can attach to cell walls or penetrate through them, damaging the intracellular components [62].

3.2.2. Fourier-Transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) analysis was carried out, as shown in Fig. 6, to identify functional groups in AgNPs synthesis. The spectra at 3320 cm^{-1} belong to N—H stretching secondary amine bonds [53] or O—H stretching alcohol bonds [58,63,64]. Weak peaks at 2112 cm-1 show C≡C stretching alkyne and N = C = S stretching bonds, while 2000 cm^{-1} shows C = C = N stretching and C—H bending aromatic compound [45]. The peak at 1636 cm-1 conforms to C = O stretching alkane and N—H bending

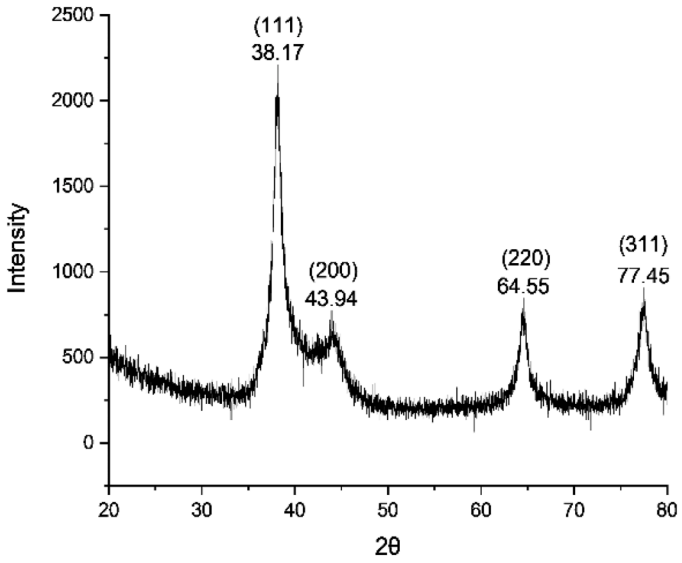

Fig. 5. XRD pattern of synthesized silver nanoparticles.

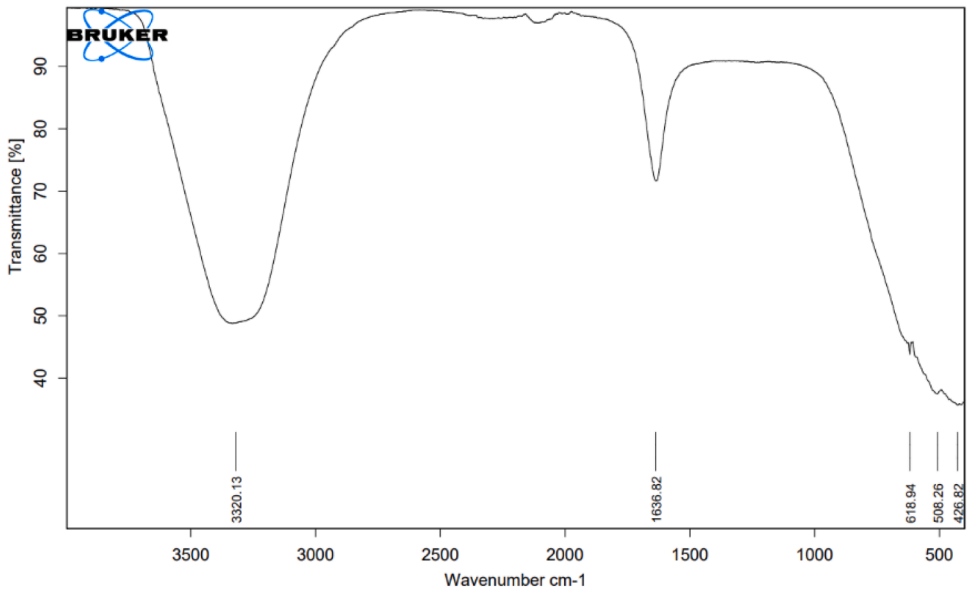

Fig. 6. FTIR spectrum of *Bentong* ginger extract synthesized silver nanoparticles.

amine bonds [65–68]. The presence of halogen and aromatic compounds is confirmed when peaks range 800–500 cm^{-1} are found [47]. The peak at 618 cm^{-1} may correspond to O—H phenolic bonds [66], and C—Cl stretch may be an alkyl halo compound. All the observed peaks indicated the presence of phenolic compounds, steroids, tannins, flavonoids, terpenoids, alkaloids, and saponin in the ginger extract, which aids in synthesizing AgNPs. FTIR analysis showed these possible bioactive molecules present in ginger extract after the synthesis of AgNPs that act as a capping agent and aid in stabilizing reduced silver ions from Ag^{+} to Ag^{0}. Regarding silver nanoparticles, the significant shift of absorbance peak with decreasing intensity from 3395 cm^{-1} to 3436 cm^{-1} shows the binding of silver ions with hydroxyl groups present in the ginger extract [58]. Besides that, the prominent shift from 1651 cm^{-1} to 1630 cm^{-1} correlates with amide linkages that verify free amino acid and carboxyl groups in ginger extracts that have reacted with AgNPs and are stabilized [67].

3.2.3. *Transmission electron microscope*

Transmission electron microscope (TEM) was used to analyze the morphology of the synthesized AgNPs. Shape and size distribution obtained confirmed that AgNPs were spherical and quasi-spherical in shape with a mean size of 15.08 ± 6 nm and size range from 5 to 29 nm (Fig. 7). This result was similar to other studies carried out using ginger extract. Yadi et al. (2022) reported an AgNPs size of 15 nm utilizing ginger root, Mohammadi et al. (2021) obtained an AgNPs size of 10 ± 4 nm using ginger rhizome extract, and 26 nm in a study by Singh et al. (2011) [69–71]. The size was smaller than other metal nanoparticles when synthesized with other plant

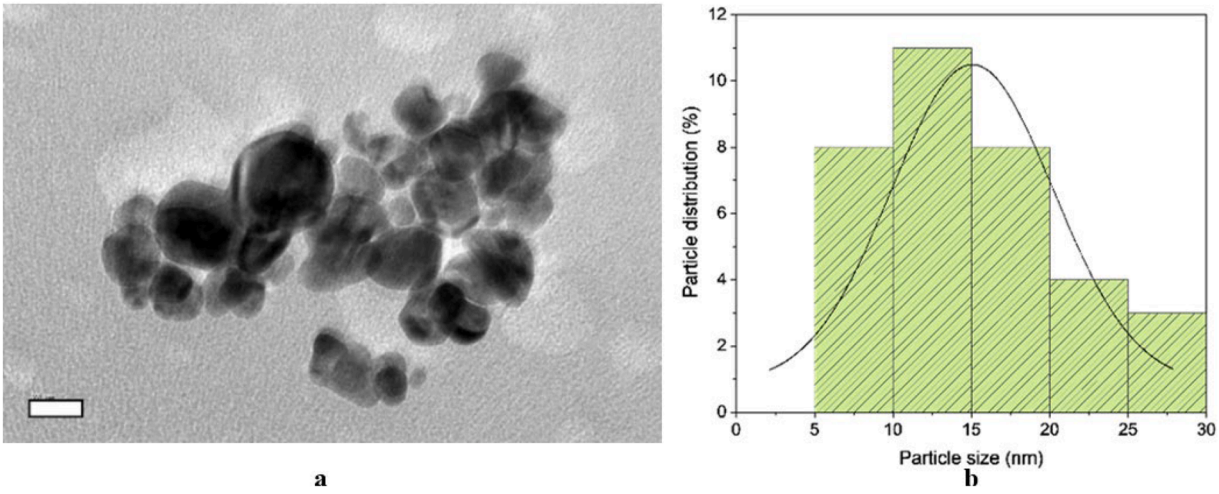

Fig. 7. TEM image (a) and size distribution (b) of green synthesized AgNPs with ginger extract.

extracts. Mortazavi-Derazkola et al. (2024) proved that magnesium oxide silver nanoparticles synthesized with pistachio leaf extract were more significant at 70–80 nm in size and in stone-like morphology [72]. This is due to adding a layer of magnesium oxide at the core of silver during synthesis. Another study by Mumtaz et al. (2022) concluded that sericin-conjugated silver nanoparticles produced 44.6 nm particles post-synthesis [73]. Although sericin acts as a favorable capping agent, the incomplete silver reduction and inherent macromolecule structure cause larger nanoparticles to form, which might cause instability in future applications. Smaller size of silver nanoparticles benefits in effective drug delivery and electric sensors. Plant extract is used as a capping and reducing agent to prevent agglomeration and size uniformity throughout the process [72]. The phytochemicals in ginger rhizome extract play some roles, which include aiding in reducing silver ions to silver nanoparticles and acting as a capping agent by binding to the surface of silver nanoparticles and providing electrostatic stabilization [73]. Through this, silver nanoparticles' size and shape are preserved when utilized for long-term application.

Based on TEM analysis, the population of AgNPs comprises spherical shape molecules with varied sizes besides some other quasi-spherical shapes. It can be justified that aggregation seen from a microscopic view was due to the direct contact of AgNPs with each other or due to the hydroxylic bond arrangements during silver nanoparticles synthesis [74]. However, AgNPs were stabilized with a functional group in bioactive molecules in the ginger extract [75–80].

3.3. Cytotoxicity assay

The cytotoxicity effect of *Bentong* rhizome ginger extract synthesized silver nanoparticles (AgNPs) was studied using brine shrimp (*Artemia salina*) lethality assay. *A. salina* nauplii was exposed to a two-fold dilution concentration of AgNPs. Lethal dose (LC_{50}) was determined by plotting probit versus log concentration of AgNPs linear correlation graph (Fig. 8). The toxicity level was calculated by comparing it with Clarkson's toxicity index (Table 1) [81]. Based on the graph, LC_{50} for AgNPs reported was 838.31 μg/mL. Based on Clarkson's index, it is considered as low toxic (500 – 1000 μg/mL). On the other hand, the LC_{50} for positive control of potassium dichromate was 0.9933 μg/mL, which was considered highly toxic (0–100 μg/mL).

Fig. 9

Brine shrimp lethality assay is one of the most common bioassays used to assess the toxicity of substances as a screening procedure to determine any pharmacological activities. This assay utilized brine shrimp to evaluate lethality by calculating the LC_{50} value, the concentration to kill 50 % of brine shrimp nauplii. This method is commonly used as preliminary screening due to its simplicity, cost-effectiveness, and fast results [82]. The result shows that brine shrimp mortality increases when the concentration of AgNPs increases. The result aligns with other plant extract studies by Kabiru et al. (2022) and Low et al. (2022) [83,84]. At AgNPs concentration of 250 μg/mL, 20 % of brine shrimp were dead overnight, and 100 % of brine shrimp were dead after 24 h exposure to AgNPs. One of the factors for the mortality could be cellular and structural damage due to high concentration exposure of AgNPs. Reduced silver ions from AgNPs cause binding to chitin, an essential component in shrimp's cuticle. This causes structural deformation of the cuticle, leading to the brine shrimp's integrity loss [85]. On the molecular level, exposure to high concentrations of AgNPs may cause apoptosis and DNA damage in brine shrimp [86]. Tissue damage and gut disruption are common when brine shrimps are exposed to AgNPs [87,88]. Based on the result, the AgNPs have a moderate risk of causing harm to human and animal health when exposed for a long time. However, it is not considered immediately lethal at lower concentrations of AgNPs. Although it is not severely toxic, caution is still required by setting safe exposure limits lower than the tested value especially when applied in food and medical industries. Due to this, utilizing low concentrations of AgNPs in a controlled environment and without direct contact with food or humans, such as in packaging and biosensors, will be a reliable and practical application. Other than that, techniques like a slow-release system and encapsulation of AgNPs could be employed to ensure a small amount of AgNPs is released over time.

4. Conclusion

In this current study, silver nanoparticles were synthesized using an economical, simple, environment-friendly green synthesis approach. Two varieties of aqueous *Bentong* ginger rhizome extract and commercialized ginger powder extract were used to optimize silver nanoparticles synthesis. The optimization results denoted that AgNPs synthesized from both varieties were similar; they had an optimum condition of pH 11, 1 mM of $AgNO_3$ concentration, and 24 h incubation time. Due to that, only *Bentong* ginger rhizome extract has proceeded with characterization analysis. The FTIR analysis result showed AgNPs were in cubic face-centered silver planes. The presence of functional groups alkane (C = O) and amine group (N—H) might aid the synthesis of AgNPs. TEM analysis revealed that AgNPs are almost spherical, with a mean size of 15.08 ± 6 nm. These results show that formed AgNPs are stable and suitable for applications such as antibacterial activity. Besides that, the safety level of synthesized AgNPs is considered safe that can be practically applied in food industries without any long-term effects on the food and consumers. However, further research must be elucidated to determine the effectiveness *in vitro* and its mechanisms for inhibiting microorganisms. Other than that, a detailed phytochemical profiling analysis such as LC-MS shall be conducted to enhance the understanding of reduction and capping mechanisms during synthesis. Extensive *in vivo* toxicological studies and safety assessments will be advantageous to determine the exact safe concentration of AgNP applied in the food and medical field.

CRediT authorship contribution statement

Nor-Azmiraah Abd Jabar: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Mahmud Ab Rashid Nor-Khaizura:** . **Siti Izera Ismail:** Writing – review

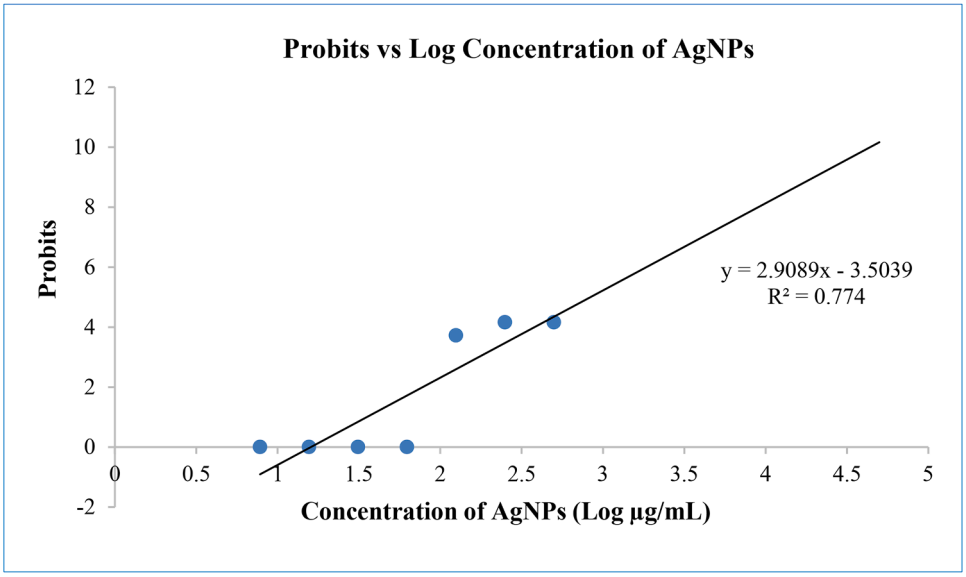

Fig. 8. Plot of probit versus log concentration for LC_{50} determination of AgNPs.

Table 1
Clarkson's toxicity index.

LC_{50} value (µg/mL)	Level of Toxicity
>1000	Non-toxic
500 – 1000	Low toxic
100 – 500	Medium toxic
0 - 100	Highly toxic

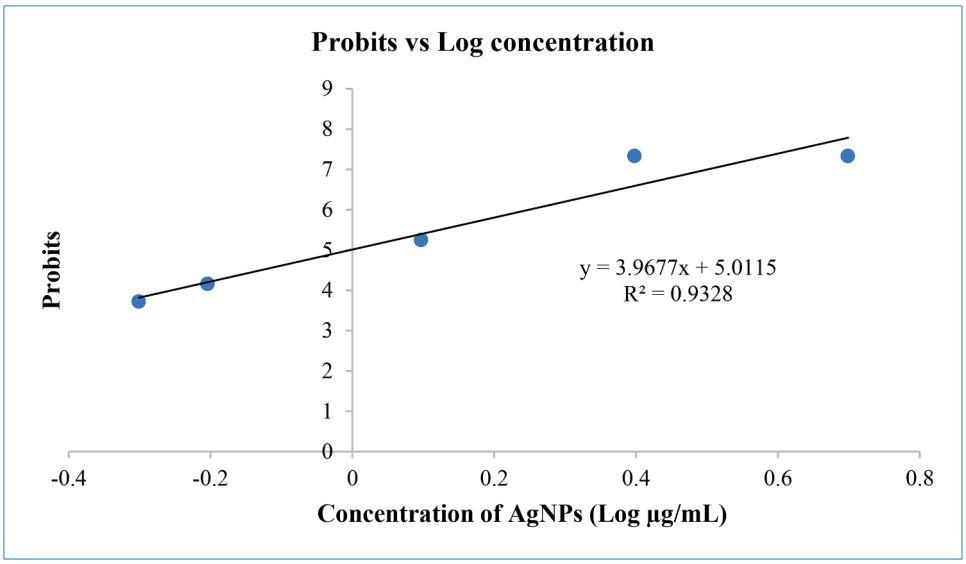

Fig. 9. Plot of probit versus log concentration for LC_{50} determination of postiitve control potassium dichromate.

& editing, Funding acquisition, Conceptualization. **Yuet Ying Loo:** Visualization, Methodology, Conceptualization. **Kah Hui Chong:** Writing – original draft, Software, Methodology. **Kousalya Padmanabhan:** Writing – original draft, Validation, Project administration, Methodology, Data curation. **Shan Jiang:** Software, Methodology, Formal analysis.

Declaration of competing interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

This work was kindly supported by the Ministry of Education Malaysia through the Higher Institution Centre of Excellence (HICoE) grant scheme—project no 800-3/8/HiCOE(FasaII)/2023/5230004, and special appreciation to the Universiti Putra Malaysia for providing research materials and equipment.

References

[1] N.E.A. El-Naggar, N.A. Abdelwahed, O.M. Darwesh, Fabrication of biogenic antimicrobial silver nanoparticles by *Streptomyces aegyptia* NEAE 102 as eco-friendly nanofactory, J. Microbiol. Biotechnol. 24 (4) (2014) 453–464, https://doi.org/10.4014/jmb.1310.10095.
[2] R. Arora, A. Grewal, Biogenic synthesis, characterization of silver nanoparticles from *Candelula officinalis* extract and evaluation of their antimicrobial activity, Nano Biomed. Eng. 7 (2) (2015) 47–51, https://doi.org/10.5101/nbe.v7i2.p47-51.
[3] L.A. Kolahalam, I.K. Viswanath, B.S. Diwakar, B. Govindh, V. Reddy, Y.L.N Murthy, Review on nanomaterials: synthesis and applications, Mater. Today: Proc. 18 (2019) 2182–2190, https://doi.org/10.1016/j.matpr.2019.07.371.
[4] M. Summer, R. Ashraf, S. Ali, H. Bach, S. Noor, Q. Noor, S. Riaz, R.R.M. Khan, Inflammatory response of nanoparticles: mechanisms, consequences, and strategies for mitigation, Chemosphere (2024) 142826, https://doi.org/10.1016/j.chemosphere.2024.142826.
[5] V.V. Singh, Green nanotechnology for environmental remediation. Sustainable Nanotechnology for Environmental Remediation, Elsevier, 2022, pp. 31–61, https://doi.org/10.1016/B978-0-12-824547-7.00017-5.
[6] M.S. Tufail, I. Liaqat, Silver nanoparticles and their applications-A comprehensive review, Pure Appl. Biol. (PAB) 11 (1) (2021) 315–330. Retrieved from, https://thepab.org/index.php/journal/article/view/2314.
[7] Z. Zulfiqar, R.R.M. Khan, M. Summer, Z. Saeed, M. Pervaiz, S. Rasheed, B. Shehzad, F. Kabir, S. Ishaq, Plant-mediated green synthesis of silver nanoparticles: synthesis, characterization, biological applications, and toxicological considerations: a review, Biocatal. Agric. Biotechnol. (2024) 103121, https://doi.org/10.1016/j.bcab.2024.103121.
[8] M.F. Akhtar, M. Irshad, S. Ali, M. Summer, M. Jawad, M.F. Akhter, M.A. Farooq, G. Asghar, Spectrophotometric, microscopic, crystallographic and X-ray based optimization and biological applications of *Olea paniculata* leaf extract mediated silver nanoparticles, South Afr. J. Botany 166 (2024) 97–105, https://doi.org/10.1016/j.sajb.2024.01.019.
[9] H. Khan, U. Shahab, A. Alshammari, A.R. Alyahyawi, R. Akasha, T. Alharazi, R. Ahmad, A. Khanam, S. Habib, Kirtanjot Kaur, S. Ahmad, & Moinuddin, Nano-therapeutics: the upcoming nanomedicine to treat cancer, IUBMB Life (2024), https://doi.org/10.1002/iub.2814.
[10] Y.A. Rani, M.N. Devamma, Metallic nanoparticles applications in medicine, Int. J. Adv. Nano Comput. Anal. 2 (2) (2023) 76–80, https://doi.org/10.61797/ijanca.v2i2.286.
[11] M. Summer, S. Ali, H.M. Tahir, R. Abaidullah, U. Fiaz, S. Mumtaz, H. Fiaz, A. Hassan, T.A. Mughal, M.A. Farooq, Mode of action of biogenic silver, zinc, copper, titanium, and cobalt nanoparticles against antibiotics resistant pathogens, J. Inorg. Organomet. Polym. Mater. 34 (4) (2024) 1417–1451, https://doi.org/10.1007/s10904-023-02935-y.
[12] H. Mhetre, D. Kanse, D. Patil, Nanomaterials: applications in Electronics, Int. J. Adv. Eng. Nano Technol. 4 (2021) 7–19, https://doi.org/10.35940/ijaent.D0464.094621.
[13] N. Hossain, M.H. Mobarak, M.A. Mimona, M.A. Islam, A. Hossain, F.T. Zohura, M.A. Chowdhury, Advances and significances of nanoparticles in semiconductor applications–A review, Results. Eng. 19 (2023) 101347, https://doi.org/10.1016/j.rineng.2023.101347.
[14] S. Nazir, J.M. Zhang, M. Junaid, S. Saleem, A. Ali, A. Ullah, S. Khan, Metal-based nanoparticles: basics, types, fabrications and their electronic applications, Zeitschrift für Physikalische Chemie (0) (2024), https://doi.org/10.1515/zpch-2023-0375.
[15] A. Sharma, D. Manocha, A. Arora, A. Kumar, C.M. Srivastava, V. Rawat, H. Kim, M. Verma, G.K. Rao, Applications of green nanomaterials in electronic and electrical industries. Green Nanomaterials for Industrial Applications, Elsevier, 2022, pp. 397–421, https://doi.org/10.1016/B978-0-12-823296-5.00016-2.
[16] L.V. Srinivasan, S.S. Rana, A critical review of various synthesis methods of nanoparticles and their applications in biomedical, regenerative medicine, food packaging, and environment, Discover Appl. Sci. 6 (7) (2024) 371, https://doi.org/10.1007/s42452-024-06040-8.
[17] Rosario del, M. Herrera-Rivera, S.P. Torres-Arellanes, C.I. Cortés-Martínez, D.C. Navarro-Ibarra, L. Hernández-Sánchez, F. Solis-Pomar, E. Pérez-Tijerina, R. Román-Doval, Nanotechnology in food packaging materials: role and application of nanoparticles, RSC. Adv. 14 (30) (2024) 21832–21858, https://doi.org/10.1039/d4ra03711a.
[18] B. Naseer, G. Srivastava, O.S. Qadri, S.A. Faridi, R.U. Islam, K. Younis, Importance and health hazards of nanoparticles used in the food industry, Nanotechnol. Rev. 7 (6) (2018) 623–641, https://doi.org/10.1515/ntrev-2018-0076.
[19] S. Dhiman, A. Kumari, R. Sharma, R. Jasrotia, J. Ahmed, A. Kandwal, N. Lakshmaiya, V. Raja, R. Sharma, K. Sharma, A. Sharma, Green synthesis of Ag-doped ZnO nanoparticles using Bael (*A. marmelos*) leaves for wastewater remediation, ECS J. Solid State Sci. Technol. 13 (11) (2024) 117001, https://doi.org/10.1149/2162-8777/ad8c81.
[20] C. Chen, W. Li, X. Liu, J. Yu, S. Xing, J. Yang, Q. Han, Silver nanoparticles/graphene oxide arranged on polytetrafluoroethylene substrate hydrophilic modified with TiO2 to construct efficient air purification material, J. Environ. Chem. Eng. 11 (6) (2023) 110848, https://doi.org/10.1016/j.jece.2023.110848.
[21] D.S. Idris, A. Roy, Synthesis of bimetallic nanoparticles and applications—an updated review, Crystals. (Basel) 13 (4) (2023) 637, https://doi.org/10.3390/cryst13040637.
[22] S. Shamaila, A.K.L. Sajjad, S.A. Farooqi, N. Jabeen, S. Majeed, I. Farooq, Advancements in nanoparticle fabrication by hazard free eco-friendly green routes, Appl. Mater. Today 5 (2016) 150–199, https://doi.org/10.1016/j.apmt.2016.09.009.
[23] N.T.T. Nguyen, L.M. Nguyen, T.T.T. Nguyen, T.T. Nguyen, D.T.C. Nguyen, T.V. Tran, Formation, antimicrobial activity, and biomedical performance of plant-based nanoparticles: a review, Environ. Chem. Lett. 20 (4) (2022) 2531–2571, https://doi.org/10.1007/s10311-022-01425-w.
[24] R.W. Mohamed, M. Sharaky, S.H. Ismail, H.S. NN, Pivotal role of copper nanoparticles shelled by turmeric or sumac on Huh-7 cell line cytotoxicity, apoptosis and antioxidant capacity, Pakistan J. Biol. Sci.: PJBS 25 (10) (2022) 952–960, https://doi.org/10.3923/pjbs.2022.952.960.
[25] Y.S. Alqahtani, A. Bahafi, K.K. Mirajkar, R.R. Basavaraju, S. Mitra, S. Shailaja, S.S. More, U.M. Muddapur, A.A. Khan, P.A. Sudarsha, I.A. Shaikh, In vitro antibacterial activity of green synthesized silver nanoparticles using *Mangifera indica* aqueous leaf extract against multidrug-resistant pathogens, Antibiotics 11 (11) (2022) 1503, https://doi.org/10.3390/antibiotics11111503.
[26] A.A. Subhani, M. Irshad, S. Ali, M. Jawad, M.F. Akhtar, M. Summer, UV-spectrophotometric optimization of temperature, pH, concentration, and time for *Eucalyptus globulus* capped silver nanoparticles synthesis, their characterization and evaluation of biological applications, J. Fluoresc. 34 (2) (2024) 655–666, https://doi.org/10.1007/s10895-023-03260-w.
[27] M. Sharifi-Rad, E.M. Varoni, B. Salehi, J. Sharifi-Rad, K.R. Matthews, S.A. Ayatollahi, D. Mnayer, Z.A. Zakaria, M. Sharifi-Rad, Z. Yousaf, M. Irit, A. Basile, D. Rigano, Plants of the genus *Zingiber* as a source of bioactive phytochemicals: from tradition to pharmacy, Molecules. 22 (12) (2017) 2145, https://doi.org/10.3390/molecules22122145.
[28] A. Sethiya, P. Teli, D.K. Agarwal, S. Agarwal, Insight view on the pharmacological potential and bio-active components of ginger, Lett. Drug Des. Discov. 21 (12) (2024) 2195–2209, https://doi.org/10.2174/1570180820666230807152019.

[29] Dubey, S., & Kushwaha, S. A review on pharmacological properties of *Zingiber officinale*. https://doi.org/10.18231/j.ijnmhs.2022.003.
[30] S. Xiang, Q. Jian, W. Chen, Q. Xu, J. Li, C. Wang, R. Wang, D. Zhang, J. Lin, C. Zheng, Pharmacodynamic components and mechanisms of ginger (*Zingiber officinale*) in the prevention and treatment of colorectal cancer, J. Ethnopharmacol. (2024) 117733, https://doi.org/10.1016/j.jep.2024.117733.
[31] G.A. Otunola, A.J. Afolayan, E.O. Ajayi, S.W. Odeyemi, Characterization, antibacterial and antioxidant properties of silver nanoparticles synthesized from aqueous extracts of *Allium sativum, Zingiber officinale*, and *Capsicum frutescens*, Pharmacogn. Mag. 13 (Suppl 2) (2017) S201, https://doi.org/10.4103/pm.pm_430_16.
[32] M. Ramzan, B. Zeshan, Assessment of the phytochemical analysis and antimicrobial potentials of *Zingiber zerumbet*, Molecules 28 (1) (2023) 409, https://doi.org/10.3390/molecules28010409.
[33] M.A. Alshiekheid, Y. Dwiningsih, A.A. Sabour, J. Alkahtani. Phytochemical Composition and Antibacterial Activity of *Zingiber cassumunar* Roxb. against Agricultural and Foodborne Pathogens, 2022, https://doi.org/10.20944/preprints202208.0511.v1.
[34] M.Y. Suhaimi, A.M. Mohamad, M.N.F. Hani, Potential and viability analysis for ginger cultivation using fertigation technology in Malaysia, Int. J. Innov. Appl. Stud. 9 (1) (2014) 421.
[35] B. Zhu, S. Zhu, J. Li, X. Hui, G.X. Wang, The developmental toxicity, bioaccumulation, and distribution of oxidized single walled carbon nanotubes in *Artemia salina*, Toxicol. Res. (Camb) 7 (5) (2018) 897–906, https://doi.org/10.1039/c8tx00084k.
[36] O.T. No, 203: fish, acute toxicity test, OECD Guidelines for the Testing of Chemicals, Section 2 (1992) 1–24.
[37] P. Mulvaney, Surface plasmon spectroscopy of nanosized metal particles, Langmuir. 12 (3) (1996) 788–800, https://doi.org/10.1021/la9502711.
[38] N. Gautam, N. Salaria, K. Thakur, S. Kukreja, N. Yadav, R. Yadav, U. Goutam, Green silver nanoparticles for phytopathogen control, Proc. National Acad. Sci., India Section B: Biol. Sci. 90 (2020) 439–446, https://doi.org/10.1007/s40011-019-01115-8.
[39] H. Muhammad Tahir, F. Saleem, S. Ali, Q.U. Ain, A. Fazal, M. Summer, R. Mushtaq, M.D. Zahid, I. Liaqat, G Murtaza, Synthesis of sericin-conjugated silver nanoparticles and their potential antimicrobial activity, J. Basic Microbiol. 60 (5) (2020) 458–467, https://doi.org/10.1002/jobm.201900567.
[40] H. Barabadi, F. Mojab, H. Vahidi, B. Marashi, N. Talank, O. Hosseini, M. Saravanan, Green synthesis, characterization, antibacterial and biofilm inhibitory activity of silver nanoparticles compared to commercial silver nanoparticles, Inorg. Chem. Commun. 129 (2021) 108647, https://doi.org/10.1016/j.inoche.2021.108647.
[41] V.P. Skulachev, Bacterial energetics at high pH: what happens to the H+ cycle when the extracellular H+ concentration decreases?, in: Novartis Foundation Symposium 221-Bacterial Responses to pH: Bacterial Responses to pH: Novartis Foundation Symposium 221, Chichester, UK John Wiley & Sons, Ltd., 2007, pp. 200–217.
[42] T. Tanveer, S. Ali, N.M. Ali, et al., Evaluating the effect of ph, temperature and concentration on antioxidant and antibacterial potential of spectroscopically, spectrophotometrically and microscopically characterized *Mentha Spicata* capped silver nanoparticles, J. Fluoresc. 34 (2024) 1253–1267, https://doi.org/10.1007/s10895-023-03322-z.
[43] D. Vanhauteghem, G.P.J. Janssens, A. Lauwaerts, S. Sys, F. Boyen, E. Cox, E. Meyer, Exposure to the proton scavenger glycine under alkaline conditions induces *Escherichia coli* viability loss, PLoS. One 8 (3) (2013) e60328, https://doi.org/10.1371/journal.pone.0060328.
[44] P. Velmurugan, K. Anbalagan, M. Manosathyadevan, K.J. Lee, M. Cho, S.M. Lee, J.H. Park, S.G. Oh, K.S. Bang, B.T. Oh, Green synthesis of silver and gold nanoparticles using *Zingiber officinale* root extract and antibacterial activity of silver nanoparticles against food pathogens, Bioprocess. Biosyst. Eng. 37 (2014) 1935–1943, https://doi.org/10.1007/s00449-014-1169-6.
[45] M.S. Mehata, Green route synthesis of silver nanoparticles using plants/ginger extracts with enhanced surface plasmon resonance and degradation of textile dye, Mater. Sci. Eng.: B 273 (2021), https://doi.org/10.1016/j.mseb.2021.115418.
[46] M. Ramzan, M.I. Karobari, A. Heboyan, R.N. Mohamed, M. Mustafa, S.N. Basheer, V. Desai, S. Batool, N. Ahmed, B. Zeshan, Synthesis of silver nanoparticles from extracts of wild ginger (*Zingiber zerumbet*) with antibacterial activity against selective multidrug resistant oral bacteria, Molecules. 27 (6) (2022) 2007, https://doi.org/10.3390/molecules27062007.
[47] H. Tahir, F. Rashid, S. Ali, M. Summer, M. Afzal, Synthesis, characterization, phytochemistry, and therapeutic potential of *Azadirachta indica* conjugated silver nanoparticles: a comprehensive study on antidiabetic and antioxidant properties, Biol. Trace Elem. Res. (2024) 1–16, https://doi.org/10.1007/s12011-024-04293-3.
[48] C. Krishnaraj, R. Ramachandran, K. Mohan, P.T. Kalaichelvan, Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi, Spectrochimica Acta Part A: Mol. Biomol. Spectros. 93 (2012) 95–99, https://doi.org/10.1016/j.saa.2012.03.002.
[49] R. Rajkumar, G. Ezhumalai, M. Gnanadesigan, A green approach for the synthesis of silver nanoparticles by *Chlorella vulgaris* and its application in photocatalytic dye degradation activity, Environ. Technol. Innov. 21 (2021) 101282, https://doi.org/10.1016/j.eti.2020.101282.
[50] M. Yadi, M. Azizi, H. Dianat-Moghadam, A. Akbarzadeh, M. Abyadeh, M. Milani, Antibacterial activity of green gold and silver nanoparticles using ginger root extract, Bioprocess. Biosyst. Eng. 45 (12) (2022) 1905–1917, https://doi.org/10.1007/s00449-022-02780-2.
[51] M.M. Khalil, E.H. Ismail, K.Z. El-Baghdady, D. Mohamed, Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity, Arab. J. Chem. 7 (6) (2014) 1131–1139, https://doi.org/10.1016/j.arabjc.2013.04.007.
[52] S.M. Mousavi, S.A. Hashemi, Y. Ghasemi, A. Atapour, A.M. Amani, A. Savar Dashtaki, A. Babapoor, O. Arjmand, Green synthesis of silver nanoparticles toward bio and medical applications: review study, Artif. Cells Nanomed. Biotechnol. 46 (sup3) (2018) 855–872, https://doi.org/10.1080/21691401.2018.1517769.
[53] Y. Kato, M. Suzuki, Synthesis of metal nanoparticles by microorganisms, Crystals. (Basel) 10 (7) (2020) 589, https://doi.org/10.3390/cryst10070589.
[54] A.K. Mittal, Y. Chisti, U.C. Banerjee, Synthesis of metallic nanoparticles using plant extracts, Biotechnol. Adv. 31 (2) (2013) 346–356, https://doi.org/10.1016/j.biotechadv.2013.01.003.
[55] Abdullah, T. Hussain, S. Faisal, M. Rizwan, M.M. Almostafa, N.S. Younis, G Yahya, *Zingiber officinale* rhizome extracts mediated in nanoparticles and its promising biomedical and environmental applications, BMC. Complement. Med. Ther. 23 (1) (2023) 349, https://doi.org/10.1186/s12906-023-04182-7.
[56] I. Hazim, K.Y. Abd, F.T. Abachi, Newly formulated extract of *Zingiber officinale* as reducing agent for silver nitrate Nanoparticals, Pharma Innov. J 9 (5) (2020) 232–238.
[57] C.V. Restrepo, C.C. Villa, Synthesis of silver nanoparticles, influence of capping agents, and dependence on size and shape: a review, Environ. Nanotechnol. Monit. Manage 15 (2021) 100428, https://doi.org/10.1016/j.enmm.2021.100428.
[58] N. Almasoud, T.S. Alomar, M.A. Awad, M.F. El-Tohamy, D.A. Soliman, Multifunctional green silver nanoparticles in pharmaceutical and biomedical applications, Green. Chem. Lett. Rev. 13 (4) (2020) 316–327, https://doi.org/10.1080/17518253.2020.1839572.
[59] P.N. Kirdat, P.B. Dandge, R.M. Hagwane, A.S. Nikam, S.P. Mahadik, S.T. Jirange, Synthesis and characterization of ginger (*Z. officinale*) extract mediated iron oxide nanoparticles and its antibacterial activity, Mater Today: Proc. 43 (2021) 2826–2831, https://doi.org/10.1016/j.matpr.2020.11.422.
[60] S.A. Nayem, N. Sultana, M.A. Haque, B. Miah, M.M. Hasan, T. Islam, M.M. Hasan, A. Awal, J. Uddin, M.A. Aziz, A.S. Ahammad, Green synthesis of gold and silver nanoparticles by using *Amorphophallus paeoniifolius* tuber extract and evaluation of their antibacterial activity, Molecules. 25 (20) (2020) 4773, https://doi.org/10.3390/molecules25204773.
[61] S. Mathew, A. Prakash, E.K. Radhakrishnan, Sunlight mediated rapid synthesis of small size range silver nanoparticles using *Zingiber officinale* rhizome extract and its antibacterial activity analysis, Inorganic Nano-Metal Chem. 48 (2) (2018) 139–145, https://doi.org/10.1080/24701556.2017.1373295.
[62] H. Tahir, F. Rashid, S. Ali, M. Summer, R. Abaidullah, Spectrophotometrically, spectroscopically, microscopically, and thermogravimetrically optimized TiO2 and ZnO nanoparticles and their bactericidal, antioxidant and cytotoxic potential: a novel comparative approach, J. Fluoresc. 34 (5) (2024) 2019–2033, https://doi.org/10.1007/s10895-023-03367-0.
[63] S. Gopi, K. Varma, S. Jude, Study on temperature dependent conversion of active components of ginger, Int. J. Pharma Sci 6 (1) (2016) 1344–1347.
[64] B. Venkatadri, E. Shanparvish, M.R. Rameshkumar, M.V. Arasu, N.A. Al-Dhabi, V.K. Ponnusamy, P. Agastian, Green synthesis of silver nanoparticles using aqueous rhizome extract of *Zingiber officinale* and *Curcuma longa*: in-vitro anti-cancer potential on human colon carcinoma HT-29 cells, Saudi. J. Biol. Sci. 27 (11) (2020) 2980–2986, https://doi.org/10.1016/j.sjbs.2020.09.021.
[65] N. Yang, F. Li, T. Jian, C. Liu, H. Sun, L. Wang, H. Xu, Biogenic synthesis of silver nanoparticles using ginger (*Zingiber officinale*) extract and their antibacterial properties against aquatic pathogens, Acta Oceanologica Sinica 36 (2017) 95–100, https://doi.org/10.1007/s13131-017-1099-7.

[66] A.H. Alkhathlan, H.A. Al-Abdulkarim, M. Khan, M. Khan, A. AlDobiy, M. Alkholief, A. Alshamsan, H.Z. Alkhathlan, M.R.H. Siddiqui, Ecofriendly synthesis of silver nanoparticles using aqueous extracts of *Zingiber officinale* (ginger) and *Nigella sativa* L. seeds (black cumin) and comparison of their antibacterial potential, Sustainability. 12 (24) (2020) 10523, https://doi.org/10.3390/su122410523.
[67] T.I. Shalaby, O.A. Mahmoud, G.A. El Batouti, E.E. Ibrahim, Green synthesis of silver nanoparticles: synthesis, characterization, and antibacterial activity, Nanosci. Nanotechnol 5 (2) (2015) 23–29, https://doi.org/10.5923/j.nn.20150502.01.
[68] N. Cyril, J.B. George, L. Joseph, A.C. Raghavamenon, S. VP, Assessment of antioxidant, antibacterial and anti-proliferative (lung cancer cell line A549) activities of green synthesized silver nanoparticles from *Derris trifoliata*, Toxicol. Res. (Camb) 8 (2) (2019) 297–308, https://doi.org/10.1039/c8tx00323h.
[69] M. Yadi, M. Azizi, H. Dianat-Moghadam, A. Akbarzadeh, M. Abyadeh, M. Milani, Antibacterial activity of green gold and silver nanoparticles using ginger root extract, Bioprocess. Biosyst. Eng. 45 (12) (2022) 1905–1917, https://doi.org/10.1007/s00449-022-02780-2.
[70] M. Mohammadi, L. Zaki, A. KarimiPourSaryazdi, P. Tavakoli, A. Tavajjohi, R. Poursalehi, H. Delavari, F. Ghaffarifar, Efficacy of green synthesized silver nanoparticles via ginger rhizome extract against *Leishmania major* in vitro, PLoS. One 16 (8) (2021) e0255571, https://doi.org/10.1371/journal.pone.0255571.
[71] R.P. Singh, S. Magesh, C. Rakkiyappan, Ginger (*Zingiber officinale*) root extract: a source of silver nanoparticles and their application, Int J Bio-Eng Sci Technol 2 (3) (2011) 75–80.
[72] S. Mortazavi-Derazkola, A. Dehghani-Ashkezari, P. Mohammadparast-Tabas, M. Yousefi, A facile green synthesis route to novel MgO-Ag nanoparticles using *Pistacia atlantica* leaf extract (MgO-Ag@ PALE NPs) and its photocatalytic and antibacterial activity, BioMass Convers. Biorefin. (2024) 1–11, https://doi.org/10.1007/s13399-024-05788-8.
[73] S. Mumtaz, S. Ali, A. Pervaiz, M.Z. Qureshi, K. Kanwal, T. Saleem, Apoptotic and antiproliferative effects of silk protein sericin conjugated-AgNO3 nanoparticles in human breast cancer cells, Saudi. J. Biol. Sci. 30 (2) (2023) 103551, https://doi.org/10.1016/j.sjbs.2022.103551.
[74] S. D'Addato, D. Pinotti, M.C. Spadaro, G. Paolicelli, V. Grillo, S. Valeri, L. Pasquali, L. Bergamini, S. Corni, Influence of size, shape, and core–shell interface on surface plasmon resonance in Ag and Ag@ MgO nanoparticle films deposited on Si/SiOx, Beilstein. J. Nanotechnol. 6 (1) (2015) 404–413, https://doi.org/10.3762/bjnano.6.40.
[75] M. Sarmin, S. Gurung, S. Sarkar, S. Das, M. Hoda, Photocatalysis-enhanced synthesis and stabilization of silver nanoparticles by methanol-based phytochemicals extract of *Trigonella foenum-graecum* seeds, JCIS Open (2024) 100116, https://doi.org/10.1016/j.jciso.2024.100116.
[76] A. Labanni, S. Arief, D. Handayani, *Uncaria gambir* Roxb. mediated synthesis of stable silver nanoparticles in the presence of triethanolamine as the capping agent, in: IOP Conference Series: Earth and Environmental Science, IOP Publishing, 2023 012085, https://doi.org/10.1088/1755-1315/1201/1/012085. Vol. 1201, No. 1.
[77] F. Ali, S. Ali, S. Shahbaz, M. Summer, N. Mazhar Ali, M. Adeel Farooq, T. Tanveer, A. Hassan, H. Abdullah Shakir, Bactericidal and antioxidant potential of *Moringa oleifera* capped silver nanoparticles under varied conditions, ChemistrySelect. 8 (43) (2023) e202301889, https://doi.org/10.1002/slct.202301889.
[78] M. Summer, S. Ali, H.M. Tahir, R. Abaidullah, H. Tahir, S. Mumtaz, S. Mumtaz, S.A. Butt, M. Tariq, Silk Sericin protein: turning discarded biopolymer into ecofriendly and valuable reducing, capping, and stabilizing agent for nanoparticles synthesis using sonication, Macromol. Chem. Phys. 224 (17) (2023) 2300124, https://doi.org/10.1002/macp.202300124.
[79] A.J. Kora, R.B. Sashidhar, J. Arunachalam, Aqueous extract of gum olibanum (*Boswellia serrata*): a reductant and stabilizer for the biosynthesis of antibacterial silver nanoparticles, Process Biochemistry 47 (10) (2012) 1516–1520, https://doi.org/10.1016/j.procbio.2012.06.004.
[80] A.J. Kora, L. Rastogi, Catalytic degradation of anthropogenic dye pollutants using palladium nanoparticles synthesized by gum olibanum, a glucuronoarabinogalactan biopolymer, Ind. Crops. Prod. 81 (2016) 1–10, https://doi.org/10.1016/j.indcrop.2015.11.055.
[81] C. Clarkson, V.J. Maharaj, N.R. Crouch, O.M. Grace, P. Pillay, M.G. Matsabisa, N. Bhagwandin, P.J. Smith, P.I. Folb, In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa, J. Ethnopharmacol. 92 (2–3) (2004) 177–191, https://doi.org/10.1016/j.jep.2004.02.011.
[82] C. Arhafna, T.M. Sarjani, E.S. Pandia, Test the toxicity of the ethanol extract of lacum leaves (*Cayratia trifolia* L.) using the brine shrimp lethality test (BSLT) method, Jurnal Biologi Tropis 23 (1) (2023) 471–479, https://doi.org/10.29303/jbt.v23i1.6200.
[83] H.D. Kabiru, K.B. Ahmad, N.M. Bello, C.E. Chiegero, Biogenic silver nanoparticles from *Stereospermum kunthianum cham* stem bark extract: synthesis, characterization, in vitro antimicrobial study, and cytotoxicity effects against brine shrimp artemia, Int. J. Sci. Global Sustain. 8 (4) (2022), https://doi.org/10.57233/ijsgs.v8i4.371, 9-9.
[84] S.Y. Low, J.S. Lean, K.X. Yu, C.L. Wong, J.F. Ng, Synthesis of *Padina tetrastromatica* seaweed mediated silver nanoparticles and its toxicological evaluation against brine shrimp *Artemia*, in: Materials Science Forum, Trans Tech Publications Ltd, 2022, pp. 9–14, https://doi.org/10.4028/p-48s1r8. Vol. 1058.
[85] S.K. de Paiva Pinheiro, A.K.M. Lima, T.B.A.R. Miguel, A.G. Souza Filho, O.P. Ferreira, M. da Silva Pontes, R. Grillo, E de Castro Miguel, Assessing toxicity mechanism of silver nanoparticles by using brine shrimp (*Artemia salina*) as model, Chemosphere 347 (2024) 140673, https://doi.org/10.1016/j.chemosphere.2023.140673.
[86] C. Arulvasu, S.M. Jennifer, D. Prabhu, D. Chandhirasekar, Toxicity effect of silver nanoparticles in brine shrimp *Artemia*, Sci. World J. 2014 (1) (2014) 256919, https://doi.org/10.1155/2014/256919.
[87] S. Kachenton, V. Whangpurikul, N. Kangwanrangsan, T. Tansatit, W. Jiraungkoorskul, Siver nanoparticles toxicity in brine shrimp and its histopathological analysis, Int. J. Nanosci. 17 (06) (2018) 1850007, https://doi.org/10.1142/S0219581X18500072.
[88] M.C. Chávez-Sánchez, S. Abad-Rosales, R. Lozano-Olvera, L. Montoya-Rodríguez, M.Á. Franco-Nava, C.H. Mejía-Ruíz, C.H. Mejía-Ruíz, A. Pestryakov, N. Bogdanchikova, Silver nanoparticles induce histopathological alterations in juvenile *Penaeus vannamei*, Environ. Sci. Pollut. Res. 28 (2021) 8224–8234, https://doi.org/10.1007/s11356-020-11175-3.