

Applications of Silver Nanoparticles and Zinc Oxide Nanoparticles from *Spirulina platensis*

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Abstract

In Myanmar, the natural *Spirulina platensis* is produced from the natural lake of Yae Kharr lake. The aim of this research was to achieve silver and zinc oxide nanoparticles from *Spirulina platensis* and its utilizations in biomedical, waste water and balm, some lotions, and face cream for cosmetic products. *Spirulina platensis* (blue green algae) plays a very important role for health food. Silver and zinc oxide nanoparticles from *Spirulina* were obtained by green synthesis. Silver and zinc oxide nanoparticles from *Spirulina* were obtained by calcinations at 60⁰ C and characterized by XRD, SEM, AFM and UV visible spectrophotometer techniques. Average crystallite size of silver and zinc oxide nanoparticles from *Spirulina* was found to be 23.93 nm and 12 nm by using Debye Scherer equation. The antimicrobial activity of silver and zinc oxide nanoparticles against both gram positive bacteria (*Bacillus subtilis*, *Bacillus pulamis*, *Staphylococcus aureus*, and gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and *Candida albicans* fungus strain were done by agar well diffusion method. Among these strains, *Escherichia coli* and *Candida albicans* showed the highest antimicrobial activity of silver and zinc oxide nanoparticles from *Spirulina platensis*. Rhodamine G dye was used as model solution and removal percent of Silver and zinc oxide nanoparticles were found to be 84.58% and 75. 12%. Silver and zinc oxide nanoparticles were applied and made for the formation of face cream, body lotion and balm. It was observed that these products can be used safely for body lotion, balm for skin and face cream because the pH value and microbial testing (total plate and yeast and mold counts) of these cosmetic products were found under acceptable level compared to literature values. The main advantages of zinc oxide nanoparticles coated with fabrics can be used in textile industry due to uniform distribution of nanoparticles on the fabric surface to obtain long elasticity.

Keywords: Antimicrobial activity, Cosmetic products, Microalgae, *Spirulina platensis*, Waste water.

I. Introduction

Spirulina is microalgae and are found in tropical and subtropical areas with high pH. *Spirulina* is one of the most important medical drugs and cosmetic products. It is used as medicine around the world. It contains vitamins especially vitamin A, and vitamin C. The natural spirulina is also produced from the natural lake of Yae Kharr which is located between Sagaing Hill and Min Wun Hill in Sagaing Region of central part of Myanmar. Yae Kharr Lake produces natural spirulina used in the production of medicines and consumer goods. *Spirulina* is a blue-green microalgae which grows in alkaline water. It is highly nutritious and actually a total food for human nutrition. *Spirulina* is a blue-green microalgae which grows in alkaline water. It is highly nutritious and actually a total food for human nutrition. Human can survive with spirulina and water without taking any other food (Ghaeni and Roomiani 2016). *Spirulina* can be found in the volcanic crater lakes and the natural lakes, having high pH level. *Spirulina* makes you healthy, have long life and free from diseases because it contains nearly all vitamins proportionately.

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Green synthesis of nanomaterials

Green Chemistry is the design of chemical products and processes that reduces or eliminates the use and generation of hazardous substances. It prevents waste, contains less hazardous chemical synthesis, and safer solvents. It does not need organic solvents to obtain nanomaterials. Green Nanotechnology makes nanomaterials and their products those cause no harm.

Sample collection of Spirulina Platensis

The samples were collected from Sagaing June Pharmaceutical and Foodstuff Industry Ltd, Yae Khar Lake, Sagaing Region located at North Latitude 22° 02' 57.4" and East Longitude 95° 53' 17.4". Yae Kharr Lake produces spirulina platesis naturally.

II. Materials and methods

Identification of Spirulina Platensis

Botanical identification of spirulina sample was confirmed at the Department of Botany, University of Yangon.

Apparatus

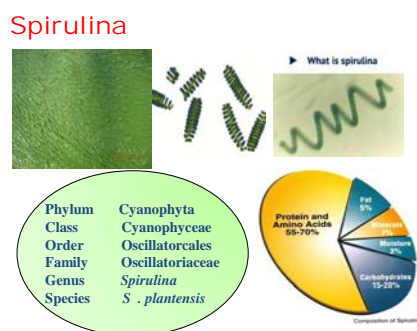
Volumetric flask (100, 50 and 25 mL), measuring cylinder (100 and 10 mL), graduated pipettes (10 and 1 mL), glass rods, glass tubes, micro pipettes, an electric balance (AY 120, Shimadzu, Japan) and UV-visible spectrophotometer (UV-visible spectrophotometer, 1800) were used.

Chemicals

Silver nitrate (BDH), cetyl alcohol, stearic acid, beeswax, olive oil, lanolin, titanium dioxide, triethanolamine, ethanol (HPLC grade), Petroleum jelly 10g, Paraffin wax, Camphor, Menthol, Eucalyptus oil, Peppermint oil, and deionized water, Rhodamine B dye (basic dye, $C_{28}H_{31}ClN_2O_3$; molecular weight: 479.02 g/mol) from BDH, doubled distilled water were used in this work.

Characterization Techniques

The silver and zinc oxide nanoparticles were characterized by using X-ray diffraction (XRD) (Rigaku Multiflex 2kW X ray diffractometer, Japan), Scanning electron microscopy (SEM) (ZEISS, Germany), Fourier transform infrared spectrometer (FT IR) (Perkin Elmer 1600), EDXRF Energy dispersive X ray fluorescence Spectrometer Shimadzu EDX 700) and Double beam Shimadzu UV-Vis spectrophotometer (1800) equipped with 1cm quartz cell and computer, Atomic Force Microscope (AFM) (Bruker), and N8 Rados (Germany).

Figure 1. *Spirulina platensis*Figure 2. Location of *spirulina platensis* in Myanmar

Synthesis and characterization of silver and zinc oxide nanoparticles

Dried powder *Spirulina platensis* (10g) was extracted with deionized water 100 mL and centrifuged for 30 mins and supernatant solution was taken and mixed with 100mL of 1mM silver nitrate and 1 M zinc nitrate solution adjusted to reach pH 7 and shaken and stirred for 30 mins with a magnetic stirrer at 100 rpm at room temperature. This solution was sonicated for one hour and taken the precipitate and kept in the refrigerator at 4°C overnight and kept until dried at room temperature to obtain silver nanoparticles and heated in an oven (Tactical 308, Gallenkamp, England) for 5 hrs at 50°C to obtain zinc oxide nanoparticles (Ali et al., 2015).

Determination of wavelength of maximum absorption of organic dyes solution Rhodamine B dye (10ppm)

A 0.01 g of *Rhodamine B dye* solution was dissolved in deionized water and made up to 100 mL as 10 ppm stock solution. A 10 ppm stock solution was diluted with distilled water to make 10 ppm solution. This instrument was warmed up for 10 minutes prior to use. The UV visible spectrophotometer was firstly adjusted to zero balance value with deionized water as reference solution. The absorbance values of different concentrations of *Rhodamine B dye* solutions (2, 4, 6, 8, and 10 ppm) were measured at different wavelengths ranging from (500-700 nm). The wavelengths of maximum absorption of *Rhodamine B dye* solution were found at 526 nm (Figure 5).

Construction of standard calibration curve for Rhodamine B dye

To obtain 2, 4, 6, 8 and 10 ppm *Rhodamine B dye* solutions 2 mL, 4 mL, 6 mL, 8 mL and 10 mL, respectively of 100 ppm of *Rhodamine B dye* solutions were diluted with 100 mL with deionized water. The absorbance values of different concentrations of *Rhodamine B dye* solution (2, 4, 6, 8, and 10 ppm) were measured at the maximum wavelength (526 nm) by using UV-visible spectrophotometer (1800).

Preparation of body lotion by using silver and zinc oxide nanoparticles

Beeswax (1g) was placed in a 250 mL beaker and heated at 50°C. (2 g) of stearic acid, (10% V) of olive oil and 2 g of cetyl alcohol were added into the mixture and then the mixture was stirred at 80°C for 20 mins. The oil phase was obtained. Then, 5 g of *Spirulina platensis* was dissolved with 100 mL of distilled water and 2g of triethanolamine was added into a 250 mL beaker and stirred for 10 minute until the water phase was obtained. The water phase was added into the oil phase at 80°C. The mixture was continuously stirred and cooled to room temperature. After cooling, (2 mL) of fragrance oil was added into the mixture and stirred by using magnetic stirrer at a rate of 410 rpm. The body lotion was formed and filled into a bottle and then packed. Similarly, silver nanoparticles 0.1 g were used for preparation of body lotion as mentioned above (Sudhahar and Balasubramanian 2013).

Preparation of face cream by using silver and zinc oxide nanoparticles

Beewax (1% wt) was placed in a 250 mL beaker and heated at 50°C. (9 % wt) of stearic acid, (5% wt) of lanolin, (10% V) of olive oil and (0.1% wt) of titanium dioxide were added into the mixture and then the mixture was stirred at 80°C for 20 mins. The oil phase was obtained. Then *Spirulina platensis* 5 g was dissolved in distilled water (60% mL), and (2 mL) of triethanolamine were added into a 250 mL beaker and stirred for 10 mins until the water phase was obtained. The water phase was added into the oil phase at 80°C. The mixture was continuously stirred and cooled to room temperature. After cooling, (2% V) of fragrance oil was added into the mixture and stirred by using magnetic stirrer at the rate of 400 rpm. The nano face cream was filled into a bottle and then packed. Similarly, silver and zinc oxide nanoparticles 0.1 g for face cream was produced as mentioned above.

Preparation of nano balm by using silver and zinc oxide nanoparticles

Petroleum jelly and Paraffin wax are mixed and heated for 10 mins. It is marked as solution A. Camphor, Menthol, Eucalyptus oil and Peppermint oil are mixed and 0.1 g of zinc oxide Nanoparticles was added. It is marked as solution B. Solution A and B were mixed for 15 minutes and kept to solidfy to obtain Nano balm.

Characteristics of body lotion, face cream and nano balm by using silver and zinc oxide nanoparticles

Characteristics of body lotion, face cream and nano balm were done. pH was determined by pH meter. Some metals were measured by the atomic absorption spectrophotometer at

Universities' Research Center. Arsenic was determined by Arsenic Test Kit by Lovibond Tintometer GmbH. Sun Protection Factor (SPF) of Face Cream was measured by Ultraviolet Spectrophotometry. Moisture Content were done by oven method. Total Plate Count, Yeast and Mold Count on body lotion, face cream and nano balm were performed by 3 M petri film method (AOAC).

Antimicrobial test of silver and zinc oxide nanoparticles by using six microorganisms

The strains of *Bacillus subtilis* (N.C.T.C-8236), *Bacillus pumilus* (N.C.I.B- 8982), *Candida albicans*, *Staphylococcus aureus* (N.C.P.C-6371), *Pseudomonas aeruginosa* (N.C.T.C-6749) and *Escherichia coli* (N.C.I.B-8134) from Pharmaceutical Research Department (PRD), Ministry of Industry were used in this work. Each strain was incubated in a temperature controlled shaker (1000 rpm) at 30°C overnight. Antibiotic (amoxicillin) used for the analysis was purchased from Sigma Aldrich, India. 100 µl of 1mg/mL concentration of silver and zinc oxide nanoparticles were used and done by Agar well diffusion method. The plates were incubated for 24 hrs at 37°C. The maximum zone of inhibition (in diameter) was determined (Sudha *et al.*, 2011).

Application of zinc oxide nanoparticles for textile industry

Tensile strength and elongation percentage at break for cotton and polyester fabric samples were measured by using a Shimadzu Universal Tester, Japan. The measurements were done according to standard method for tensile properties of textile (ASTMD1984)

III. Results and discussion

Characterization of silver nanoparticles by UV visible spectroscopy

UV-visible spectroscopy is one of the most widely used techniques for structural characterization of SNPs. Reduction of the silver ion to SNPs during exposure could be monitored by UV visible spectrophotometer. Silver nanoparticles were characterized by UV visible spectrophotometer within the range of 200–800 nm. Figure 3 shows the UV visible spectrum of the nano silver formation and the change in the colour of the reaction mixture to brown, indicating the bio transformation of ionic silver to reduced silver. It is observed that the maximum absorbance is observed at 400nm (Figure 3).

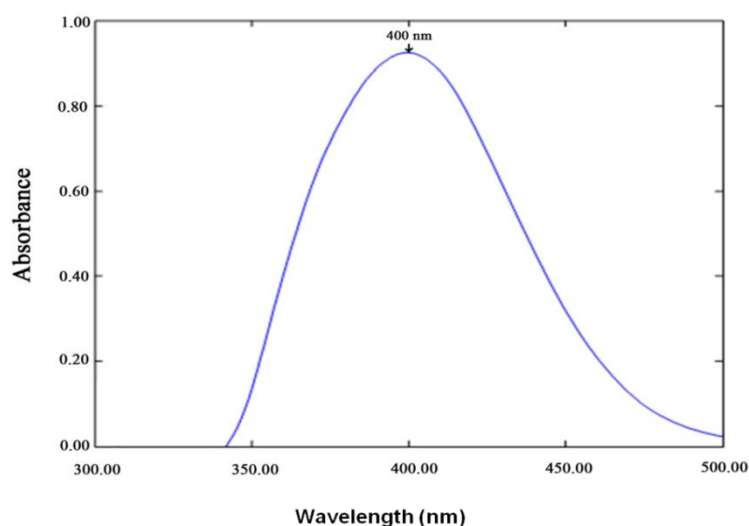


Figure 3. UV spectrum of silver nanoparticles from *Spirulina platensis* at 400 nm

Qualitative Analysis by Energy Dispersive X-ray Fluorescence (EDXRF) Analysis

Energy dispersive X-ray fluorescence (EDXRF) spectrometer can analyse the elements from Al to U under non-vacuum condition. It can be utilized for qualitative identification and quantitative estimation of elements in solid, powder and liquid with appropriate sample preparation techniques. EDXRF spectra showed the presence of K, Ag, P, S, Fe, Zn, Cu for silver nanoparticles and Zn, Al, S, K, Ca, Fe and Cu for zinc oxide nanoparticles and presented in Figures 4 and 5.

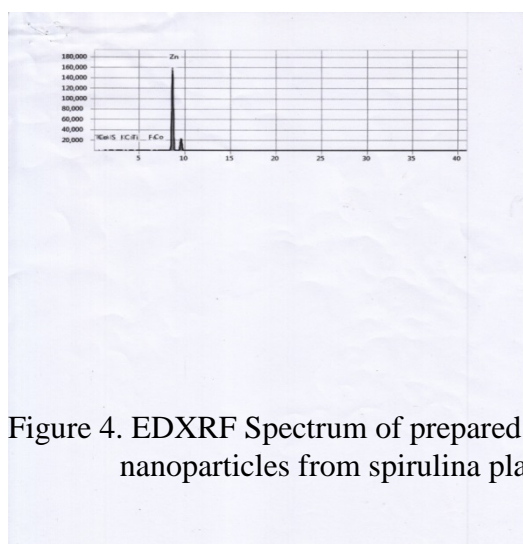


Figure 4. EDXRF Spectrum of prepared silver nanoparticles from spirulina platensis

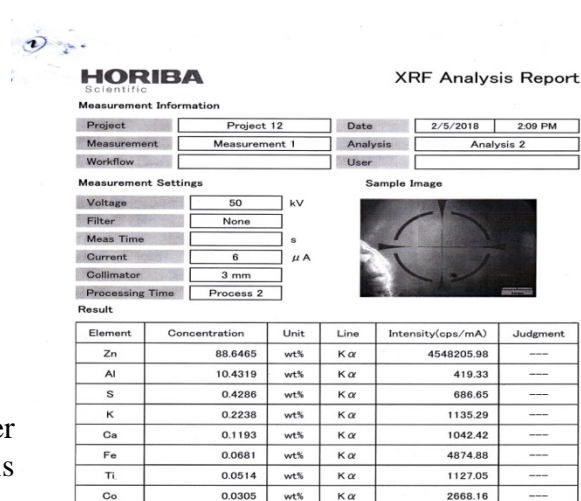


Figure 5. EDXRF Spectrum of prepared zinc oxide nanoparticles from spirulina platensis

X ray Diffraction Analysis

Powder X-ray diffraction is one of the powerful techniques for the characterization of core-shell nanoparticles. It could also be used for calculating mean particle size. It was observed that the sharp peaks of the silver nanoparticles indicated well-defined Miller

indices of (111), (200), and (220). These peaks are well matched with standard library data of (PDS 04-0783) and showed in Figure 6 and 7. The required angle at specific counts was presented and scanned the sample with a start angle at 10 °C and a stop angle at 70°C. From the results obtained, the average crystallite size of the nanoparticles was calculated using Debye Scherrer's formula. The crystal structure of silver nanoparticles was found to be cubic according to lattice parameters (a= b= c= 4.11Å) and two theta values 37.798, 43.96 and 64.120.

$$D = 0.9 \lambda / B \cos \theta$$

Where λ is wavelength of copper K α line (1.546 Å), θ is diffraction angle, B is full width at half maximum of peak (FWHM), and D is the average crystallite size. It was found that the average crystallite size of silver nanoparticles was observed to be 25.30 nm (Figure 6 and 7).

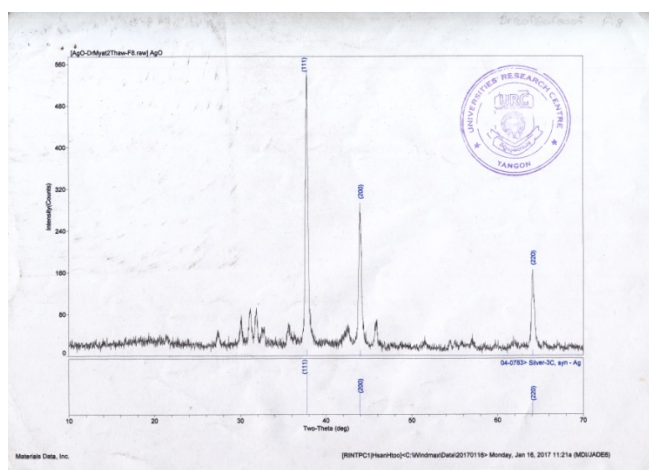


Figure 6. X ray diffractogram of prepared silver nanoparticles from spirulina platensis

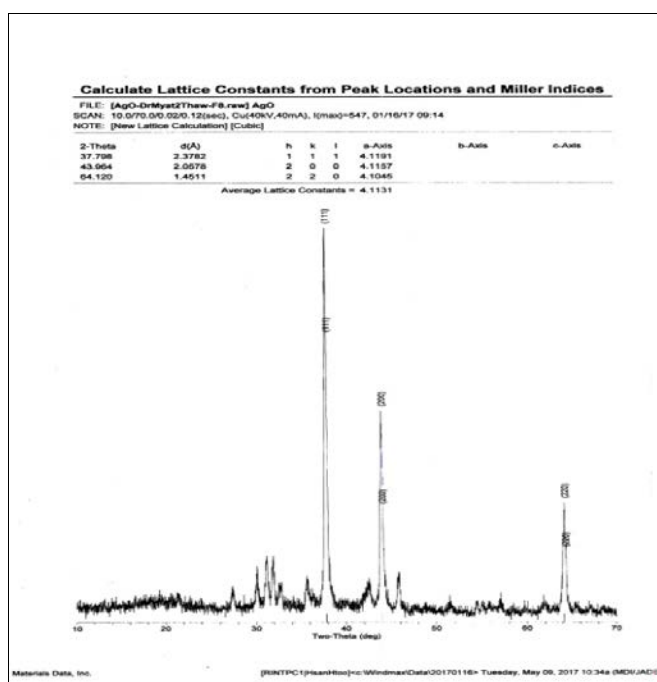


Figure 7. XRD data of silver nanoparticles

The crystal structure of zinc oxide nanoparticles was found to be hexagonal according to lattice parameters ($a= b= 3.2687$, $c = 5.2201 \text{ \AA}$) and the average crystallite size was observed to be 12.30nm. The sharp peaks of the zinc oxide nanoparticles indicated well-defined Miller indices of (100,002,101,102,110,103,200 and 201). These peaks are well matched with standard library data of (PDS 653411).

$$D = 0.9 \lambda / \beta \cos \theta$$

Where λ is wavelength of copper $K\alpha$ line (1.546 \AA), θ is diffraction angle, β is full width at half maximum of peak (FWHM), and D is the average crystallite size. It was found that average crystallite size of zinc oxide nanoparticles was observed to be 12.30 nm (Figure 8).

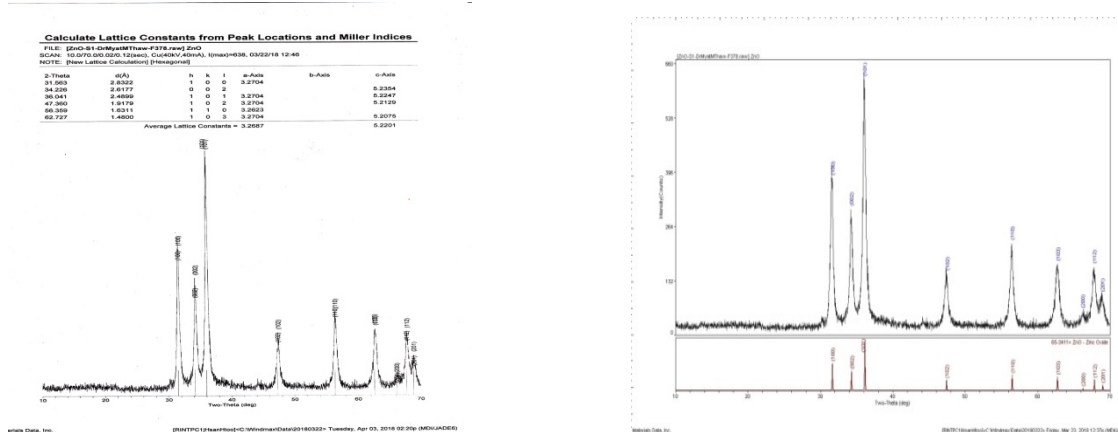


Figure 8. XRD data of zinc oxide nanoparticles

Characterization of silver and zinc oxide nanoparticles by using Scanning Electron Microscope and Atomic Force Microscope

SEM is important to assess the dimensions, morphology of the structures fabricated and the materials prepared nanoparticles when characterizing device structures. The scanning electron microscopy results clearly indicate that the morphologies and particle sizes of silver and zinc oxide nanoparticles were observed spherical shape with its size ranging between 40 nm and 50 nm as shown in Figures 9 and 10.

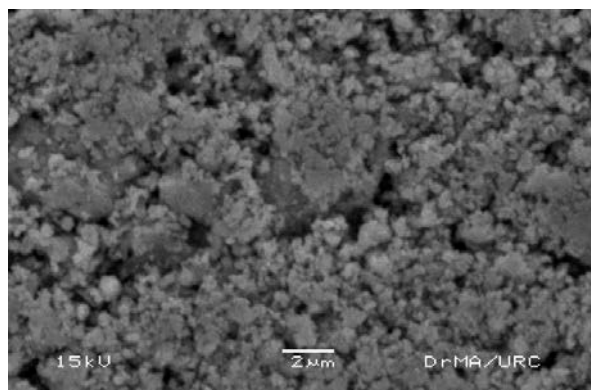
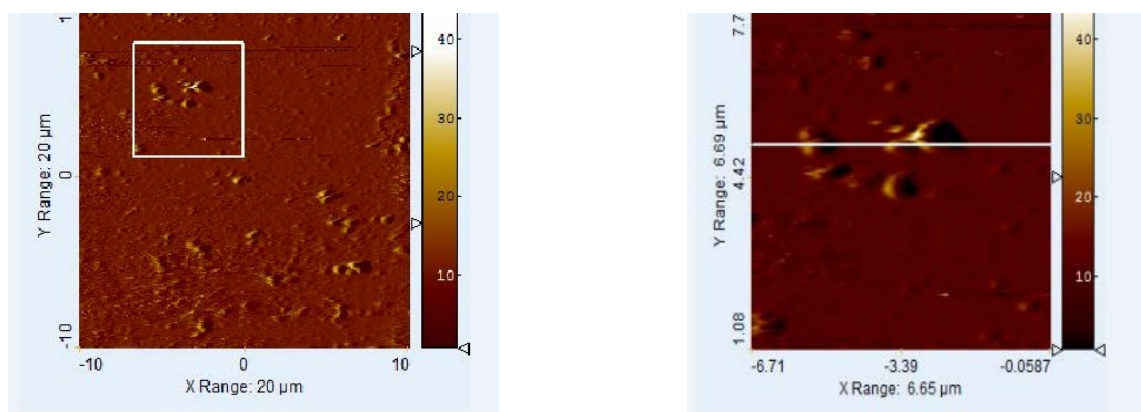
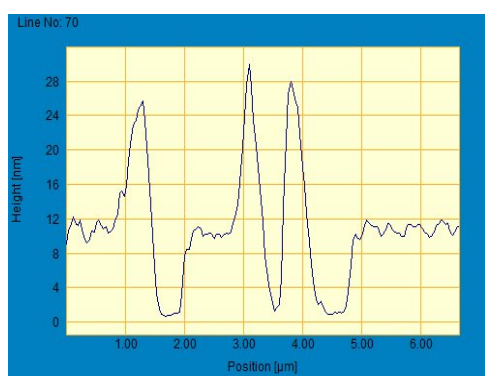


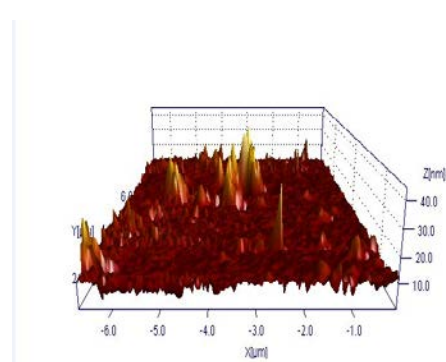
Figure 9. SEM image of zinc oxide nanoparticles



(a) 2 D structure of silver nanoparticles



(b) Particle size of silver nanoparticles



(c) 3 D structure of silver nanoparticles

Figure 10. AFM images of silver nanoparticles

In this work, the particle size of silver nanoparticles was determined by Atomic force microscopy. In the 2 D structure, there are some particles on the substrate. It was assessed that the highest particle size was approximately 30.5 nm by Figure 10 (b). In the 3 D structure, the particle is very small and it was observed that the highest particle size is 30.5 nm. It shows that AFM images showed the silver nanoparticles from spirulina was found the nano range (Figure 10 b and c). Quantitative information regarding individual nanoparticles and groups of particles such as size (length, width, and height), morphology, and surface texture can be evaluated. The size and shape of metal nanoparticles are typically measured by analytical techniques atomic force microscopy (AFM). Unlike SEM and TEM, AFM images presented three-dimensional images so that particle size and height can be assessed (Figure 10 c).

Wavelength of Maximum Absorption of Rhodamine B dye

In this work, the wavelength of maximum absorption of Rhodamine B dye was determined by using UV- visible absorption spectroscopy. The (basic dye) Rhodamine B dye was used as model solutions. The catalytic degradation properties of silver and zinc oxide nanoparticles were measured in organic dye contaminated water. Rhodamine B dye is an intense red color. In the present research, the wavelength of maximum absorption of rhodamine B red was recorded between 300- 800 nm. The maximum wavelength of absorption was found to be 526 nm and shown in Figure 11.

Standard Calibration Curves of Rhodamine B Dyes

Quantitative analysis of a substance was measured by the spectroscopic method. The standard calibration curves for methylene blue and Rhodamine B dye dye were constructed by using different concentrations of **Rhodamine B Dye** solution (2,4,6,8,10 ppm) and were measured and found the absorbance at 526nm (Figure 12). The straight line passes through the origin and shows to obey the Lambert- Beer’s law ($r^2 = 0.999$). Data were shown in Table1 and Figure 12.

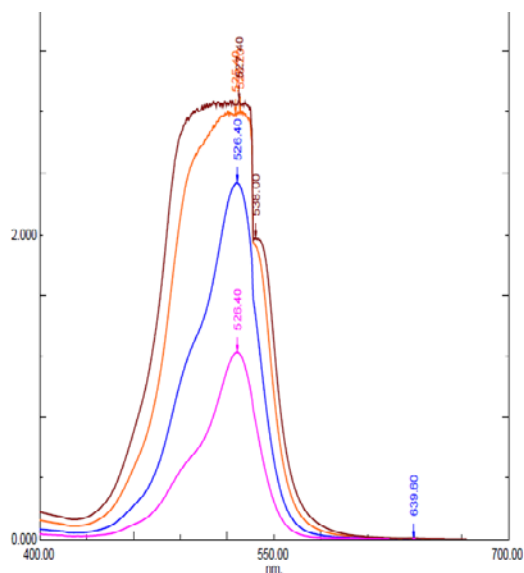


Figure 11. Visible absorption spectra of rhodamine B dye solution at different concentrations

A= 10 ppm, B= 8 pm, C= 6 ppm, D= 4ppm, E= 2 ppm

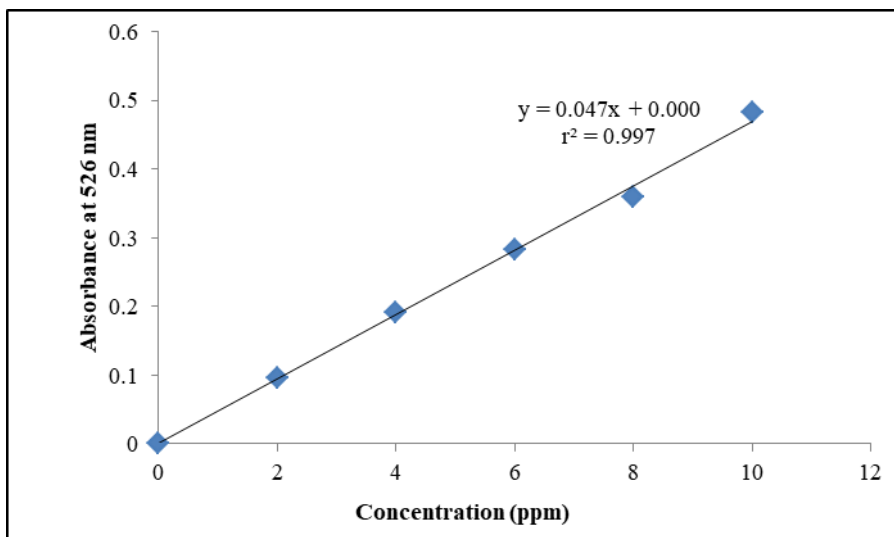


Figure 12. Standard calibration curve for the Rhodamine B dye solution

Table 1.

No	Contact time(min)	Absorbance at 526nm	Removal percent (%)
1	0	0	0
2	15	0.615	56.56
3	30	0.499	64.71
4	60	0.345	75.60
5	120	0.218	84.58
6	180	0.235	83.38

Table 2. Degradation Percent of Rhodamine G Dye by Zinc oxide Nanoparticles from *Spirulina Platensis*

No	Contact time(min)	Absorbance at 526nm	Removal percent (%)
1	0	0	0
2	15	0.905	34.52
3	30	0.804	41.58
4	60	0.558	59.50
5	120	0.345	75.12
6	180	0.350	74.23

Application of silver and zinc oxide nanoparticles for the colour removal of textile dyes by using waste water samples

In this research, waste water samples were obtained from textile dyes factories in South Okakala Industrial Zone. A 50 ml of water sample solution was mixed with 0.1 g of silver and zinc oxide nanoparticles in 250mL volumetric flask. It was stirred and shaken for 2 h. Then, the solutions were filtered by centrifugation. The resultant filtrates were taken and the absorbance of before and after treatment were measured by using UV-visible spectrophotometer. Absorption spectra of the dyes waste water samples were measured by using UV-visible spectrophotometer. Figure 13 showed the dye waste water samples before and after treatment with silver and zinc nanoparticles. It can be seen clearly that the colour of the dye solutions decreased significantly after treatment with silver and zinc oxide nanoparticles. It was found that absorbance of these sample solutions decreased significantly after treatment with silver and zinc oxide nanoparticles and shown in Figures 13.

Antimicrobial activity of silver and zinc oxide nano nanoparticles by using six microorganisms

The antimicrobial activities of silver and zinc oxide nanoparticles were tested by using six microorganisms. It was found that silver nanoparticles showed a better characteristic inhibition zone of zinc oxide nano particles. Among these strains, it was observed that *E coli* and *Candida albicans* are found the higher inhibition zone as shown in Table 5.

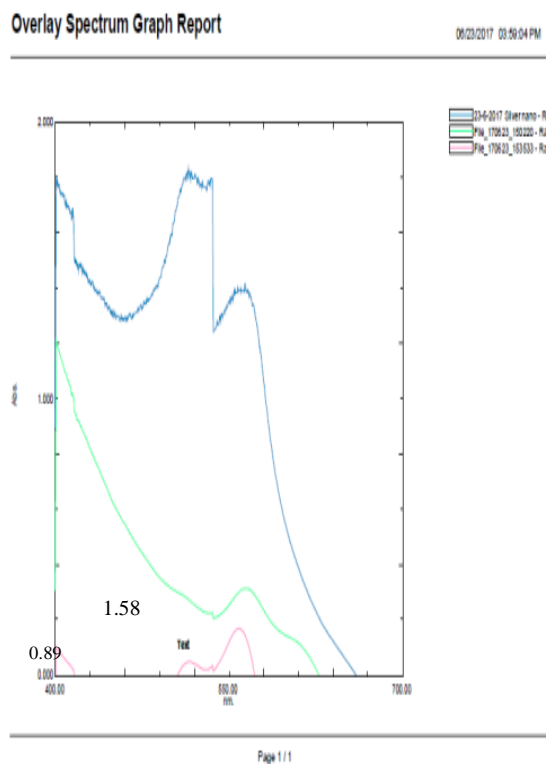


Figure 13. Photograph of dye waste water sample II (a) before treatment, (b) after treatment with silver and zinc oxide nanoparticles for 2 h

Application of silver and zinc oxide nanoparticles for body lotion, face cream and nano balm

The most favorable condition for the ingredients of prepared silver nano body lotion, zinc oxide face cream and nano balm were successfully achieved in this work. Quality and hazard of characteristics of zinc oxide and silver nano body lotion, face cream and nano balm were investigated by determining pH, moisture, total plate count, yeast and mold, SPF, lead and arsenic. The prepared silver and zinc oxide nanoparticles and commercial products are shown in Table 3 and 4. It can be observed that the SPF values found in the prepared silver and zinc oxide nanoparticles are lower than labeled SPF in the commercial product. These data variations can be due to the various reasons like the type of emulsion used for the formulations, and the emulsion properties. There is no irritation according to dermatological test.

Table 6. Tensile strength (kg/force) of zinc oxide nanoparticles

ZnO nano(%)	Polyester		Cotton	
	Warf	Weft	Warf	Weft
Blank	41.08	38.90	19.23	11.78
0.5	57.34	48.60	45.79	33.60
1	60.21	51.42	55.02	49.08

Table 7. Elongation % of zinc oxide nanoparticles

ZnO nano(%)	Polyester		Cotton	
	Warf	Weft	Warf	Weft
Blank	5.80	4.90	3.53	2.78
0.5	9.50	8.60	8.79	7.60
1	10.21	9.42	8.02	5.08

IV. Conclusion

In this work, silver and zinc oxide nanoparticles were obtained by using green synthesis. *Spirulina platensis* was used as starting materials. Green synthesis is found to be environmental friendly, simple and low cost effective technique. The average crystallite size of silver and zinc oxide nanoparticles was found to be 23.59 nm and 12 nm by using Debye Scherrer equation. In this study, degradation percents of Rhodamine B dye were investigated by using silver and zinc oxide nanoparticles and were found to be 83.38 % and 74.23 %. Silver and zinc oxide nanoparticles were applied for the removal of textile dyes by using waste water samples. It can be clearly presented that colour of the textile dyes solution decreased significantly after treatment with silver and zinc oxide nanoparticles for 2 h. Preparation of face cream, body lotion and nanobalm by using with silver and zinc oxide nanoparticles from *spirulina platensis* were done for cosmetic applications. Sun protection factor on face cream of zinc oxide and silver nano body lotion was determined and observed to be 14.54 and 8.579. The prepared face cream and body lotion have no skin irritation effect, and lead, free alkali and arsenic are in this product from for 2 months to till now. There is no hazard for human being according to the microbial profiles of all prepared skin lotion. Therefore, microbiological testing is essential to ensure the quality and integrity of the products. In textile industry, zinc oxide nanoparticles can be used to coat in textile fabrics namely, cotton, and polyester by pad dry cure method and the improvement of in mechanical properties was found according to the order: polyester > cotton.

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