IOSR Journal of Engineering (IOSRJEN) **www.iosrjen.org**
ISSN (e): 2250-3021, ISSN (p): 2278-8719
Vol. 07, Issue 10 (October. 2017), ||V2|| PP 64-69

Synthesis and study of antibacterial activity of Ag nanoparticles using kumquat extract and under supporting of microwave

Tran Thi Bich Quyen[1*], Bui Thi Anh Mai[1], Tran Thi Xuan Mai[2]
[1]*Nanomaterials Laboratory, Department of Chemical Engineering, College of Technology, Can Tho University, 3/2 Street, Ninh Kieu District, Can Tho City, Vietnam*
[2]*Genetic Technology Laboratory, Biotechnology Research and Development Institute, Can Tho University, 3/2 Street, Ninh Kieu District, Can Tho City, Vietnam*
**Corresponding Authors: Dr. Tran Thi Bich Quyen*; Email: *ttbquyen@ctu.edu.vn*

Abstract: In this work, a simple and rapid green approach was employed for the preparation of silver nanoparticles that were successfully synthesized using kumquat aqueous extract and under supporting of microwave as biological reducing agents. It proves to be an eco-friendly, rapid green method for the synthesis providing a cost effective and an efficient route for the Ag nanoparticles' synthesis. The shape and size of nanoparticles were controlled by the experimental conditions (reaction time (only 13 min for the conversion of silver ions into silver nanoparticles), ratio of kumquat extract and silver nitrate, ect…). Herein, the synthesized Ag nanoparticles were characterized using UV-vis, XRD, TEM and EDS. This green method has been obtained Ag nanoparticles quite uniformly with average diameter around 10-25 nm. Moreover, the synthesized Ag nanoparticles also showed efficient antimicrobial activity against of *E. coli* bacteria. This eco-friendly method could be a competitive alternative to the conventional physical/chemical methods used for the synthesis of Ag nanoparticles. Thus, it has a potential to use in biomedical applications, opto-electronics and medical devices in future.

Keywords: Silver nanoparticles (Ag NPs); Green synthesis; Kumquat extract; Microwave; Bioreduction; Anti-bacterial activity (antimicrobial activity).

Date of Submission: 02-11-2017 Date of acceptance: 16-11-2017

I. INTRODUCTION

In the past decade, development of simple methods for the nanosized metal particles' preparation has attracted significant attention of many scientists because of their importantly future applications due to difference in physiochemical properties and surface to volume ratio [1]; unusual size-dependent optical and electronic properties.

In last years, silver nanoparticles (Ag NPs) have received deeply attention of the scientists due to their antimicrobial properties (i.e, the emergence of microorganisms resistant to multiple antimicrobial agents) [2]; and high conductivity [3]. The especial characteristics of Ag NPs have made them applicable in many various fields such as biomedical, drug delivery, detection of biomarkers or dye molecules, water treatment, agricultural-seafood preservation; electronic devices, inks, adhesives, pastes, etc [4-9]. Ag NPs have been synthesized by physio-chemical methods (i.e, chemical reduction, electrochemical, autoclave, microwave, and photochemical reduction, etc.) [10-14]. These methods have significantly high yield; however, they are also related to the limitations as using of toxic chemicals, high operational cost and energy needs. Therefore, considerable interest has been paid to the preparation of metallic nanoparticles by green synthesis in recent years [15-19]. Green synthesis is the green environment friendly processes in chemistry, in chemical technology and engineering; which are becoming more popular and much needed since the global's concern is about environmental problems [20].

To overcome the drawbacks of physio-chemical methods, green synthetic methods have been used new alternative for metal nanoparticles as well as Ag NPs synthesis using natural polymers (chitosan, etc.), sugars, enzymes, microorganisms, plant extracts as reductants (e.g, lemon aqueous extract, Azadirachita indica aqueous leaf extract,…) and capping agents. They are simple, one step, cost-effective, energy efficient, more stable materials and environment friendly [21-23]. Moreover, use of plant extracts also reduces the cost of microorganisms isolation and significant enhancing the cost competitive feasibility for synthesis of nanoparticles by microorganisms [24]. Since, the association of nanotechnology and green chemistry will open up the range of biologically and cytologically compatible metallic nanoparticles [25, 26].

As we known, using kumquat aqueous extract and under supporting of microwave for the synthesis of silver nanoparticles have not been reported previously. In this work, plant aqueous extract of kumquat a species of family lemon or orange was used as a reducing agent for bioconversion of silver ions (Ag^+) to nanoparticles (Ag^0). Silver nanoparticles (Ag NPs) can be generated at low concentration of kumquat aqueous extract and under supporting of microwave without using any additional harmful chemical/physical methods. The effect of reaction time, temperature (microwave's power), ratio between concentration of silver ions and concentration of kumquat aqueous extract quantity were also evaluated to optimize route to synthesize silver nanoparticle. Herein, the synthetic method used here is simple, rapid reaction time, cost effective, easy to perform, uniform particle size, environment friendly, stable and sustainable. Moreover, the synthesized silver nanoparticle was also test its antibacterial activity on *E. Coli* bacteria. Since, it indicates that the synthesized silver nanoparticles have a significant promise as bactericidal agent for applications (i.e, biomedical, food, agriculture and cosmetics, etc…) in the current time and in future.

II. MATERIALS AND METHODS

2.1. Materials

Kumquat fruit (~3 months old, green shell) was purchased from a garden at Phong Dien, Can Tho City in Vietnam. Silver nitrate ($AgNO_3$), poly(vinyl pyrrolidone) (PVP, MW≈55,000) were purchased from Acros. *Escherichia coli (E. coli)* bacteria was bought from Sigma-Aldrich. All solutions were prepared using deionized (DI) water from a MilliQ system.

2.2. Methods

2.2.1. Preparation of kumquat aqueous extract

Fresh kumquat was squeezed and obtained the kumquat juice mixture. After that, the kumquat juice was filtered, centrifuged and washed with DI water for three times to obtain a juice extract from kumquat. This kumquat aqueous extract was used for synthesis of silver nanoparticles (Ag NPs) in following steps.

2.2.2 Synthesis of silver nanoparticles using kumquat aqueous extract and under supporting of microwave

In typical, 5 mL of lemon extract is added into 10 mL of $AgNO_3$ solution (0.003 M) and stirred at room temperature for 5 min. After that, 2 mL of PVP solution (100 mM) was added into the above solution mixture after stirring for 2 min and heated at 80 W for 13 min under supporting microwave. The solution mixture was centrifuged and washed several times with DI water to remove excess kumquat extract and PVP. And then redispersed in deionized water and obtained silver nanoparticle (Ag NPs). The average particle size of the Ag NPs as prepared were about 10-25 nm.

2.2.3. Characterization

The absorbance spectra of particle solutions (Ag nanoparticles) were examined by UV–vis spectrophotometry (UV-675; Shimadzu). Fourier transform infrared spectroscopy (FTIR) spectra of Ag nanoparticles were obtained by using a Renishaw 2000 confocal Raman microscope system. The crystallinity of silver nanoparticle was determined by a X-ray diffractometer (Rigaku Dmax-B, Japan) with Cu K_α source operated at 40 kV and 100 mA. A scan rate of 0.05 deg^{-1} was used for 2θ between 20^o and 90^o. The particle size and surface morphology of Ag nanoparticles were examined by scanning electron microscope (SEM) and transmission electron microscope (TEM) with a Philips Tecnai F20 G2 FEI-TEM microscope (accelerating voltage 200 kV). The EDX measurement was performed with a JSM 6500 EDX analyzer.

2.2.4 Preparation for investigating the antibacterial activity of silver nanoparticles on E.Coli bacteria

The antibacterial activity of Ag nanoparticles was tested on the *Escherichia coli (E. coli)* strain by using standard disk diffusion method. The bacterial suspension of *E. coli* (100 µL of 10^6 $CFU.mL^{-1}$) was applied uniformly on the surface of a nutrient agar plate before placing the disks on the plate (5 per plate). Small disks were uniform size (6 mm diameter) containing 0 µL; 10 µL; 20 µL; 30 µL; and 40 µL of Ag nanoparticles solution (0.5×10^5 $particles.mL^{-1}$), respectively. The plates were incubated at 37^oC for 17 h, after which the average diameter of the inhibition zone surrounding the disk was measured by a ruler with up to 1 mm resolution. The mean and standard deviation (SD) reported for silver nanoparticles (Ag NPs) and with microbial strain (*E. coli)* were based on three replicates.

III. RESULTS AND DISCUSSION

3.1. Morphology and characterization of silver nanoparticles using kumquat extract and under supporting of microwave

The UV-vis spectra of silver nanoparticles (Ag NPs) exhibited with the maximum absorption peak in the range from 408-426 nm, respectively – see Figure 1(A). Herein, the plasmon resonance peaks are quite match with the surface absorption of Ag nanoparticles [27, 28]. Since, it is demonstrated that Ag nanopaticles are generated in

the solution. The maximum absorption peaks of Ag nanoparticles measured in the range 408-435 nm, which can be predicted the particle size of Ag nanoparticles being ~10-25 nm [29].

The presence of free ions in the kumquat extract solution and under supporting of microwave have greatly accelerated for the polyol synthesis of silver nanoparticles. During the synthesis, we could easily monitor the progress of the nanoparticles production through its color changes from colorless to yellow and red-brown due to a dramatic increase in the reduction rate of silver ions (Ag^+). The absorption intensity of synthesized samples tend to proportional increase to the Ag nanoparticles' solution color, corresponding to increasing the Ag nanoparticles' concentration. It demonstrated that reaction rate of reducing agent using kumquat extract and microwave affect to create of Ag nanoparticles.

Through the analytical result of UV-vis spectra shows silver nanoparticles is generated with a large amount and obtained a maximum value at ($V_{kumquat\ extract}$:V_{AgNO3}) ratio of 1:1 and reaction time for 13 min at 80 W in microwave, respectively – see in Figure 1A (b). Besides, the absorption peaks of these experiments has not much changed for several reactions again, which is indicated the nanoparticles' size approximately. When the initial concentration of $AgNO_3$ is larger than that amount of kumquat extract (ratio of $AgNO_3$ (Ag^+) > kumquat extract (H^+)), leading to decrease generation of silver ions (Ag^+) and reduce amount as well as form concentration of silver nanoparticles (Ag NPs). And the reaction time is increased more long, leading to create parallel of Ag_2O in the Ag nanoparticles' solution. Thus, maximum absorption peaks are also gradually shifted to the near infrared (from 424 nm to 436 nm), leading to particle size of Ag nanoparticles' increase due to the agglomeration of Ag nanoparticles and decreased the absorbed intensity - see in Figure 1B (d, e).

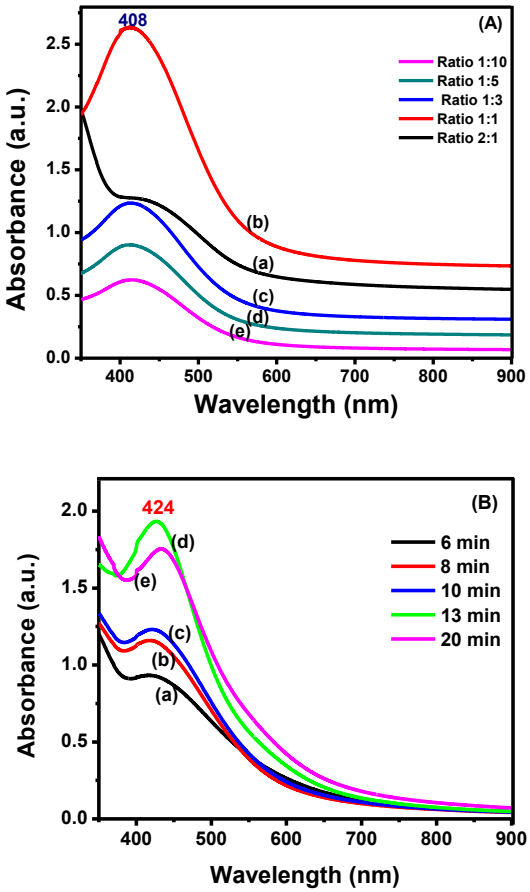

Figure 1. UV-vis spectra of Ag nanoparticles with difference about (A) volume ratios of $V_{kumquat\ extract}$:V_{AgNO3} of: (a) 2:1; (b) 1:1; (c) 1:3; (d) 1:5; and (e) 1:10, and (B) reaction times: (a) 6 min; (b) 8 min; (c) 10 min; (d) 13 min; and (e) 20 min, respectively.

The X-ray diffraction (XRD) pattern of Ag nanoparticle (Figure 2) is compared and interpreted with standard data of JCPDS (No. 04-0783). The characteristic peaks for Ag nanoparticles appear at 38.14°, 44.28°, 65°, and 78°, which correspond to crystal facets of {111}, {200}, {220}, and {311} of silver. Each crystallographic facet contains energetically distinct sites based on atom density. Besides, the EDS result of Ag nanoparticles confirm the presence of silver in pure form with Ag content ~94.1%. No additional impurities are detected in the EDS result – see in Figure 3.

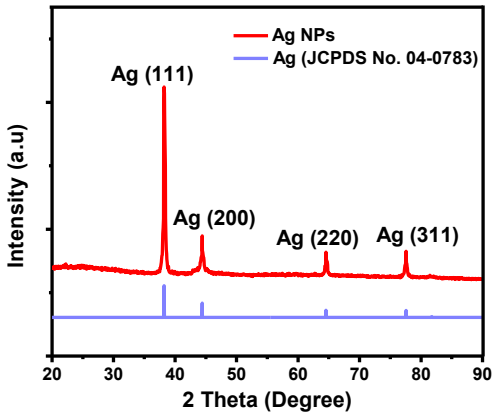

Figure 2. XRD pattern of Ag nanoparticles using kumquat extract as a reducing agent.

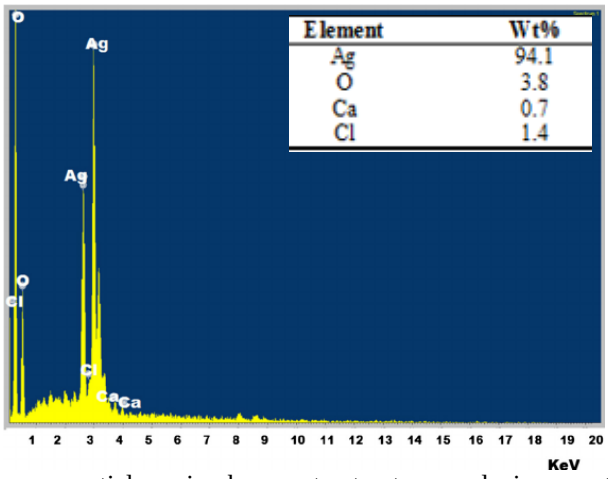

Figure 3. EDS profile of Ag nanoparticles using kumquat extract as a reducing agent and quantitative analysis.

Figure 4 shows representative TEM images of Ag nanoparticles sample. From result image analysis confirms the Ag nanoparticles reveal that the nanosize and that they are well dispersed and spherical in shape. The nanoparticles are homogeneous and spherical which conforms to the shape of SPR band in the UV-visible spectrum. The particle size of Ag nanoparticles are uniformly quite generated with average diameter about 10-25 nm.

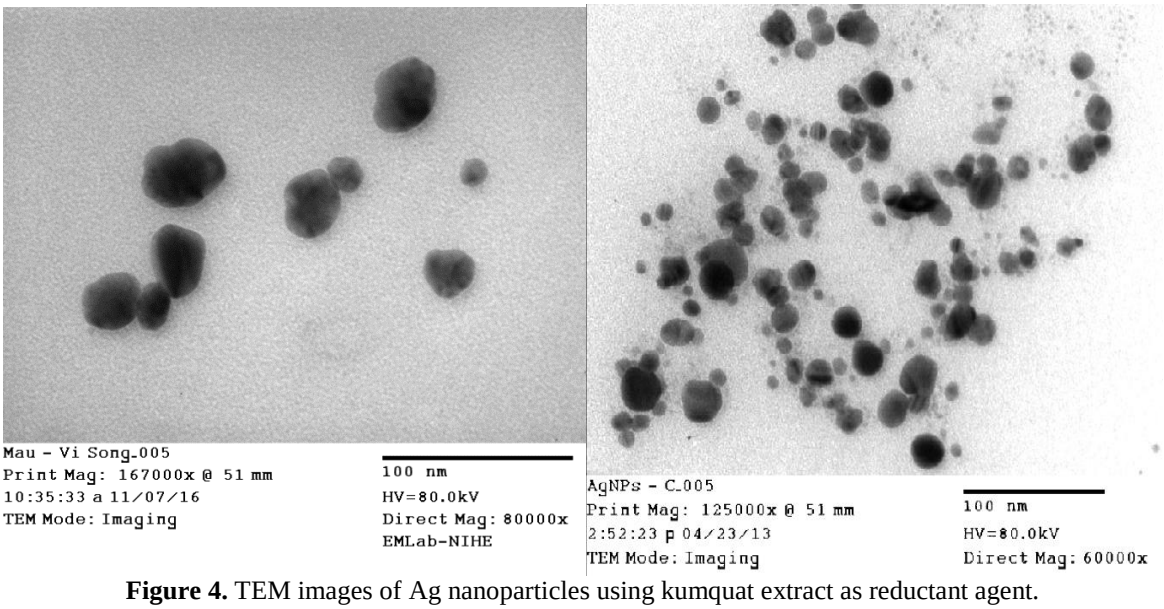

Figure 4. TEM images of Ag nanoparticles using kumquat extract as reductant agent.

3.2. Antibacterial activity measurements of Ag nanoparticles on E. coli bacteria

The antibacterial activity of Ag nanoparticles was compared for *E. coli* strain using the diameter of inhibition zone in disc diffusion test with initial concentration of *E. coli* bacteria (10^6 CFU.mL^{-1}). The diameter of inhibition zone (DIZ) reflects magnitude of sensitivity of the microorganism. Based on the zone of inhibition genergated, synthesized Ag nanoparticles prove to exhibit good antibacterial activity against *E. coli* – see in Figure 5. On the other hand, control alone did not exhibit any antibacterial activity. DIZ was measured on agar plates using a ruler with 1 mm resolution. The results of antibacterial activities of prepared Ag nanoparticles evaluated from the disc diffusion method are given details in Table 1.

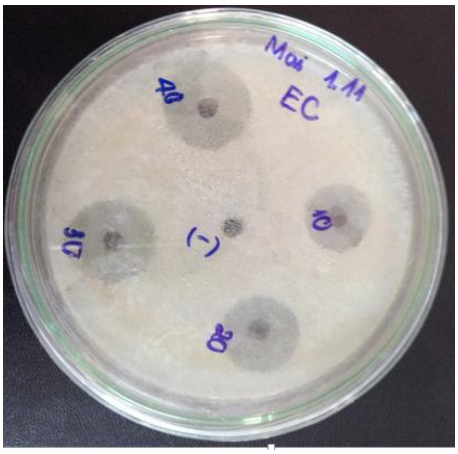

Figure 5. Representative images of agar plates containing Ag nanoparticles with various volumes of Ag nanoparticles solution: 0 µL; 10 µL; 20 µL; 30 µL; and 40 µL, respectively.

Table 1. Zone of inhibition (mm) obtained by disc diffusion method.

Components	**Zone of inhibition (mm)**			
Control (*E. coli*, 10^6 CFU.mL^{-1}) (-)			NZ	
Ag nanoparticle (µL): 10; 20; 30; and 40 µL, respectively	8	10	11	11.5

IV. CONCLUSION

A simple and rapid green method of Ag nanoparticles using kumquat extract and under supporting of microwave have been successfully synthesized in this study. It proves to be an eco-friendly, rapid green approach for the synthesis providing a cost effective and an efficient route for the Ag nanoparticles' synthesis. This green method has been obtained Ag nanoparticles with average diameter about 10-25 nm and uniformly formed. Moreover, the synthesized Ag nanoparticles showed efficient antimicrobial activity against of *E. coli* bacteria in this work. Besides, the synthesis of Ag nanoparticles using kumquat extract and under supporting of microwave have brought many benefits such as energy efficient, cost effective, rapid reaction time, protecting human health (non-toxic to humans in minute concentrations) and environment leading to safer products and lesser waste. Since, it has plenty potential and promising to use for applications in biomedical, opto-electronics and medical devices in the current time and in future.

ACKNOWLEDGEMENTS

This research is funded by Vietnam Ministry of Education and Training under grant number B2017-TCT-28ĐT.

REFERENCES

[1]. Mody V V, Siwale R, Singh A and Mody H R 2010 *J. Pharm. Bioall. Sci.* **2** 282-289.
[2]. Sharma V K, Yngard R A and Lin Y 2009 *Adv. Colloid Interface Sci.* **145** 83-96.
[3]. Park K, Seo D and Lee J 2008 *Colloids Surf. A.* **313–314** 351-354.
[4]. Chaloupka K, Malam Y and Seifalian A M 2010 *Trends Biotechnol.* **28** 580-588.
[5]. Prow T W, Grice J E, Lin L L, Faye R, Butler M, Becker W, Wurm E M T, Yoong C, Robertson T A, Soyer H P and Roberts M S 2011 *Adv. Drug Deliv. Rev.* **63** 470-491.
[6]. Gray T A D a D G 2011 *Environ. Sci. Technol.* **45** 1992–1998.
[7]. Bich Quyen T T, Su W N, Chen C H, Rick J, Liu J Y and Hwang B J 2014 *J. Mater. Chem. B* **2** 5550-5557.
[8]. Quyen T T B and Hwang B J 2016 *Procedia CIRP* **40** 551-556.

[9]. Nair R, Varghese S H, Nair B G, Maekawa T, Yoshida Y and Kumar D S 2010 *Plant Sci.* **179** 154-163.
[10]. Khan Z, Al-Thabaiti S A, Obaid A Y and Al-Youbi A O 2011 *Colloids Surf. B.* **82** 513-517.
[11]. Reicha F M, Sarhan A, Abdel-Hamid M I and El-Sherbiny I M 2012 *Carbohydr. Polym.* **89** 236-244.
[12]. Yang J and Pan J 2012 *Acta Mater.* **60** 4753-4758.
[13]. Khan A, El-Toni A M, Alrokayan S, Alsalhi M, Alhoshan M and Aldwayyan A S 2011 *Colloids Surf. A.* **377** 356-360.
[14]. Alarcon E I, Udekwu K, SkognM, Pacioni N L, Stamplecoskie K G, González-Béjar M, Polisetti N, Wickham A, Richter-Dahlfors A, Griffith M and Scaiano J C 2012 *Biomater.* **33** 4947-4956.
[15]. Kundu S, Mandal M, Ghosh S K and Pal T 2004 *J. Colloid Interface Sci.* **272** 134-144.
[16]. Qian L and Yang X 2005 *Colloids Surf. A.* **260** 79-85.
[17]. Huang Y F, Huang K M and Chang H T 2006 *J. Colloid Interface Sci.* **301** 145-154.
[18]. Pal A, Shah S and Devi S 2008 *Australian J. Chem.* **61** 66-71.
[19]. Pal A, Shah S and Devi S 2007 *Colloids Surf. A.* **302** 483-487.
[20]. Thuesombat P, Hannongbua S, Akasit S and Chadchawan S 2014, *Ecotoxicol. Environm. Saf.* **104** 302-309.
[21]. Shakeel Ahmed M A a S I 2014 *J. Appl. Chem.* **3** 493-503.
[22]. Ahmed S, Ahmad M, Swami B L and Ikram S 2016 *J. Adv. Res.* **7** 17-28.
[23]. Kharissova O V, Dias H V R, Kharisov B I, Pérez B O and Pérez V M J 2013 *Trends Biotechnol.* **31** 240-248.
[24]. Singhal G, Bhavesh R, Kasariya K, Sharma A R and Singh R P 2011 *J. Nanopart. Res.* **13** 2981-2988.
[25]. Philip D 2010 *Spectrochim. Acta. A.* **75** 1078-1081.
[26]. Philip D 2010 *Spectrochim. Acta. A.* **77** 807-810.
[27]. Tsuji T, Kakita T and Tsuji M 2003 *Appl. Surf. Sci.* **206** 314-320.
[28]. Phuoc T X, Soong Y and Chyu M K 2007 *Opt. Lasers Eng.* **45** 1099-1106.
[29]. Aswathy B, Avadhani G S, Sumithra I S, Suji S and Sony G 2011 *J. Mol. Liq.* **159** 165-169.

Tran Thi Bich Quyen Synthesis and study of antibacterial activity of Ag nanoparticles using kumquat extract and under supporting of microwave." IOSR Journal of Engineering (IOSRJEN) , vol. 7, no. 10, 2017, pp. 64-69.