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BIOSYNTHESIS OF SILVER NANOPARTICLES USING MARINE MICROALGAE *ISOCHRYSIS sp.*

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ABSTRACT

Biosynthesis of metal nanoparticles has received a remarkable attention due to their eco-friendly and potential applications in pharmaceutical and medical fields. The searches for natural alternatives to replace biosynthetic nanoparticles have resulted in extensive studies of microalgal derived metal nanoparticles. Since there are very limited reports on *Isochrysis* sp. in synthesising metal nanoparticles, a novel initiative was taken to induce an environmentally friendly and low cost technique to biosynthesise the silver nanoparticles (AgNPs) using marine microalgae, *Isochrysis* sp. Further, the synthesised silver nanoparticles were screened against human pathogen for antimicrobial effects. The characterisation of nanoparticles were confirmed by UV-visible spectroscopy, field emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR), and X-ray powder diffraction (XRD). The results obtained from characterisations indicate that the AgNPs have an almost spherical shape with a various size of 98.1 to 193 nm. The synthesised nanoparticles exhibited outstanding antioxidant and antimicrobial activities.

Keywords: silver nanoparticles; nanoparticles; nanobiotechnology; *Isochrysis*.

1.0 INTRODUCTION

Nanotechnology is one of the most interesting studies in the current research world due to its vast applications in pharmaceuticals, medicine, and electronic fields. The synthesis of nanoparticles involves the particles size ranging from 1 to 100 nm (Naveena and Prakash, 2013). Electrochemical, sonochemical, and photochemical methods are some of the approaches being used to synthesise metallic nanoparticles. However, chemical method is not suitable due to the utilisation of toxic and hazardous chemicals, and involves difficult purification steps (Pak *et al*., 2016). Biological method combines the principles of biology with chemical and physical procedures to generate nanosized particles, which is termed as nanobiotechnology. Nanobiotechnology emphasises on economic alternatives and promotes nontoxic, ecofriendly, clean, and green synthesis method of nanoparticles. Biomolecules such as proteins, amino acids,

carbohydrates, and sugars from microorganisms such as bacteria, fungi, and algae are used to biologically synthesise the metal nanoparticles (Chinnappan *et al*., 2015). Silver, gold, zinc, aluminium, titanium, palladium, iron, fullerenes, and copper are some of the metallic nanoparticles commonly synthesised via bionanotechnology procedures.

Silver nanoparticles (AgNPs) are one of the most important nanoparticles extensively studied in recent years due to its diverse beneficial properties and wide variety of applications in biological, chemical, and physical sciences. It is proved that silver nanoparticles possess unusual properties such as high antimicrobial activity, particles stability, and surface chemistry. Specific surface plasmon resonance (SPR) peak of silver nanoparticles falls in between 450 and 530 nm. These different wavelengths are able to express different particle sizes, shapes, and surface properties of nanoparticles. Different physical formations lead to AgNPs to be widely used as antimicrobial and antifungal agents in healthcare, food, textile coatings, and electronic device industries. AgNPs which attached to the microbial cell surface cause structural changes of the cells and further lead to cell death by damaging the entire cell functions. Up to date, many commercial products incorporated with AgNPs were approved by FDA (USA), SIAA (Japan), KTR and FITI (Korea) (Kuppusamy *et al*., 2016).

Chemical methods such as sonochemical, polyols, matrix chemistry, sol gel, chemical reduction, and electrolysis are some of the proven metallic nanoparticles synthesising methods. Although these methods are efficient and fast, it also produces adverse effects to human and living organisms. Therefore, a sustainable method using biological medium as an alternative to conventional methods is essential to be developed. The synthesis of metal nanoparticles from readily available biological substances will allow the development of novel eco-friendly and cost effective procedures. Such eco-friendly metal nanoparticles synthesis particularly from marine algae were reported by Azizi *et al.* (2013), Mahdavi *et al.* (2013), Naveena and Prakash (2013), Patel *et al.* (2015), Singaravelu *et al.* (2007), and Venkatesan *et al.* (2014).

Microalgae is an autotrophic microorganism that consists of large taxa in the world. *Isochrysis* sp., a marine golden-brown flagellated microalga, is one of the prominent sources renowned for its potential pharmaceutical value through phyconanotechnology. *Isochrysis* sp. belongs to the phylum Haptophyta, class Coccolithopyhceae, subclass Prymnesiophycidae, order Isochrysidales, family Isochrysidaceae. Generally, with a small size of around 4–7 μm, this microalga is able to grow very fast in a wide range of temperature, salinity, nutrients, and photoperiod. This fast growing microalga also rich with biopolymers containing glucose, galactose, mannose, xylose, arabinose, fucose, and rhamnose in various percentages. About 13% of dry matter of this microalga constituents are originated from carbohydrates derivatives. Usually, marine microalgae are prevalent in nutraceutical and pharmaceutical industries by providing high content of lipids, minerals, vitamins, secondary bioactive compounds such as polysaccharides, and proteins with potential medicinal property in fighting cancer, inflammation, allergy, and other metabolic diseases. These secondary metabolites can act as a functional group, reducing agents, and biological capped substances for all nanomaterials to play a role in antibiotic mechanisms against pathogens (Mohamed *et al*., 2012; Namvar *et al*., 2012; Zuercher *et al*., 2006). It was proven by the synthesis of cadmium nanomaterial using microalga *Phaeodactylum* sp. and biological reduction of gold nanoparticles by

Rhizoclonium sp., *Navicula* sp., and *Nitzschia* sp. (Nayak *et al*., 2006; Scarano and Morelli, 2003).

In this study, brown microalga *Isochrysis* sp. was used for the synthesis of silver nanoparticles. Analytical techniques such as UV-visible spectroscopy, field emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR), and X-ray powder diffraction (XRD) were used to characterise the biologically synthesized AgNPs using *Isochrysis* sp. Furthermore, scavenging rate of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as an indicator was used to study the antioxidant activity of AgNPs.

2.0 METHODS

Microalgae cultivation

Isochrysis sp. was cultivated aseptically at Bioprocess Laboratory, Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang. The F2 culture medium was used as microalgae nutrient support medium. The culture was grown in glass cylinders under indecent cool fluorescent light that was aided with clean sterile compressed aeration. Modified method from Naveena and Prakash (2013) was used to extract microalgal biomass cells. Microalgal cells were harvested by centrifugation for 10 min at 3,000 rpm. The collected cells were washed with sterile distilled water and then boiled with sterile distilled water. The extract was stored in a chiller until further use.

Synthesis of AgNPs

About 50 mL of *Isochrysis* sp. extract was mixed with 50 mL of freshly prepared $AgNO_3$ (1 mM) solution and briskly stirred for 30 min at room temperature. Then, the mixture was allowed to stand at room temperature for 2 h. The dark brown product was collected through centrifugation at 6,000 rpm for 20 min. The final product was dried at room temperature overnight and stored in an airtight container for further analysis (Azizi *et al*., 2013).

Characterisation of AgNPs

UV-visible spectroscopy, FESEM, FTIR, and XRD techniques were used to determine the size, shape, surface area, and dispersity of the nanoparticles produced. FESEM (JEOL JSM 7800F, USA) was used to observe the morphology of AgNPs. EDX analysis was conducted with the same FESEM instrument to determine the elemental composition of the synthesised AgNPs. For X-ray diffraction study, the synthesized silver nanoparticles were coated on XRD grid and Cu-Ka radiation of 1.541 Å at 30 kV voltage and 15 mA current were used. FTIR spectra was recorded using PERKIN Elmer model at the resolution of 1 cm^{-1} in the range of 4000 to 400 cm^{-1}. Thermogravimetric analysis (TGA) was monitored through Mettler-Toledo thermal analyser at a scanning rate of 10 °C/min.

Antioxidant activity of AgNPs

DPPH test was performed to estimate free radical scavenging activity as described by Phull *et al*., (2016). Different concentrations of silver nanoparticles were used to evaluate antioxidant activity of AgNPs. The silver nanoparticles were divided into aliquots of 2.5 mL and added into 1 mL of DPPH solution and made up to a final volume of 4 mL. Once the solution was mixed properly, the absorbance value of the tested solution was read using a UV-visible spectrophotometer. The following formula was used to calculate the percentage of DPPH radical scavenging activity.

$$\text{DPPH radical scavenging activity, \%} = \left(\frac{\text{Control OD} - \text{biosynthesized silver solution OD}}{\text{Control OD}}\right) \times 100$$

Antibacterial activity of AgNPs

Antibacterial activity of AgNPs was performed by agar well diffusion method on selected human pathogens like *Staphylococcus aureus* and *Escherichia coli*. Biologically synthesised silver nanoparticles were conjugated with streptomycin before tested on pathogens at a ratio of 1:1. An aqueous solution of streptomycin was prepared as control. The strength of inhibition zone was measured after 24 h of incubation at 37 °C (Naveena and Prakash, 2013).

3.0 RESULTS AND DISCUSSION

In this study, the silver nanoparticles were synthesised biologically using microalga *Isochrysis* sp. The sizes, shapes and functional groups of the metal nanoparticles are reported. Antioxidant and antimicrobial activities of the synthesised silver nanoparticles are ascertained.

Figure (1) shows the colour change of silver nanoparticles from pale yellow to brown. The colour of the solution changed to brown within 2 h. There are a few reports on the synthesis of silver and gold nanoparticles using macroalgae, such as *Sargassum muticum* (Azizi *et al*., 2013) and various types of green microalgae (Patel *et al*., 2015) for silver nanoparticles, and *Sargassum wightii* (Singaravelu *et al*., 2007), *Gracilaria corticata* (Naveena and Prakash, 2013); *Turbinaria conoides*, and *Sargassum tenerrimum* (Ramakrishna *et al*., 2016) for gold nanoparticles.

The UV-visible spectroscopy at light wavelengths of 200–800 nm is a commonly used technique to characterise various metal nanoparticles in the size range of 2–100 nm (Manivasagan and Kim, 2015). In this study, AgNPs solutions were subjected to different UV-spectrum wavelengths ranging from 300 to 700 nm and showed maximum absorption peak at 414 nm (Figure 2). The UV-visible absorption spectrum is a strong evidence that the metal nanoparticles formed in different sizes and shapes in the medium. Resonant peak occurs at various wavelengths for different types of nanoparticles mixture. This factor is termed as surface plasmon resonance (SPR). According to SPR theory, the maximum resonance wavelength is absorbed at resonant wavelength. Molecular stability, shape, and size of particles in the medium or upon inner particles distance and its surrounding media are the factors influencing SPR patterns of metallic nanoparticles. Various previous studies suggested a usual silver nanoparticles SPR pattern at wavelength in the range of 400–480 nm (Haghighi Pak *et al*., 2016; Manivasagan and Kim, 2015). In this UV-visible spectrum analysis, broad SPR peak was observed at 414 nm. This result confirmed the formation of AgNPs from

Isochrysis sp. extract. Past researches suggested that spherical shaped nanoparticles (Figure 3) was attributed to SPR peak between 410 and 450 nm (Azizi *et al*., 2013; Devi and Bhimba, 2012; Jyoti *et al*., 2016).

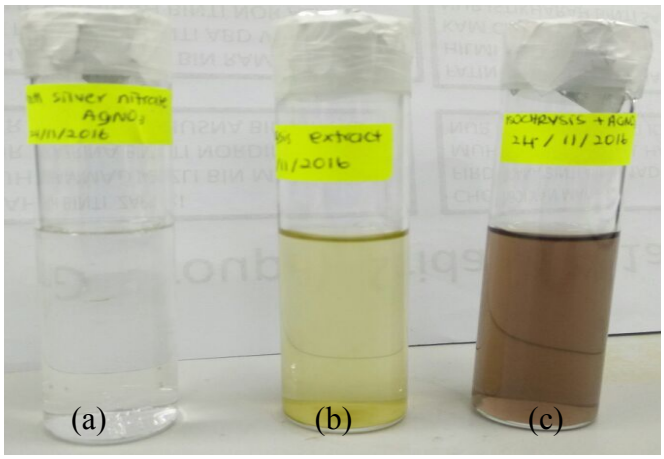

Figure 1: (a) Silver nitrate ($AgNO_3$) solution (1 mM), (b) Microalga extract, (c) Brown colour formation after the reaction of 1 mM of $AgNO_3$ with microalga extract.

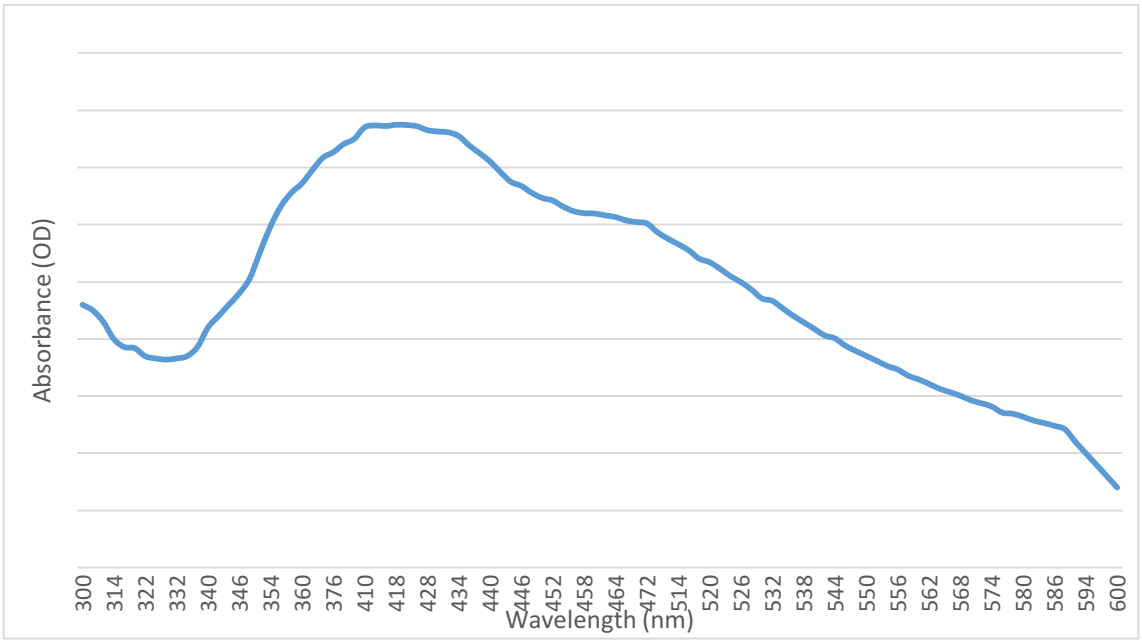

Figure 2: UV-visible absorbance curve of AgNPs synthesised using *Isochrysis* sp.

The size and morphology of the synthesised AgNPs were determined through FESEM images, as shown in Figure 3. The morphology of AgNPs is almost spherical with an average size ranges from 98.1 to 193 nm. Through FESEM images, it was confirmed that the synthesised nanoparticles were monodispersed in terms of particle size. Moreover, Pak *et al*., (2016) revealed that not indirect contact of monodispersed of AgNPs are the indication of stable nanoparticles through capping agents. The capping ligand could be of a carbonyl group, an aromatic compound, alkanes, or amines (Vivek *et al*., 2011). Such bio-capped molecules will help to prevent agglomeration of NPs and at the same time will enhance antimicrobial activity. In this study, aggregated AgNPs with indirect contact of AgNPs was observed in Figure 4.

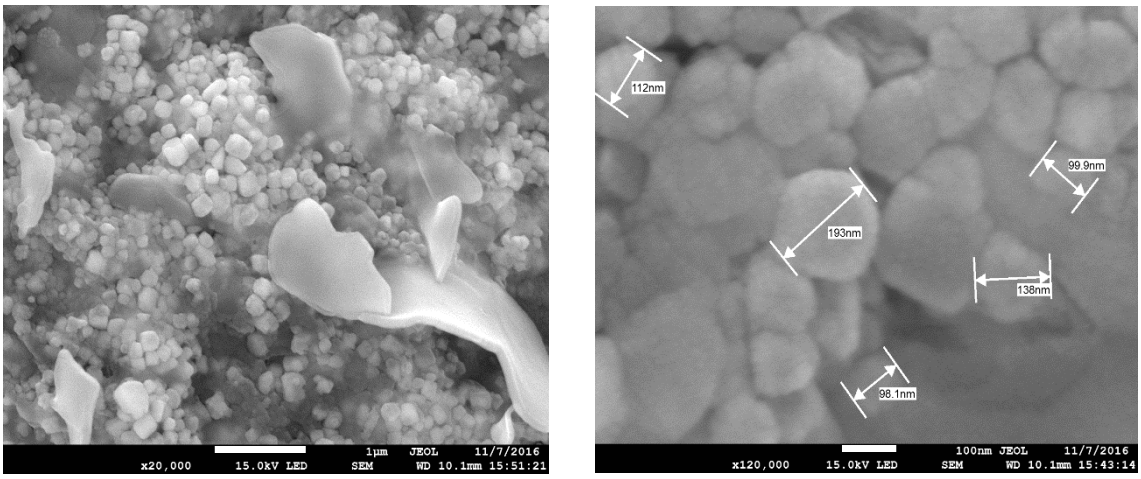

Figure 3: Field emission scanning electron microscope (FESEM) images of silver nanoparticles ($AgNO_3$) synthesised through *Isochrysis sp*.

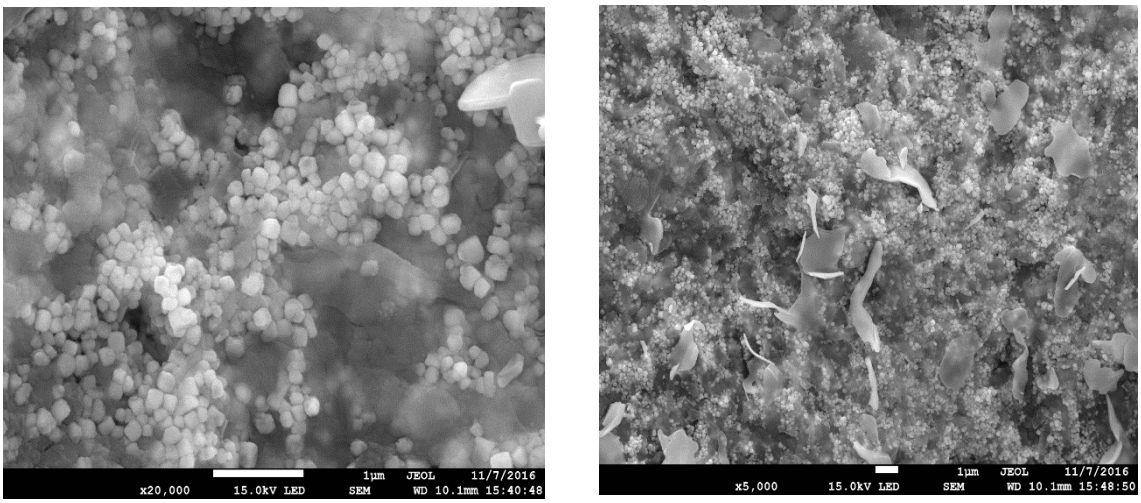

Figure 4: Monodispersed AgNPs with indirect contact of nanoparticles.

Figure 5 shows EDX spectra of the synthesised AgNPs. The spectra confirmed that Ag was expressed where the silver composition was nearly 43% in the sample. The strong signal energy peaks of silver atoms were observed in the range between 3 and 4 keV. Weaker signals are observed for C, O, N, Mg, Si, and Cl (Table 1). Carbon, oxygen, nitrogen, magnesium, silicon, and chlorine elements that shown in the EDX spectra which is dominant compounds in the secondary metabolites of the plant extract (Figure 5 a, b). Similar compounds were observed by Mahdavi *et al*., (2013) and Ravichandran *et al*., (2015).

Through X-ray diffraction pattern (XRD), four peaks were detected at 22.3°, 28.3°, 32.9°, and 46.6° which are assigned to (111), (220), (122), and (231), respectively. This observation revealed that AgNPs that were synthesised from *Isochrysis* sp. consisted of pure crystalline Ag. Similarly, silver nanostructure which was obtained from *Gelidiella acerosa* extract was confirmed by the characteristic peak observed at 2θ = 28.09° marked with (220). Thus, Bragg reflections corresponding to the (220) sets of lattice planes based on the face-centred crystal structure of silver were predicted (Vivek *et al*., 2011). In addition, similar results were recorded by Gnanajobitha *et al*., (2013) and Jyoti *et al.* (2015) with facets of face-centred cubic crystal structure of silver nanoparticles.

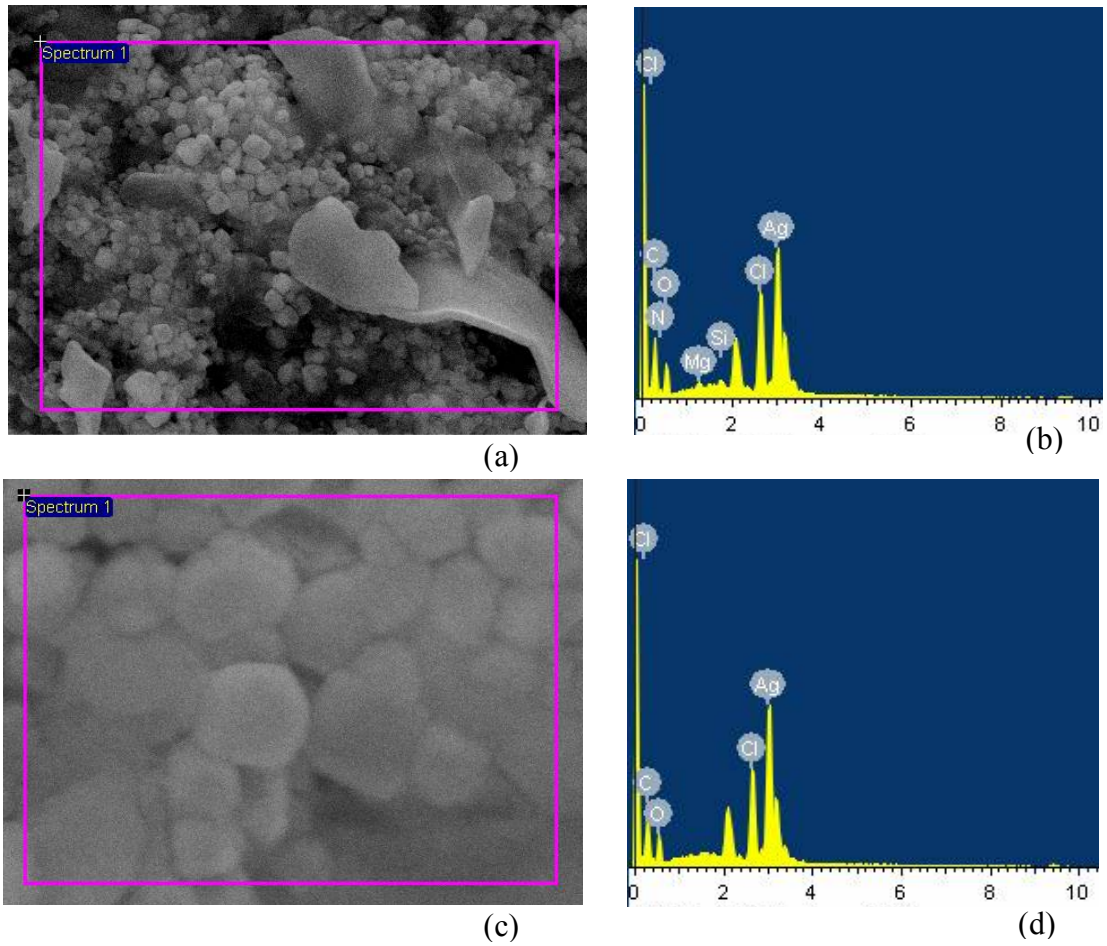

Figure 5: EDX results of (a) and (b) nanoparticles inclusive dead microalgae cells and (c) and (d) nanoparticles only.

Table 1: The elements in AgNPs determined by EDX

Element	Weight %	Atomic %
C	21.30	43.20
N	8.56	14.89
O	15.92	24.25
Mg	0.47	0.47
Si	0.61	0.53
Cl	10.10	6.94
Ag	43.05	9.72
Total	100.00	100.00

Fourier transform infrared spectroscopy (FTIR) measurement was carried out to identify the biomolecules that bound on the silver surface (Xie *et al*., 2007). Figure (6) shows the FTIR spectrum of the biosynthesised silver nanoparticles. Absorbance bands were seen at 602, 1053, 1535, 1637, 2848, 2917, and 3448 cm^{-1}. Strong absorption band at 602 cm^{-1} could be a C-H alkene band. Strong peak observed at 1053 cm^{-1} C-O could be bending esters and the peak at 1637 cm^{-1} is attributed to C=O stretching carbonyls. The high absorbance bond was observed at 3448 cm^{-1} which indicates deforming vibration of O-H strong bond stretching phenols and N-H stretching primary and secondary amines and amides. Also, C-H stretching aldehyde, C-H stretching alkene, and aromatics N-H amine were observed at 2848, 2917, and 1535 cm^{-1}, respectively. The presence of amines, amides, phenols, and carboxyl in the synthesised AgNPs are shown in FTIR analysis results. It is recommended that during the reduction with silver ion, the C-OH alcohol group is converted to the carbonyl group C=O. Carbonyl group plays a role as a stabilizing agent for Ag nanoparticles. The following equation Ag^{+} + R-OH → R=O + Ag + H^{+} explains the reaction between the microalgae extract and silver ions (Meng, 2015). Meanwhile, earlier reports indicated that the stabilisation of silver nanoparticles is also possible when free amine groups or cysteine residues in the proteins bind with nanoparticles via electrostatic attraction of negatively charged carboxylate groups (Sanghi and Verma, 2009).

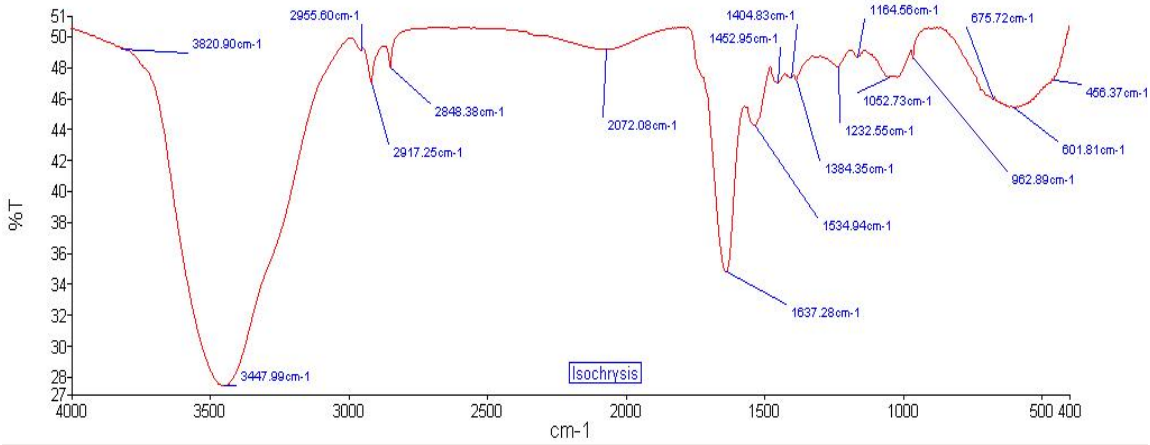

Figure 6: FTIR spectra of the synthesised AgNPs using *Isochrysis* sp. extract

TGA curve of the silver nanoparticles synthesised using *Isochrysis* sp. is shown in Figure 7. The samples was heated from 50 to 700 °C which was in between the boiling point of the solvent and the degradation temperature of polymer (Alahmad *et al*., 2013). In this study, two main weight losses were observed in the TGA graph. The first weight loss was observed at 55 °C. Water molecules losses believe for the first weight loss. The second weight loss was recorded at the temperature between 290 and 320 °C. It can be attributed to the evaporation of organic components occurred at this stage. Moreover, complete thermal decomposition and crystallisation of the sample happened concurrently. Similar trend was observed by Khan *et a.* (2011) between 200 and 300 °C.

Radical scavenging activity of a compound generally is tested via DPPH test. DPPH test is an easy and rapid method to analyse antioxidant activity. The biosynthesised silver nanoparticles were tested for the potential free radical scavenging activity. The effective free radical scavenging potential of *Isochrysis* sp. derived silver nanoparticles and *Isochrysis* sp. extract were 58.8% and 28.4%, respectively. The presence of nanoparticles improved the scavenging activity compared to normal microalga extract. Saranya *et al.* (2014) indicated that a maximum 34.18% of scavenging activity was found in methanol extract of *Isochrysis galbana*.

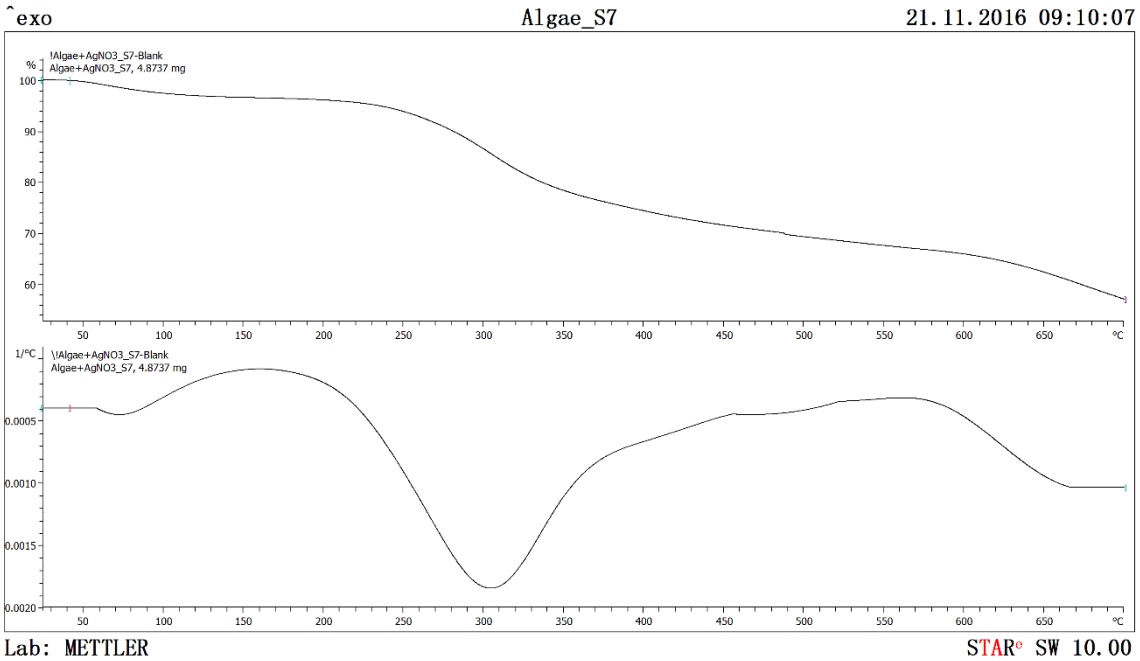

Figure 7: TGA thermogram of *Isochrysis* sp. synthesised silver nanoparticles

Silver nanoparticles are well known for their antibacterial effects. Table (2) shows the antibacterial activity of AgNPs against *S. aureus* and *E. coli*. As predicted, maximum zones of inhibition were observed in the antibiotic conjugated silver nanoparticles. A maximum antimicrobial activity was observed against *E. coli* with 15 mm inhibition zone. About 10 mm of inhibition zone was observed against *S. aureus*. Although only two types of bacterial strain used in this study, the synthesised AgNPs were able to be used against gram-positive and gram-negative bacteria. Whereas, Sriram and Pandidurai (2014) demonstrated that AgNPs synthesised from *Psidium guajava* was able to inhibit the growth of *S. aureus* and *E. coli*. Gnanajobitha *et al.* (2013) verified that

biosynthesised AgNPs from *Vitis vinifera* was able to inhibit the growth of *Bacillus subtilis* and *Klebsiella planticola*.

Moreover, Pak *et al.* (2016) validated that biologically synthesised AgNPs exhibited greater antimicrobial activity when compared with chemically synthesised AgNPs. The small size of biosynthesised nanoparticles and the presence of bioactive compound capping may contribute for enhanced activity of bio-nanoparticles. AgNPs were able to inhibit the growth of *Bacillus subtilis* and *Klebsiella planticola* (Gnanajobitha *et al*., 2013

Table 2: Antibacterial activity of biosynthesised silver nanoparticles

Bacterial Strain	Inhibition zones in mm	
	Streptomycin	Streptomycin + AgNPs
Staphylococcus aureus	6.0 ± 0.5	10.5 ± 0.5
Escherichia coli	8.0 ± 0.5	15.5 ± 0.5

4.0 CONCLUSION

In the present study, microalga *Isochrysis* sp. was used to synthesise AgNPs at room temperature. The preparation method was a simple and environmentally friendly approach. Additionally, the biosynthesised AgNPs can be used in medical and other applications. The current study demonstrated that *Isochrysis* sp. reduced silver nanoparticles were capable of inhibiting bacterial growth and showed antioxidant potential as well. On top of that, studies on *Isochrysis* sp. synthesised AgNPs will give a new insight for a new range of antibacterial and antioxidant agents. Further research is required to identify the potential of microalga derived AgNPs and compounds responsible to synthesise AgNPs from microalga. Therefore, optimisation of biosynthesis of AgNPs can be highlighted in future.

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