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Research paper

Ayurvedic Mediated Green Synthesis of Gold and Silver Nanoparticles from Marine Microalgae *Isochrysis sp.*

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Abstract

Ayurveda is an Indian traditional medicinal system. Yet Ayurveda remains in living tradition. There has been an increased global interest in traditional medicine systems. Asavas is a novel yet less exploited hydro extraction method in Ayurveda. As green synthesis of metal nanoparticles is widely under exploration in the current research world, synthesis of gold and silver nanoparticles by employing the Ayurveda method using marine microalgae is tested in this research. The characterization of metal nanoparticles was confirmed by UV-Visible Spectroscopy, field emission scanning electron microscopy (FESEM), and Fourier transform infrared spectroscopy (FT-IR). Through the Arishtas method, gold and silver nanoparticles were successfully isolated from *Isochrysis* sp. The synthesized nanoparticles exhibit excellent antioxidant and antimicrobial activities.

Keywords: *Ayurvedic, Nanoparticles, Microalgae, Isochrysis, Arishtas*

1. Introduction

Many scientists consider Ayurveda as the oldest healing science. In the Tamil language, Ayurveda means "The Science of Life" (Ayur/l = life, Veda = science of knowledge). Ayurveda emphasise on the use of vegetation as drugs. The knowledge of Ayurveda originates from India more than 5,000 years ago. Around 1,200 species of plants, 100 minerals and over 100 animal products make up the Ayurvedic Pharmacopoeia [1]. Ayurveda describes the existence of eight components of medical sciences. General medicine, the treatment for children, surgical techniques, treatment of ailments, pacification of possessing spirits, toxicology, tonics for increasing lifespan and aphrodisiacs are of those eight components branched in Ayurvedic medication

Kashayam is the water extract of a group of herbs used in Ayurvedic medication. Kashayam which is also identified as a herbal decoction is prepared by the boiling of herbs for several hours within the right intervals of time [2]. The final product is named as Arishtas. Arishtas is processed further until it is consumed directly by the patient. Currently there are 38 types of arishtas products commercialized and used for the treatment of illnesses in paediatric, nervous, and blood and circulatory system as well as many more.

In the present work, we report the synthesis of silver and gold nanoparticles using the Ayurvedic method. Kashayam preparation method was employed prior to synthesis of silver and gold nanoparticles using marine microalgae *Isochrysis* sp. Kashayam extraction method is used to extract marine microalgae and further reduce Silver Nitrate ($AgNO_3$) and Chloroauric acid ($HAuCl_4$) into silver and gold nanoparticles which seems to be the first report to best of our knowledge. Synthesis of metal nanoparticles from readily available biological substances along with eco-friendly methods allow for the development of novel eco-friendly and cost-effective procedures. Synthesized silver and gold nanoparticles exhibits good antioxidant and anti-bacterial effects.

2. Materials and Methods

2.1 Preparation of Microalgae Kashayam:

Two-week old Isochrysis sp. culture maintained at Bioprocess Laboratory, Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang was used for biosynthesis of nanoparticles. About 1,000 ml of microalgae culture was centrifuged at 8,000 rpm. Supernatant was discarded and the pallet were washed two times. Microalgal pallet was later diluted using distilled water. As per the Kashayam preparation method, the solute was further diluted using distilled water with the ratio of 1:5. The volume of microalgal solution was then brought down to its original quantity by boiling in mild heat. Boiled arishtas was cooled to room temperature before use.

2.2 Synthesis of Gold and Silver Nanoparticles:

About 20 ml of the aqueous arishtas of Isochrysis sp. was added to 80 ml of HAuCl4·3H2O (10-4) solution. For the synthesis of silver nanoparticles about 30 ml of microalgal arishtas was added to 170 ml of 1 mM AgNO3 (10-4) solution. Both mixtures were stirred for 30 minutes in room temperature. At the same time, microalgal arishtas which was mixed with water, was set as a positive control while silver nitrate and chloroauric acid were maintained under same conditions as a negative control [3].

2.3 Characterization of Metal Nanoparticles:

The formation of ruby pink and light brown color was the indication of gold and silver nanoparticles formation respectively. Both reduction could be observed within 30 minutes in room temperature. UV-vis spectrophotometer analysis, at wavelength of 300 to 800 nm was measured. The morphology of synthesized gold and silver nanoparticles was characterized using Field Emission Scanning Electron Microscope (FESEM) with the optimum voltage at 10 kV. Energy-dispersive X-ray spectroscopy (EDX) analysis was conducted to confirm the elemental composition of synthesized gold and silver nanoparticles. Further, fourier transformation infrared spectroscopy (FTIR) spectrum was recorded using PERKIN Elmer model at the resolution of 1 cm-1 in the range of 4000 to 400 cm-1.

2.4 Antibacterial Activity of Gold and Silver Nanoparticles:

Staphylococcus aureus, Escherichia coli and Bacillus subtilis are three bacterial cultures used for the antibacterial assay. Antibacterial activity of synthesized metal nanoparticles was performed by disc diffusion assay. The zone of inhibition was measured after 24 hours of incubation. Ampicillin was used as positive control.

3. Results and Discussion

In this study microalgae extraction was conducted through the Ayurvedic method. Therefore, hazardous solvents which are usually used in conventional extraction process were eliminated. Still, the Arishtas of microalgae was able to synthesise gold and silver nanoparticles successfully. So, the Kashayam preparation method was proven to be one of the best green methods to prepare plant extract. In modern theory, traditional Kashayam preparation method was referred as water extraction method.

Although water extraction was established long ago in traditional medicine and essential oil extraction from plants, conventional methods using solvents were chosen due to their compound specific nature. Water extraction also ensures that purification of products are taking place naturally. Unlike conventional solvents, once cooled the organic products will remain stable and are not soluble at ambient temperature water. This ensures faster startup and simplifies process steps. Solvent-matrix interaction was able to be influenced through higher temperature of extraction, solubility and diffusion. Higher temperature and pressure increases water diffusivity and decreases the dielectric constant of water. Thus, this condition will solubilize more non-polar molecules [4].

Kashayam extraction method is a noble synthesis method which was not only environmental friendly but it is also ensures cost effective biological synthesis of metal nanoparticles. Thus, the nanoparticles which were synthesized through this method could be used in food, pharmaceuticals and the healthcare industry without any fuss.

The UV-Vis spectra provided evidence that nanoparticles have formed through the redox reaction. Wavelength at 200 – 800 nm is commonly used to characterize various types of nanoparticles in the size range of 2 – 100 nm. The surface plasmon resonance absorption band stimulated by free electrons which was released by metal nanoparticles during the redox reaction [5]. Figure 1 and Figure 2 shows the UV-Vis spectra recorded at the peak position between 520 to 540 nm regions for gold nanoparticles and 410 to 420 nm regions for silver nanoparticles respectively. The formation of silver oxide (Ag2O) is the primary reaction and was further reduced into silver nanoparticles (AgNPs). OH-group that was present in polyphenols reacted with Ag+ to produce AgOH. The highly unstable AgOH was oxidized to Ag2O and further reduced to Ag nanoparticles.

Ag2O decomposition in alkaline aqueous/ water-enriched environments at room temperature enabled the formation of AgNPs and O2. The same article indicating that thermal decomposition of Ag2O colloids in water enriched environment offers the possibility to produce silver nanoparticles at low cost, clean, safe and green chemistry procedures Therefore, the traditional water extraction method (Kashayam preparation) is evidently a reliable method to produce AgNPs from biological materials.

Higher density of polydispersed spherical gold nanoparticles (AuNPs) was synthesized through the reaction. The sizes were estimated from 50 to 150 nm (Figure 3). There was a small percentage of rectangle shaped structures observed as well. In the meantime, almost spherical type of AgNPs with average size ranges from 98 nm to 190 nm was synthesized (Figure 4).

While AgNPs were monodipersed, AuNPs were aggregated and a few of the particles were scattered as observed under FESEM. Indirect contact of monodispersed AgNPs were derived from capping agents. Capping ligands could be a carbonyl group, an aromatic compound, alkanes or amine. Agglomeration of nanoparticles were prevented through bio-capping molecules [6].

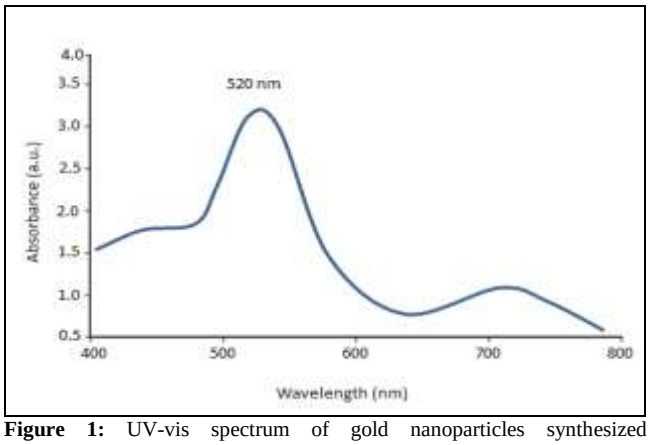

Figure 1: UV-vis spectrum of gold nanoparticles synthesized through Arishtas extracted from Isochrysis sp. mixed with $HAuCl4·3H_2O$ (10^{-4}) solution.

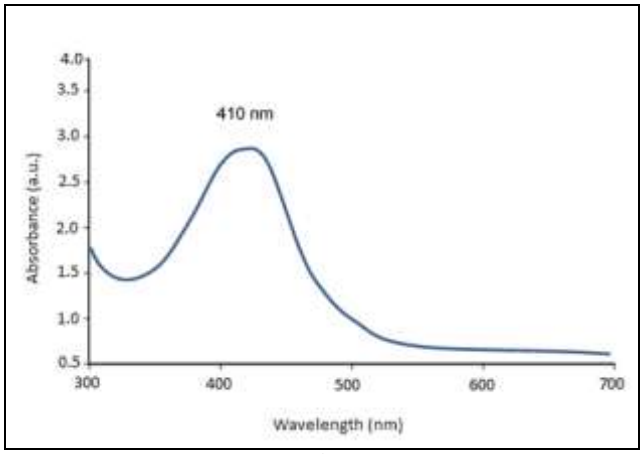

Figure 2: UV-vis spectrum of silver nanoparticles synthesized through Arishtas extracted from Isochrysis sp. mixed with 1 mM $AgNO_3$ (10^{-4}) solution.

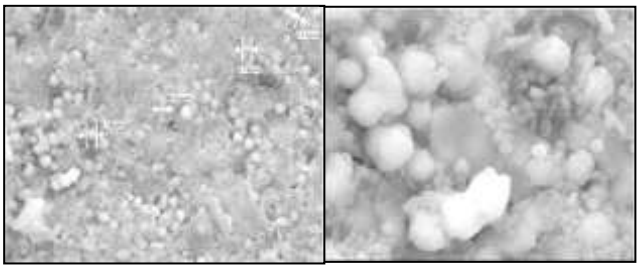

Figure 3: FESEM image of AuNPs.

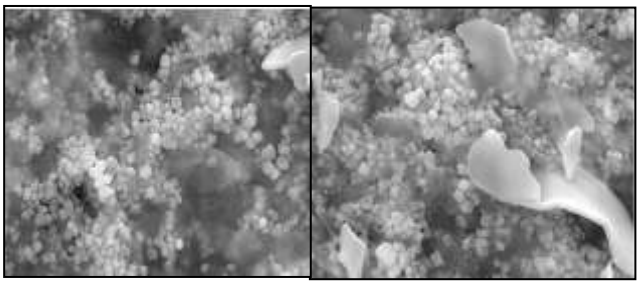

Figure 4: FESEM image of AgNPs.

Strong peaks of Au and Ag were present as per Figure 5 and Figure 6, EDX test has confirmed the presence of AuNPs and AgNPs as the major elements after redox reaction. The presence of other elements are the indication of the existence of secondary metabolites in final product.

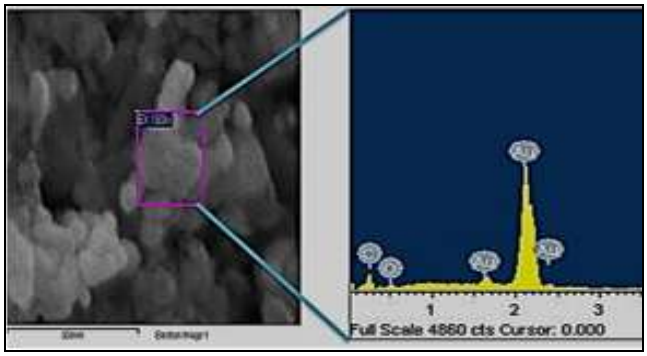

Figure 5: Presence of AuNPs

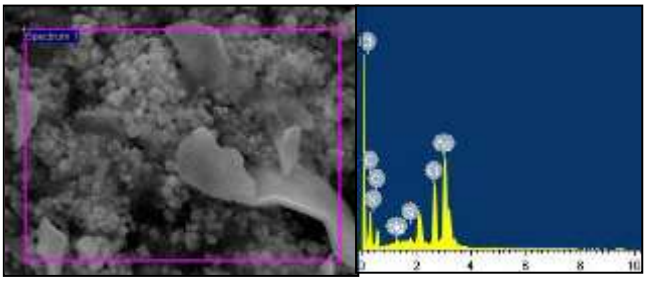

Figure 6: Presence of AgNPs

Different functional groups involved in the metal nanoparticles were investigated through FT-IR spectra. Strong absorbance band indicates N-H stretch–amides, alcohols; C=O stretch, amides; N-H bends, amides; and C-N stretch, aliphatic amines are present in the synthesis of gold nanoparticles. The FT-IR for gold synthesis concluded that amide, phenolic acids, sugar moieties and aliphatic amines predominantly contributied to the bioreduction of HAuCl4·3H2O (10-4) solution into gold nanoparticles (Figure 7).

Figure 7: FT-IR characterization AuNPs.

In the meantime, C-H alkene band; esters; C=O stretching carbonyls; O-H stretching phenols; N-H stretching primary and secondary amines and amides; C-H stretching aldehyde, C-H stretching alkene; aromatics N-H amine were predicted from silver nanoparticles (Figure 8). The carbonyl group (C=O) was synthesized through alcohol group (C-OH) during the reduction of silver nanoparticles derived from AgNO3 solution. The following equation Ag+ + R-OH → R=O + Ag + H+ was derived from the reaction equation between the microalgae extract and silver ions.

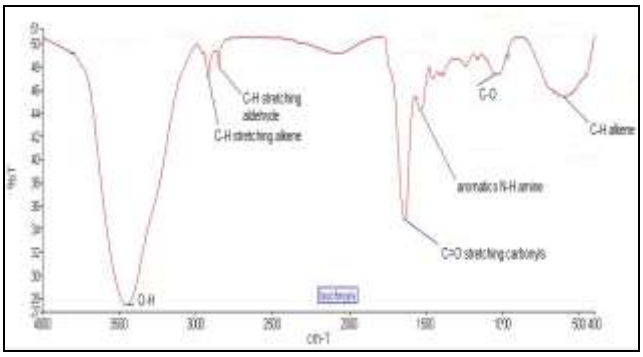

Figure 7: FT-IR characterization AgNPs.

Metal nanoparticles are known for their antibacterial potential. Table 1 shows the inhibition zones formed through the antibacterial activity of Au and Ag-NPs against Staphylococcus aureus, Escherichia coli and Bacillus subtilis. Thin cell wall made from peptidoglycan of Gram negative bacteria allow the optimal antibacterial effects to take place when tested against both Au and Ag-NPs. The antibacterial effects of metal nanoparticles are highly related to the formation of dissolved cations of Ag+ and Au+. Cations interact with thiol groups and proteins of bacterial cell walls while further deactivating enzymes [7]. Although both metal NPs are capable of possessing antibacterial effects, gold nanoparticles have limited antibacterial potential. Results indicating that silver nanoparticles are more bactericidal than gold nanoparticles [8, 9].

Table 1: Inhibition measurement of Ag and Au-NPs against bacterial strains

AgNPs Inhibition Zone (mm)			
Bacteria	20mg/ml	40mg/ml	60mg/ml
Staphylococcus aureus	6.3	7.2	7.9
Escherichia coli	8.3	8.9	9.2
Bacillus subtilis	6.5	7.6	8.0
AuNPs Inhibition Zone (mm)			
Bacteria	20mg/ml	40mg/ml	60mg/ml
Staphylococcus aureus	5.3	5.8	6.2
Escherichia coli	7.0	7.6	7.9
Bacillus subtilis	4.0	4.5	5.1

4. Conclusion

In conclusion, Arishtas obtained from the Kashayam extraction method using marine microalgae Isochrysis sp. was able to reduce HAuCl4 and AgNO3 within 30 minutes. Further the FESEM and EDX characterization has shown that the gold nanoparticles were spherical in shape with polydispersed characteristics. Both metal nanoparticles exhibited good bactericidal activity against tested pathogenic bacteria. The present data suggest that the ancient traditional method is still viable in modern science to produce antibacterial specific metal nanoparticles; Isochrysis sp. is a suitable marine microalgae source for silver and gold nanoparticles synthesis.

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