Journal of Sustainability Science and Management
Volume 17 Number 4, April 2022: 84-98
eISSN: 2672-7226

EFFICIENCY OF SILVER NANOPARTICLES (AgNPs) SYNTHESIS BASED ON SAMPLE PREPARATION OF MANGROVE BAITWORM (ANNELIDA, EUNICIDAE) *MARPHYSA MORIBIDII* IDRIS, HUTCHINGS AND ARSHAD, 2014

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Submitted final draft: 9 September 2021 *Accepted: 8 January 2022* http://doi.org/10.46754/jssm.2022.4.007

Abstract: Green synthesis of silver nanoparticles (AgNPs) is gaining interest due to its environmentally friendly, easy and biocompatible process. AgNPs synthesis using polychaete extract is relatively new but exhibits good potential. This study examines a local baitworm species, *Marphysa moribidii,* on its efficiency in synthesising AgNPs using two-sample preparations (fresh and frozen) with different weights. Samples were mixed with silver nitrate ($AgNO_3$) before recording the colour changes. The ultraviolet-visible (UV-Vis) spectroscopy analysis, scanning electron microscope (SEM) and transmission electron microscope (TEM) were undertaken to detect the quantity and quality of synthesised AgNPs. Successful synthesis of AgNPs was observed for both samples as the solution colour changed from pinkish red to brown and confirmed with surface plasmon resonance peaks. Fresh extract with 5 g, 10 g and 15 g demonstrated higher reducing capability in AgNP synthesis than those frozen based on their peak uniformity in the UV-Vis spectrum. SEM and TEM images revealed spherical AgNP formation, which is the preferred shape for antimicrobial studies. X-ray diffraction and Fourier transform infrared spectroscopy found no trace of Ag contaminants from the environment. Further analysis and optimisation are required to reveal the reducing mechanism, especially using fresh samples, as it has a higher capability in synthesising AgNPs.

Keywords: Antimicrobial, biosynthesis, green technology, nanobiotechnology, polychaete, silver nanoparticles.
Abbreviations*:* $AgNO_3$, AgNPs, BDM, FTIR, SEM, SPR, SPT, TEM, XRD.

Introduction

Metal nanoparticle synthesis is currently attracting significant attention, especially in the application of electronics, catalysis, photonics, storage information, chemical imaging and sensing, drug delivery, environmental remediation and biological labelling due to their unique physicochemical properties (Bhimba *et al.,* 2010; Nath & Banerjee*,* 2013; Burdușel *et al.,* 2018). Nanoparticles, either silver, copper, or gold are produced by a cluster of atoms, with dimensions varying between 1 nm and 100 nm (Edmundson *et al.,* 2014). Nanoparticles are gaining interest due to their large surface area per volume area relative to their bulk form. Thus, their chemical reaction is enhanced tremendously as smaller particle size increases chemical reactivity.

Among metal nanoparticles, silver nanoparticles (AgNPs) are the most widely utilised (Rao *et al.,* 2007). This is attributed to their unique physical, optical and chemical properties and them being an excellent antimicrobial agent due to their broad-spectrum antimicrobial properties (Tran *et al.,* 2013; Zhang *et al.,* 2016). AgNPs are steadily gaining demand

due to their various applications in biomedical devices, textile, personal care, electronics, food packaging and water treatment (Masimen *et al.,* 2020; Rosman *et al.,* 2021). The primary methods of synthesising AgNPs are chemical and physical approaches. Nevertheless, both methods entail issues, such as complicated condensation and evaporation processes, hazardous chemical by-products to the environment, as well as complex and high operational costs (Ravindran *et al.,* 2013; Ansari & Alzohairy, 2018). Meanwhile, some disadvantages are toxic chemicals, such as borohydride, citrate, ascorbate and elemental hydrogen, utilised in the chemical synthesis.

The usage of biological organisms overcomes these problems as the reducing agent in AgNP synthesis. In biological synthesis, biomolecules present in living organisms, such as proteins, lipids, carbohydrates and vitamins are employed as reducing agents, replacing the harmful chemicals used in the chemical method (Singh *et al.,* 2014; Hamouda *et al.,* 2019), thus making the synthesis more cost-effective and environmentally friendly. Plants and microorganisms are commonly used as reducing agents in this synthesis. However, the usage of marine organisms in the synthesis is gaining recognition due to the vast number of species found worldwide. This includes the utilisation of polychaetes in AgNP synthesis (Singh *et al.,* 2014).

Polychaetes, or commonly known as marine or bristle worms, are usually used as fish bait. Despite their high diversity, their utilisation in the scientific field is limited since previous studies primarily focused on taxonomy and diversity. Nonetheless, polychaetes are used as a pollution indicator (Dean, 2008), aquaculture food (Leelatanawit *et al.,* 2014) and fishing (Martin *et al.,* 2020). Recently, Singh *et al.* (2014) revealed the potential of polychaetes as a reducing agent in AgNP synthesis.

Research on polychaetes in Malaysia has gradually increased, reflecting the number of identified species and scopes of research (Idris & Arshad, 2013; Rosli *et al.,* 2016). One of the recently described polychaetes in Malaysia is *Marphysa moribidii,* locally known as *ruat bakau* (Idris *et al.,* 2014). This species is mainly used as bait, but recent discoveries showed its extract could be used in wound healing and as a reducing agent in metallic nanoparticles synthesis (Pei *et al.,* 2020; Rapi *et al.,* 2020; Rosman *et al.,* 2020; Soh *et al.,* 2020). Although it can act as a reducing agent, its efficiency in reducing $AgNO_3$ to AgNPs using fresh and frozen samples and different reducing weights has not been investigated. The properties of the produced AgNPs, such as shape and size, greatly depend on the sample preparation and the weight of the reducing agent (Christopher *et al.,* 2015; Khan & Jameel, 2016). Thus, this study was conducted to determine the effectiveness of fresh and frozen extract samples using different weights in synthesising AgNPs.

Materials and Methods

Sampling and Sample Preparation

The specimens were collected during the low tide as the tide recedes. Living polychaetes (fresh sample) were housed in the aquaria filled with artificial seawater. Meanwhile, dead specimens (frozen samples) were stored at -20°C. The two types of polychaetes were then prepared at different concentrations: 1 g, 3 g, 5 g, 10 g and 15 g by pulverisation for further analysis.

Synthesis of Silver Nanoparticles

10 mL of crude extract from each concentration (1 g, 3 g, 5 g, 10 g and 15 g) of fresh and frozen samples were mixed well with 90 mL of 1 mM silver nitrate ($AgNO_3$) solution. A total of 10 mL of deionised water with 90 mL of 1 mM $AgNO_3$ solution acted as the negative control. Simultaneously, the citrate stabilised AgNPs was used as a positive control and was prepared following the method indicated by van Berkel and Hawker (2010). All samples were wrapped and agitated for 24 hours and further incubated at room temperature for two weeks before observing the colour changes (Singh *et al.,* 2014). Observations on colour changes were carried out continuously every day after 24 hours of agitation.

UV-Vis Spectroscopy Analysis

Surface plasmon resonance (SPR) bands of the AgNP samples from both groups were characterised using the ultraviolet-visible (UV-Vis) spectrophotometer (UV 1800, Shimadzu) with a broad range of wavelengths, varying from 300 nm to 600 nm. The spectra were plotted using automated UV-Vis spectra software and were re-plotted through Microsoft Office Excel using raw data obtained from the spectrum. The spectra of both groups were compared to observe the effectiveness of the sample in synthesising AgNPs.

Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) Analysis

Poly-L-lysine was dropped on a dried and cleaned aluminium stub for the scanning electron microscope (SEM). After two weeks of incubation, the selected samples with different concentrations were placed onto the stub and subjected to sputter coating with gold before detection under SEM (JSM-6360LA, JEOL). For the transmission electron microscope (TEM), one drop of each sample was deposited onto the copper mesh grid and allowed to dry for observation by TEM (1200 EX II, JEOL).

X-ray Diffraction (XRD)

X-ray diffraction (XRD) was performed to detect the presence of silver (Ag) contaminant in the polychaete crude extract, which might be derived from the environment and the polychaete body itself. It was operated at a voltage of 30 kV and a current of 30 mA using the MiniFlesx II diffractometer (Rigaku, Japan) equipped with an X'celerator using Cu Kα radiation in the range between zero and 110 (2) (Moteriya *et al.,* 2017).

Fourier Transform Infrared Spectroscopy (FTIR)

The chemical composition of the polychaete crude extract was characterised by Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer of Spectrum 100). The IR absorbance of samples was measured with a Nicolet 520 FTIR spectrophotometer. All measurements were carried out in the range of 400 cm^{-1} to 4000 cm^{-1} at a resolution of 4 cm^{-1} (Rosman *et al.,* 2020).

Antimicrobial Study

Broth dilution method (BDM) and spread plate technique (SPT) were utilised to evaluate the antimicrobial activity of AgNPs from the polychaete extract (Balouiri *et al.,* 2016; Wiegand *et al.,* 2008). Penicillin and Amphotericin B were used as positive controls for bacteria and yeast, respectively. The bacteria utilised in this assessment were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *S. epidermidis;* meanwhile, *Candida albicans* was used as a yeast sample. For BDM, AgNPs were diluted using a serial dilution of 0.1%, 1%, 10%, 50% and 100% of AgNP concentrations from the stock solution (Akinduti *et al.,* 2019). A 0.5 McFarland bacterial inoculum and an antimicrobial agent were added into Muller Hinton (MH) broth and then incubated in an incubator shaker at 37°C overnight. The turbidity of the culture was then measured at 600 nm using the Eppendorf BioPhotometer. The same method was applied to yeast, but the incubation time was around two to three days at 30°C. For SPT, the method was slightly modified from the typical SPT, where the MH agar was supplemented with 50 µL of an antimicrobial agent. The MH agar was replaced with Sabouraud dextrose agar for yeast and 100 µL of microbial inoculum was spread on the agar. Inoculated agar without an antimicrobial agent acted as a negative control. The plates were incubated following the method above. The colony formation was observed after incubation.

Statistical Analysis

Each experiment was conducted in triplicate and calculated using Microsoft Excel, ImageJ and Origin Pro 9 software. The statistical significance of the experimental results (significance level of $p<0.05$) was computed using IBM SPSS V.22 (IBM Corporation, Endicott, NY, USA) using the student *t*-test.

Results and Discussion

Colour Changes of AgNPs

Colour variations were observed in both groups (fresh and frozen) at all concentrations (1 g, 3 g, 5 g, 10 g and 15 g) after 24 hours of incubation with the $AgNO_3$ solution. For the 24-hour incubation, only 5 g, 10 g and 15 g concentrations turned from brick red to a brown-coloured solution. This is probably due to a sufficient amount of reducing agent in the sample to reduce $AgNO_3$. After 24 hours of agitation followed by two weeks of incubation, the colour gradually changed from brown to dark brown for all concentrations except frozen samples for 1 g and 3 g (Figure 1). This is probably due to the lack of bioactive compounds present in the frozen samples for the reduction process (Hussain *et al.,* 2018).

These colour variations from brown to dark brown were assumed to indicate the reduction reaction of $AgNO_3$ to AgNPs (Ayad *et al.,* 2019; Rosman *et al.,* 2020). The observed colour was parallel to the dark brown colour described by other researchers (Ansari & Alzohairy, 2018; Bhatnagar *et al.,* 2019; Hamouda *et al.,* 2019). It occurred due to the reduction of Ag^+ of $AgNO_3$, which indicated the synthesis of AgNPs (Hussain *et al.,* 2018). Moreover, after two weeks of incubation, the colour of positive control changed from light yellow to dark yellow, indicating further AgNP synthesis. However, no colour changes were detected as far as a negative control was concerned.

UV-Vis Spectroscopy

The UV-Vis spectroscopy then verified the formation of AgNPs in both groups. The peak formed in the spectroscopy spectrum appeared due to the surface plasmon resonance (SPR) band exhibited by AgNPs (Mlalila *et al.,* 2016; Ider *et al.,* 2017). The SPR phenomenon occurs when the light at a specific wavelength hits the electrons on the metal surface and undergoes collective oscillation. Several factors greatly influence the SPR, including the morphology, size and shape of the synthesised AgNPs (Salem

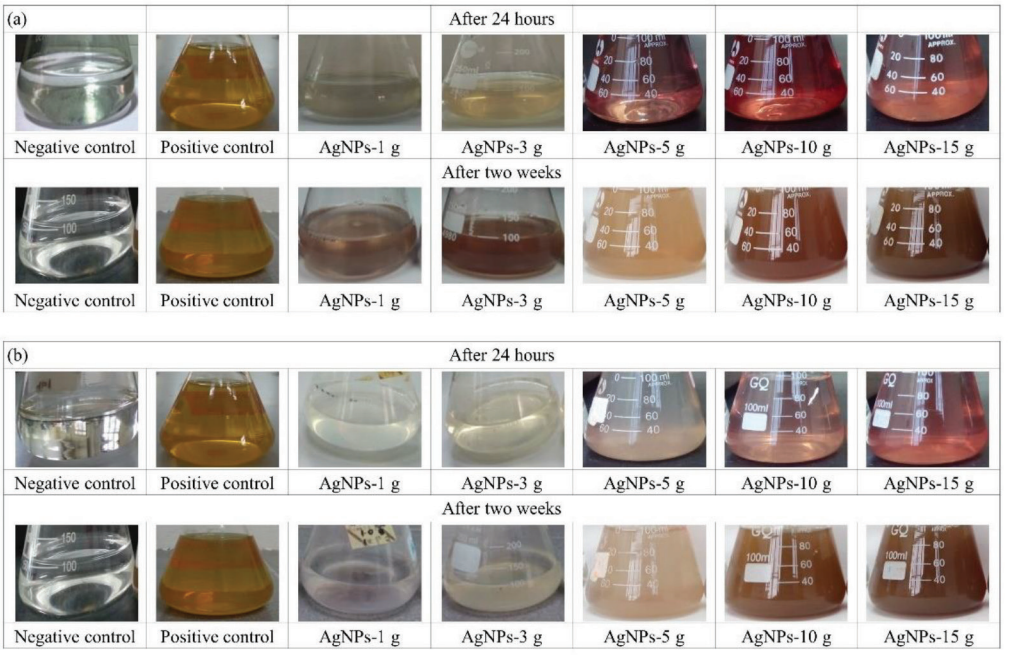

Figure 1: Colour observation in control and synthesised AgNPs after 24 hours and two weeks of incubation. (a) AgNPs synthesised from freshly prepared polychaete crude extract and (b) AgNPs synthesised from frozen polychaete crude extract

et al., 2016; Zhang *et al.,* 2016). From the absorption spectra, the SPR band for AgNPs occurred at a wavelength of 402 nm to 404 nm for the freshly prepared sample and a wavelength of 398 nm to 409 nm for the frozen samples.

The uniform wavelengths indicated no significant shift in the absorbance intensity that contributed to the synthesis of a uniform particle size throughout the experiment (Figure 2). The frozen polychaete crude extract might be synthesised to different sizes of AgNPs, attributed to the significant shift in the absorbance intensity. However, the spectrum revealed that the AgNPs synthesised from polychaete extract from fresh and frozen samples had a broader peak than the positive control, which exhibited a sharp peak. The sharp peak indicated that the sample was homogenous with a single AgNP produced (Umadevi *et al*., 2012; Srinath & Rai, 2015; Hussain *et al*., 2018). In contrast, the broader peak indicated the formation of large aggregation that contributes to a bigger size of AgNPs. However, the broadness of the peak formed from freshly prepared samples was lesser than the peak formed from frozen samples. The results indicated that freshly prepared samples' tendency to synthesise single AgNP was higher than frozen samples.

Generally, both fresh and frozen groups at concentrations of 5 g, 10 g and 15 g exhibited a significant SPR band, which indicated AgNP formation (Figure 2). However, at lower concentrations (1g and 3 g), no SPR peak can be detected in both samples throughout the incubation period. It may be because the amount of reducing agents in the samples to reduce Ag^{+} to AgNPs completely were not enough (Hussain *et al.,* 2018). Besides that, the spectral data strongly suggested that AgNPs from this synthesis were almost spherical. The results were supported by previous studies, indicating that spherical AgNPs were observed approximately at the range of 400 nm in the UV-Visible spectra (Maiti *et al.,* 2013; Rosman *et al.,* 2020).

SEM and TEM Analysis

SEM images revealed spherical like particles collected from the AgNP synthesis in both polychaete groups (Figure 3). The SEM image also showed AgNPs agglomerated, especially at the highest concentration (15 g). AgNPs have the potential to aggregate in both freshly prepared and frozen samples. It also may be due to the error during the complicated preparation of SEM samples (Hussain *et al.,* 2018; Rosman *et al.,* 2020). Nevertheless, the SEM images validated the outcome of spectral reading on the morphology of synthesised AgNPs, confirming that most of the particles were of a relatively spherical-like form.

TEM further validated the shape and size of AgNPs as TEM magnification was more significant than SEM. For this analysis, only samples from fresh polychaetes were chosen

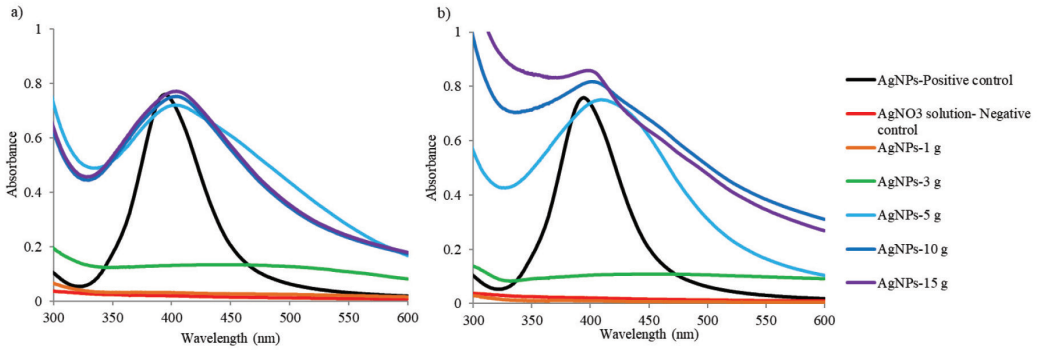

Figure 2: UV-Vis spectroscopy analysis of synthesised AgNPs at five different concentrations from (a) freshly prepared and (b) frozen polychaete crude

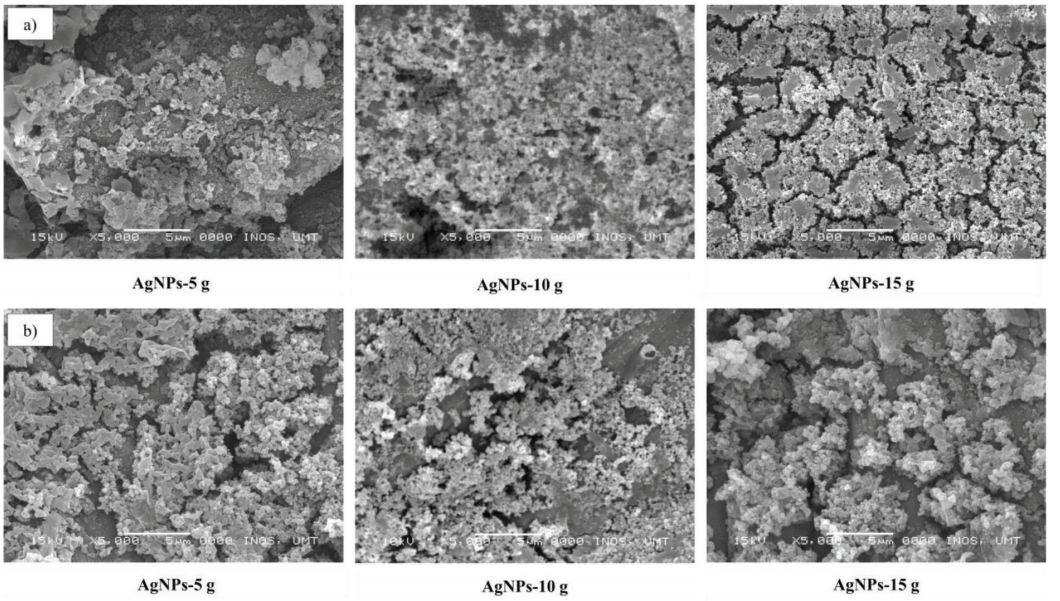

Figure 3: Magnified AgNP images of (a) freshly prepared and (b) frozen samples viewed under SEM Scale: x5000 magnification

as it has higher efficiency in synthesising AgNPs based on the SPR peak. TEM images confirmed that the polychaete-synthesised AgNPs were spherical (Figure 4). The sizes of AgNPs synthesised by citrate reduction and 15 g of polychaete extract were bigger than 50 nm. Meanwhile, 5 g of polychaete extract-synthesised AgNPs were 30 nm to 50 nm. The 10 g of polychaete demonstrated the most potential, with AgNPs in sizes of 15 nm to 30 nm. Smaller sizes of AgNPs are desired due to their massive surface area per volume ratio that enhances the AgNP properties (Zhang *et al.*, 2016). This also showed that the weight of the reducing agent could significantly influence the size of AgNPs, as previous studies also imply similar results when different reducing agent weights were used (Chartarrayawadee *et al.,* 2020; Shahzad *et al.,* 2019). When the optimum weight of the reducing agent for the optimum synthesis is exceeded, it will lead to competition between Ag^+ and bioactive compounds, which causes larger AgNPs to form due to aggregations (Shahzad *et al.,* 2019).

X-ray Diffraction

XRD was used to analyse the presence of Ag metal contaminant that probably existed in the polychaete crude extract. Analysis was conducted to validate the initiated synthesis by the biomolecules in the extract and not from the polychaete body or contaminant from the sampling site. This analysis was also done to ensure consistency with our previous experiments using the same reducing agent (Pei *et al.,* 2020; Rosman *et al.,* 2020). The XRD pattern of polychaete extract in the whole spectrum of 2θ values ranging from 10 to 120 showed the amorphous hump of polychaete extract without the presence of sharp diffraction peaks, which usually indicates the crystalline nature of the product in this case, silver metal (Figure 5). This confirmed that the bioactive compounds present in the polychaete extract are responsible for the reducing activity of Ag^+ to AgNPs and are consistent with our previous studies (Pei *et al.,* 2020; Rosman *et al.,* 2020).

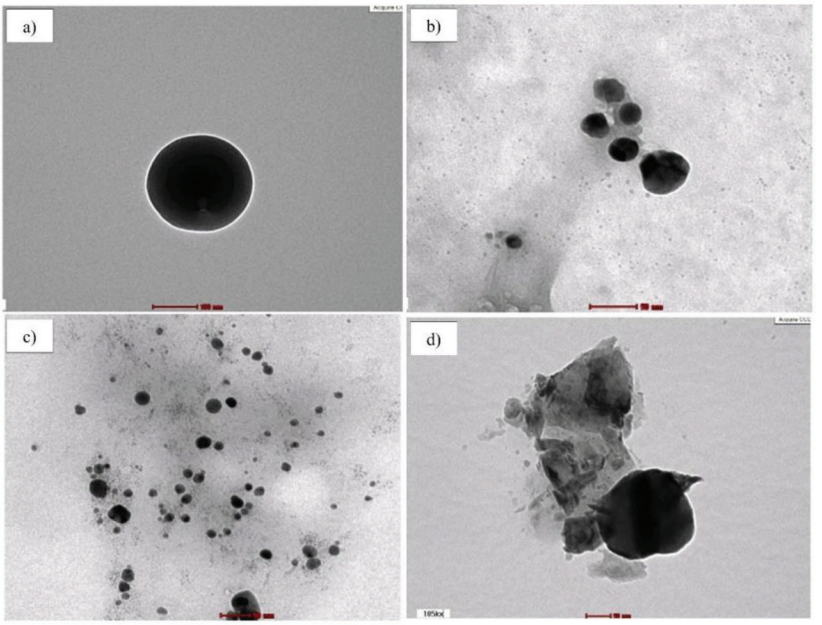

Figure 4: TEM analysis of AgNPs synthesised using (a) $AgNO_3$ solution with citrate (Positive control), (b) AgNPs-5 g, (c) AgNPs-10 g and (d) AgNPs-15 g. Scale bar represents 50 nm, except for (a), where the scale bar represents 100 nm

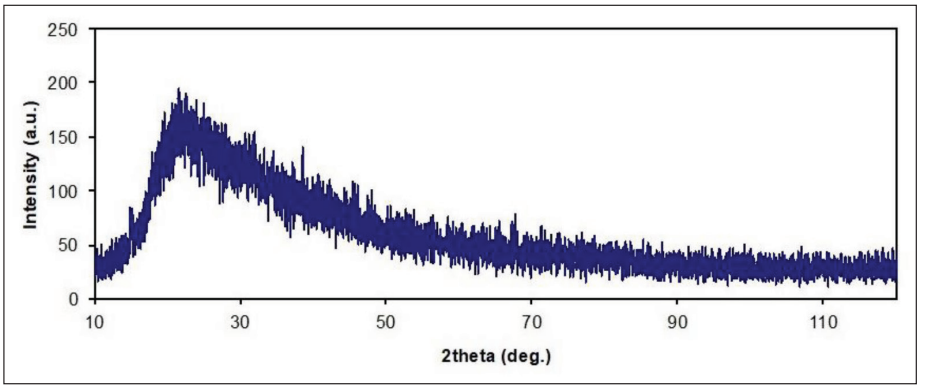

Figure 5: XRD patterns for polychaete crude extract

Fourier Transform Infrared Spectroscopy

FTIR was also redone in this experiment to validate the consistency in results with our previous studies using the same reducing agent (Rosman *et al.,* 2020). The IR spectrum of polychaete in Figure 6 showed the presence of prominent peaks with specific chemical compounds at 3318 cm^{-1} (O-H band), 2091 cm^{-1} (C≡N band), 1642 cm^{-1} (C=C band), 1402 cm^{-1} (CH_2 band), N-H band at 1556 cm^{-1}, 1226 cm^{-1}, 1079 cm^{-1} and 1045 cm^{-1} and C-Br band at 619 cm^{-1}.

The 3318 cm^{-1} band represented the hydroxyl (-OH) group, commonly present in the carboxylic acid and phenols groups. These groups can possibly be found in carbohydrates, sterols, lipids, proteins and ethers present in polychaetes (Figure 7). Simultaneously, the (−CN)

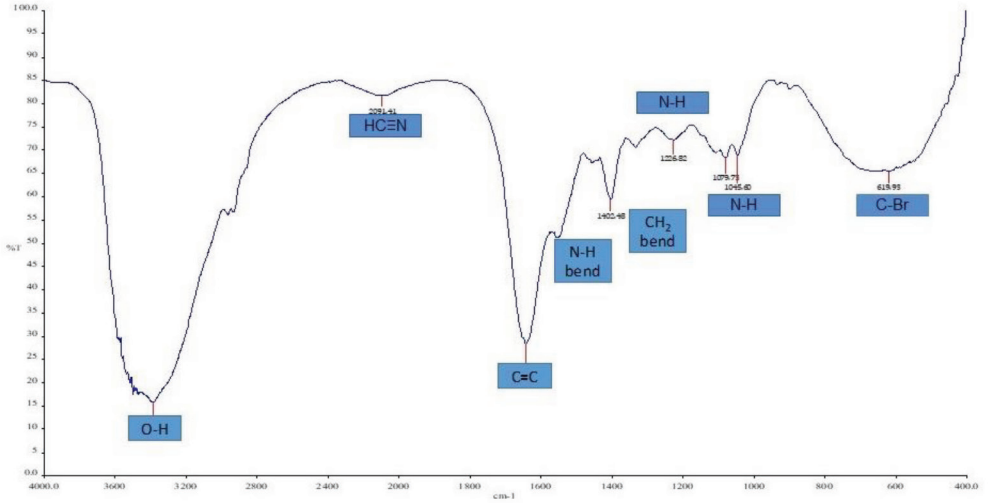

Figure 6: FTIR spectral representation of the crude polychaete extract

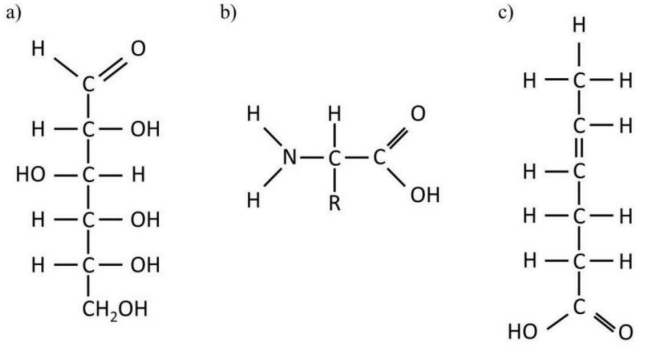

Figure 7: The basic chemical structure of important bioactive compounds that are probably involved in the biosynthesis of AgNPs, (a) carbohydrate monomer (glucose), where the hydroxyl group can be seen, (b) a general structure of amino acid, where amino and carboxyl groups are present in the structure and (c) fatty acid structure, where alkene and carboxyl functional group can be found

band at 2091 cm^{-1} can be found in nitrile. The alkene group was also detected at 1642 cm^{-1}, which exists in proteins and lipids. While the 1556 $cm^{-1,}$ 1226 cm^{-1}, 1079 cm^{-1}, 1045 cm^{-1} and at 619 cm^{-1} bands represent (-NH), which can be found in amine groups, from amino acids and peptides to proteins. Alkane was present at 1402 cm^{-1} ($-CH_2$) and alkyl halides were detected at 619 cm^{-1} (C-Br bond).

The exact mechanisms in the biological synthesis of AgNPs are still unclear, although extensive studies have been conducted. However, it is suggested that the organic compounds present in the polychaete extract, such as alkene, phenols, carboxylic acid and amine, are responsible for the synthesis process (Singh *et al.,* 2014; Bhatnagar *et al.,* 2019). The presence of the hydroxyl group in carbohydrate, amino acid and fatty acids are able to initiate the reduction of $AgNO_3$ to AgNPs and stabilise it. The carboxylic group also reduced Ag^+ to AgNPs and bound to its surface and capped around it, thus stabilising the product (Figure 8). Other functional groups such as amine, alkene and alkyl halides are also postulated to be involved in the AgNP synthesis and stabilisation process (Rosman *et al.,* 2020; Singh *et al.,* 2014). Thus, more advanced studies are required to identify the bioactive compounds that are responsible for the reduction process by the polychaete extract.

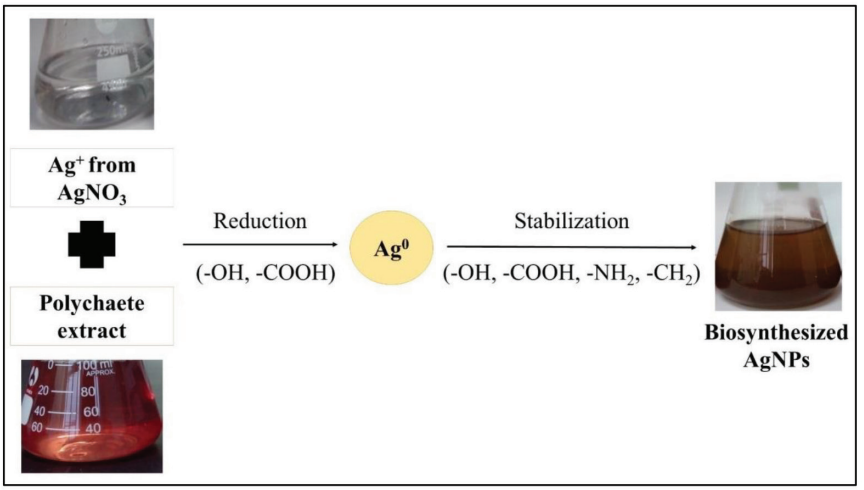

Figure 8: Proposed AgNPs synthesis mechanism using polychaete extract as the reducing agent. Functional groups, such as -OH and –COOH, present in the polychaete extract are responsible for reducing Ag^+ to AgNPs before stabilisation. While stabilisation occurred, the aid of -OH, -COOH, NH_2 and -CH_2 function groups cap the AgNPs, thus stabilised it

Antimicrobial Study

BDM and SPT assessed the efficiency of AgNPs from fresh polychaete samples against certain bacteria and yeast. In BDM, only a 100% concentration of AgNPs synthesised by polychaete extract inhibited microbial growth relative to the negative control (Figure 9). Meanwhile, only AgNPs from fresh samples were used in SPT due to their UV-Vis spectroscopy forming uniform peak wavelength and SPT was used to observe the bacterial colony reduction once the bacteria was spread into the AgNP-infused plate. Based on the results, AgNPs from polychaete extract reduced microorganism growth compared with the negative control plate (Figure 10). The results showed that the AgNPs produced from the polychaete extract could be a promising antimicrobial agent in combating microorganisms, as silver is noted for its broad-spectrum activity (Franci *et al.,* 2015; Qing *et al.,* 2018; Masimen *et al.,* 2020). Its nano-size attribute also aids in microbial growth reduction due to a large contact surface area with microorganisms, as silver is toxic to them (Keat *et al.,* 2015; Qing *et al.,* 2018; Mikhailova, 2020). Thus, AgNPs are more effective compared with their bulk form. Further experiments using several methods of antimicrobial assessments will be conducted once the production of AgNPs has been optimised.

Based on the results, AgNPs from 10 g of polychaete extract inhibited bacteria slightly better than the 5 g and 15 g. This is probably due to the AgNP size, which allows the nanomaterial to interact with the bacteria since it has a larger surface area per volume ratio (Raza *et al.,* 2016; Kora & Sashidhar, 2018; Shahzad *et al.,* 2019). The mechanism of AgNPs in inhibiting microbial growth is still unclear. However, AgNPs interact with bacteria and yeast by adhering to the cell membrane and disrupting membrane integrity. Subsequently, it forms "pits" on the membrane surface and causes cellular leaking (Prabhu & Poulose, 2012; Senthil *et al.,* 2018; Salleh *et al.,* 2020). Thus, the bacterial cells cannot regulate the normal transport system across the membrane, inducing cell death. Moreover, AgNPs can penetrate the cell membrane and interact with vital enzymes to deactivate the microbe (López-Heras *et al.,* 2015; Dakal *et al.,* 2016). AgNPs can also induce excessive reactive oxygen species in the bacterial cell, which will inhibit the cell's growth (Rai *et al.,* 2012; Liao *et al.,* 2019). AgNPs can also alter

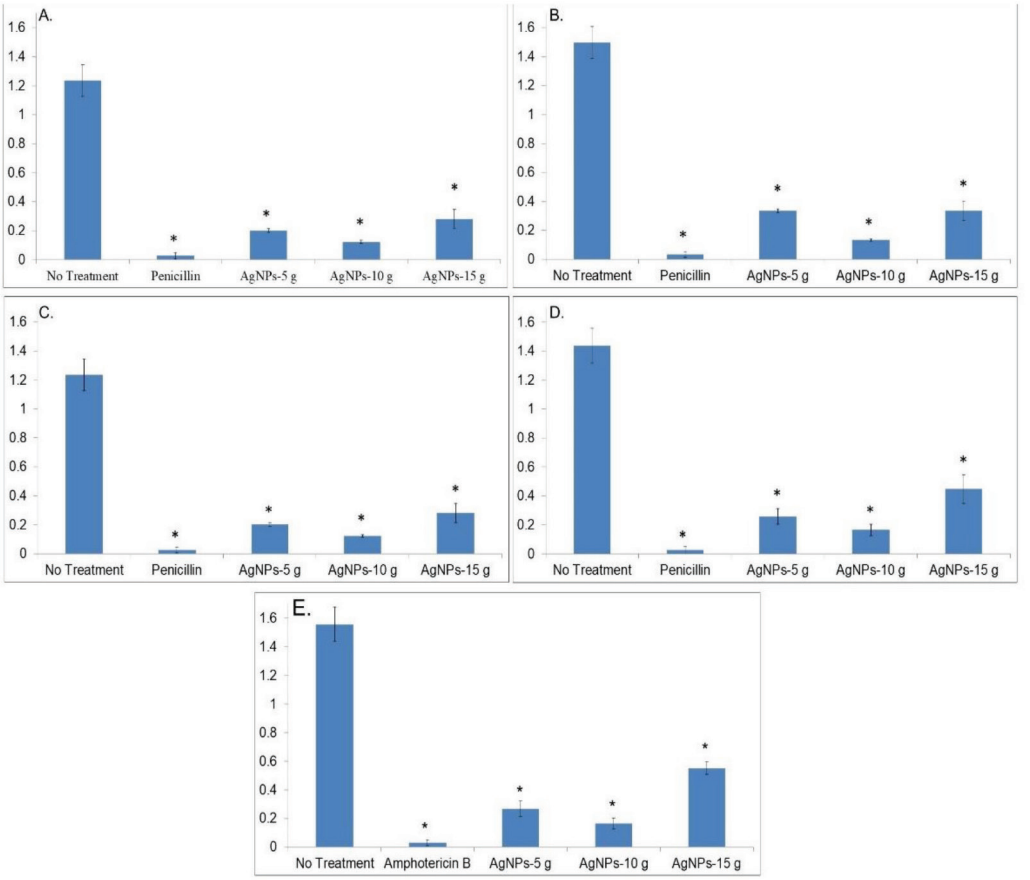

Figure 9: BDM assessment of a 100% concentration of fresh polychaete extract AgNPs compared with the positive control (bacteria: penicillin, yeast: amphotericin B) and no treatment (microbial growth in Muller Hinton (MH) broth without an antimicrobial agent). BDM assessment against (a) bacteria *E. coli*, (b) bacteria *S. aureus*, (c) bacteria *P. aeruginosa*, (d) bacteria *S. epidermidis* and (e) yeast *C. albicans*. *Significant difference compared with a group of no treatment, $p < 0.05$. n = 3

phosphotyrosine profiles vital for the bacterial life cycle, which will inhibit signal transduction for the cell growth process (Shrivastava *et al.,* 2007). In order to confirm the hypotheses, in-depth studies are required to investigate the mechanism of action by the polychaete-based AgNPs in inhibiting bacterial growth in the future.

Conclusion

This study revealed the potential application of local polychaetes in the synthesis of AgNPs. Based on the results, both freshly prepared and frozen polychaete samples demonstrated positive AgNP formation based on their colour changes. From a UV-Vis spectroscopy analysis, freshly prepared polychaete crude extracts at concentrations of 5 g, 10 g and 15 g showed more consistent AgNP formation than the frozen samples. It was attributed to the significant peak and uniform wavelengths formed in the spectrum. The spherical shape of synthesised AgNPs was recorded using SEM and TEM. Based on the XRD analysis, the AgNPs were synthesised by the polychaete extract reducing activity with $AgNO_3$ and not from environmental contaminants. The chemical compositions detected from the polychaete extract were O-H, C≡N, C=C, N-H, C-Br and CH_2, which

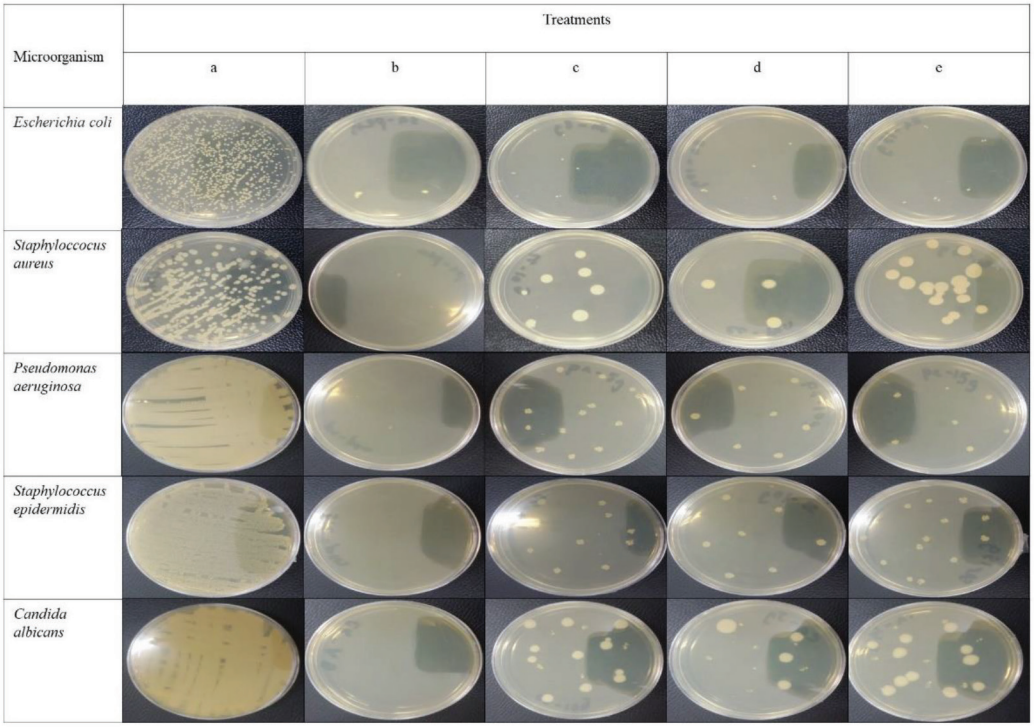

Figure 10: Spread plate technique (SPT) results of antimicrobial properties of fresh polychaete extract AgNPs, where reduction of the bacterial colony can be observed on the AgNPs infused agar. (a) Negative control, (b) positive control, agar supplemented with penicillin for bacteria and amphotericin B for yeast, (c) bacteria or yeast growth on agar supplemented with AgNPs-5 g, (d) bacteria or yeast growth on agar supplemented with AgNPs-10 g and (e) bacteria or yeast growth on agar supplemented with AgNPs-15 g. n = 3

may play a significant role in synthesising AgNPs. Antimicrobial results also showed that AgNPs from fresh polychaete samples could inhibit selected bacteria and yeast growth. Thus, from this study, AgNPs synthesised from fresh samples and 10 g of polychaete weight are preferred due to their smaller AgNP size formation that is more favourable, especially in antimicrobial activity studies. Further studies on the effects and underlying mechanisms involved in the reactions, especially fresh polychaete samples, must be investigated intensively.

Acknowledgements

The authors extend their gratitude to the Faculty of Science and Marine Environment and Institute of Oceanography and Environment, Universiti Malaysia Terengganu, for their continuous support in this research. Special thanks to the local indigenous people at the sampling site for their assistance during the sampling process. This project is funded by the Fundamental Research Grant Scheme (FRGS/1/2016/WAB09/UMT/02/2) under the Ministry of Higher Education, Malaysia.

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