Application of Silver Oxide Nanoparticles from *Spirulina Platensis* and Its Nutritional Values

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Abstract

Spirulina platensis L is belong to a genus of filamentous cyanobacteria (commonly called bluegreen algae). In this study, Spirulina platensis L sample were collected from Sagaing June Pharmaceutical Ltd., Yae Kharr Inn, Sagaing Region. Silver oxide nanoparticles from Spirulina platensis L were obtained by green synthesis and characterized by X ray Diffraction Analysis (XRD) and Atomic Force Microscopy (AFM) instruments. Average crystallite size of silver oxide nanoparticles from spirulina was observed to be 23.93 nm by using Debye Scherrer equation and AFM tehniques. The preliminary photochemical test and nutritional values were carried out by AOAC method. The phytochemical analysis of Spirulina platensis indicated that the highly content of Carbohydrates and Glycosides, Amino acid, Protein, Alkaloids and Saponins by AOAC method. Flavonoids, Terpenes, Steroids and Tannins were found to be absent. The antimicrobial activities of silver oxide nanoparticles from Spirulina platensis was studied on 8 microorganisms by both agar well and paper disc diffusion method. The highest antimicrobial activities order of silver oxide nanoparticles and Spirulina platensis were observed to be Candida albicans, Pseudomonas fluorescens. Escherichia coli, Aspergillus flavus, Bacillus pumalis, Klebsiella pneumoniae, Staphylococcus aureus and Bacillus subtilis according to their inhibition zones.

Keywords: Antimicrobial activities, AFM, nutritional values, phytochemical analysis, XRD

Introduction

Spirulina platensis L is a microalgae that appeared on earth there are over 3.5 billion years. They are found in tropical and subtropical areas with high pH values with high level of carbonate and bicarbonate.

Scientific Classification

| Domain | : | Bacteria |
|---------|---|---------------------|
| Kingdom | : | Eubacteria |
| Phylum | : | Cyanophyta |
| Class | : | Cyanophyceae |
| Order | : | Oscillatories |
| Family | : | Phormidiaceae |
| Genus | : | Spirulina |
| Species | : | Spirulina platensis |
| | | |

Genus of *Spirulina* have 58 species. *Spirulina* is a non-heterocystous, composed of vegetative cells that undergo binary fusion in a single plane, show easily-visible transverse cross-walls. Major commercial *Spirulina* procedures are Myanmar, India, Thailand, Taiwan, Greece, Chad, United States, Bangladesh and Chile. The four places (Twin Taung lake, Taung Pyauk lake, Twin Ma lake and Yae Kharr lake in Myanmar. *Spirulina* benefits are; 1. detoxes heavy metals, 2. Eliminates *Candida*, 3. improves HIV/AIDS, 4. helps prevent Cancer, 5. lowers blood pressure, 6. reduces cholesterol, 7. lower chance of stroke, 8. boosts energy, 9.

speeds up weight loss, 10. alleviates sinus issues. This study aimed to investigate the application or obtain silver oxide nanoparticles from *Spirulina platensis* and its nutritional values. In this study, the Ethanolic and Watery extract of *Spirulina platensis* L were studies the presence and absence of phytochemical constituents. *Spirulina platensis* L is one of the most important medicinal drugs. *Spirulina platensis* L is used as medicine in around over the world. *Spirulina* contains vitamins, especially vitamin A in the form of betacarotene, Vitamin C and

Spirulina have the potential to produce a large number of antimicrobial substances, so they are considered as suitable organisms for exploitation as biocontrol agents of pathogenic bacteria and fungi. Antimicrobial compounds found in cyanobacterial include polyphenols, fatty acids, glycolipids, terpenoids, alkaloids and a verity of yet to be described bacteriocins. Antimicrobial effects are shown as visible zone of growth inhibition. Aspergillus flavous, Candida albicans, Bacillus pumalis, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas fluorescens are used to detect antimicrobial activity in this research.

Microbiological Aspect

A microorganism or microbe is a microscopic organism, which may be single-celled or multicellular. Microorganisms are very diverse and include all bacteria, and most protozoa. This group also contains some fungi, algae, and some micro-animals such as rotifers.

Escherichia Coli

Escherichia coli form short-plump rods, cell grouping occur singly in pairs or in short chains. Size 0.5 microbes broad to 1 to 3 microbes long. Gram-negative, non-sporing, motile or non-motile strain have peritrichous flagella usually non-encapsulated. It found in the intestinal tract of man and animals also in water, milk and soil.

Bacillus Subtilis

Bacillus subtilis form rods, straight or curved with rounded ends, cell grouping occurs singly or in short chains. Size to 4 microbe by 1 m. These 1.2 microbe by 0.6 m and appear agar in 18 hrs. Gram positive and non-acid fast. Motility by 8 to 12 peritrichous flagella. Most strains of the organism are non-pathogenic. *Bacillus subtilis* may give rise to conjunctivitis, iridochroiditis and panopthalmittis in man. It occasionally invades the bloodstream in cachectic diseases (Bandow, 2002).

Bacillus Pumalis

Bacillus pumalis is a spore-forming bacterium that is rod-shaped. Gram-positive and aerobic. It resides in soils and some colorize in the root area of some plants where *Bacillus pumalis* has antibacterial and antifungal activity. The use of *Bacillus pumalis* plasmids in gene transfer systems. The proteases from *Bacillus pumalis* are used in various industries. Food, chemical detergent, and leather industries can benefit from the proteases from *Bacillus pumalis*.

Aspergillus Flavus

Aspergillus flavus is a saprotrophic and pathogenic fungus. *Aspergillus flavus* colonies are commonly powdery masses of yellow-green spores on the upper surface and reddish-gold on the lower surface. Hyphal growth usually occurs by thread-like branching and produces mycelia. Hyphae are septate and hyaline. *Aspergillus flavus* grows on leaves after damage by leaf-feeding insects (Katsuya Gomi , 2010).

Candida Albicans

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Vitamins of group B. The importance of vitamin B and Vitamin C as antioxidants in the prevention of numerous degenerative diseases.

Candida albicans is a dimorphic fungus that grows both as yeast and filamentous cells and one of the few species of the *Candida* genus that cause the infection candidiasis in humans. *Candida albicans* is responsible for 50-90% of all cases of candidiasis in humans. *Candida albicans* is a diploid, naturally heterozygous, opportunist pathogen. *Candida albicans* normal habitat is the mucosal membranes of humans and various other mammals including the mouth, gut, vagina and sometimes the skin (Dan Otho ,1952).

Klebsiella Pneumoniae

Klebsiella pneumoniae is a Gram-negative, non motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar. It naturally occurs in the soil, and about 30% of strains can fix nitrogen in anaerobic conditions.

Staphylococcus Aureus

Staphylococcus aureus is a Gram-positive, round-shaped bacterium and frequently found in the nose, respiratory tract, and on the skin. It is often positive catalyses and nitrate reduction and a facultative anaerobe that can grow without the need for oxygen. *Staphylococcus aureus* is not always pathogenic, it is a common cause of skin infections such as skin abscess, respiratory infections such as sinusitis and food poisoning.

Pseudomonas Fluorescens

Pseudomonas fluorescens is a common Gram-negative, rod-shaped bacterium. *Pseudomonas fluorescens* has multiple flagella. It has a extremely versatile metabolism, and can be found in the soil and in the water.

Classification of Microalgae

The main groups of microalgae differ primarily in terms of pigment composition, biochemical constituents, ultra structure, and life cycle. The groups include diatoms (Class Bacillariophyceae), green (Class Chlorophyceae), golden brown (Class Chrysophyceae), primnesio- phytes (Class Prymnesiophyceae), eustagmatophytes (Class Eustagmatophyceae) and blue-green or cyanobacteria (Class Cyanophyceae) (Sheehan et al., 1998).

Cell Structure and Metabolism

Spirulina are Gram-negative, with soft cell walls that consists of complex sugars and protein. They are undifferentiated and filamentous. *Spirulina* can be rod or disk shaped. Their main photosynthesis pigment is phycocyanin, which is blue in color. These bacteria also contain chlorophyll and carotenoids. Some contain the pigment phycoythrin, giving the bacteria a red or pink color. *Spirulina* also have gas vesicles, giving them buoyancy in the aquatic environments they inhibit. *Spirulina* are photosynthetic, and therefore autotrophic *Spirulina* reproduce by binary fission (Masanori, 2014).

Materials and Methods

Sample Collection of Spirulina platensis L

The samples were collected from Sagaing June Pharmaceutical Ltd, Yae Kharr Inn, Sagaing Region located at North latitude 22° 02'57.4" and East longitude 95° 53'17.4".

Identification of Spirulina platensis L

Botanical identification of *Spirulina* Sample was confirmed by using available literature in library, book of Botany Algae Vishishta, Department of Botany, University of Yangon.



Figure 1. Location of

Spirulina platensis L

Nutritional Values

Nutritional values of *Spirulina platensis* L were done in laboratory of Union of Myanmar Federation of Chambers of Commerce and Industry (UMFCCI).

Preparation of Silver Oxide 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 min 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 dried at room temperature until silver oxide nanoparticles was obtained.

Preparation of Watery Extract of Spirulina platensis L

Freshly dried *Spirulina platensis* L was mixed with ethanol (30mL/2g of sample) and extracted for 60 minutes. The extract was filtered and solvent was removed by air drying. The extract were stored in an airtight glass bottle in a refrigerator for analysis of phytonutrients and other finding.

Phytochemical Analysis Test for Carbohydrates

A small quantity of extract was dissolved separately in 5mL of distilled water and filtered. The filtrate was tested to detect the presence of carbohydrates .

Molisch's Test

2 mL of algal extract and 2mL of Molisch's reagent were mixed. Then, 2 mL of concentrated sulfuric acid was added along the sides of the test tubes. Disappearance in color on the addition of excess solution indicated the presence of carbohydrates.

Benedict's Test

0.5 mL of extract and 5 mL of Benedict's reagent were mixed. The mixture is then boiled for 5 minutes. Presence of a bluish green precipitate indicated the presence of carbohydrates.

Test for Glycosides

2 mL of algal extract and 1mL of aqueous NaOH solution were mixed. The appearance of yellow color indicated the presence of glycosides.

Test for Proteins and Amino Acids

Ninhydrin Test

A small quantity extract solution was boiled with 0.2% solution of ninhydrin. Purple color indicated the presence of free amino acids.

Test for Phytosterols and Triterpenoids

Salkowski Test

To 2 mL of algal extract, 1 mL of concentrated sulfuric acid added. Chloroform was added along the sides of the test tube. A red color produced in the chloroform layer indicated the presence of Phytosterols or if it is yellow in color at the lower layer indicated the presence of triterpenoids.

Test for Flavonoids

Zinc Hydrochloride Reduction Test

The extract was treated with mixture of zinc dust and concentrated hydrochloric acid. Red color indicated the presence of flavonoids.

Test for Alkaloids

A small portion of the solvent free extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with Mayer's reagent (Potassium mercuric iodide solution). The cream precipitate indicates the presence of alkaloids.

Test for Tannins

Gelatin Test

5 mL of algal extract, few drops of 1% lead acetate were added. Absence of a yellow or red precipitate indicated the absence of tannins.

Test for Saponins

5 mL of the algal extract, and 5 drops of sodium bicarbonate were added. It was shaken vigorously and kept undisturbed for 3 minutes. Appearance of a honey comb like froth indicated the presence of Saponins.

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

The silver oxide nanoparticles were characterized by using X-ray diffraction (XRD) (Rigaku Multiflex 2kW X ray diffractometer, Japan) for average crystallite size determination and Atomic Force Microscope (AFM)(Bruker), N8 Rados (Germany) for morphology of silver oxide nanoparticles.

Preparation of Spirulina platensis L extract for Antimicrobial Test

The *Spirulina platensis* L materials were ground to a fine powder. 5 gram of powdered *Spirulina platensis* were extracted respectively with 50 mL of water solvent in 5 days upon the shaker to dissolve constituents. The extracts were filtered and the solvent was removed by air drying.

Diffusion Method

The antimicrobial activity was determined by diffusion method. Disc diffusion method is the most widely used method. It is a simple and reliable test especially applicable in routine clinical laboratory. It consists of impregnating small discs of a standard filter paper with given amounts of a chosen range of antibiotics. These are placed on plates of culture medium with previously incubate of bacterial and fungi isolated to the tested. After incubation, the degree of sensitivity is determined by measuring the easily visible areas of inhibition of growth produced by the diffusion of the antibiotic from the discs the surrounding medium (Cruickshank, 1968).

Paper Disc Diffusion Method (Tamura, 1968)

Six mm diameter discs were prepared by using sterile filter paper. *Spirulina platensis* L extract obtained using water solvent were mixed with 0.01g of 5% Dimethyl sulfoxide (DMSO). 0.5 mL of 0.1 g of silver oxide nanoparticles was added into the discs with 30 micro liter by using capillary tube to check their antimicrobial activity.

Agar Well Diffusion Method (Boyanova, 2005)

The steried agar medium with 0.5mL of 0.1g of silver oxide nanoparticles are poured on the petri dishes at around 45 degree Celsius and then the media was allowed solidify. The antimicrobial activity of silver oxide nanoparticles and *Spirulina platensis* L water extract were determined by well division method. A well of 8 mm diameter was cut on the agar with sterile bores. 0.1 mL of silver oxide nanoparticles were added to the wells using micropipettes. The plates were incubated at 37 degree Celsius for 24 to 72 hours and examined. The diameters of the inhibition zone (mm) were measured for each bacterial and fungi species.

Preparation of Disc

The disc of No.4 Whatman filter paper were punched 6 mm in diameter with cock borer and sterilized by autoclaving followed by dry heating at 60°C for one hour. The paper disc were done by autoclaving in 3 to 4 times for aseptic. Each of them was impregnated with extract (2 mg/disc) and then allowed to be dried at 37°C.

Preparation of Bacterial and Fungal Suspension

A few colonies of the organisms to be tested were checked with 0.1 mL of silver oxide nanoparticles by micropipette from the original test tube and introduced into a test tube containing 5 mL of nutrient broth. To obtain a bacterial and fungal suspension of moderate cloudiness it was incubated previous day before the actual testing of the sample.

Preparation of Mueller Hinton and Media for Pathogenic Microorganisms

Mueller Hinton agar were used for antibacterial test and Sabouraud's dextrose agar were used for antifungal test. Both the agar mediums were done to autoclave at 121°C for 15 minutes. The agar medium are allowed to cool around 45°C. And the 0.5 mL of test organism were added and to shake slowly for uniform spread of test organisms. 40 mL of this mediums are poured into sterilized petri dishes. The medium allowed to dry. The extract paper disc placed onto the plates. The plates were incubated at 37°C for 24-72 hours. The inhibition zones of inhibition were observed and measured in millimeters.

Compositions of Mueller-Hinton Agar (Pollock, 1986) (per/Liter)

| Beef extract | 2.0 g |
|--------------------|---------|
| Casein hydrolysate | 17.5 g |
| Starch | 1.5 g |
| Agar | 17 .0 g |

Compositions of Sabouraud's Dextrose Agar (Sabouraud, 1910) (per/Liter)

| | (per/Liter) |
|----------|-------------|
| Dextrose | 40 g |
| Peptone | 10 g |
| Agar | 17 g |
| pН | 3.5 |

Results and Discussion

Spirulina is a multicellular filamentous cyanobacterium. *Spirulina* is a ubiquitous organism. *Spirulina* appeared as blue green filaments composed of cylindrical cells arranged in unbranched, helicoidal trichomes under a microscopic observation. *Spirulina* is a non-heterocystous, composed of vegetative cells that undergo binary fusion in a single plane, show easily-visible transverse cross-walls. Filaments are solitary, free-floating and display gliding motility.







(b) Figure 2 (a, b). Spiral shape of *Spirulina platensis* L under the 40X of the microscope

Nutritional Values of Spirulina platensis L

Nutritional values of *Spirulina platensis* L showed in Table 1 and 2. The nutritional values of *Spirulina* contains Energy 305 kcal, Protein 43 g, Fat 1g and Carbohydrate 31 g are is based on 100g.

| Table 1. | Compositions | of Nutritional | Values in 100g |
|----------|--------------|----------------|----------------|
|----------|--------------|----------------|----------------|

| Nutritional Values | | | |
|--------------------|----------|--|--|
| Energy | 305 kcal | | |
| Protein | 43 g | | |
| Fat | 1 g | | |
| Carbohydrate | 31 g | | |

| No. | Tested parameters | Tested method | Results |
|-----|--------------------------|------------------------|---------|
| 1 | Moisture | AOAC (2000) 934.01 | 12.05% |
| 2 | Ash | AOAC (2000) 942.05 | 13.50% |
| 3 | Protein (crude) | AOAC (2000) 920.152 | 42.56% |
| | | (Kjeldahl method) | |
| 4 | Crude fiber | AOAC (2000) 978.10 | 0.37% |
| | | (Fiber Cap Method) | |
| 5 | Ether extract | AOAC | 0.77% |
| | (crude fat) | (Buchi Soxhlet Method) | |
| 6 | Carbohydrate | By Difference | 30.75% |

Table 2. Nutritional Values of Spirulina platensis L

Phytochemical Analysis

The phytochemical analysis of *Spirulina platensis* L indicated that the highly presence of Carbohydrates, Glycosides, Amino acid, Protein, Saponin. Terpens, Alkaloids and Saponins. Flavonoids, Steroids and Tannins were found to be absent.

| No | Tests | Extract | Test Reagents | Observation | Results |
|----|---------------|---------|-------------------|-------------------------------|---------|
| 1 | Carbohydrates | EtOH | Molisch's reagent | Disappearance in color | ++ |
| | | EtOH | Benedicts reagent | Bluish green precipitate | + + |
| 2 | Glycosides | H_2O | Killer-Killanis | Appearance in yellow color | + + |
| 3 | Amino acids | EtOH | Ninhydrin | Appearance in purple color | + + |
| 4 | Protein | EtOH | Ninhydrin | Appearance in purple color | + + |
| 5 | Terpenes | H_2O | Salkowski | Yellow color at lower layer | + + |
| 6 | Steroids | EtOH | Salkowski | Red color at chloroform layer | |
| 7 | Flavonoids | H_2O | Reduction of Zinc | Precipitate in red color | + |
| | | | hydrochloride | | |
| 8 | Alkaloids | H_2O | Meyer's reagent | Cream precipitate | ++ |
| 9 | Tannins | H_2O | Gelatin | White precipitate | |
| 10 | Saponins | H_2O | Frothing | Frothing | + + |
| | 1 | | - | | |

Table 3. Results of Phytochemical Analysis on Spirulina platensis L

++ = Highly present

+ = Present

-- = Absent



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

It was observed that the sharp peaks of the silver oxide 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 Figure 4. 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 oxide 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°.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 average crystallite size of silver oxide nanoparticles was observed to be 23.93 nm.



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

Figure 4. X ray diffractogram of obtained silver oxide nanoparticles from Spirulina platensis

Characterization of Silver Oxide Nanoparticles by using AFM

In this work, the particle size of silver oxide nanoparticles was determined by Atomic force microscopy. It was assessed that the highest particle size was approximately 30.5 nm by looking the colour scale bar as shown in Figure 5 (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 silver oxide nanoparticles from *Spirulina platensis* was found to be within the nano range by AFM (Figure 5 a and b). Morphology and surface texture of silver oxide nanoparticles can be evaluated. The size and shape of metal oxide nanoparticles are typically measured by analytical techniques atomic force microscopy (AFM).



Figure 5.(a) Particle size of silver oxide nanoparticles (b) 3Dstructure of silver oxide nanoparticles from *Spirulina platensis* by AFM

Antimicrobial Activities of Silver Oxide Nanoparticles from Spirulina platensis L

The antimicrobial activities of silver oxide nanoparticles and water solvent extract of *Spirulina platensis* L were tested on 8 pathogenic organisms, *Aspergillus flavus, Candida albicans, Bacillus pumalis, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus Pseudomonas fluorescens.* The antimicrobial activities of silver oxide nanoparticles from *Spirulina platensis* L was carried out by both paper disc diffusion method and agar well method. The diameter of inhibition zones of silver oxide nanoparticles and *Spirulina platensis* showed in Table 4. The higher antimicrobial activity of *Candida albicans, Pseudomonas fluorescens. Escherichia coli, Aspergillus flavus, Bacillus pumalis , Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus subtilis* were observed in this study (Table 4) and Figures 6 to 13.

 Table 4. Inhibition Zone Diameter(mm) of Silver Oxide Nanoparticles and Spirulina platensis L on

 Antimicrobial Activity

| Sample | Pseudomonas fluorescens | Candida albicans | Bacillus pumalis | Bacillus subtilis | E coli | Klebsiella Pneumoniae | Staphylococcus aureus | Aspergillus flavus |
|-------------------------------|----------------------------|---------------------|---------------------|----------------------|-----------|--------------------------|--------------------------|-----------------------|
| Silver oxide Nanoparticles | 36.33 | 51.01 | 28.24 | 19.56 | 33.71 | 24.32 | 21.43 | 31.16 |
| Spirulina platensis | 34.80 | 40.34 | 23.30 | 16.26 | 30.85 | 19.48 | 15.13 | 26.06 |





Figure 6.(a) and (b). Inhibition zone of *Candida albicans* on silver oxide nanoparticles and *Spirulina platensis*





Figure 7.(a) and (b). Inhibition zone of *Pseudomonas fluorescens* on silver oxide nanoparticles and *Spirulina platensis*





Figure 8.(a) and (b). Inhibition zone of *E coli* on silver oxide nanoparticles and *Spirulina platensis*





Figure 9.(a) and (b). Inhibition zone of *Aspergillus flavus* on silver oxide nanoparticles and *Spirulina platensis*





Figure 10.(a) and (b). Inhibition zone of *Bacillus pumalis* on silver oxide nanoparticles and *Spirulina platensis*





Figure 11.(a) and (b). Inhibition zone of *Klebsiella Pneumoniae* on silver oxide nanoparticles and *Spirulina platensis*





Figure 12.(a) and (b). Inhibition zone of *Staphylococcus aureus* on silver oxide nanoparticles and *Spirulina platensis*





Figure 13.(a) and (b). Inhibition zone of *Bacillus subtilis* on silver oxide nanoparticles and *Spirulina platensis*

Conclusion

Silver oxide nanoparticles were synthesized by using green synthesis. This method provides an environmental friendly, simply, and efficient technique for the production of silver oxide nanoparticles. Average crystallite size of silver oxide nanoparticles was found to be 23.93 nm by using XRD and AFM instruments. *Spirulina* appeared as blue green filaments composed of cylindrical cells arranged in unbranched, helicoidal trichomes under a microscopic observation. It was observed that the phytochemical analysis of *Spirulina platensis* L indicated that the highly presence of Carbohydrates, Glycosides, Amino acid, Protein, Saponins. Terpens, Alkaloids and Sapovins. Flavonolds, Steroids and Tannins were observed to be absent. The order of antimicrobial activities on silver oxide nanoparticles and *Spirulina platensis* showed the highest effect on *Candida albicans, pseudomonas fluorescens, Escherichia coli, Aspergillus flavus, Bacillus pumalis, Klebsiella pneumonia and Bacillus subtilis respectively* due to the presence of peptides, alkaloids and lipopolysaccharides.

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