BIOSYNTHESIS OF SILVER NANOPARTICLES BY *SPIRULINA PLATENSIS* AND ITS UTILIZATIONS

Myat Myat Thaw[1], Khin Hla Mon[2], Hla Hla Win[3], Ohn Mar Kyi[4],New Nwe Aung[5]

Abstract

In Myanmar, the natural *Spirulina* is produced from the natural lake of Yae Khar lake. The aim of this research was to prepare silver nanoparticles from *Spirulina platensis* and to study its utilizations in biomedical, waste water treatment and some lotions for cosmetic products. Microalgae are microscopic photosynthesis organisms that are found in both marine and fresh environments. *Spirulina platensis* (blue green algae) plays very important role for health food. Silver nanoparticles were prepared by green synthesis of silver nitrate with spirulina at 50˚C for 3 h and characterized by XRD, SEM, AFM, UV visible spectrophotometer and FTIR techniques. Average crystallite size of silver nanoparticles from spirulina were found to be 23.93 nm by using Debye-Scherrer equation. The antimicrobial activity of silver nanoparticles against both gram positive bacteria (*Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus and* gram negative bacteria *(Escherichia coli and Pseudomonas aeruginosa)* and *Candida albicans* , a fungus strain was done by agar well diffusion method. Among these strains, silver nanoparticles from *Spirulina platensis* on *Escherichia coli* and *Candida albicans* showed the highest antimicrobial activity. Silver nanoparticles were applied for the removal of textile dyes in waste water samples. Utilizations of *Spirulina platensis* and silver nanoparticles were performed for the formulation of face cream and body lotion and their sun protection factors (SPF) were observed to be 11.34 and 8.57. It was observed that these products can be used safely for face cream and body lotion because of the pH value and their characteristics of microbiological parameters (total plate count and yeast and mold count) for the cosmetic products compared with commercial products.

Keywords: Microalgae, *Spirulina platensis*, antimicrobial activity, cosmetic products, waste water

[1] Professor, Dr, Department of Chemistry, University of Yangon
[2] Professor and Head, Dr, Department of Industrial Chemistry, Dagon University
[3] Lecturer, Dr, Department of Chemistry, University of Yangon
[4] Assistant Lecturer, Department of Industrial Chemistry, Dagon University
[5] Lecturer, Dr, Department of Botany, University of Yangon

Introduction

Spirulina is microalgae and found in tropical and subtropical areas at high pH. Spirulina is one of the most important source of medical drugs and cosmetic products as well. 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 Khar which is located between Sagaing Hill and Min Wun Hill in Sagaing Region of central part of Myanmar. Yae Khar lake produces natural spirulina which is used in the production of medicines and consumer goods. Spirulina is a blue-green microalgae 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). The spirulina can be found in the volcanic crater lakes and the natural lakes, having high pH level. Spirulina makes healthy, long life and free from diseases because nearly all vitamins are proportionately present in it.

Green Synthesis

Green chemistry is the designof chemical products and processes that reduce or eliminate the use and/or generation of hazardous substances. Green chemistry can also be described assustainable chemistry. This work aims to focus on development of more convenient methods by using green synthesis for production of eco-friendly, non toxic, and environmental nanoparticles (Mahdieh *et al*., 2012). Green synthesis methods include biological synthesis of nanoparticles by microorganisms and plants, irradiation, polysaccharide. Eco friendly bio-organics in plants extract contain proteins, which act as both reducing and capping agents forming stable and shape controlled silver nanoparticles. The green synthesis of silver nanoparticles involves three main criteria, which must be evaluated based on the green chemistry perspectives, including (1) selection of solvent medium (2) selection of environmentally reducing agent, and (3) selection of nontoxic substances for the silver nanoparticles stability (Mahdavi *et al*., 2013).

Cosmetics

Cosmetics include skin care creams, lotion, powders, perfumes, lipsticks, hair colours, hair sprays and gels, deodorants, hand sanitizers, baby products, bath oils, bubble baths, bath salts, shower cream and many other

types of products. Lotion is applied to external skin with bare hands, a brush, a clean cloth, cotton wool or gauze. While lotion may be used as a medicine delivery system, many lotions, especially hand lotions and body lotions are meant instead to simplify smooth, moisture and soften the skin. These may be used in anti-aging lotions, which can also be classified as a cosmetic in many cases and may contain fragrances. Face cream is applied to the skin to provide a smooth emollient base before the application of face powder and other make-up preparations. The original foundation creams were known as vanishing cream so called because they disappear when rubbed into the skin (Sathish *et al*., 2012).

They are based on stearic acid which is partially saponified with alkali, when the bulk of the acid is emulsified with the soap thus formed. The functions of lotion and cream are to protect the skin against harshness from the environment and any dry conditions of the skin. Manufacturing lotion and face cream can be completed in two cycles: (1) emollient and lubricants are dispersed in oil with blending and thickening agents and (2) perfume, colour and preservatives are dispersed in the water cycle. Active ingredients are broken up in both cycles depending on the raw materials involved and the desired properties of the lotion or cream.

Materials And Methods

Sample Collection of *Spirulina platensis*

The samples were collected from Sagaing June Pharmaceutical and Foodstuff Industry Ltd, Yae Khar Inn, Sagaing Region located at North Latitude 22˚ 02' 57.4" and East Longitude 95˚ 53'17.4". Yae Khar lake produces *Spirulina platesis* naturally (Figure 1).

All experiments and measurements were carried out at the Department of Chemistry, University of Yangon. *In vitro* screening of antibacterial activities of silver nanoparticles from *Spirulina platensis* were carried out by agar well diffusion method against six microorganisms (*Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* fungus strain at Pharmaceutical Research Department (PRD), Ministry of Industry, Yangon, Myanmar.

Chemicals

Silver nitrate (BDH), cetyl alcohol, stearic acid, beeswax, olive oil, lanolin, titanium dioxide, triethanolamine, ethanol (HPLC grade) and deionized water were used.

Instruments used in the Characterization of Silver Nanoparticles from *Spirulina platensis*

The silver 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), N8 Rados (Germany).

Instruments used in the Determination of COD in Waste Water by using Silver Nanoparticles

Lovibond Photometer System MD 200 (Lovibond Water Testing) (Germany) was used to determine Chemical Oxygen Demand (COD).

Apparatus used in the Characterization of Silver Nano Body Lotion and Face Cream

Arsenic Test Kit by Lovibond Tintometer GmbH (Germany), Autoclave, Stomacher (Homogenizer), Colony counter (magnifier-illuminator), 3 M petrifilm aerobic count plate, 3 M yeast and mold count plate, Incubator, and Bunsen burner were used in this work**.** Incubator, and Bunsen burner.

Identification of *Spirulina platensis*

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

Ye Kharr Lake

Figure 1 Location of *Spirylina platensis* in Myanmar

Synthesis and Characterization of Silver Nanoparticles

Dried powder *Spirulina platensis* (5 g) was extracted in 100 mL of deionized water in 250 mL beaker and mixed with 100mL of 1mM silver nitrate solution and adjusted to reach pH 7 and shaken and stirred for 30 minutes in a magnetic stirrer at 100 rpm at room temperature. Supernatant solution was removed and the pellet of this solution was taken and it was concentrated and heated in an oven (Tactical 308, Gallenkamp, England) for 3 hours at 50˚C (Ahmed et al.,2015).

Characterization of Silver Nanoparticles

The synthesized silver nanoparticles were characterized by UV visible spectrophotometer, XRD, SEM, AFM and FT IR analysis for detecting size, structure, shape and morphology.

UV Visible Spectrophotometry

0.01g silver nanoparticles was dissolved in deionized water and mixed with small amount of ethanol and added into the quartz cell and measure the wavelength of silver nanoparticles by UV visible spectrophotometer. The reduction of pure silver ions was recorded by measuring the UV–visible spectrum of the solution at room temperature with a Perkin Elmer Lambda 1234 UV–visible spectrophotometer at the wavelength of 200–800 nm.

Determination of Particle Size of Silver Nanoparticles from *Spirulina platensis* by Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM)

1mg of silver nanoparticles were dissolved in 90% ethanol and dispersed in a sonicator for 30 minutes and put in glass plate and measured the particles size of silver nanoparticles by AFM and SEM.

Antimicrobial Test by using Microorganisms for the Analysis

The *strains* of *Bacillus subtilis*(N.C.T.C-8236), *Bacillus pumilus* (N.C.I.B- 8982), *Candia 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. Nutrient agar was prepared to culture theses microorganisms. Some plates for each organism were inoculated and four wells (10 mm diameter) were made by using cork borer. First well was made with 100 µL of distilled water. Second well was made with 100 µl of 1mg/mL concentration of silver nanoparticles. Third well was done with 100 µl of 1mg/mL of reference antibiotics (Amoxicillin).The fourth well was done with 100 µL of silver nanoparticles with equal amounts of antibiotics (0.5mg/mL). The plates were incubated for 24 h at 37˚C. The maximum zones of inhibition (in diameter) was determined (Sudha *et al*., 2011).

Determination of Chemical Oxygen Demand in Waste Water

Deionized water (2 mL) was used as a blank. 2 mL of waste water sample was taken and 0.1 g of silver nanoparticles was added into the tubes and mixed with COD reagents and closed tightly and mixed until became hot during mixing for several times and digested these tubes and heated in the reactor for 120 minutes at a temperature of 150˚C .The vials containing the sample tubes were removed from the reactor and allowed to cool down to 60˚C . Then the contents were mixed by inverting each vials for several times before

measuring. Then COD concentration was measured in waste water samples by Lovibond Photometer System MD 200 (Lovibond Water Testing)(Germany).

Preparation of Body Lotion on *Spirulina platensis* and Silver Nanoparticles

Beeswax (1g) was placed in a 250 mL beaker and heated at 50˚C. (2 g) of stearic acid, (10% V/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 minutes. The oil phase was obtained. Then, *Spirulina platensis* **(**5 g) was dissolved with 100 mL of distilled water and 2g of triethanolamine was added into a 250 mL beaker and stirred for 10 minutes. 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.

Preparation of Face Cream on *Spirulina platensis* and silver nanoparticles

Beeswax (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/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 minutes. 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 minutes 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/V) of fragrance oil was added into the mixture and stirred by using magnetic stirrer at the rate of 400 rpm. The spirulina face cream was filled into a bottle and then packed. Similarly, silver nanoparticles (0.1 g) for face cream was carried out as mentioned above.

Characteristics Properties of Prepared Body Lotion and FaceCream

Determination of pH

The pH of prepared body lotion and face cream were determined by using digital pH meter (Pen Type pH meter, Range: 0.0-14.0). The glass electrode was first standardized by using standard buffer solution of pH 4 and 7 and the electrode was adjusted to that value. Then, the values of prepared sample were measured with pH meter.

Determination of Some Metals

Ash sample (0.1)g was placed in a 250 mL beaker and 8 mL of concentrated nitric acid were slowly added. The solution was evaporated to dryness and the residue, after cooling, was dissolved in 6 mL of 25 % (v/v) nitric acid solution. The solution was transferred to a 100 mL volumetric flask and the volume was made up to the mark with distilled water. Lead and Arsenic content of these elements in the body and face lotion were determined by the atomic absorption spectrophotometer at Universities' Research Center.

Determination of Arsenic Test Kit by Lovibond Tintometer GmbH

Body lotion and face cream 10 % solution was made by using deionized water and one drops of arsenic reagent (1) , 2 mL of arsenic reagent (2) and 2 mL of arsenic reagent (3) were added in the reagent bottle and kept for 20 min and then arsenic test strips were kept on the reagent and grapped the gas. It did not show the yellow colour. It indicated the absence of arsenic content (Table 4).

Determination of Sun Protection Factor (SPF) of Face Cream by Ultraviolet Spectrophotometry

Sample Preparation for Determination of Sun Protection Factor

0.1g of sample was weighed, transferred to a 100 mL volumetric flask, diluted to volume with ethanol, followed by ultrasonication for 10 min and then filtered through Whatman No1 filter paper and collect the filtrate by rejecting the ten first mL of the filtrate. A 5.0 mL aliquot was taken to 50 mL

volumetric flask and diluted to volume with ethanol. Then a 5.0 mL of the diluted solution was transferred to a 25 mL volumetric flask and the volume made up with ethanol. The absorption spectra of samples in solution were obtained in the range of 290 to 320 nm for every 5 nm, and three determinations by using 1 cm quartz cell, and ethanol as a blank and calculated the SPF values by UV spectrophotometry. The Mansur equation was applied to calculate SPF values (Sudhahar *et al.,* 2013). The SPF of the samples were calculated using the equation (a mathematical expression derived by Mansur) below and the relationship between erythemogenic effect and radiation intensity at each wavelength, (EE X I) was determined as shown below.

$$SPF_{spectrophotometric} = CF \times \sum_{290}^{320} EE\ (\lambda) \times I(\lambda) \times Abs(\lambda)$$

EE – erythemal effect spectrum; I – solar intensity spectrum; Abs - absorbance of sunscreen product; CF – correction factor (= 10). The values of EE x I are constants.

Determination of Moisture Content

The sample (5) g was weighed accurately in a clean and dry moisture dish which was previously weighed. The dish was placed in an oven for 2 hours at 105˚C. The dish was removed from the oven, cooled in a dessicator at room temperature and weighed. The loss in weight of the sample was recorded. Drying, cooling and weighing were repeated until the loss in weight became constant. The moisture content of the sample was calculated as follows.

$$\text{Moisture \%} = \frac{\text{Loss in weight x 100 \%}}{Weigh\ \ of\ sample}$$

Determination of Emulsion Type

The type of emulsion was detected by dispersability of sample in water or oil. 1 g of sample was added into 10 mL of water and stirred. If the sample is oil in water emulsion type, the sample will be soluble in water. On the other hand, water in oil emulsion type is insoluble in oil.

Determination of Total Plate Count and Yeast and Mold Count

2.5g of sample was aspetically weighed into another sterile plastic bag. Each pieces of sample was aspetically cut into pieces in sterile plastic bag. 225mL of phosphate buffer was added and blended for 2 minutes at high speed. Dried film aerobic count plate was placed on flat surface. Top film was lifted and inoculated 1 mL test portion into center on film base.

Top film was carefully put down on inoculums. Test portion was distributed over prescribed growth area with downward pressure in center of plate spreader device. The plate was incubated for 24 hours for determination of total plate count and Coliform Plate for 48 hours at 35℃ was promptly counted after incubation and measured and counted by using colony counter.

Dermatological Test

The dermatological test of spirulina cream and lotion was determined using the open diagnostic patch test. The open patch test was best formed on the sensitive part of the skin like the bend of elbow, popliteal space, the skin behind ears in some instances, skin of the upper eyelid. The suspended cosmetic as actually was applied to 1 inch square of the skin and left uncovered. The subject was instructed not to wash it off or remove it in any other way. The site of the skin was inspected at the end of 6 h and if there was no reaction, the cosmetic may be applied to some sites of the skin. These tests were performed with persons and the results are tabulated in Table 6.

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 was 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 turns

brown, indicating in the bio transformation of ionic silver to reduced silver. It is observed that the maximum absorbance was found at 426 nm.

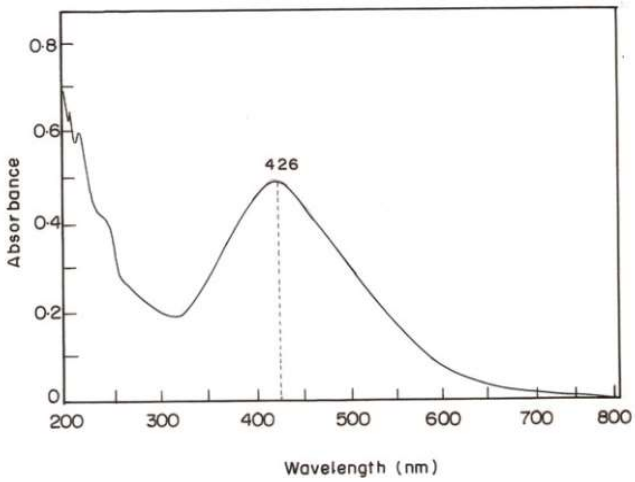
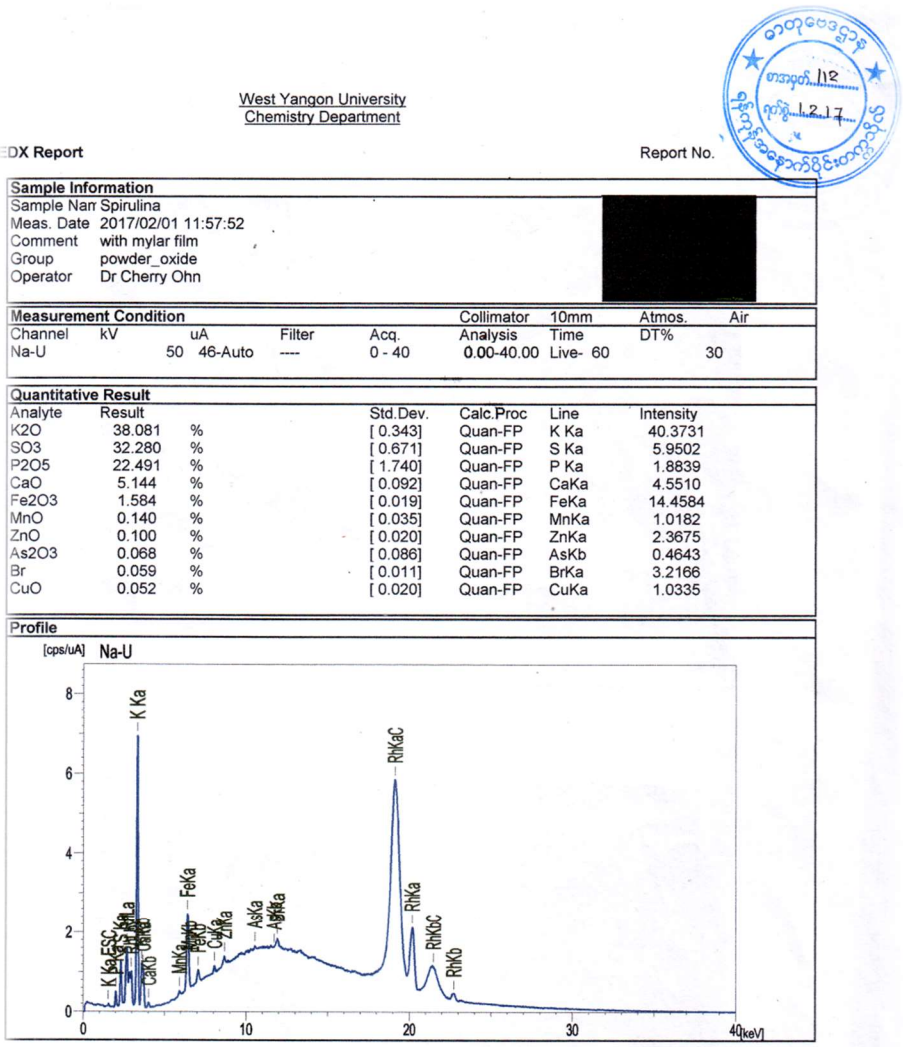


Figure 2 UV spectrum of silver nanoparticles from *Spirulina platensis*

Characterization of Spirulina by Energy Dispersive X-ray Fluorescence(EDXRF) Analysis

Figures 3 shows the EDXRF spectrum of the spirulina. The presence of K_2O, SO_3, P_2O_5, CaO, Fe_2O_3, MnO, ZnO, As_2O_3 and CuO were found in *Spirulina platensis* according to EDXRF data.



West Yangon University
Chemistry Department

DX Report Report No.

Sample Information

Sample Nam Spirulina
Meas. Date 2017/02/01 11:57:52
Comment with mylar film
Group powder_oxide
Operator Dr Cherry Ohn

Measurement Condition

Channel	kV	uA	Filter	Acq.	Collimator	10mm	Atmos.	Air
Na-U	50	46-Auto	----	0 - 40	Analysis 0.00-40.00	Time Live- 60	DT%	30

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
K2O	38.081	%	[0.343]	Quan-FP	K Ka	40.3731
SO3	32.280	%	[0.671]	Quan-FP	S Ka	5.9502
P2O5	22.491	%	[1.740]	Quan-FP	P Ka	1.8839
CaO	5.144	%	[0.092]	Quan-FP	CaKa	4.5510
Fe2O3	1.584	%	[0.019]	Quan-FP	FeKa	14.4584
MnO	0.140	%	[0.035]	Quan-FP	MnKa	1.0182
ZnO	0.100	%	[0.020]	Quan-FP	ZnKa	2.3675
As2O3	0.068	%	[0.086]	Quan-FP	AsKb	0.4643
Br	0.059	%	[0.011]	Quan-FP	BrKa	3.2166
CuO	0.052	%	[0.020]	Quan-FP	CuKa	1.0335

Profile

Figure 3 EDXRF spectrum of *Spirulina platensis*

Characterization of Silver Nanoparticles by X ray Diffraction Analysis (XRD)

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 shown in Figures 4 and 5. 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= 4.11 Å) and two theta values 37.798, 43.96 and 64.120˚.

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

where λ is wavelength of copper Kα line (1.546 Å), θ is diffraction angle, Ḃ is full width at half maximum of peak (FWHM), and D is the average crystallite size. It was found that average crystallite size of silver nanoparticles was observed to be 23.93 nm.

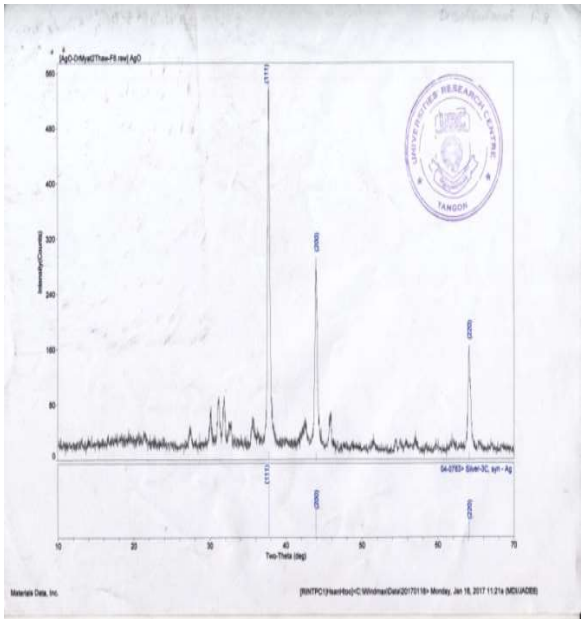


Figure 4 X ray diffractogram of prepared silver nanoparticles

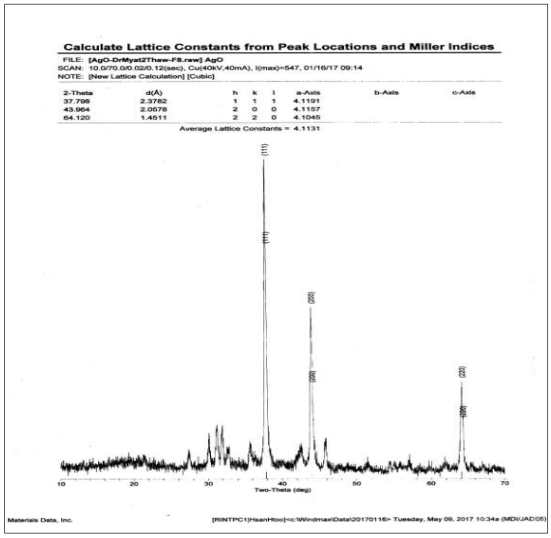


Figure 5 XRD data of silver nanoparticles

Characterization of Silver Nanoparticles by using Scanning Electron Microscope

SEM is important to know the dimensions of the structures fabricated and the materials prepared when characterizing device structures. The scanning electron microscopy results clearly indicate that the formation of spherical silver nanoparticles with its size ranging between 40 nm and 50 nm as shown in Figure 6.

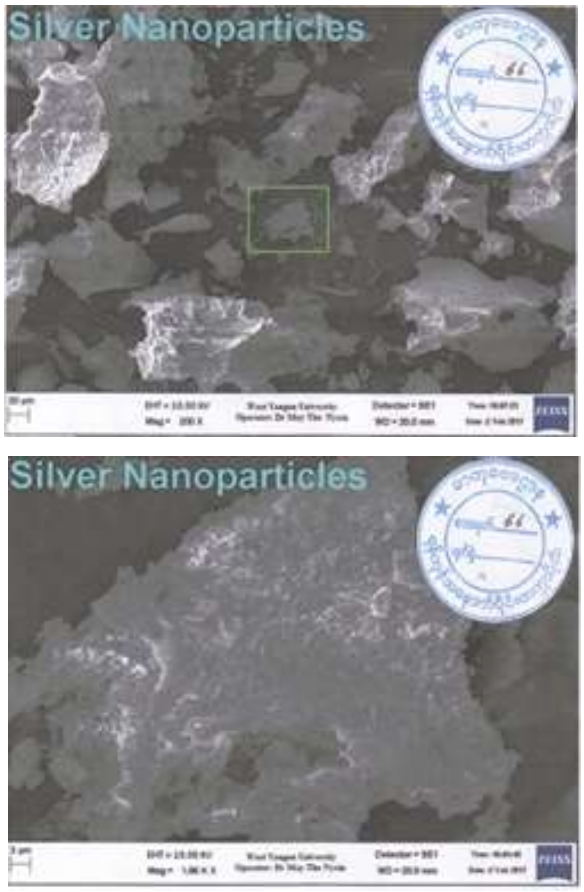
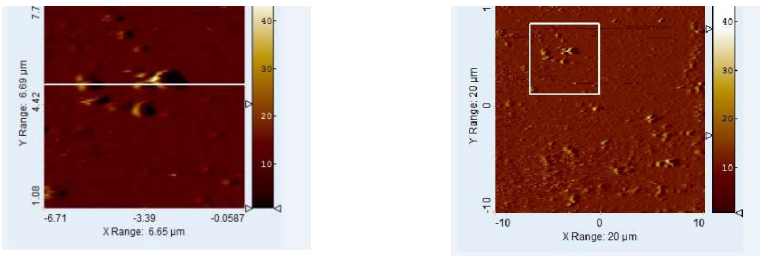
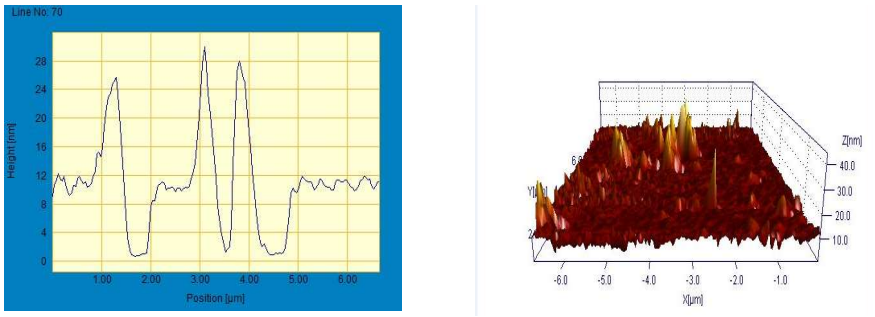


Figure 6 SEM images of silver nanoparticles

Characterization of Prepared Silver Nanoparticles by Atomic Force Microscopy (AFM)



(a) 2 D structure of prepared silver nanoparticles



(b) Particle size of silver nanoparticles (c) 3Dstructure of silver nanoparticles

Figure 7 AFM images of prepared 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 looking the colour scale bar as shown in Figure 7 (a). In the 3 D structure, the particles are very small and it was observed that the highest particle size is 30. 5 nm. It shows that the prepared silver nanoparticles was within the nano range by AFM(Figure 7 b and c). This method is capable of ultra-high resolution for particle size measurement by using AFM, 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). AFM is used to study the morphology of nanoparticles. Unlike SEM and TEM, AFM produces three-dimensional images so that particle size and height can be assessed.

Characterization of Silver Nanoparticles by using FT IR Analysis

In this work, the prepared silver nanoparticles from *Spirulina platensis* were characterized by FT IR technique**.** FT IR technique was used for evaluation the type of organic and inorganic complexes in plants. The infrared spectrum shows a frequency ranges from 3500-3200 cm^{-1} representing the O-H stretching vibration, presence of alcohol, phenol. The frequency ranges from 3000- 2800 cm^{-1} peaks are representing aliphatic C-H stretching vibration present in alkenes. The bands at 1634 and 1651cm^{-1} represent in the C=C stretching vibration present in the alkenes. The bands at 1084 and 1041cm^{-1} may be due to the C-N stretching due to, presence of C-H aliphatic amines. The bands at 447 and 410cm^{-1} may be assigned due to the metal oxygen bond and shown in Table 1 and Figure 8.

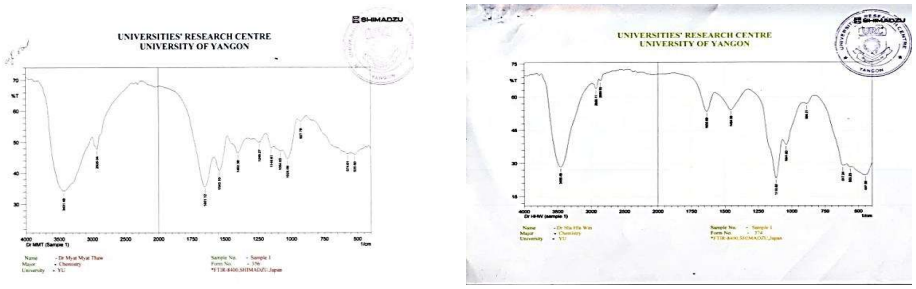


Figure 8 FT IR spectra of prepared silver nanoparticles

Table 1 FT IR Data of Prepared Silver Nanoparticles

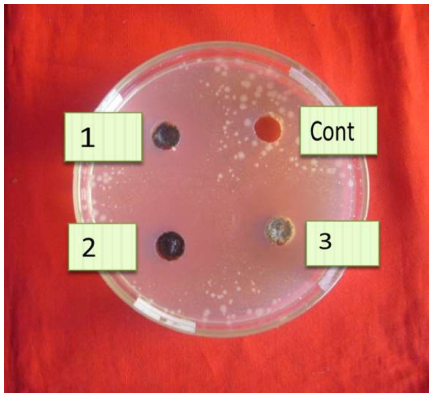
Wave number			
Observed Value for spirulina(cm^{-1})	Observed Value for silver nanoparticles (cm^{-1})	Literature Value (cm^{-1})*,**	Assignments
3431	3458	3560-3500	OH Stretching vibration, presence of carbohydrate and amino acids
2928	2926	2925-2875	Aliphatic C-H stretching vibration (ester and amino acids)
1651	1634	1680-1640	C= C stretching vibration present in the alkenes
1084	1041	1020	C-N stretching, presence of C-H aliphatic amines
532	563	515	C-H stretching vibration presence of alkyl halides compounds
	447	410	Stretching vibration of Ag-O bond

* (Ali *et al*.,2015)
** (Ahmed *et al*., 2015)

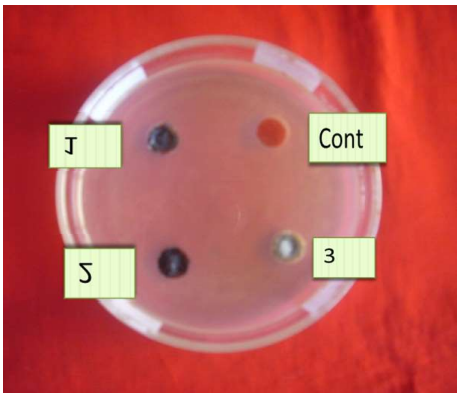
Antimicrobial Activity of Spirulina Prepared Silver Nanoparticles

The antimicrobial activities of spirulina and silver nanoparticles were tested by using six microorganisms. Silver nanoparticles showed a characteristic inhibition zone of 23.06 mm and 28.19 mm diameter against *Bacillus subtilis*, 18.50 mm and 23.29 mm against *staphylococcus aureus*, 20.87 mm and 28.05 mm against *Pseudomonas aeruginosa*, 15.90 mm and 21.43 mm against *Bacillus pumilus*, 35.06 mm and 49.49 mm against *Candida albicans* and 31.70 mm and 38.98 mm against *E coli* respectively. Among

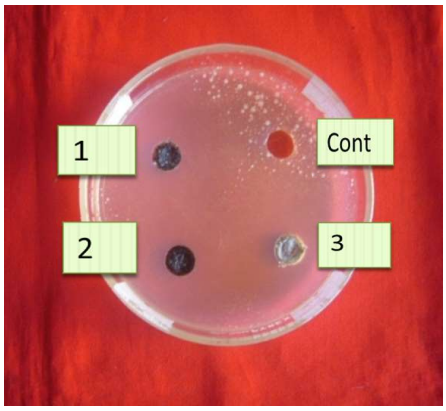
these strains, it was observed that *E coli* and *Candida albicans* are found the higher inhibition zone when silver nanoparticles . These results refer the sensitivity of the microbial species towards the nanoparticles for 100 µg MIC (minimum inhibitory concentration) and shown in Table 2 and Figures 9 and 10.



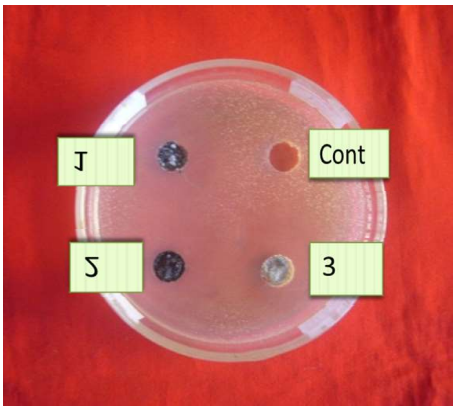
(a) *Bacillus subtilis*



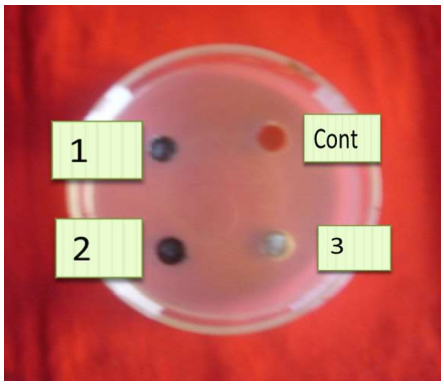
(b) *Staphylococcus aureus*

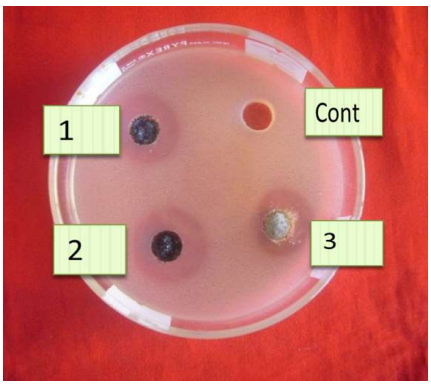


(c) *Pseudomonas aeruginosa*



(d) *Bacillus pumilus*





(e) *Candida albicans* (f) *Escherichia coli (E coli)*

Figure 9 Inhibition zone produced by (a), (b), (c), (d), (e) and (f)

Table 2 Comparison of Maximum Inhibition Zone Diameter of *Spirulina platensis* and Prepared Silver Nanoparticles against Six Microorganisms

Sample	**Diameter of inhibition zone (mm) against six microorganisms** *Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus pumilus*	*Candida albicans*	*E coli*
Spirulina	23 (+++)	18 (+++)	20 (+++)	15 (++)	35 (+++)	31 (+++)
Silver nanoparticles from spirulina	28 (+++)	23 (++)	27 (+++)	21 (+++)	49 (+++)	38 (+++)
Control	10	10	10	10	10	10

Agar well- 10 mm
10 mm – 14 mm (+)
15 mm – 19 mm (++)
20 mm above (+++)

Organisms
1) *Bacillus subtilis* (N.C.T.C- 8236)
2) *Staphylococcus aureus* (N.C.P.C-6371)
3) *Pseudomonas aeruginosa* (N.C.T.C-6749)
4) *Bacillus pumilus* (N.C. I.B-8982)
5) *Candida albicans*
6) *E coli* (N.C.I.B-8134)

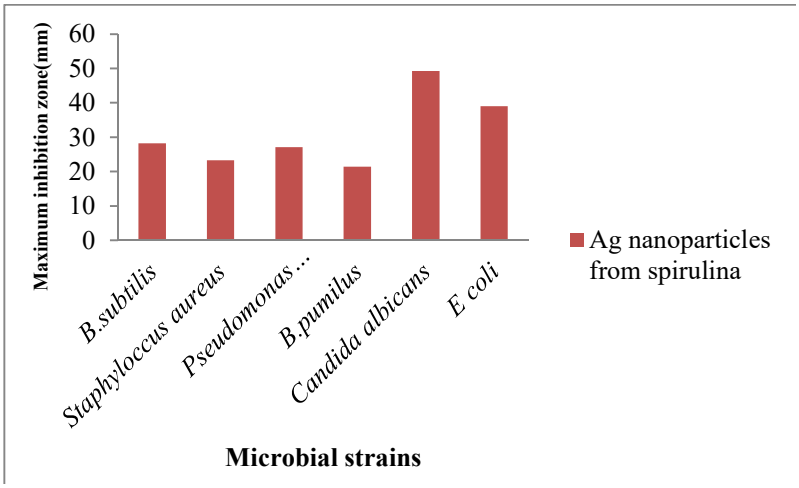


Figure 10 Maximum inhibition of silver nanoparticles from *Spirulina platensis*

Application of Silver Nanoparticles for the Colour Removal of Textile Dyes in Waste Water Samples

Figure 11 shows the presence of dye in waste water samples before and after treatment with silver nanoparticle. It can be seen clearly that colour of the dye solutions decreased gradually after treatment with silver nanoparticles. It was found that absorbance of these sample solutions decreased significantly after treatment with silver nanoparticles Figures 11 and 12.

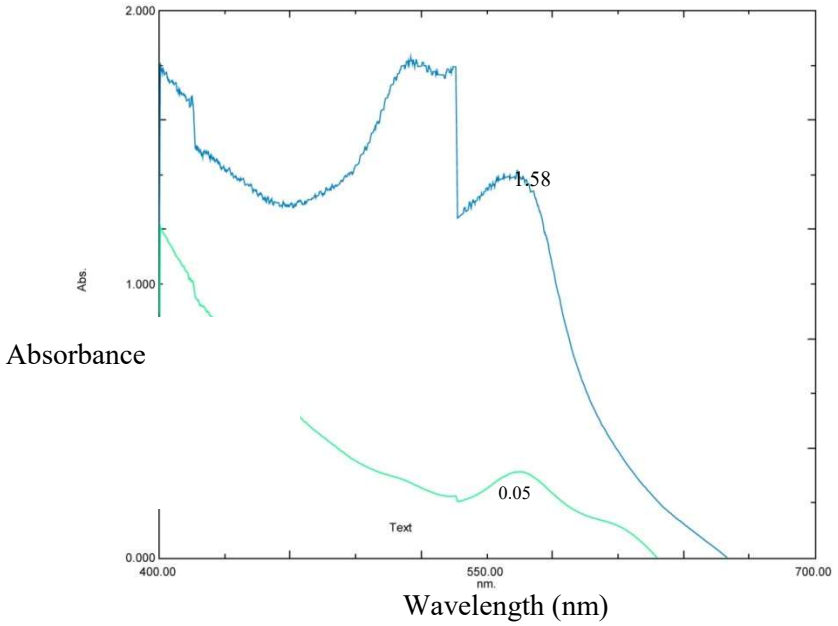


Figure 11 Absorption spectrum of dye waste water sample

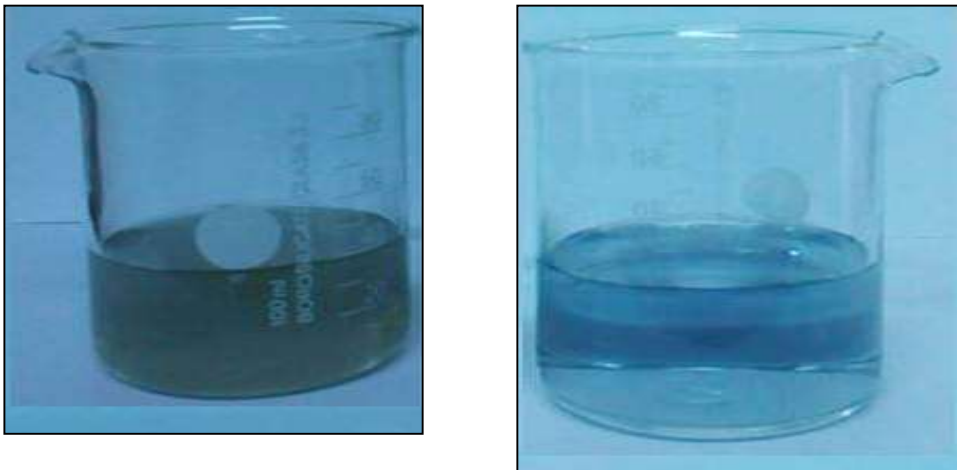


Figure 12 Photograph of dye waste water sample (a) before treatment (b)after treatment with silver nanoparticles for 2 hours

Treatment of Waste Water from Wundwin, Meiktila District by *Spirulina platensis* and Prepared Silver Nanoparticles

Table 3 shows the values and percentage of COD values in waste water treatment with *Spirulina platensis* and silver nanoparticles. It was found that chemical oxygen demand (COD) observed to be 55.5 mg/L by spirulina and 44.8 m/L by silver nanoparticles in the first day and increased to reach 74.35 % and 89.99 % in the 7 day. It showed good efficiency on the reduction of COD from waste water during the treatment period. Prevention and treatment of dyeing wastewater pollution are complementary. The higher the chemical oxygen demand, the higher the amount of pollutant in the water sample. However, COD is considered one of the important quality control parameter of an effluent in wastewater treatment.

Table 3 COD in Waste Water Samples from Wundwin Textile Factory

Sample		COD (mg/L)/Days				Treated with Spirulina and Silver nanoparticles Reduction after 7 days
		1	3	3	7	
Spirulina	85	55.5	42.30	30.8	21.80	74.35 %
Silver nanoparticles from spirulina	85	44.8	35.20	23.8	8.58	89.90 %

Application of Silver Nanoparticles by Body Lotion and Face Cream

The most favorable condition for the ingredients of prepared spirulina lotion and silver nano skin lotion were successfully achieved in this work. Quality and hazard of characteristics of spirulina and silver nano skin lotions were investigated by determining pH, moisture, total plate count, yeast and mold, emulsion type, lead and arsenic . These results are shown in Table 4.

Table 4 Characteristics of Body Lotion and Face Cream Prepared by Silver Nanoparticles

Sr. No.	Characteristics	Experimental Values	
		Silver nano body lotion	Silver nano face cream
1.	pH	6.8	7
2.	Moisture (%)	6	8
3.	Total Plate Count (cfu/g)	$<10^3$	$<10^3$
4.	Yeast and Mold (cfu/g)	$< 10^2$	1×10^3
5.	Emulsion	Water-in-oil	Water-in-oil
6.	Lead	ND	ND
7.	Arsenic	ND	ND
8	Free Alkali	ND	ND

ND = not detected
cfu = coliform unit

Sun Protection Factor (SPF) of Face Cream by Ultraviolet Spectrophotometry

In this research, two different commercial available sunscreen products (Nivea body lotion and Nivea face cream) were studied by UV spectrophotometry by using Mansur mathematical equation. The SPF labeled values were in the range of 8 to 30 according to literature values (Sudhahar and Balasubramaniam,2013). The prepared silver nanoparticles and commercial products are shown in Table 5. It can be observed that the SPF values found for the prepared silver 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, for the use of different solvents in which the sunscreens are dissolved.

Table 5 Observed and Labeled SPF in the Prepared Silver Nanoparticles

Samples	SPF		
	Observed SPF	Labeled SPF	Literature values*(SPF)
Nivea body lotion (Germany)	20.114	30	(8- 30)
Plate snail body lotion (Thailand)	21.54	50	
Nivea face cream	39.11	30	
Silver Nanoparticles for face cream	*11.344*		
Silver nanoparticles for body lotion	*8.579*		

**(Abreu et al., 2004)*

The SPF is

Sample –Face Cream (SPF content) λ – (290~320nm)
Value of EE (λ) x 1(λ) x Abs (λ)
At 290nm
EE (290nm) x 1 (290nm) Abs (290nm) = 0.0150 x 0.926 = 0.0138
At 295 nm
EE (295 nm) x 1 (295 nm) x Abs (295 nm) = 0.0817 x 0.805 = 0.0657
At 300 nm
EE (300 nm) x 1 (300 nm) x Abs (300 nm) = 0.2874 x 0.664 = 0.1908
At 305 nm
EE (305 nm) x 1 (305 nm) x Abs (305 nm) = 0.3278 x 0.536 = 0.1757
At 310 nm
EE (310 nm) x 1 (310 nm) x Abs (310 nm) = 0.1864 x 0.420 = 0.0782
At 315 nm
EE (315 nm) x 1 (315 nm) x Abs (315 nm) = 0.0837 x 0.314 = 0.0262
At 320 nm
EE (320 nm) x 1 (320 nm) x Abs (320 nm) = 0.0180 x 0.229 = 0.0041
SPF = C x $\sum_{290}^{320} EE$ (λ) x 1 (λ) x Abs (λ)

= 10 (0.0537+.0.2214+0.1015+0.3966+0.2013+0.082+0.0144)
= 10 x 1.0826
= 11.3445

SPF of Face cream = 11.3445

Dermatological Test for body lotion was performed with 10 girls practically. It was observed that there is no irritation on these girls by using these lotions Table 6.

Table 6 Dermatological Test for Lotion on Spirulina and Silver Nanoparticles

Size of skin = 1 square inch
Testing time = 2 hours

Sr. No.	Males/ Females	Age (years)	Observation
1.	female	23	no irritation
2.	female	23	no irritation
3.	female	23	no irritation
4.	female	23	no irritation
5.	female	22	no irritation
6.	female	22	no irritation
7.	female	22	no irritation
8.	female	22	no irritation
9.	female	22	no irritation
10.	female	22	no irritation

Dermatological tests were performed with ten girls.

Conclusion

In this work, silver nanoparticles were synthesized by using method of green synthesis. This method provides an environmental friendly, simple, and efficient technique for the preparation of silver nanoparticles. From the technological point of view, silver nanoparticles have potential applications in the biomedical field and some advantages of cosmetic products for commercial production. *Spirulina platensis* was used as starting materials and capping agent. After the calcination at 500˚C for 3 hours, silver nanoparticles became the crystalline nature. Average crystallite size of silver nanoparticles was found to be 23.93 nm by using Debye-Scherrer equation. The highest antimicrobial activity of silver nanoparticles was observed on *Candida albicans* and *E coli* strains. Silver nanoparticles can reduce the COD content in waste water from Textile Factory. It can be clearly seen that colour of the textile dyes solution decreased significantly after treatment with silver nanoparticles for 2hours. The most suitable amounts of ingredients for preparation of face cream and body lotion by using spirulina platensis and silver nano body lotion and face cream were achieved and obtained as one of the cosmetic products (body lotion and face cream). Sun protection factor on face cream and body lotion was determined, and observed to be 11.34 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. These applied research work shows that one of the cosmetic products (body lotion and face cream) by using silver nanoparticles was successfully achieved for applications.

Acknowledgements

The authors would like to thank deep appreciation to Rector Dr Pho Kaung, University of Yangon and Dr Hnin Hnin Aye, Professor and Head, Department of Chemistry, University of Yangon for allowing this paper and also special thanks to Department of Medical Research, Ministry of Health and Sports for giving this opportunity of external grant and the Myanmar Academy of Arts and Science for allowing to read at the 17th Research Conference, Yangon ,Myanmar.

References

Abreu,D.E., Oliveira,D.A, Hackmann, E.R and M.I.Santoro, (2004) " Determination of Sun Protection Factor (SPF) of Sunscreen by Ultraviolet Spectrophotometry." *Journal of Pharmaceutical Sciences*, Vol. 40, pp.381-385.

Ahmed, A.E., H. Ekhal Hafez Abdel, F. Amel M. Ismail, M.elsonbaty Sawsan, and S. Heba Abbas, A. Raw Heya, E.Salah Din, (2015) " Biosynthesis of Silver Nanopartilces by *Spirulina platensis* and Nostoc Sp." *Global Advanced Journal of Microbiology*, Vol. 4 (4), pp. 036-049.

Ali, M.I.,Sharma,G., Kumar, M. and Dut Jasuja,N. (2015) " Biological Approach of Zinc Oxide Nanoparticles Synthesis by Cell Free Extract of *Spirulina platensis*." *International Journal of Current Engineering and Technology*, Vol.4, pp. 35-39**.**

Ghaeni, M., and Roomiani, (2016) " Review for Application and Medicine Effect of Spirulina, *Spirulina platensis* Microalgae." *Journal of Advanced Agricultural Technologies*, Vol. 3, pp. 114-117.

Madhieh, M., A. Zolanvari and A.S. Azimee, (2012) " Green Biosynthesis of Silver Nanoparticles by *Spirulina platensis*." *Scientia Iranica* F. (1993), pp.926-929.

Mahdavi,M., Namvar.F, Ahmad. M.B, and Mohamad. R. (2013). "Green Biosynthesis and Characterization of Magnetic Iron Oxide (Fe3O4) Nanoparticles using Seaweed (Sargassummuticum) Aqueous Extract". *Journal of molecules*, Vol.18,pp.5954-5964.

Sathish.S., C. Mahesh, D.Saikat, D.S.B. Lavanyav, (2012) " Preparation and Evaluation of Salicylic Acid and Medicated Lipstick." *Journal of Advacned Pharmaceutical Sciences*, Vol. 2. pp. 254- 261.

Sudha. S. S., R. Karthic, J. Rengaramanujam and Athulya, (2011) " Antimicrobial Activity of *Spirulina platensis* and Aphanothece sp.on Selected Clinical Bacterial Isolate and its Antioxidant Activity." *South Ase. J.Biol. Sci.* Vol. 1(2), pp. 87-98.

Sudhahar,V., and V. Balasubramanian, (2013) " Sun Production Factor (SPF), Determination of Marketed Suncreen Formulation by *In- Vitro* Method using UV-Visible Spectrophotometer." *Archives of Applied Science Research*, Vol. 5(6), pp.119-122.