

# HOMOEOPATHIC MEDICINE AS A NANOMEDICINE – SPIRULINA

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## **ABSTRACT**

The paper examines the growing evidence supporting the adaptive network nanomedicine model for homoeopathic medicines and their actions. Multiple laboratories have identified nanostructure in homoeopathically manufactured medicines at low and high potencies. Homoeopathic mother tincture and potencies of *Spirulina* was capable precipitating silver nanoparticles at ambient temperature. *Spirulina* has been used as a dietary supplement by astronaut which contain more protein and fibres. In homoeopathically it is used as a dietary supplement to boost immunity. The nanoparticles were characterised by UV spectrometer, where the peaks obtained are 631nm for mother tincture, 371 nm for 6C, 373 nm for 12C, 379 nm for 15C, 208 nm for 30C (self-potencies) respectively. SEM shows the shape and size of particles and by FTIR the functional groups of mother tincture. The synthesised silver nanoparticles were generally found to be effective as antimicrobial agent against some important pathogen like *Escherichia coli*, *Bacillus anthracis*, *Klebsiella pneumoniae*, *Staphylococcus aureus* which causes disease. The antioxidant activity of *Spirulina* mother tinctures and potencies are also accessed in this study. Scanning electron microscope (SEM) image of *Spirulina* mother tincture shows nanomaterials as spherical in shape in 100 nm. The values of *Spirulina* mother tincture were compared with the potencies of *Spirulina* in these studies. The work was concluded as *Spirulina* contains nanoparticles even in higher dilutions beyond Avogadro's number, hence it can be a qualified nanomedicine.

KEYWORDS – *SPIRULINA*, SILVER NANOPARTICLE, FOURIER TRANSFORM INFRARED MICROSCOPE(FTIR), UV SPECTROMETER, SCANNING ELECTRON MICROSCOPY(SEM)

## **I. INTRODUCTION**

The process of manufacturing homeopathic medicines employs extreme dilutions even beyond what the atomic theory of matter provides, leading to doubts regarding the existence of starting materials in these formulations. Homoeopathy, since its inception in the late eighteenth century, has been used extensively for the treatment of various ailments while also being the epicentre of controversy. This therapy is based on the tenet that a solution of a particular substance when diluted several folds, accompanied by a vigorous shaking process called succussion at every dilution step, imparts a potent activity with medical value. The scientific question often posed is, how are the homoeopathic medicines active when they are used at extreme dilution, often well beyond Avogadro's number, where in the presence of even remnants of the starting materials is unimaginable? (2)

Nanomedicine and nanotechnology accept that particles smaller than 100 nm are capable of showing new chemical and physical properties absent in particle of bigger dimensions. It is observed that these smaller particles have different electronic structure, conductivity, reactivity, melting temperature and mechanical properties. (1)

*Spirulina*, is a medicine prepared from blue green algae. Dried spirulina contains 60% proteins and less fibres. It contains chlorophyll, which helps remove toxin from the blood and boost the immune system. It has ability to modulate immune functions and exhibits anti-inflammatory properties by inhibiting the release of histamine by mast cells. In homeopathy it is used as a dietary supplement as well as a whole food. (3)

## **II.METHODOLOGY**

### **2.1.1 COLLECTION OF MATERIALS**

Mother tincture collected from Homoeopathic pharmacy, Mangalore

### **2.1.2 PREPARATION OF POTENCIES**

1 drops of mother tincture and 99 drops of distilled water was added to phial and 10 strokes of equal strength was given, 1st potency was prepared. To another clean phial one drop from previous and 99 drops of distilled water added and 10 equal strength strokes are given, forms the 2nd potency. Continue the same step till 5th potency. From 5th potency 1 ml is taken and 99 ml of dispensing alcohol is added and 10 strokes are given, forms the 6 th potency. Like all the potencies 12C,15C and 30C are prepared.

### **2.2.1 SYNTHESIS OF SILVER NANOPARTICLE PREPARATION**

Homoeopathic mother tincture *SPIRULINA* was used for the synthesis of silver nanoparticles 100mg of silver nitrate was added to 20 ml of distilled water. Vigorously stirred with 5ml mother tincture. A change in the colour of solution was observed after 25 minutes. The extract was stored at room temperature for further use.

### **2.2.2 CHARACTERISATION OF SILVER NANOPARTICLES**

The synthesised particles were characterized using UV spectroscopy, FTIR analysis and SEM. The UV –spectroscopy analysis used to study the absorption peak of synthesized particles. The FTIR analysis is used to study the functional groups present in the synthesized Nano particles the SEM analysis is used to study the size of the particles.

### 2.3 U V SPECTROPHOTOMETER

The reduction of pure Ag<sup>+</sup> ion was monitored by measuring the UV VIS spectrum of the reaction medium at 5 hours after diluting a small amount of the sample into distilled water, UV-VIS spectral analysis was done by using UV-VIS spectrophotometer.

### 2.4 SCANNING ELECTRON MICROSCOPY (SEM)

SEM is a type of electron microscope that images a sample by scanning it with a high energy beam of electrons in a raster scan patterns. SEM technique was employed to visualize the size and shape of silver nanoparticles. The pattern of dependence of the morphology and size of nano sized objects is described.

### 2.5 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The Fourier transform infrared spectroscopy is used to study the infrared absorption of particles. IR spectra were recorded using KBr pellets, on a Perkin – Elmer GX FTIR spectrophotometer

### 2.6 PHYTOCHEMICAL ANALYSIS

Spirulina mother tincture ,6CH ,12CH, 15CH and 30CH have been used for all studies. All reagents have been prepared following standard protocols. Spirulina mother tincture has been used as the positive control. A preliminary phytochemical study to determine the phytoconstituents present was undertaken for the synthesised solutions and homoeopathic formulations.

### 2.7 ANTIMICROBIAL ACTION

#### 2.7.1 PREPARATION OF MICROBIAL CULTURES AND ISOLATION

*Escherichia coli*, *Bacillus anthracis*, *Klebsiella pneumoniae*, *Staphylococcus aureus* were used as test from P.G department of Alva's college and microscopic examination was done for the confirmation and were maintained in slants.

#### 2.7.2 ANTIMICROBIAL ASSAY

Petri dishes were plated with Nutrient Agar media and allowed to solidify for 30 mins. The last organism was spread on surface of the media using sterile swab stick. Cork borer (7mm) was used to bore wells in media. The silver nanoparticle extract in different potencies (6C,12C,15C,30C, Mother tincture, sac lac) were dispensed into the wells using a micropipette of 10 microlitre. A positive control of amoxicillin (30mcg/disc) was kept and the extract was allowed to diffuse for 1 hr at room temperature. Then the plates were incubated at 37°C for 24 hrs. Zones of inhibition were measured.

### 2.8 ANTIOXIDANT ACTION

Antioxidant property was determined by DPPH assay.

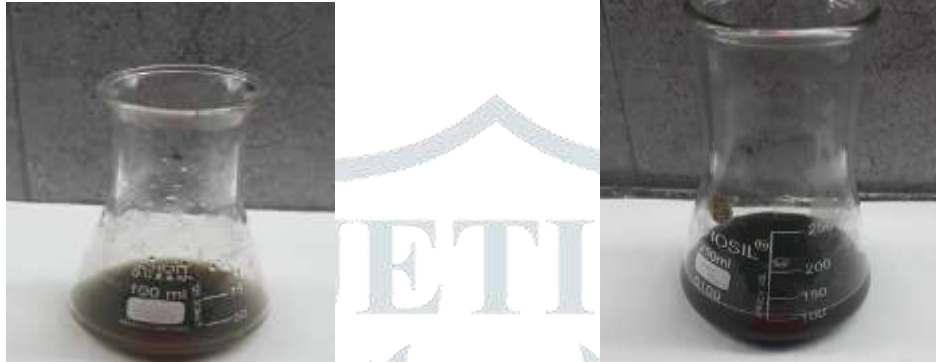
The scavenging activity of homoeopathic mother tinctures was checked with DPPH radical technique. A volume of 0.1 mM solution of DPPH was prepared by adding 25 mg of DPPH in 100 ml of methanol. A micro assay of antioxidant evaluation was used with slight modifications [9]. A volume of 90 µl of DPPH solution with subsequent different serial dilutions (5, 2.5, and 1.25 µl) of mother tincture were placed in wells of a 96-well micro plate. The wells containing DPPH and 10 µl of ethanol were used as negative control. Ascorbic acid was taken as standard control. (8)

$$\% \text{ of inhibition} = \frac{\text{OD of control} - \text{OD of sample} \times 100}{\text{OD of control}}$$

### III. RESULTS

#### 3.1 SYNTHESIS OF SILVER NANOPARTICLE IN *SPIRULINA*

The silver nanoparticles were synthesized by the reduction of silver ions. This was shown by the changes in colour of solution from dark green to black colour. The colour change in the reaction mixer was observed after 45 minutes.



(a) Before the reaction

(b) After the reaction

#### 3.2 Characterization of synthesised nanoparticles.

UV -Visible Spectroscopy:

The change in the colour was visually observed which indicates the presence of silver nanoparticles. The change in colour is mainly because of surface Plasmon resonance. The UV -visible spectroscopy is an important technic to study the metal nanoparticles.

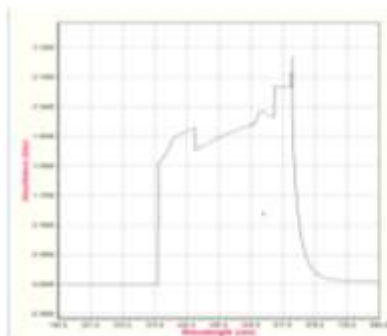


FIGURE 1: UV Spectrometer of Spirulina mother tincture

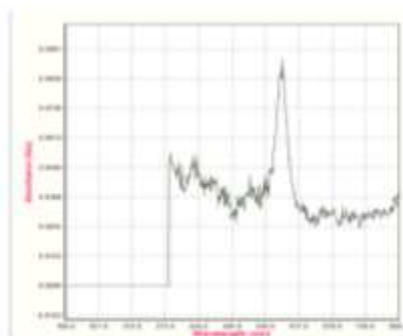


FIGURE 2 : UV Spectrometer of Spirulina 6C potency

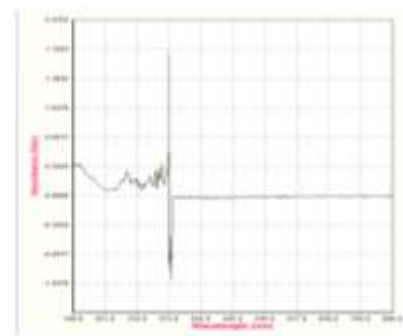


FIGURE 3 : UV Spectrometer of Spirulina 12C potency

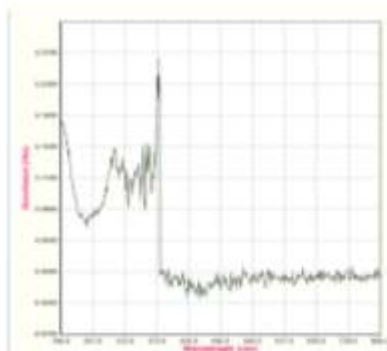


FIGURE 4 : UV Spectrometer of Spirulina 15C potency



FIGURE 5 : UV Spectrometer of Spirulina 30C potency

Peaks obtained are 631nm for mother tincture, 371 nm for 6C, 373 nm for 12C, 379 nm for 15C, 208 nm for 30C (self-potencies) respectively.

### 3.3 SCANNING ELECTRONE MICROSCOPY

The sample showed presence of particle in mother tincture and chlorella powder. particles are spherical in shape at 100 nm

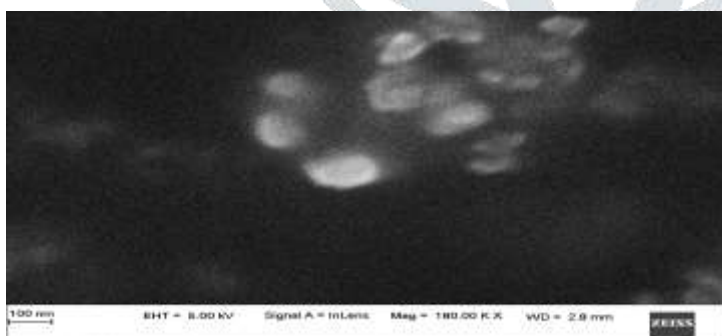


Figure 10: Spirulina mother tincture scanning electron microscope image at 100 nm

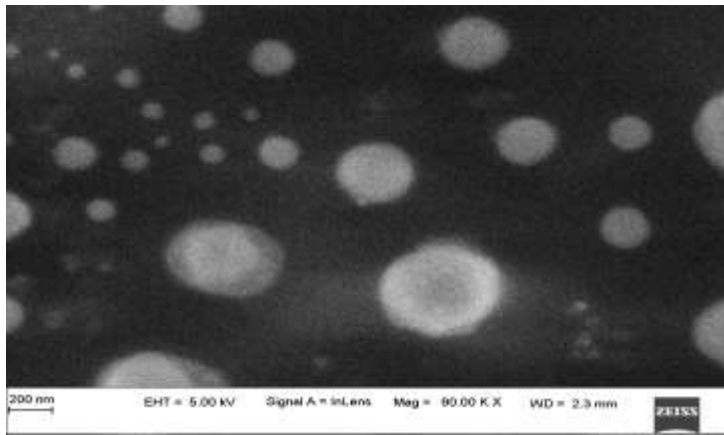


Figure 11: Spirulina mother tincture scanning electron microscope image at 200 nm

### 3.4 FTIR

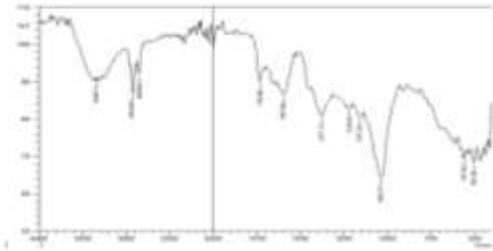


Figure 12: FTIR spectra of produced SNPs of MOTHER TINCTURE

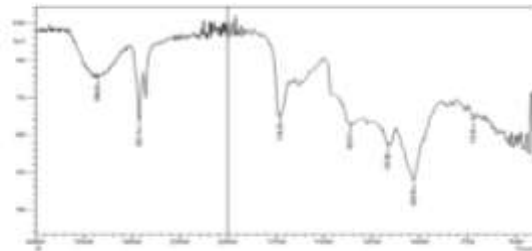


Figure 13: FTIR spectra of produced SNPs of spirulina MOTHER TINCTURE

The IR spectrum for sample of *Spirulina* showed bands at  $3340.71\text{cm}^{-1}$ ,  $2924.09\text{cm}^{-1}$ ,  $2854.65\text{cm}^{-1}$ ,  $1732.08\text{cm}^{-1}$ ,  $1597.06\text{cm}^{-1}$ ,  $1377.17\text{cm}^{-1}$ ,  $1219.01\text{cm}^{-1}$ ,  $1157.29\text{cm}^{-1}$ ,  $1035.77\text{cm}^{-1}$ ,  $557.43\text{cm}^{-1}$ ,  $501.49\text{cm}^{-1}$  for figure 1.

The band at  $3340.71\text{cm}^{-1}$  represent N-H and O-H bonds. The band at  $2924.09\text{cm}^{-1}$  corresponds to C-H bond. The band at  $1732.08\text{cm}^{-1}$  represents C=O bond. The band at  $1597.06\text{cm}^{-1}$  N-H bond. The band at  $1377.17\text{cm}^{-1}$  O-H bond. The band at  $1219.01\text{cm}^{-1}$  C-O bond. The band at  $1157.29\text{cm}^{-1}$  C-N bond. The band at  $1035.77\text{cm}^{-1}$  C-H bond. The band at  $557.43\text{cm}^{-1}$  C-I and C-Cl bond. The bond at  $501.49\text{cm}^{-1}$  C-Cl bond.

The IR spectrum for sample of SNPs showed bands at  $3360\text{cm}^{-1}$ ,  $2922.16\text{cm}^{-1}$ ,  $1726.29\text{cm}^{-1}$ ,  $1363.67\text{cm}^{-1}$ ,  $1165\text{cm}^{-1}$ ,  $1029.99\text{cm}^{-1}$ ,  $719.45\text{cm}^{-1}$  for figure 2

The band at  $3360\text{cm}^{-1}$  represent O-H bond. The band at  $2922.16\text{cm}^{-1}$  C-H bond. The band at  $1726.29\text{cm}^{-1}$  represent C=O bond. The band at  $1363.67\text{cm}^{-1}$  represent C-N bond. The band at  $1165\text{cm}^{-1}$  represent C-N bond. The band at  $1029.99\text{cm}^{-1}$  represent C-H bond. The band at  $719.45\text{cm}^{-1}$  represent C-H bond.

3.5 PHYTOCHEMICAL ANALYSIS

Sl.no	Phytoconstituents	Mother tincture	6c potency	12 c potency	15 c potency	30 c potency	Mother Tincture synthesised	6c synth esised	12 c synt hesis ed	15 c Synt hesis ed	30 c synth esise d
1	Flavonoid	-ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
2	Alkaloid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3	Saponin	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4	Amino acid	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5	Tannins	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
6	Carbohydrate	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
7	Steroids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8	Cardiac glycoside	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
9	Anthraquinone glycoside	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
10	Proteins	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Table 1: Results of Phytochemical analysis

3.6 ANTIMICROBIAL ACTION

Sl.no	Micro organism	Mother tincture(cm)	6C potency(cm)	12C potency(cm)	15 C potency(cm)	30 C potency(cm)	Amoxicillin (cm)
1	<i>Escherichia coli</i>	1.2 cm	0.8 cm	0.8 cm	0.7 cm	0.6 cm	0.5 cm
2	<i>Staphylococcus aureus</i>	0.9 cm	0.7 cm	0.7 cm	0.5 cm	0.5 cm	0.5 cm
3	<i>Klebsiella pneumoniae,</i>	0.9 cm	0.7 cm	0.6 cm	0.5 cm	0.6 cm	0.5 cm
4	<i>Bacillus anthracis</i>	0.8 cm	0.6 cm	0.6 cm	0.6 cm	0.5 cm	0.5 cm

Table 2. Antimicrobial action with Homoeopathic medicine

Sl.no	Micro organism	Mother tincture SYN(cm)	6 C potency(cm)	12 C potency(cm)	15 C potency(cm)	30 C potency(cm)	Amoxicillin (cm)
1	<i>Escherichia coli</i>	1.4 cm	0.9 cm	0.8 cm	0.9 cm	0.9 cm	0.5 cm
2	<i>Staphylococcus aureus</i>	1.1 cm	0.6 cm	0.6 cm	0.7 cm	0.8 cm	0.5 cm
3	<i>Klebsiella pneumoniae,</i>	1.1 cm	0.8 cm	0.7 cm	0.7 cm	0.7 cm	0.5 cm
4	<i>Bacillus anthracis</i>	0.9 cm	0.8 cm	0.9 cm	0.8 cm	0.6 cm	0.5 cm

Table 3. Antimicrobial action with synthesised silver nanoparticles in Homoeopathic medicine

### 3.7 ANTIOXIDANT PROPERTY

DPPH Radial scavenging activity of mother tincture of spirulina

$$\% \text{ inhibition} = \frac{\text{Absorbance (blank)} - \text{Absorbance (sample)}}{\text{Absorbance (blank)}} \times 100 = \frac{0.12 - 0.06}{0.12} \times 100$$

$$= 60 \%$$

DPPH Radial scavenging activity of synthesised mother tincture of spirulina

$$\% \text{ inhibition} = \frac{\text{Absorbance (blank)} - \text{Absorbance (sample)}}{\text{Absorbance (blank)}} \times 100 = \frac{0.12 - 0.04}{0.12} \times 100$$

$$= 66.6 \%$$

## IV. DISCUSSION

*Spirulina* has been used to support a number of health conditions, including fatigue, high cholesterol, high triglycerides and infectious conditions. It is used as a dietary supplement which contains more proteins and less fibres. *Spirulina* is medicine prepared from blue green algae that contains number nutrients, including vitamins, minerals, antioxidant and common source of vegan protein. (5) properties of chlorella and Spirulina medicine were compared in this study and it showed that most of the properties of the spirulina medicine were more or similar with the pure form of algae. Homoeopathy has occasionally been ‘lambasted’ in published literature for using dosages considered too small to have an effect. (9) The colour change in synthesis of silver nanoparticle observed visually. UV spectrometer peaks showed that reduction has taken place in solution. This study using Scanning electron microscope the size and shape of the particle in nanometers has been obtained for mother tincture and potencies. The functional groups present in the homoeopathic medicine *Spirulina* are studied by FTIR. Antimicrobial action was studied on organism like *Escherichia coli*, *Bacillus anthracis*, *Klebsiella pneumoniae*, *Staphylococcus aureus* which causes disease and a positive result with all potencies, mother tincture, has been observed. Which confirms that these medicines have antimicrobial properties. The antioxidant properties of the medicine were also assed in this study. The biosynthesised silver nanoparticle was shown to have antioxidant property of 60% for mother tincture. Many recent studies proved that nanotechnology has a new therapeutic modality in silver particles for use in medicine. (2) This study proved that spirulina as a nanomedicine it has good antioxidant and antimicrobial properties.

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