Green synthesis of silver nanoparticles using *Zanthoxylum chalybeum* and their antiprolytic and antibiotic properties

Courtie Mahamadi and Tinashe Wunganayi

**Abstract:** In the present study, silver nanoparticles were synthesized using aqueous extract of *Zanthoxylum chalybeum* roots. The root extract was used both as the silver salt reducing agent as well as the post-synthesis stabilizing agent. The synthesis of the silver nanoparticles was optimized for pH, time, *Z. chalybeum* extract concentration, silver nitrate concentration, and temperature. The shape, morphology and size of the synthesized silver nanoparticles were characterized using UV-vis spectrometry, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscope (TEM), whilst elemental analysis was performed using Energy dispersive X-ray spectroscopy (EDS). Investigation of anti-proteolytic activity of the silver nanoparticles using egg albumin showed that the particles inhibited digestion of albumin by *Bitis arietans* snake venom. The minimum concentration of silver nanoparticles required for 100% inhibition of the proteolytic activity was 3.28 mg/L. Antimicrobial activity of the silver nanoparticles against both gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains was confirmed by zone of inhibition observed for the strains. The findings of...
this work show that *Z. chalybeum*-mediated silver nanoparticles have great potential both as anti-snake venom and antibacterial agents.

**Subjects:** Drug Discovery; Natural Products; Biotechnology; Materials Chemistry; Medicinal Chemistry;

**Keywords:** green synthesis; silver nanoparticles; *Zanthoxylum chalybeum*; antimicrobial; anti-proteolytic

1. Introduction

Due to its wide and profound applications, silver has emerged as one of the most commercialized nano-material with large quantities produced annually (Larue et al., 2014). Such applications include use in catalysis, biosensor technology, medicine, and high-sensitivity biomolecular detection. Typical catalytic applications of silver include its use in the oxidation of methanol to formaldehyde and ethylene to ethylene oxide (Sharma, Yngard, & Lin, 2009). More recently, silver nanoparticles have been reported to show strong inhibitory, antimicrobial, antifungal, and anti-inflammatory activities (Veerasamy et al., 2011).

Generally, silver nanoparticles can be prepared through a top-down or bottom-up approach. The top-down approach involves chopping down the bulk metals by mechanical means and the resulting particles are subsequently stabilised by colloidal protecting agents (Ahmed, Ahmad, Swami, & Ikram, 2016). However, obtaining a narrow particle size distribution is problematic, and installation of metal vapor machines is highly demanding, which present some limitations to the approach. The bottom-up approach produces nanoparticles through processes such as reduction of metal salts by chemical means, electrochemically, or through the use of chemical and biological methods to control the decomposition of metastable organometallic compounds in solution (Elghanian, Stohoff, Mucic, Letsinger, & Mirkin, 1997).

A survey of current nanomaterial research reveals that the bottom-up approach is widely used compared to the top-down approach (Kumar, Palanichamy, & Roopan, 2014; Sharma, Yngard, & Lin, 2009). This is because the bottom-up approach is advantageous as it offers better flexibility and versatility in terms of material design. Although progress has been made on the synthesis of nanoparticles, generally many of the synthetic methods are limited due to the use of hazardous chemicals or due to high-energy requirements, rendering the processes costly and unfriendly to the environment (Ahmed, Ahmad, Swami, & Ikram, 2016). As a result, researchers have devoted attention to studying more eco-friendly and sustainable ways of producing nanoparticles.

Recently, the use of naturally occurring materials such as sugars, plant extracts, and microorganisms as reductants and stabilizing agents, generally termed “green synthesis” has been found to be an attractive alternative approach (Alomri, Kloub Fares, & Moustafa, 2018; Ahmed & Ikram, 2015). A number of researchers have reported their findings on the use of plant extracts in nanoparticle synthesis including *Ziziphora tenuior* leaves (Sadeghi & Gholamhoseinpoor, 2015), *Acorous calamus* rhizome (Nakkala, Mata, Kumar, & Rani, 2014), and *Alternanthera dentate* leaves (Kumar, Palanichamy, & Roopan, 2014). The findings have revealed the vast potential of plants as sources of input material for the synthesis of nanoparticles to warrant continued research.

In the current study, silver nanoparticles were synthesized using *Z. chalybeum* root extract both as a reducing and capping agent. This plant is readily available in Western Zimbabwe, and its root extracts have been shown to contain phytochemicals capable of inhibiting proteolytic activity of *B. arietans* venom (Chayamit et al., 2013). The synthesis of silver nanoparticles was optimized with respect to pH, contact time, *Z. chalybeum* extract concentration, silver nitrate concentration, and temperature. The silver nanoparticles were tested for their anti-proteolytic activity against *B. arietans* venom and antimicrobial activity against selected gram positive and gram negative bacteria.
2. Experimental

2.1. Preparation of root extract
Z. chalybeum roots were collected from Sanyati area in Mashonaland West, Zimbabwe. The roots were selected and washed thoroughly thrice with tap water to remove both epiphytes and necrotic plants, followed with sterile distilled water to remove any remaining debris. Root samples were debarked and the barks shade-dried for 4 weeks followed by crushing into powder using a domestic blender. Finely crushed root bark powder (12 g) was placed in a 250 mL conical flask containing 100 mL of distilled water and allowed to sit at room temperature for 24 h. The resulting mixture was then filtered thoroughly until no insoluble particles were visible in the filtrate. The concentration of root extract was determined by evaporating 2.0 mL of liquid extract in an oven at 100 °C, averaging three measurements. The filtrate was stored at 4 °C for further usage.

2.2. Phytochemical screening equipment
Thermo Fisher Scientific Genesy 10S UV-VIS Spectrophotometer was used in scanning the produced silver nanoparticles at the wavelength range: 200–800 nm. Thermo Fisher Scientific GmbH, ATR Diamond, Model-Nicolet IS5 FTIR spectrometer, operated at a resolution of 4 cm⁻¹ in the region 4000–400 cm⁻¹ was used to obtain information on the functional groups present in the prepared silver nanoparticles and the spectrum recorded.

2.3. Green preparation of silver nanoparticles
For each of the following experiments, root bark extract was added to a solution of silver nitrate and the reduction of Ag(I) ions to Ag(0) was monitored by measuring the UV-visible spectra of the solution at regular intervals. The procedure was repeated for nanoparticles at various conditions.

2.4. Fixation of different parameters
To study the effect of AgNO₃ concentration, 100 mL of varying salt concentrations (0.5–5.0 mM) at pH 9 were mixed with 10 mL of 2.5 mg/mL plant extract solution and incubated at 60 °C under agitation (200 rpm) for 18 h. Other parameters were varied as follows: plant extract concentration (1–5 mg/mL), and contact time (1–23 h).

2.5. Proteolytic activity
Proteolytic activity of B. arietans was measured by modifying the method of Kunitz (1947). One millilitre of 8 mg/mL albumin in 0.1 M saline phosphate buffer pH 7.4 and 1 mL of venom (0–2 μg/mL) in the phosphate buffer was incubated for 30 min at 37 °C. The undigested albumin was precipitated and the reaction was terminated by adding 3 mL of 5% trichloroacetic acid. After centrifugation at 1500 rpm for 15 min, 1 mL of supernatant was mixed with 1 mL of biuret reagent and the absorbance was measured at 547 nm. The undigested albumin was precipitated. After centrifugation at 1500 rpm for 15 min, 1 mL of supernatant was mixed with 1 mL of biuret reagent and the absorbance was measured at 547 nm. A calibration curve of absorbance of albumin versus concentration was used to determine the variation of albumin concentration when varying concentration of venom is added. The proteolytic activity was determined as the ratio of the absorbance of albumin relative to the absorbance of albumin minus venom/albumin mixture. A plot of proteolytic activity against venom concentration was used to determine the minimum venom concentration that brings about 50% reduction in albumin concentration, LD₅₀.

2.6. Inhibition of proteolytic activity
Anti-proteolytic activity of the synthesized silver nanoparticles was investigated using modified methods of Kunitz (1947). One millilitre of 8 mg/mL albumin in 0.1 M Saline phosphate buffer pH 7.4 and 1 mL of venom 0.1 μg/mL in the phosphate buffer were mixed with varying concentrations of silver nanoparticles (0.041–4.1 mg/L) and incubated for at 37 °C for 30 min. The reaction was terminated by adding 3 mL of 5% trichloroacetic acid, and the undigested albumin was precipitated. After centrifugation at 1500 rpm for 15 min, 1 mL of supernatant was mixed with 1 mL of biuret reagent, and the absorbance was measured at 547 nm. The percentages inhibition was determined as shown in Equation 1:
where $A_{\text{max}}$ is the absorbance of albumin not exposed to venom, $A_{\text{min}}$ is the absorbance of albumin exposed to venom plus an equal volume of buffer, and $A_{\text{sum}}$ is the absorbance of albumin exposed to venom plus an equal volume of sample containing inhibitor (Girish & Kemparaju, 2005). In each case, the test-tubes were incubated at 37 °C and scanned after every 1 min using a Shimadzu UV1601 spectrophotometer.

2.7. Disc diffusion test
The antibacterial activities of silver nanoparticles were carried out against pathogenic strains of *Bacillus subtulis*, *Escherichia coli*, and *Pseudomonous aeruginosa*, by disc diffusion method Sushmita (2014). Nutrient agar medium plates were prepared, sterilized, and solidified. After solidification, the bacterial cultures were swabbed on these plates. The sterile discs were dipped in solutions of different silver nanoparticle concentrations, placed in the nutrient agar plate and kept for incubation at 37 °C for 24 hr. This was allowed to stay for 1 h to allow the extracts to be absorbed by the agar. The absorbed nutrient agar was incubated at 37 °C for 24 hr. Zones of inhibition for control and silver nanoparticles were measured.

2.8. Minimum inhibitory concentration determination
The silver nanoparticles were tested to determine the minimal inhibitory concentration (MIC) for each bacterial strain according to Datta, Shreya, and Mukesh (2011). The lowest concentration of silver nanoparticles preventing appearance of turbidity is considered to be the MIC, and at this concentration, the silver nanoparticles are bacteriostatic.

3. Results and discussion

3.1. UV-Vis spectroscopy
Addition of *Z. chalybeum* root extract to aqueous solution of silver nitrate resulted in change of color from yellowish to reddish brown. The colour change was attributed to excitation of plasmon vibrations in silver nanoparticles (Ahmed et al., 2016; Veeraasamy et al., 2011). Figure 1(a) shows that the surface plasmon resonance peak of silver nanoparticles observed between 436 and 446 nm became distinct with increasing extract concentration. This is due to an increase in the active sites available for silver reduction and subsequent complexing.

It can also be observed from Figure 1(b) that increasing the initial concentration of AgNO$_3$ resulted in an increase in the absorption peak, with maximum intensity obtained when 5 mL of extract was used, similar to what was reported by Ahmed et al. (2015). Figure 1(c) shows that the surface plasmon resonance peak of silver nanoparticles became distinct with increasing time of incubation, reaching the maximum peak intensity after 18 h with no significant change thereafter. In related studies, Castrol et al., (2011) and Chandran, Chaudhary, Pasricha, Ahmad, and Sastry (2006) reported an increase in the number of nanoparticles and sharpening of peaks with incubation time.

These results show that root extract of *Z. chalybeum* was effective in reducing Ag(I) to Ag(0), and the UV-vis spectra showed that silver nanoparticles were easily formed using the “green” approach.

3.2. FT-IR analysis
The potential functional groups present in *Z. chalybeum* responsible for the reduction of silver ions were identified using FT-IR spectroscopy. The typical FT-IR spectra obtained for dried *Z. chalybeum* root extract, solid silver nitrate and dried silver nanoparticles are shown in Figure 2. Interaction of the plant extract with silver nitrate was confirmed by the change in the spectra obtained. For example, the peaks identified at 3262, 1405, and 519 cm$^{-1}$ for the *Z. chalybeum* extract
Figure 1. UV-Vis spectra showing absorbance with different (a) plant extract (1–5 mg/mL), (b) AgNO₃ (0.5–5 mM), and time (0–23 h).

(A)

(B)

(C)

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disappeared on the formation of the silver nanoparticles. Some of the peaks were slightly shifted, for example, the peak at 2359 cm$^{-1}$ for silver nitrate shifted to 2363 cm$^{-1}$, whilst the peak at 1596 cm$^{-1}$ shifted to 1506 for Z. chalybeum extract. These bands denote stretching vibrational bands responsible for compounds like flavonoids and terpenoids (Ahmed et al., 2015). Interestingly, a new peak which was not initially present in the Z. chalybeum extract and silver nitrate solution appeared at 1291 cm$^{-1}$. The peaks at 2363, 1506, and 1291 cm$^{-1}$ can be attributed to the presence of NH$_2^+$, NH$^+$ or N$^+$H- groups bonding with the silver nanoparticles, which takes place during the stabilization following reduction from Ag(I) to Ag(0). The absorption observed at 3262 cm$^{-1}$ was characteristic of the O-H stretching, absorption at 2917 cm$^{-1}$ was due to the interlayer C-H stretching, absorption at 1590 cm$^{-1}$ for carboxylate group C = O, absorption at 1405 cm$^{-1}$ for C-O stretch, and absorption at 925 cm$^{-1}$ for C-C (Pereira, Amado, Critchley, Van de Velde, & Ribeiro-Claro, 2009) and (Pourjavadi, Harzandi, & Hosseinzadeh, 2004).

### 3.3. XRD, EDS, SEM, and TEM characterization

The XRD patterns of silver nanoparticles synthesized using 5 mM AgNO$_3$ and 5 mL of Z. chalybeum extract at 26⁰C and pH 9 are shown in Figure 3. The patterns show 20 values ranging from 20 to 80 with four distinct diffraction peaks at 38.08°, 46.14°, 64.41°, and 77.40°. These peaks can be indexed to (111), (200), (220) and (311) planes for silver nanoparticles and indicate that the particles are crystalline in nature. This agrees with what has been reported elsewhere (Kumar & Yadav, 2009; Laura, Vivekanandhan, Misra, & Kumar, 2011; Prasad & Elumalai, 2011). The sharp
bands of Bragg's peak indicate that the particles are in the nano region and are stabilized by the reducing agents in the root extract. In addition to the Bragg's peak representation of silver nanocrystals, the XRD pattern also shows additional peaks due to the organic compounds present in the root extract and responsible for silver reduction and stabilization of the resulting nanoparticles.

Figure 4 shows characteristic EDS peak of silver at 3.0 keV with additional peaks of carbon, oxygen, phosphorous, silicon, and chlorine, which may originate from the biomolecules that are bound to the surface of the silver nanoparticles. This confirms the formation of silver nanoparticles, in agreement with findings reported by Sushmita (2014). Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance (Kumar et al., 2013).

The morphology of the nanoparticles synthesized using Z. chalybeum extract was examined by SEM and TEM. The SEM micrograph (Figure 5) reveals that the nanoparticles synthesized using Z. chalybeum had rough surface, and the TEM micrograph (Figure 6) showed monodispersed silver nanoparticles with spherical shape. These results are consistent with the findings reported by Kumar et al. (2013). The average size from the TEM micrograph indicates that the shape of the silver nanoparticles synthesized using root extract of Z. chalybeum were spherical, and their size ranged from 50 to 100 nm.

### 3.4. Proteolytic activity of B. arietans venom on egg albumin

The effect of venom concentration was studied by varying concentration of venom (0–2 μg/mL) in the phosphate buffer, which was incubated for 30 min at 37 °C. From Figure 7, the venom activity increased with increasing venom concentration. Optimal activity of 90% was achieved at approximately 0.4 mg/mL of venom and a maximum value of about 96% at 2 mg/mL of venom. The minimum venom dose required to bring about a 50% proteolytic activity was found to be 0.1 μg/mL. Chayamiti et al. (2013) reported similar trend in the digestion of casein by the B. arietans venom and observed minimum venom dose that resulted in 100% caseinolytic activity to be 0.56 μg/mL.
To investigate the inhibition of *B. arietans* venom, a fixed volume of silver nanoparticles of varying concentrations (0.04–4.0 mg/L) was added to a mixture of 1 mL of 8 mg/L albumin in 0.1 M Saline phosphate buffer at pH 7.4 and 1 mL of 0.1 μg/mL venom in the phosphate buffer, and the mixture was incubated for 30 min at 37 °C. Figure 8 shows that inhibition increased with increase in concentration of silver nanoparticles, and the minimum concentration of synthesized silver nanoparticles required to achieve 100% inhibition was found to be 3.28 mg/L.

The root extract and mediated silver nanoparticles were tested for respective antimicrobial activities toward both gram positive (*B. subtilis*) and gram negative (*E. coli* and *P. aeruginosa*)
bacterial strains. Based on the zone of inhibition produced, synthesized silver nanoparticles proved to exhibit good antibacterial activity against \textit{E. coli} and \textit{S. aureus}. Control antibiotic drug and plant extract alone did exhibit antibacterial activity. However, the zone of inhibition increased as the silver nanoparticles synthesized using \textit{Z. chalybeum} were added, and the zones also increased with increase in the concentration of silver nanoparticles. In related studies, Sushmita (2014) reported that \textit{S. aureus} was inhibited at low concentration of silver nanoparticles, whereas the growth-inhibitory effects on \textit{E. coli} were mild. However, silver nanoparticles synthesized using \textit{Z. chalybeum} were able to inhibit gram positive \textit{B. subtilis} and gram negative \textit{E. coli} and \textit{P. aeruginosa}.

The results of antibacterial activities of prepared silver nanoparticles evaluated from the disc diffusion method are given in Table 1. The silver nanoparticles showed efficient antimicrobial
The graph shows the variation of zone inhibited with concentration of silver nanoparticles.

Table 1. Zones of exhibition for B. subtilis, E. coli, and P. aeruginosa

| Components     | B. Subtilis zone of inhibition | E. coli zone of inhibition | P. aeruginosa zone of inhibition |
|----------------|-------------------------------|----------------------------|----------------------------------|
|                | Average ±sd                   | Average ±sd                | Average ±sd                      |
| Control        | 0.90 ± 0.00                   | 0.90 ± 0.01                | 0.90 ± 0.00                      |
| Root extract   | 0.86 ± 0.06                   | 1.07 ± 0.06                | 0.83 ± 0.06                      |
| 4.1 mg/L SN    | 2.37 ± 0.06                   | 2.67 ± 0.05                | 2.23 ± 0.04                      |
| 2.1 mg/L SN    | 2.20 ± 0.10                   | 2.47 ± 0.04                | 2.07 ± 0.06                      |
| 0.8 mg/L SN    | 2.00 ± 0.10                   | 2.30 ± 0.17                | 1.63 ± 0.05                      |
| 0.4 mg/L SN    | 1.77 ± 0.12                   | 1.97 ± 0.06                | 1.57 ± 0.06                      |
property compared to the antibiotic and root bark extract due to their extremely large surface area providing better contact with cell wall of microorganisms.

4. Conclusion
In conclusion, the study showed that *Z. chalybeum* root extract can be used to synthesize silver nanoparticles with valuable properties. The synthesis of the silver nanoparticles was optimized for pH, root extract concentration, silver nitrate concentration, temperature, and incubation time. The UV-Vis spectrum indicated the absorption peak at around 430 nm. FTIR results showed that *Z. chalybeum* root extract can reduce silver ions to silver metal and complex the metal to form stable nanoparticles. The *Z. chalybeum* silver nanoparticles can inhibit the action of *B. arietans* snake venom on protein albumin, giving 3.28 mg/L as the minimum concentration of silver nanoparticles for 100% inhibition. The *Z. chalybeum* root-mediated silver nanoparticles showed ability to inhibit the growth of both gram positive (*B. subtilis*) and gram negative (*E. coli* and *P. aeruginosa*) bacterial strands. These findings indicate that *Z. chalybeum*-mediated silver nanoparticles present an alternative for the development of green, low-cost, and effective anti-bacterial drugs and snakebite anti-dote.

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Competing interests
The authors declare no competing interests.

Author details
Courtie Mahamadi
E-mail: cmahamadi@buse.ac.zw
Tinashe Wunganayi
E-mail: tinashewunganai27@gmail.com

1 Chemistry Department, Bindura University of Science Education, P. Bag 1020, Bindura, Zimbabwe.

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References
Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *Journal of Advanced Research*, 7, 17–28. doi:10.1016/j.jare.2015.02.007
Ahmed, S., & Ikram, S. (2015). Chitosan and its derivatives: A review in recent innovations. *International Journal Pharmaceutical Sciences Research*, 6(1), 14–30.
Alomri, A. A., Kloub Fores, K. E., & Moustafa, N. E. (2018). Green synthesis of assembled silver nanoparticles in nano capsules of Peganum harmala L leaf extract. Antimicrobial activity and conjugate investigation. *Cogent Chemistry*, 4(1), 1523274. doi:10.1080/23312009.2018.1532374
Castro, L., Blázquez, M. L., Muñoz, J. A., González, F., García-Belboa, C., & Ballester, A. (2011). Biosynthesis of gold nanowires using sugar beet pulp. *Process Biochemistry*, 46, 1076–1082. doi:10.1016/j.procbio.2011.01.025
Chandran, S. P., Chaudhary, M., Parasricha, R., Ahmad, A., & Sastry, M. (2006). Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. *Biotechnology Progress*, 22, 577–583. doi:10.1021/bp0501423
Chayamit, T., Mwenje, E., & Mahamadi, C. (2013). Spectrophotometric study of the anti-caseinolytic activity of root extracts of Telcirc roblis and Vepris zambesiaca on Bitis arietans venom. *African Journal Pharmaceutical Pharmacology*, 7, 1420–1425. doi:10.5897/AJPP2013.3514
Datta, A., Shreya, G., & Mukesh, S. (2011). Antimicrobial property of Piper betel leaf against clinical isolates of bacteria. *International Journal Pharmaceutical Sciences Research*, 2, 104–109.
Elgharian, R., Stohoff, J. J., Mucic, R. C., Letsinger, R. L., & Mirkin, C. A. (1997). Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science*, 277, 1078–1081.
Girish, K., & Kemparaju, K. (2005). Inhibition of Naja naja venom hyaluronidase by plant derived bioactive components and polysaccharides. *Biochemistry*, 70, 948–952.
Kumar, D. A., Palanichamy, V., & Roopan, S. M. (2014). Green synthesis of silver nanoparticles using Alternanthera dentata leaf extract at room temperature and their antimicrobial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 127, 168–171. doi:10.1016/j.saa.2014.02.058
Kumar, S., Daimary, R. M., Swargiary, M., Brahma, A., Kumar, S., & Singh, M. (2013). Biosynthesis of silver nanoparticles using Premna herbacea leaf extract and evaluation of its antimicrobial activity against bacteria causing dysentery. *International Journal Pharma Bio Sciences*, 4, 378–384.
Kumar, V., & Yadav, S. K. (2009). Plant-mediated synthesis of silver and gold nanoparticles and their applications. *Journal Chemical Technological Biotechnology*, 84, 151–157. doi:10.1002/jctb.v84i2
Kunitz, M. (1947). Isolation of a crystalline protein compound of trypsin and of soybean trypsin-inhibitor. *The Journal of General Physiology*, 30, 311–320.
Larue, C., Castillo-Michel, H., Sobanska, S., Cécillon, L., Bureau, S., Barthès, V., & Sarret, G. (2014). Foliar exposure of the crop Lactuca sativa to silver nanoparticles: Evidence for internalization and changes in Ag speciation. *Journal of Hazardous Materials*, 264, 98–106. doi:10.1016/j.jhazmat.2013.10.053
Lauré, C., Vivekanandan, S., Misra, M. A., & Kumar, M. M. A. (2011). Biosynthesis of silver nanoparticles using Murraya koenigii (curry leaf): An investigation on the
broth concentration in reduction mechanism and particle size. Advanced Materials Letters, 2, 429–434. doi:10.5185/amlett

Nakkala, J. R., Mata, R., Kumar, G. A., & Rani, S. S. (2014). Biological activities of green silver nanoparticles synthesized with Acorus calamus rhizome extract. European Journal of Medicinal Chemistry, 85, 784–794. doi:10.1016/j.ejmech.2014.08.024

Pereira, L., Amado, A. M., Critchley, A. T., Van de Velde, F., & Ribeiro-Claro, P. J. (2009). Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-raman). Food Hydrocolloids, 23, 1903–1909. doi:10.1016/j.foodhyd.2008.11.014

Pourjavadi, A., Harzandi, A., & Hosseinzadeh, H. (2006). Synthesis of a novel polysaccharide-based superabsorbent hydrogel via graft copolymerization of acrylic acid onto kappa-carrageenan in air. European Polymer Journal, 40, 1363–1370. doi:10.1016/j.eurpolymj.2004.02.016

Prasad, T., & Elumalai, E. (2011). Biofabrication of Ag nanoparticles using moringa oleifera leaf extract and their antimicrobial activity. Asian Pacific Journal Tropical Biomedical, 1, 439–442. doi:10.1016/S2221-1691(11)60096-8

Sadeghi, B., & Sholamhoseynoor, F. (2015). A study on the stability and green synthesis of silver nanoparticles using Ziziphus tenuior (Zt) extract at room temperature. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 134, 310–315. doi:10.1016/j.saa.2014.06.046

Sharma, V. K., Yngard, R. A., & Lin, Y. (2009). Silver nanoparticles: Green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science, 145, 83–96. doi:10.1016/j.cis.2008.09.002

Sushmita, D. (2014). Synthesis of silver nano particles using Murraya koenigii (green curry leaves), Zea mays (baby corn) and its antimicrobial activity against pathogens. International Journal Pharmtech Researcher, 6(1), 91–120. doi:10.1016/j.jscs.2010.06.004