

Green synthesis of silver nanoparticles as an antibacterial agent using *Rhodomyrtus tomentosa* acetone extract

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ABSTRACT

The capability of *Rhodomyrtus tomentosa* acetone extract (RAE) for the production of silver nanoparticles (AgNPs) has been explored for the first time. Silver nanoparticles with a surface plasmon resonance band centered at 420-430 nm were synthesized by reacting RAE with AgNO₃. Reaction time, temperature, concentration of AgNO₃ and RAE could accelerate the reduction rate of Ag⁺ and affect AgNPs size. The nanoparticles were found to be 10-30 nm in size and spherical in shape. XRD data demonstrated crystalline nature of AgNPs dominated by (200) facets. FTIR results showed decrease in intensity of peaks at 3394, 1716 and 1618 cm⁻¹ indicating the involvement of O-H, carbonyl group and C=C stretching with the formation of AgNPs with RAE, respectively. The C-O-C and C-N stretching suggested the presence of many phytochemicals on the surface of the nanoparticles. High negative zeta potential values confirmed the stability of AgNPs in water. *In vitro* antibacterial activity of AgNPs was tested against *Staphylococcus aureus* using broth microdilution method. AgNPs capped with RAE demonstrated profound antibacterial activity against the organisms with minimum inhibitory concentration and minimum bactericidal concentration in the range between 3.1-6.2 and 6.2-50 µgmL⁻¹, respectively. The synthesized nanoparticles could be applied as an effective antimicrobial agent against staphylococcal infections.

Keywords: antibacterial activity, *Rhodomyrtus tomentosa*, silver nanoparticle

1. INTRODUCTION

Resistance to antibiotics has been reported in several microorganisms. The problems can be overcome either by searching new antibiotics or targeting drug delivery systems. Silver nanoparticles have drawn high research interest due to its antimicrobial properties. Several plants have been explored for the synthesis of AgNPs including medicinal plants [1-4]. Downy rose myrtle, *Rhodomyrtus tomentosa* (Aiton) Hassk., is an evergreen shrub native to Southeast Asia including Thailand. The plant exhibits significant antibacterial activity against Gram-positive bacteria [5] as well as antioxidant activity [6]. In this communication, use of *Rhodomyrtus tomentosa* acetone extract as reducing as well as capping agent in the synthesis of silver nanoparticles was investigated.

2. MATERIALS AND METHODS

Synthesis of silver nanoparticles using *Rhodomyrtus tomentosa* acetone extract

RAE was dissolved in dimethyl sulfoxide and vortex for 5 min followed by centrifugation at 5000 rpm, 10 min to discard any insoluble material. The supernatant was used as stock solution for all the experiments. For synthesis of AgNPs, 1 mM aqueous solution of AgNO₃ was reacted with 0.01 % (w/v) acetone extract, as a final concentration. The setup was incubated at 28 °C in dark on rotator shaker at 150 rpm. The bioreduction of pure Ag⁺ ions was monitored by measuring the UV-visible spectrum of the reaction medium at 0, 2, 4, 6, 12, 24, 48 and 72 h.

The effect of the silver salt on nanoparticles synthesis was determined by varying the concentration of AgNO₃ (0, 0.5, 1.0, 1.50, and 2.0 mM) while keeping the RAE concentration 0.01 % (w/v). To see the effect of temperature and acetone extract concentration on AgNPs synthesis, 1 mM AgNO₃ solution was incubated

with 0.01, 0.05 and 0.1 % (w/v) RAE and incubated at 28 and 50 °C, 150 rpm in dark for 48 h. The AgNPs were assigned the name as A1, A2 and A3 for the samples synthesized using 0.01, 0.05 and 0.1 % RAE (w/v) at 28 °C, respectively. Another set B1, B2 and B3 were assigned for the samples synthesized by 0.01, 0.05 and 0.1 % RAE (w/v) at 50 °C, respectively.

Synthesis of silver nanoparticles using sodium borohydride

For chemical synthesis of AgNPs, 0.2 ml of ice cold 0.1 M NaBH₄ was added to 10 ml of 1 mM AgNO₃ solution drop by drop with continuous shaking at 28 °C.

Purification of silver nanoparticles

Silver nanoparticle solution obtained after 48 h was purified by repeated centrifugation at 14,500 rpm for 1 h and redispersed the pellet in sterile distilled water. The above steps were repeated 3 times to remove unreduced AgNO₃ ions and unbound RAE/NaBH₄ present in solution if any. The pellet was resuspended in sterile distilled water and used for further studies. The concentration of silver nanoparticles was determined by atomic absorption spectrophotometer after digesting with HNO₃.

To estimate the amount of RAE capped on the surface of nanoparticles, purified nanoparticles were centrifuged at 14,500 rpm for 1 h and the precipitates obtained were resuspended in 1 ml DMSO and vortex for 5 min. The nanoparticles were washed with DMSO for 3 times to ensure the removal of RAE from the surface of nanoparticles. The amount of RAE was estimated by spectrophotometer by taking the absorbance at 670 nm.

Characterization of nanoparticles

UV-visible absorption spectra were measured using Perkins Elmer LAMBDA 25 UV/Vis spectrophotometer. The bioreduction of AgNO₃ was monitored by periodic sampling of 1 ml aliquot and absorbance was recorded in range of 200-800 nm. Crystalline nature of metallic AgNPs was examined by X-ray diffraction. The film of AgNPs was prepared on glass slide by drop-coating with nanoparticle solution and air dried. The films on glass slide was then subjected to X-ray diffraction, which were performed on a transmission mode on a Philips PW 1830 instrument operated at 40 kV and a current of 30 mA with Cu K α radiation. Shape and size of AgNPs were determined by transmission electron microscopy (TEM). The image analysis of AgNPs was done using JEOL-1010 instrument operated at accelerating voltage of 120 kV. The FTIR spectra for synthesized nanoparticles were obtained at resolution of 4 cm⁻¹ in transmission mode at frequency ranged 4000–400 cm⁻¹ with EQUINOX 55 spectrophotometer (Bruker, Germany) using KBr pellet method. Zeta potential and polydispersity index were measured by Zeta PALS-zeta potential analyzer (Brookenhaven Instruments Corporation) by DLS method with respect to refractive index of deionised water.

Antimicrobial assay

Antimicrobial assay of the synthesized nanoparticles against *S. aureus* was performed using a modified broth microdilution method recommended by Clinical Laboratory Standardization Institute (CLSI) guideline [7]. *Staphylococcus aureus* ATCC 25923 was used for antibacterial study. *S. aureus* was cultured on Mueller-Hinton agar (MHA) (Difco, France) at 35°C for 24 h. Mueller-Hinton broth (MHB) (Difco, France) was used for testing antibacterial activity. The growth was assessed with a microtitre plate reader by taking the optical density (OD) at 595 nm.

3. RESULTS

UV-visible spectrograph of the colloidal solution of AgNPs has been recorded as a function of time (Figure 1). The rate of synthesis of AgNPs was faster when higher concentrations of AgNO₃ were used (Figure 2). The effects of RAE concentration and temperature on nanoparticles synthesis were also observed after 48 h (Figure 3). Silver nanoparticles formed were spherical in shape and size ranged between 10-30 nm (Figure 4). To further find out the crystallinity of AgNPs, analysis through X-ray diffraction was carried out. Figure 5 represents the XRD patterns of the drop cast film of AgNPs on a glass substrate. A comparison of XRD spectrum with the standard confirmed that the AgNPs formed in the experiments were composed of crystalline silver, as evidenced by the peaks at 2θ values of *ca.* 38, 43, 64, and 82 corresponding to (111), (200), (220) and (222), respectively.

FTIR analysis of the nanoparticles was carried out to investigate the chemical interaction between the phytochemicals present in RAE and AgNPs and how it led to stabilization of AgNPs. The FTIR spectrum recorded from RAE, A3 and B3 are shown in Figure 6. Significant changes observed between the vibrational frequencies and intensity of RAE before and after nanoparticles formation. The O-H stretching frequency observed at 3394 cm⁻¹ in case of RAE was shifted to 3440 cm⁻¹ and intensity decreased after the formation of silver nanoparticles. Such decrease in intensity is attributed to the increase in intermolecular hydrogen bond between the various phytochemicals anchored on the surface of nanoparticles. Pure extract exhibit a strong peak centered at 1716 cm⁻¹, which corresponds to the carbonyl groups. After the nanoparticles formation the intensity of the peak was decreased, a clear indication of carboxylic acid group

binding on the surface of silver nanoparticles. It is well known that carboxylic acid groups interact with silver nanoparticles surface strongly. In addition, there is significant changes in the C=C stretching intensity appeared at 1618 cm^{-1} in the case of RAE was decreased and shifted to 1623 , which is a sign of the phenyl rings proximity to the silver nanoparticles surface. Apart from these changes, significant changes were observed in the case of C-O-C and C-N

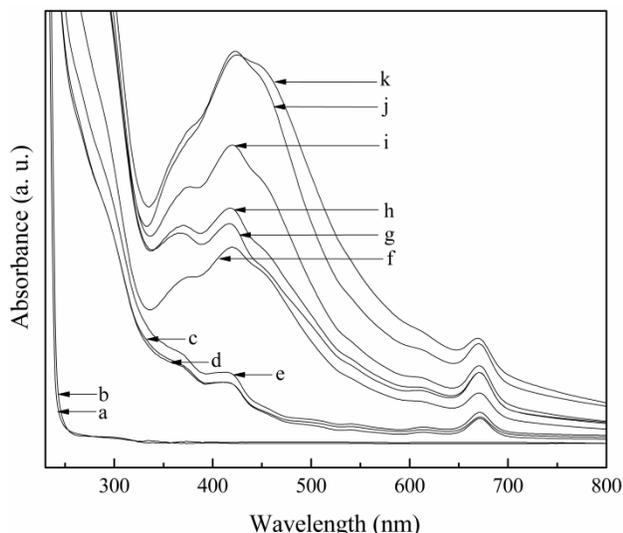


Figure 1. UV-visible absorption spectra of silver nanoparticles synthesized by 0.01% (w/v) *Rhodomyrtus tomentosa* acetone extract (RAE), 1 mM silver nitrate (AgNO_3) at $28\text{ }^\circ\text{C}$ at different time (a) control (AgNO_3), (b) AgNO_3 + DMSO, (c) RAE, (d) 0 h, (e) 2 h, (f) 4 h, (g) 6 h, (h) 12 h, (i) 24 h, (j) 48 h, and (k) 72 h.

4. CONCLUSIONS

The size of AgNPs was found in the range of 10-20 nm with higher concentration of RAE. The data from UV-visible, FTIR, and TEM micrograph supports the formation and stability of biologically synthesized AgNPs. The antimicrobial activity of RAE was increased when used in combination with AgNPs.

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