Spent Coffee Ground-derived Silver Nanoparticles Against UTI-causing Bacteria

Kai Song-aaron Ng[1], Kien Woh Kow[2], Sheri-Ann Tan[1]*

[1]Department of Bioscience, Faculty of Applied Science, Tunku Abdul Rahman University College, Jalan Genting Kelang, 53300 Kuala Lumpur, Malaysia
[2]Department of Chemical and Environmental Engineering, Faculty of Science and Engineering, The University of Nottingham Ningbo China, Ningbo 315100, China
*Corresponding author: tansw@tarc.edu.my

Abstract

Silver nanoparticles (AgNps) are incorporated in a variety of medical applications due to their antimicrobial abilities. Green synthesis of AgNps is relatively new, and the investigation of this phenomenon has acquired attention lately as there is an increase in bacterial resistance towards antibiotics. Hence, the purpose of this research was to determine the antimicrobial activities of AgNps synthesized through the green synthesis method using spent coffee ground (SCG). First, AgNps were generated by mixing hydro-alcoholic SCG extract with silver nitrate solution. The AgNps were characterized using Malvern Zetasizer analyzer for size determination. Antibacterial potentials of AgNps produced were elucidated using both disc diffusion and broth dilution assays. The nanoparticle sizes obtained exhibited unimodal distribution that centered around 164-190 nm. Disc diffusion assay showed that the AgNps (0.144mg) were able to display broad-spectrum inhibition against Gram-negative Urinary Tract Infections (UTIs)-causing pathogens: *Escherichia coli* (zone of inhibition = 10.17 ± 0.58 mm) > *Pseudomonas aeruginosa* (zone of inhibition = 10 ± 0.71mm) > *Proteus mirabilis* (zone of inhibition = 7.33 ± 0.62 mm) > *Klebsiella pneumoniae* (zone of inhibition = 7.25 ± 0.75 mm) (according to size of inhibition zone). However, in broth dilution, *K. pneumoniae* (MIC = 200µg/ml) demonstrated the highest susceptibility towards the synthesized silver nanoparticles, while *P. mirabillis* (MIC = 500µg/ml) was the least susceptible. In conclusion, silver nanoparticles generated by the green synthesis method using spent coffee ground are promising antibacterial agents against the tested UTI-causing bacteria, and this warrants further investigation.

Keywords: Green synthesis, Silver nanoparticles, Spent coffee ground, Antimicrobial, Urinary tract infection

1. Introduction

The new Global Antimicrobial Surveillance System (GLASS) launched by World Health Organization has acknowledged the increased presence of drug resistance in bacterial populations especially *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella sp*, *Streptococcus pneumoniae*, and *Staphylococcus aureus.* (WHO, 2019). Among the bacteria reported, some are known as causal agents of urinary tract infections (UTI). In the United States, this infection is the most typical microbial infection, with approximately eleven million cases reported each year (Griebling, 2005). The symptoms of UTI consist of urethritis, pyelonephritis, and cystitis. If treatment was not provided, it could lead to renal scarring and eventually kidney malfunction (O'Brien et al., 2016). Drug resistance in UTI bacteria is related directly to the use and abuse of antibiotics (Taneja et al., 2008). Hence, there is an urgent need for new antibiotics for UTI bacteria especially for those that have acquired resistance towards most available antibiotics.

Silver nanoparticles (AgNps) have sizes generally ranging between 1 to 100 nm in diameter and exist in one dimension (Murphy et al., 2015). These nanoparticles possess many applications and have been used often in fields of material sciences (Haider & Kang, 2015), electronics (Bohr, 2002), and biotechnology (Sarmast & Salehi, 2016). Besides, the AgNps are also utilized in various medical applications due to their antimicrobial properties and non-toxic nature in clinical studies (Deshmukh et al., 2019).

Many methods could be used to synthesize AgNps but the green synthesis technique has piqued the interest of the scientific community. Green synthesis is more advantageous than the conventional chemical depletion method where costly chemical reducing agent is substituted by natural product such as plant extracts for the generation of metal or metal oxide nanoparticles. Therefore, this eco-friendly technique is certainly

sustainable (Gopinath et al., 2014) and cost-effective (Mittal et al., 2013) and reduces chemical contaminations (Chandran et al., 2006).

Coffea arabica seed as well as green coffee bean extracts had been used to generate AgNps that demonstrated strong antimicrobial activities against *Escherichia coli* and *Staphylococcus aureus* (Wang et al., 2017; Dhand et al., 2016). Besides coffee beans, spent coffee grounds (SCG) produced by the coffee industry are also capable to biosynthesize AgNps due to the presence of abundant phenolic compounds such as chlorogenic acid and its derivatives along with the alkaloid caffeine (Chien et al. 2019), which are potent reducing and capping agents in nanoparticles formation (Jacob et al., 2008). The interaction between the carboxyl and hydroxyl functional groups of these phytocompounds with the metal ions causes the reduction of the metal nucleus that eventually develops into silver nanoparticles (Chien et al., 2019). The repurposing of SCG will certainly reduce the environmental impact of this by-product generated at the domestic and industrial levels worldwide. The bioactive compounds contained in the SCG are reported to be soil pollutants once disposed into landfills (Klangpetch, 2017). Hence, this research proposes the utilization of SCG to biosynthesize silver nanoparticles as an effective antibiotic towards common UTI-causing bacteria; *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis,* and *Klebsiella pneumoniae*.

2. Objectives

1) To biosynthesize silver nanoparticles (AgNps) using hydro-alcoholic extract of spent coffee grounds (SCG)

2) To determine the antibacterial properties of SCG-derived AgNps against UTI-causing bacteria; *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis,* and *Klebsiella pneumoniae*

3. Materials and Methods

Drying of spent coffee ground (SCG)

Spent coffee grounds (SCG) were obtained from a coffee house, Beam Specialty Sdn. Bhd. The materials were placed into an oven and dried at 60ºC until a constant weight was achieved (Ballesteros et al., 2014). After drying, the spent coffee grounds were transferred to an airtight bottle and stored inside the -20ºC freezer.

Preparation of hydro-alcoholic coffee extract

Dried SCG weighed at 10 g, were mixed with a 1:1 ratio of absolute ethanol and deionized water in a beaker. The mixture was heated to 60ºC for 1 hour under constant stirring. After 1 hour, the mixture was filtrated using a vacuum pump and Whatman No.1 filter paper to acquire the extract (Dhand et al., 2016).

Synthesis of silver nanoparticles using SCG

Silver nitrate solution ($AgNO_3$) at 0.05M was prepared. The silver nanoparticles were synthesized using a 4:1 ratio of the prepared $AgNO_3$ solution with the hydro-alcoholic SCG extract. Both $AgNO_3$ and hydro-alcoholic SCG extract solutions were mixed and incubated for 3 hours in a dark condition under constant stirring with room temperature. After 3 hours, the mixture was purified by using centrifugation at 8000 rpm for 10 minutes and repeated 3 times. After that, the supernatant in the centrifuge tube was discarded, and the remaining pellet was dried in an incubator at 37ºC for a week (Dhand et al., 2016).

Characterization of biosynthesized silver nanoparticles

Dynamic light scattering technique was implemented for the determination of particle size distribution of nano-particles, where a Malvern Zetasizer (Model ZS90) was applied for the particle size distribution analysis. In the sample preparation, 0.1g of dried nanoparticles were dispersed in 250 ml of ethanol by using an ultrasonic probe for 30 seconds before pipetting into micro-cuvette. The analyses of dispersed samples were performed in two replicates with a total of six measurements in every replicate. The results of particle size were presented in average values and particle size distribution (shown in volume fraction).

Antimicrobial activity of biosynthesized silver nanoparticles (AgNps) on pathogenic bacteria

Antimicrobial activity of AgNps was tested on 4 different Gram-negative bacteria such as *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 10145), *Proteus mirabilis* (ATCC 43071), and *Klebsiella pneumoniae* (ATCC 4352). These bacteria were cultured using Luria-Bertani agar and stored in a 4ºC fridge. The antibacterial properties of silver nanoparticles were tested using both the agar disk diffusion method and broth dilution assay.

Agar disc diffusion

The bacteria were grown in broth culture by inoculating at least 3-5 colonies with well-isolated and same morphological type from an agar plate culture. Each of the colonies was inoculated and transferred into a 15 ml falcon tube that contains 5 ml of LB broth. Then, the broth culture was incubated at 35ºC for 6 hours. After incubation time, the broth culture's turbidity was adjusted with LB broth to obtain turbidity comparable to 0.5 McFarland standard optically. After that, 150 µl of bacteria suspension were pipetted onto the Müeller-Hinton agar plate. A cotton swab was used to streak repeatedly on the surface of MH agar homogeneously. After streaking, the MH agar plate was left in the biosafety chamber for 5 minutes to absorb the excess surface moisture. Next, 6 mm discs were impregnated with 20 µl of silver nanoparticles (0.144 mg) and applied onto the MH agar. Gentamicin disc (10µg, Oxoid disk) was used as positive control while distilled water was served as a negative control. Lastly, the plate was inverted and placed in an incubator at 35ºC for 18 hours. After 18 hours, the inhibition zone was measured using a transparent ruler (Lalitha, 2009).

Broth dilution assay

This assay was performed using 96-well microtiter plates. The different volume of silver nanoparticles was transferred into each well before adding the bacterial suspension to produce various concentrations of nanoparticles ranging from 50 µg/ml to 500 µg/ml. The 96-well microtiter plates were placed in an incubator at 35ºC for a day. After that, 40 µl of 0.2 mg/ml p-iodonitrotetrazolium chloride was transferred into each well except for color control, and the plates were incubated again in the incubator at 35ºC for 30 minutes. Chloramphenicol served as positive control while distilled water was used as the negative control. The MIC values of each bacteria were identified by observing the color transition of p-iodonitrotetrazolium chloride (INT) in the plate (Perumal et al., 2012). Cells that viable were able to reduce yellow dye to pink, while no color change indicated that bacterial growth was inhibited (Kuete et al., 2012).

Statistical analyses

Each assay was performed in triplicates and the results acquired were expressed as mean ± standard deviation. Statistical analyses of data were as followed: prior to the analysis, the data were tested for homogeneity of variances by the test of Levene; for multiple comparisons, one-way analysis of variance (ANOVA) was performed. The p-value < 0.05 was used for the level of significance. SPSS version 26.0 was used in the statistical analyses.

4. Results

Biosynthesis and characterization of silver nanoparticles (AgNps) using spent coffee ground (SCG) extract

Figure 1 shows the formation of silver nanoparticles (AgNps) initiated when spent coffee ground (SCG) extract was blended with 0.05 M of silver nitrate solution ($AgNO_3$). The biogeny reduction of silver ions and their aggregation to form silver nanoparticles could be observed from the color change of the mixture. The original light brown colored solution turned to dark brown and this demonstrated the successful synthesis of the AgNps. Malvern Zetasizer analyzer utilized the dynamic light scattering technique for identifying the size distribution of particles. Based on the results in **Figure 2**, the average particle size of the two replicates was 216 nm. The particle sizes exhibited a unimodal distribution that centered around 164-190 nm.

(a) **(b)**

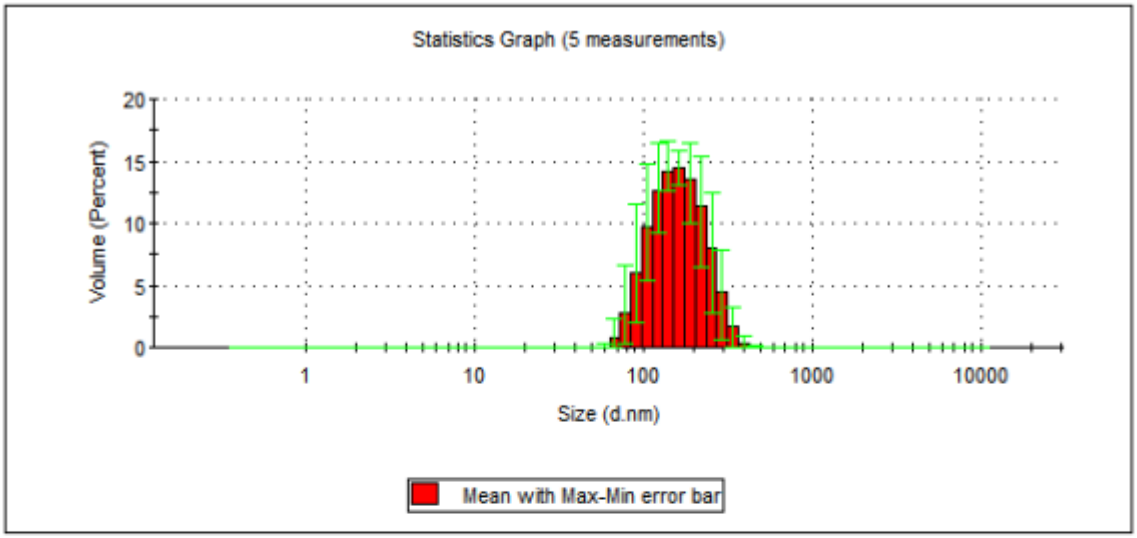
Figure 1 The mixture of 0.05 M $AgNO_3$ solution and SCG extract before (a) and after (b) 3 hours of incubation and constant stirring

Antimicrobial activity of biosynthesized AgNps on pathogenic bacteria

The antimicrobial activity of the AgNps was determined by applying agar disc diffusion and broth dilution methods. In agar disc diffusion, the inhibition zone of each plate was measured using a transparent ruler. On the other hand, the minimum inhibitory concentration (MIC) values of the bacteria in broth dilution assay using 96-wells plates were determined by observing for growth inhibition at different concentrations of nanoparticles.

Agar disc diffusion

Based on the results shown in Figure 2, the inhibition zone of *Escherichia coli* was 10.17 ± 0.58 mm, while *Pseudomonas aeruginosa* had an inhibition zone of 10 ± 0.71 mm. On the other hand, both *Proteus mirabilis* and *Klebsiella pneumoniae* both possessed inhibition zones of 7.33 ± 0.62 mm and 7.25 ± 0.75 mm, respectively. Therefore, AgNps displayed greater antibacterial properties against *E. coli* and *P. aeruginosa* when compared with *P. mirabilis* and *K. pneumoniae*.



Sample Name: Sample 5 Repeat
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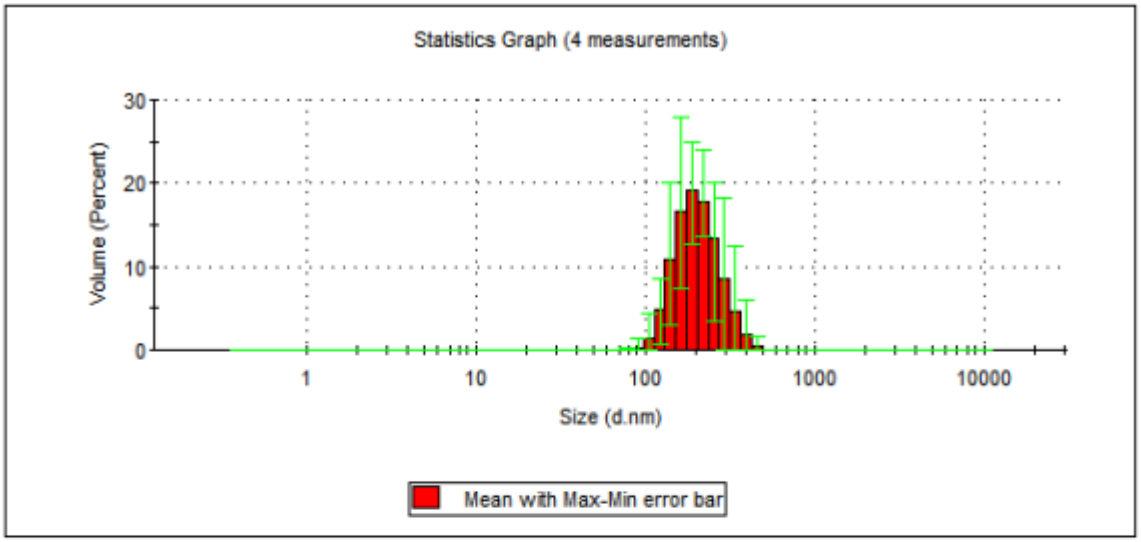
SOP Name: mansettings.nano
Measurement Date and Time: Thursday, 20 September, 2018 3:19:43 PM

Z-Average (nm):	168.0947	**Derived Count Rate (kcps):**	9249.22747802
Standard Deviation (nm):	21.70829	**Standard Deviation (kcps):**	5659.29982229
%Std Deviation:	12.91432	**%Std Deviation:**	61.1867297646
Variance:	471.25	**Variance:**	32027674.4786

Size d.nm	Mean Volume Percent	Std Dev Volume Percent	Size d.nm	Mean Volume Percent	Std Dev Volume Percent	Size d.nm	Mean Volume Percent	Std Dev Volume Percent	Size d.nm	Mean Volume Percent	Std Dev Volume Percent
0.4000	0.0	0.0	5.615	0.0	0.0	78.82	2.7	2.7	1106	0.0	0.0
0.4632	0.0	0.0	6.503	0.0	0.0	91.28	6.0	4.2	1281	0.0	0.0
0.5365	0.0	0.0	7.531	0.0	0.0	105.7	9.7	4.4	1484	0.0	0.0
0.6213	0.0	0.0	8.721	0.0	0.0	122.4	12.5	3.4	1718	0.0	0.0
0.7195	0.0	0.0	10.10	0.0	0.0	141.8	14.2	1.8	1990	0.0	0.0
0.8332	0.0	0.0	11.70	0.0	0.0	164.2	14.5	1.1	2305	0.0	0.0
0.9649	0.0	0.0	13.54	0.0	0.0	190.1	13.6	2.7	2669	0.0	0.0
1.117	0.0	0.0	15.69	0.0	0.0	220.2	11.4	4.2	3091	0.0	0.0
1.294	0.0	0.0	18.17	0.0	0.0	255.0	8.1	4.6	3580	0.0	0.0
1.499	0.0	0.0	21.04	0.0	0.0	295.3	4.4	3.4	4145	0.0	0.0
1.736	0.0	0.0	24.36	0.0	0.0	342.0	1.7	1.8	4801	0.0	0.0
2.010	0.0	0.0	28.21	0.0	0.0	396.1	0.4	0.4	5560	0.0	0.0
2.328	0.0	0.0	32.67	0.0	0.0	458.7	0.0	0.0	6439	0.0	0.0
2.696	0.0	0.0	37.84	0.0	0.0	531.2	0.0	0.0	7456	0.0	0.0
3.122	0.0	0.0	43.82	0.0	0.0	615.1	0.0	0.0	8635	0.0	0.0
3.615	0.0	0.0	50.75	0.0	0.0	712.4	0.0	0.0	1.000e4	0.0	0.0
4.187	0.0	0.0	58.77	0.1	0.2	825.0	0.0	0.0			
4.849	0.0	0.0	68.06	0.7	1.0	955.4	0.0	0.0			

(a)

Figure 2 Particle size distribution of AgNps replicates a) 1 and b) 2 determined by Malvern Zetasizer (Model ZS90)



Sample Name: Sample 6 Repeat
File Name: Losha2.dts
SOP Name: mansettings.nano
Measurement Date and Time: Thursday, 20 September, 2018 3:23:50 PM

Z-Average (nm):	263.7052	**Derived Count Rate (kcps):**	6448.25091705
Standard Deviation (nm):	22.15852	**Standard Deviation (kcps):**	4887.90015807
%Std Deviation:	8.402761	**%Std Deviation:**	75.8019534436
Variance:	491	**Variance:**	23891567.9552

Size d.nm	Mean Volume Percent	Std Dev Volume Percent	Size d.nm	Mean Volume Percent	Std Dev Volume Percent	Size d.nm	Mean Volume Percent	Std Dev Volume Percent	Size d.nm	Mean Volume Percent	Std Dev Volume Percent
0.4000	0.0	0.0	5.615	0.0	0.0	78.82	0.0	0.1	1106	0.0	0.0
0.4632	0.0	0.0	6.503	0.0	0.0	91.28	0.3	0.6	1281	0.0	0.0
0.5365	0.0	0.0	7.531	0.0	0.0	105.7	1.5	2.0	1484	0.0	0.0
0.6213	0.0	0.0	8.721	0.0	0.0	122.4	4.8	4.2	1718	0.0	0.0
0.7196	0.0	0.0	10.10	0.0	0.0	141.8	10.7	7.2	1990	0.0	0.0
0.8332	0.0	0.0	11.70	0.0	0.0	164.2	16.6	8.5	2305	0.0	0.0
0.9649	0.0	0.0	13.54	0.0	0.0	190.1	19.2	6.1	2669	0.0	0.0
1.117	0.0	0.0	15.69	0.0	0.0	220.2	17.7	4.5	3091	0.0	0.0
1.294	0.0	0.0	18.17	0.0	0.0	255.0	13.4	7.3	3580	0.0	0.0
1.499	0.0	0.0	21.04	0.0	0.0	295.3	8.6	7.5	4145	0.0	0.0
1.735	0.0	0.0	24.36	0.0	0.0	342.0	4.7	5.6	4801	0.0	0.0
2.010	0.0	0.0	28.21	0.0	0.0	396.1	1.9	2.8	5560	0.0	0.0
2.328	0.0	0.0	32.67	0.0	0.0	458.7	0.5	0.8	6439	0.0	0.0
2.696	0.0	0.0	37.84	0.0	0.0	531.2	0.0	0.1	7456	0.0	0.0
3.122	0.0	0.0	43.82	0.0	0.0	615.1	0.0	0.0	8635	0.0	0.0
3.615	0.0	0.0	50.75	0.0	0.0	712.4	0.0	0.0	1.000e4	0.0	0.0
4.187	0.0	0.0	58.77	0.0	0.0	825.0	0.0	0.0			
4.849	0.0	0.0	68.06	0.0	0.0	955.4	0.0	0.0			

(b)

Figure 2 Particle size distribution of AgNps replicates a) 1 and b) 2 determined by Malvern Zetasizer (Model ZS90) (Cont.)

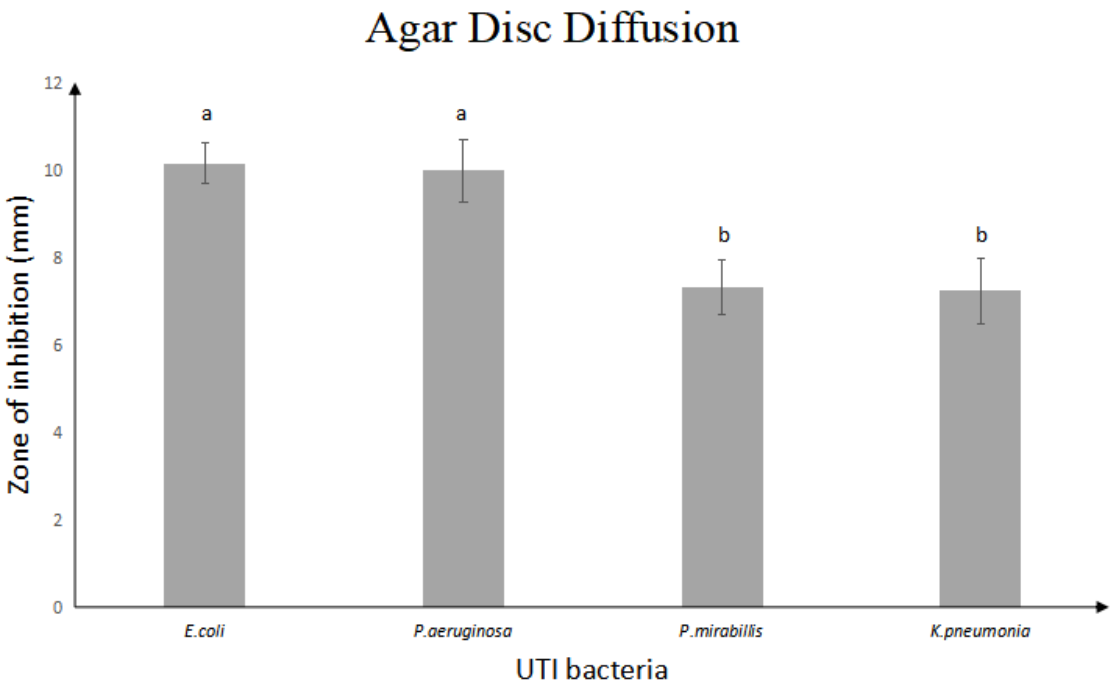


Figure 3 Growth inhibition of urinary tract infection (UTI)-causing bacteria by SCG synthesized silver nanoparticles. Gentamicin and sterile distilled water served as positive and negative controls, respectively. Values are means ± SD, n=3. The data with different alphabet letter are significantly different with a p-value < 0.05

Broth dilution assay

Broth dilution assay was applied to acquire minimum inhibitory concentrations (MIC) of AgNps. Based on Table 1, *K. pneumoniae* possessed the lowest MIC value (200 µg/ml), followed by *P. aeruginosa* and *E. coli* with the same MIC value (400 µg/ml), while *P. mirabilis* showed the highest MIC value of 500 µg/ml. Besides, the MIC value of Chloramphenicol against *K. pneumoniae, P. aeruginosa, and P. mirabilis* were found to be the same (50 µg/ml). Meanwhile, this antibiotic recorded the lowest MIC value of 7.5 µg/ml towards *E. coli*.

Table 1 The minimum inhibitory concentration (MIC) of silver nanoparticles against Gram-negative bacteria obtained from broth dilution assay. Chloramphenicol served as positive control and distilled water was used as the negative control

UTI Bacteria	**MIC (µg/ml)**	**Positive Control (µg/ml)**
Klebsiella pneumoniae	200	50
Escherichia coli	400	7.5
Pseudomonas aeruginosa	400	50
Proteus mirabillis	500	50

Discussion

The biogenic reduction of the aqueous state of silver ions, Ag^+ was established when 0.05 M of silver nitrate (AgNO3) solution was mixed with spent coffee grounds (SCG) extract. The biogeny reduction of silver ions and their aggregation to form silver nanoparticles (AgNps) could be observed from the color change of the mixture (Figure 1). Phenolic compounds present in the SCG facilitated the reduction process of silver nitrate to silver ions and at the same time stabilized the silver ions to form AgNps (Baiocco et al., 2016). SCG is reported to be rich in polyphenolic compounds such as chlorogenic acid. The interaction between the carboxyl and hydroxyl functional groups of these phytocompounds with the metal ions causes the reduction of the metal nucleus that eventually develops into silver nanoparticles (Chien et al., 2019).

According to Figure 2, the average size of the nanoparticles obtained was 216 nm, exhibiting a unimodal distribution that centered around 164-190 nm. The nanoparticles' dimension and morphology are greatly influenced by factors such as pH, temperature, as well as reaction time. Larger particles are more likely to be produced at an acidic condition compared with a basic condition (Dubey et al., 2010). Shorter reaction time, on the other hand, reduces the dimension of the nanoparticles (Sathishkumar et al., 2010). Finally, the higher the temperature during the synthesis process generates a smaller average size of particles (Kaviya et al., 2011).

The antibacterial properties of AgNps were investigated by using disc diffusion. Based on our results (Figure 3), the inhibition zone of AgNps against *Escherichia coli* was 10.17 ± 0.58 mm, while *Pseudomonas aeruginosa* had an inhibition zone of 10 ± 0.71 mm. On the other hand, both *Proteus mirabilis* and *Klebsiella pneumoniae* possessed smaller inhibition zones of 7.33 ± 0.62 mm and 7.25 ± 0.75 mm, respectively. Therefore, AgNps displayed greater antibacterial properties against *E. coli* and *P. aeruginosa* as compared with *K. pneumoniae* and *P. mirabilis*. Nevertheless, findings from this work demonstrated that the nanoparticles formed were able to induce inhibition on the growth of the tested Urinary Tract Infections (UTIs)-causing pathogens. Silver nanoparticles synthesized using other plant extracts also showed similar bioactivities. In recent studies, AgNps produced using *Anogeissus acuminata* (Mishra & Padhy, 2018) and *Aloe vera* (Yadav & Kumar, 2016) leaves extracts exhibited antibacterial actions against the same bacterial species; *E. coli*, *P. aeruginosa*, *P. mirabilis*, and *K. pneumoniae*.

Minimum inhibitory concentrations (MIC) of AgNps against UTI bacteria were obtained using broth dilution assay (Table 1). Interestingly, *K. pneumoniae* charted the lowest MIC value (200 µg/ml) followed by *E. coli* and *P. aeruginosa* with a MIC value of 400 µg/ml, while *P. mirabilis* had the highest MIC (500 µg/ml). The growth inhibition results for *K. pneumoniae* from the disc diffusion and broth dilution assays were not complementary unlike for the other bacteria. This phenomenon could be due to the formation of microcolonies or biofilm in the agar-grown *K. pneumoniae*, protecting the cells from the antimicrobial effects of the AgNps leading to a smaller zone of inhibition (Hachicho et al., 2017).

Hydro-alcoholic extract of *Coffea arabica* seeds had been utilized in the biosynthesis of AgNps that showed potent antimicrobial activities towards *Escherichia coli* and *Staphylococcus aureus* based on well diffusion assay (Dhand et al., 2016). Furthermore, green synthesized AgNps (particle size ranging from 4-50 nm) using culture of *Lactobacillus acidophilus*, reported a MIC value of 60 µg/ml on *K. pneumoniae* (Rajesh et al., 2014) three times lower than our study. Pu-erh tea leaves extract also acted as a reducing agent in the generation of spherical silver nanoparticles at a measured size of 4 nm that were tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella Typhimurium*, and *Salmonella Enteritidis* (Loo et al., 2018). The tea leaves-derived AgNps recorded MIC values ranging from 3.9 to 7.8 µg/mL for all bacteria understudy indicating higher antibiotic efficacy. One of the main factors leading to a lower MIC in previous studies as compared with ours may be due to the size of the nanoparticles obtained in this work that was significantly larger at 216 nm. A smaller particle size conferred a higher surface ratio, which indirectly exhibits greater antimicrobial activity. The novelty of this research is using spent coffee ground, a by-product of the coffee industry, to biosynthesize these silver nanoparticles as an antibacterial agent against pathogens that cause urinary tract infections.

The authors postulated that our synthesized AgNps anchored themselves onto the bacterial cell walls and infiltrated the intracellular environment, thus, creating a series of events leading to cell death. Deformation in the bacterial membrane resulted in leakage of cellular contents and eventually death of bacteria. Moreover, the AgNps present intracellularly could form interaction with biomolecules and cellular structures such as DNA, lipids, and proteins. Denaturation of ribosomes by the AgNps ultimately halted protein synthesis (Seong & Lee, 2017; Khalandi et al., 2016). Besides, the AgNps were able to cause oxidative stress within the cells by generating large amounts of reactive oxygen species (ROS) and free radical species such as superoxide anion and hydroxyl radical (Qing et al., 2018) while disrupting the expression of the bacterial antioxidant enzymes (Yuan et al., 2017). The competency of the AgNps to discharge silver ions (Ag^+) into the bacterial community served as another mode of antibacterial action (Kim et al., 2016). According to Klueh et al., (2000), the Ag^+ released was found to attach itself to the proteins at the cell membrane, which gives rise to protein deactivation.

5. Conclusion

Spent coffee grounds (SCG) could be employed as an alternative substrate to synthesize silver nanoparticles (AgNps) via the green synthesis approach. The AgNps produced by this method were found to display antibacterial properties against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Klebsiella pneumoniae*. Nevertheless, optimization of nanoparticle synthesis should be performed to generate particles of smaller sizes with stronger antimicrobial action. Mechanistic actions of the AgNps towards growth inhibition of the UTI-causing bacteria are to be investigated as well.

6. Acknowledgements

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