GREEN SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITIES OF TUNGSTEN, TIN AND LEAD NANOPARTICLES BY *CUCURBITA MAXIMA* (PUMPKIN) FLOWER EXTRACT

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Abstract:

In this paper, a green synthetic route was developed to synthesize tungsten, tin and lead nanoparticles mediated by the *Cucurbita maxima* flower extract, *Cucurbita* is monoecious (unisexual flowers, with male and female on the same plant), bearing solitary actinomorphic flowers (10-20 cm across) that are open for a single day *Cucurbita maxima* was a traditional vegetable food for south Indian. The flower of *Cucurbita maxima* aqueous extract is analyzed for the phytochemical constituent. The synthesized nanoparticles are characterized by UV, FTIR, XRD and SEM with EDS. The synthesized three nanoparticles also screened by gram positive and negative bacterial strains.

Keywords: Green synthesis, WNP, SnNPs, PbNPs, Characterization, Antibacterial activity

1. INTRODUCTION

Nanotechnology aims the production of very small sized particles that have nanoscale dimension [1-2]. A number of chemical and physical approaches are used to synthesize nanoparticles (NPs) with desired properties, but these methods are very expensive, harmful for the humans and environment, labor intensive, toxic and high amount of energy and pressure is required for their proper completion [3]. NPs are biologically produced by plants, algae, fungi, bacteria, diatoms and human cells. Plants are preferred among all the green synthesis routes for nanoparticles [4-5]. Characteristics of nanoparticles as well as their stability, size and shape are greatly affected by the temperature, concentration, pH and many other factors during the formation process [6]. Different kinds of NPs including gold, iron, zinc, silver, copper, platinum as well as oxides of metals and non-metals etc are formed from prokaryotes and eukaryotes [7]. Among all, Zn NPs are preferred because they can generate ROS and are good semiconductors [8-9]. Characteristics of Zn NPs depend on their size, concentration and shape [10]. Extracts of the plants are used as bioreductant and capping agent for producing NPs [11]. Phytochemicals [12] and secondary metabolites present in plants extract play vital role during nanoparticle synthesis [13-14]. Green synthesis is based on redox reaction, in which metal ions are reduced to stable NPs by the components of an organism or its extract. Although living organisms, such as algae, fungi, bacteria and plants, could synthesize NPs *in vivo* [15-19], plant extract-mediated, *in vitro* green synthesis of NPs has gained popularity due to its simplicity, low cost, eco-friendly nature, and easiness to scale up. Although plant extract-mediated green synthesis is a recent technology, a contrasting process has been used for the preparation of Bhasma (ash of metal) in Ayurveda, a traditional Indian medicine practiced for more than 2000 years. In the former, metal ions are reduced and stabilized as NPs [20-23] by the components of the extract (bottom-up approach); by contrast, in the latter, NPs are produced by the calcination of metal sheets (top-down approach), which are macerated with medicinal plant extracts to obtain therapeutic potential. We report the green synthesis of tungsten, tin and lead nanoparticles mediated by tradition plant of *Cucurbita maxima* flower extract, characterization and also reported antimicrobial activities against three gram positive and three gram negative bacterial strains.

Figure:1:Images of *Cucurbita maxima* (Pumpkin)flowers

2. Materials and Methods

2.1 Sample collection

The fresh flower of *Cucurbita maxima* are collected early morning in winter season (November and December) from the C. Mutlur village of Chidambaram, Cuddalore district, Tamil Nadu, India

2.2 Reagents

All analytical grade chemical and solvents used in the sample preparation were purchased from local suppliers of Singma-Aldrich chemical company-Pondicherry, Precession scientific company-Trichy.

2.3 Extraction

Extraction from dried flower of *Cucurbita maxima*, these flowers are done using water as the extraction solvents. via direct hot extraction (DHE) method [24], 100 ml of solvent with 100 gm of the sample (flower extract) in around bottomed flask was kept on a heating mantle covering at 60°C for two days followed by ensuing filtration by means of whatman filter paper # 1. Then, the obtained filtrates were collected and dried with vacuum evaporator pending a rudimentary glutinous extract is obtained. Then, the collective supernatants are filtered and the filtrates are cooled and dried using hot air oven until a crude glutinous extract is obtained. After vanishing of organic solvents, these are stored at – 20°C till scrutiny.

2.3.1 PHYTOCHEMICAL SCREENING

The majority bioactive constituents of plants are steroids, tannins, flavonoid, alkaloids and phenolic compounds. To know the phyto-constituents of plants, the extracts are subjected to macro molecules and secondary metabolites analysis by using paradigm methods.

2.3.1.1 PRELIMINARY QUALITATIVE ANALYSIS

The preliminary qualitative analysis [25] of *Cucurbita maxima* (Pumpkin)flower extract, macro molecules was done as per the procedure given below.

DETECTION OF CARBOHYDRATES

A little amount of the extract is dissolved in 5ml of water and filtered. The filtrate is subjected to the subsequent test.

Molisch's test

All soluble carbohydrates give a purple colour when treated with molisch's reagent (alcohol, naphthol solution and conc. H₂SO₄) a soluble carbohydrates will form violet ring at the junction of the liquids.

Fehling's test

To a heated solution of extract mixture of equal parts of Fehling's solution A and Fehling's solution B was added drop by drop. Brick red precipitate shows the presence of reducing sugar.

Barfoed's test

To a portion of the extract, 2ml of Barfoed reagent is added and kept in boiling for less than 5 minutes. A coloured residue at the side's upper surface of the liquid is formed. Extract is hydrolyzed with dilute Hydrochloric acid for a few hours on water bath.

Benedict's test

To a portion of the extract, 5ml Benedict reagent is added and kept in boiling for less than 5 minutes. Reducing sugar indicates the brick red precipitate.

DETECTION OF FREE AMINO ACIDS AND PROTEINS

Little quantity of alcoholic and aqueous extract is dissolved in few ml of water and subjected to following test.

Millon's test

To the above extract Millon's reagent is added. Amino acids are confirmed by red colour formation.

Biuret test

To the above extract same volumes of 5% NaOH and 3 or 1% of solution of CuSO_4 are added. A violet colour formation indicates the amino acids present in the solution.

Ninhydrin test

To the extract ninhydrin reagent is added. The amino acid is identified by violet colour formation.

DETECTION OF ALKALOIDS

The small portion of the extract is dissolved in suitable solvent and each extract is stirred separately with only some drops of dilute HCl and filtered. The filtrate was examined for alkaloids by using following reagents.

Mayer's reagent

To the extract, potassium mercury iodide was added. Alkaloid indicates by formation of cream precipitate.

Wagner's reagent

To the extract, solution of iodine in potassium iodide is added. Formation of reddish brown colour, indicates the presence of alkaloid.

Hager's reagent

To the extract, saturated solution of picric acid and water was added. Formation of yellow precipitate indicates the presence of alkaloid.

Dragandroff's reagent

To the extract KI solution of $\text{Bi}(\text{NO}_3)_2$ was added. Formation of reddish brown colour evidenced the incidence of alkaloid.

2.3.1.2 QUALITATIVE ANALYSIS OF SECONDARY METABOLITES

DETECTION OF PHYTOSTEROLS

Libermann Burchard test

The small amount of extract is dissolved in 2ml of CHCl_3 in a dry test tube. Then 10 drops of $(\text{AcO})_2\text{O}$ and 2 drops conc. H_2SO_4 are added. The solution became red then blue and finally bluish green colour indicated the presence of steroids.

Salkowasky test

The few drops of extract are treated with 2ml of chloroform and equal volume of conc. H_2SO_4 . Bluish red to cherry red colour is observed in chloroform layer; whereas the acid layer assumes marked green fluorescence evident the presence of steroids.

DETECTION OF PHENOLIC COMPOUNDS AND TANNINS

The few drops of alcoholic and aqueous extract are treated in water and tested for the presence of phenolic compounds and tannins with FeCl_3 solution (5%), solution of gelatine containing 10% NaOH, 10% $\text{Pb}(\text{OAc})_2$ and aqueous bromine water.

DETECTION OF FLAVANOIDS

Extract is treated with sodium hydroxide the formation of yellow colour, evident that the presence of flavone.

Extract is treated with concentrated sulphuric acid, the formation of yellow to orange colour, indicates the presence of flavones

DETECTION OF TRITERPINOIDS

LibermannBurchard test

The few amount of the extract is dissolved in 2ml of chloroform in a dry test tube. Then 10drops of acetic anhydride and 2drops of conc. H_2SO_4 are added. Formation of wine red colour indicates the presence of tri-terpenoids.

DETECTION OF GLYCOSIDES

Legal test

Small amount of the extract is dissolved in pyridine and sodium nitro prusside solution is added to make alkaline. Pink colour reveal that the presence of glycosides.

Baljet test

To the extract, sodium picrate solution is added. Yellow to orange colour indicated the presence of glycosides.

Borntrager's test

The extract was mixed with dilute sulphuric acid and filtered. Then filtrate was shaken with chloroform and the chloroform layer was separated and added dilute ammonia. Pink red to violet colour indicates the presence of glycosides.

DETECTION OF SAPONINS

Foam Test

1ml of the test solution is taken in a measuring cylinder. To this, 20 ml of distilled water is added and shaken well.

Hameolysis test

The extract of the plant is spreaded over a glass slide to form a thin film layer on which a drop of human blood was placed and spreaded over the extract layer. After 30 minutes slide was examined under microscope for change in structure and shape of red blood cells. Control was always maintained to see the change in red blood cells structure for hemolysis.

DETECTION OF FIXED OILS AND FATS

Extracts are taken and they are pressed between filter paper and the paper is noted.

Few drops of 0.5N alc.KOH are added to various extracts with few drops of phenolphthalein. The mixture is heated on a water bath for 1-2 hours. Table:2 shows the result of preliminary tests of *Cucurbita maxima* flower extract.

2.4. Preparation of *Cucurbita maxima* flower extract for synthesis of nanoparticles

Cucurbita maxima flower washed are several times with de-ionized water and tried five days in room conditions, then grained and powdered. A 20 g of powdered flower is taken in 250

mL round bottomed flask and stirred with 100 mL de-ionized water, heated at 80°C for three minutes and filtered by using whatmann 1 μ filter paper to get the extract. The filtrate is used in the further synthesis process.

Table:1 Green Synthesis of WNP, SnNPs and PbNPs

S.No	Metal Sources	Metal solution	Flower extract	MNPs
1	tungstic acid (H ₂ WO ₃)
		0.1M tungstic acid	CMFE	WNPs
2	Stannous chloride SnCl ₂
		0.1M Stannous chloride	CMFE	SnNPs
3	lead acetate Pb(CH ₃ COO) ₂
		0.1M lead acetate	CMFE	PbNPs

2.4.1 Synthesis of tungsten nanoparticles (WNPs)

Synthesis of WNP is carried out by reducing tungstic acid (M.F:H₂WO₃, M.W: 249.85 g/mol) using *Cucurbita maxima* flower extract (CMFE). To achieve this purpose, 100 mL of saturated solution of tungstic acid is steadily mixed with 10mL of *Cucurbita maxima* flower extract (CMFE). The colour of the solution turns from pale yellow to chocolate brown in colour forming tungsten nanoparticles. (**Table:1**). Further, obtained suspension is transferred to 250mL separating funnel to collect the suspended enriched part, the suspended enriched part is centrifuged at 4000 rpm/20 min. Supernatant is discarded and pellet containing WNP is carefully washed 3 times with distilled water to remove unwanted materials. The obtained powder assumed as WNP [26] is incubated at ~100 °C until the water evaporated completely. Finally, WNP were systematically characterized.

2.4.2 Synthesis of tin nanoparticles (SnNPs)

For the fabrication of SnNPs by reducing stannous chloride using *Couropita maxima* flower extract (CMFE). To achieve this intention, 100 mL of homogenous solution of stannous chloride is steadily mixed with 10 mL of *Couropita maxima* flower extract (CMFE), followed by continuous heating (70°C) and stirring at 500 rpm for 2 h in a magnetic stirrer with heating instrument, to achieve precipitate containing final solution (**Table:1**). The white colour reactant solution is turned to golden yellow in colour. The obtained brown colour solution was centrifuged at 4000 rpm/20 min. Supernatant was discarded and pellet containing SnNPs [27] is carefully washed 3 times with double distilled water (DDW) to remove uninvolved phyto-constituent and other chemicals. The obtained wet precipitate is dried, collected and stored in the airtight clicklock tube. Finally, SnNPs are methodically characterized.

2.4.3 Synthesis of Lead nano particles (PbNPs)

The synthesis of lead nanoparticles (PbNPs) carried out by 100 mL of 0.1 M of lead acetate solution is taken in a 250 mL conical flask. Then, 10 mL of *Cucurbita maxima* flower extract was added drop wise into lead acetate solution under continuous stirring for 30 min at the room temperature, the milky white solution of lead acetate is turned to pale pinkish in colour [28]. The resultant residue is filtered by using Whatman 1st filter paper, the precipitate is washed several times in double distilled water, finally obtained the glittering crystalline product. The obtained final product is dried and characterized.

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis

3.1.1 Yield of extraction

The yields of dried extract based on their dry weights are calculated using the following equation:

$$\% \text{ Yield (g / 100 g of arid plant material)} = (W_1 / W_2 \times 100)$$

Where, W_1 was the weight of the extract after the evaporation of solvent and W_2 was the weight of the dry plant material. The yield of aqueous extract of *Cucurbita maxima* flower is 4.28%

3.1.2 PRELIMINARY PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical study [31-32] of aqueous extract of *Cucurbita maxima* flower is conceded elsewhere to characterize the chemical constituents present in the extracts following standard procedures.

The results obtained from qualitative phytochemical analysis revealed the presence of biomolecules like carbohydrate, fats along with other important secondary metabolites like alkaloids, flavonoids, saponins, phenols and steroids, which are known for their varied pharmaceutical roles. The use of preliminary data obtained was extended to extract the relevant biomolecules from the aqueous extract of *Cucurbita maxima* flower. The results of preliminary phytochemical analysis in **Table: 2**

Table: 2 Phytochemical constituents of extract of *Cucurbita maxima* flower

S.No	Phytoconstituent	Water	Ethyl alcohol	Benzene	Methanol	CHCl ₃
1	Carbohydrates	+	-	+	+	+
2	Protein	-	-	+	-	+
3	Glycosides	+	-	-	+	-
4	Flavonoids	+	+	-	+	+
5	Alkaloids	-	-	+	+	-
6	Fixed oil/ Fat	+	-	+	-	+
7	Spaonins	-	-	-	+	+
8	Tannis	-	+	+	-	-
9	Phenols	+	-	-	+	+
10	Steriods	+	+	+	-	+

(+) indicate the existence of phytoconstituents

(-) indicate the nonexistence of phytoconstituents

3.2 Characterization

3.2.1 UV-Vis Spectroscopy analysis

Formation of nanoparticles is generally determined by optical properties which is one of the main criteria for their synthesis. Free electrons in the nanoparticles are excited by visible light and transmitted to a higher energy state, but the electron is wobbly in an excited state and returns to the base energy level and as a result a photon is emitted. Concurrently resonance frequency of surface plasmon in the metallic nanoparticles depends on shape, size and environment maintained during blend of nanoparticles. The UV-Vis spectrum of tungsten nanoparticles (Figure 2a) gave absorbance peaks around 385.5nm and it showed strapping band at this wavelength. The spectrum figure 3a has shown maximum absorption around 334 nm for tin nanoparticles and lead nanoparticles figure 4a have shown in absorption peak at 330nm.

3.2.2 FT-IR Spectroscopy analysis

FTIR is applied in order to investigate possible biomolecules bearing different functional groups responsible for reduction, capping and efficient stabilization of newly synthesized tungsten nanoparticles (Figure.2b). The absorption bands at 3440, 1596, 1459, 874 and 487 cm⁻¹ were observed. The strong peaks at 3440 cm⁻¹ corresponds to hydroxyl group, H-bonded OH stretches. The band at 2498 cm⁻¹ was attributed to methyl CH asym/sym stretches and 487 cm⁻¹ has represented W-O stretching vibrations. The strong band at 1420 cm⁻¹ is due to COO⁻ symmetric stretching and the carboxylate ion is responsible for the reduction of tungstic acid to W-O nanoparticles, which is confirmed by the reduced intensity of 1420 cm⁻¹ band.

FTIR spectrum of green synthesized SnNPs. The band between 420 cm⁻¹ can be assigned to Sn—O. The peak located at 728, 880, and 2390 cm⁻¹ can be assigned to aromatic CH₂ out of plane bending [63-68], aromatic ring stretching of cyclic compound, respectively (Figure 3b). The weak absorption at 3490 cm⁻¹ denotes N-H stretching vibration of the secondary amine. The strong band at 1440 cm⁻¹ is due to COO⁻ symmetric stretching and the carboxylate ion is responsible for the reduction of stannous chloride to Sn-O nanoparticles, which is confirmed by

Figure:2 (a) UV (b)FTIR(c)XRD spectrum images of WNPs

Figure:3 (a) UV (b)FTIR(c)XRD spectrum images of SnNPs

Figure:4 (a) UV (b)FTIR(c)XRD spectrum images of PbNPs

The reduction of intensity of 1440 cm^{-1} band. FTIR spectra for the lead nanoparticles are appeared in figure 4b. The strong band at 416 cm^{-1} demonstrates the nearness of Pb-O extending and furthermore the band at 582 cm^{-1} shows the nearness of oxides. These two pinnacles are sharp and is affirmed that the last item is the nearness of lead and oxide. The weak band at 1460 cm^{-1} is due to COO^- symmetric stretching and the carboxylate ion is responsible for the strong reduction of lead acetate to Pb-O nanoparticles, which is confirmed by the reduction of intensity of 1460 cm^{-1} band. The yield of the PbNPs is very high compared with WNPs and SnNPs.

3.2.3 XRD analysis

X-ray diffraction (XRD) studies are carried out to confirm the synthesis of WNP and characterize crystallinity and the phase pattern of synthesized tungsten nanoparticles. It was observed that 2θ (in degrees) are in the range of 28.4 to 66.5° (Figure. 2c). The said 2θ values of peaks are in harmony with the paradigm of JCPDS. The XRD study confirms that the consequential particles are nanoparticles. Still, it also confirms so as to the synthesized nanoparticles are free of impurities as no other description XRD peaks were observed. The mean grain crystalline size of green synthesized tungsten nanoparticles was calculated using the Debye–Scherrer formula $D = K\lambda / 6 \cos \theta$ where D is the average crystalline diameter size (Å), K is a constant (0.9), λ is the wavelength of the X-ray used ($\lambda = 1.54$ Å), ' θ ' is the angular line width [63] at the half maximum of diffraction (radians) and ' θ ' is the Bragg's. The diffraction peaks at 22.08°, 28.21°, 30.13°, 39.21°, 41.35°, 42.27°, 51.02°, 64.09°, 71.02°, and 80.25° can be indexed to (100), (111), (101), (121), (200), (222), (001), and (110), crystal planes of tetragonal WNP (JCPDS card No. 46-1043). The crystal phase of the biosynthesized SnO₂ NPs was analyzed using XRD. Figure 3c shows the XRD pattern of the biosynthesized SnNPs, which exhibits a series of well-defined diffraction peaks. The diffraction peaks at 27.05°, 37.21°, 48.99°, 52.02°, 54.19°, 58.05°, 61.89°, 65.06°, 65.74°, 69.21°, 72.01° and 78.98° be capable of indexed to (110), (200), (111), (211), (311) and (321) crystal planes of cubic SnO₂ (JCPDS card No. 41-1445). Moreover, the XRD pattern of the biosynthesized SnO₂ NPs also presents the peak at 27.05°, 37.21° and 48.99°, which is analogous to the carbon signals. As mentioned above, the continuation of carbon is likely to originate from the organic encapsulation of SnNPs. The green synthesized PbNPs is analyzed using XRD. Figure 4c, shows the XRD pattern of the biosynthesized PbNPs, which exhibits a sequence of well-defined diffraction peaks. The diffraction peaks at 30.05°, 38.21°, 48.99°, 58.05°, 62.89 and 64.98° are indexed to (111), (110), (102), (002), (001), (200) and (121) crystal planes of crystalline cubic PbNPs (JCPDS card No. 04-0784). Furthermore, the XRD pattern of the biosynthesized PbNPs also presents the peak at 30.05° and 38.21°. As mentioned before, the existence of carbon is probable to instigate from the organic encapsulation of PbNPs.

3.2.4 SEM analysis

The SEM uses a beam of high-energy electrons to fabricate a collection of signals at the exterior of specimens used. The signals demonstrate in sequence about the sample as well as chemical composition, and crystalline structure, external morphology (texture) and compass appraisal of materials which build up the illustration. SEM analysis is in wide-ranging precise to be non-destructive since the x-rays spawn do not pilot to loss of volume of the mock-up, so it becomes possible to frequently investigate the same materials. A scanning electron microscope is a category of electron microscope which images a sample by scanning it using a high-energy electron beam. The electrons then cooperate with the atoms making up the sample, consequently producing signals which divulge in sequence regarding the sample's opus, surface topography and other properties such as electrical conductivity.

The SEM analysis is used to resolve the structure of the reaction products that are fashioned. Thin films of the sample are equipped on a gold coated copper lattice by just dipping a very small amount of the taster on the net, extra solution was removed using a blotting paper and then the film on the SEM grid are endorsed to dry by putting it under a mercury lamp for 5 minutes. SEM image has showed individual tungsten particles as well as a quantity of aggregates. SEM analysis is done to envisage outline and size of nanoparticle. The SEM image of synthesized tungsten nanoparticles (WNPs) are shown in figure 5. From the SEM images are

seen in diverse intensification ranges like $5\mu\text{m}$ – $50\mu\text{m}$ which clearly demonstrated the presence of rod shaped nanoparticles.

Figure:5 SEM images of Tungsten Nanoparticles(WNPs)

Figure:6 SEM images of Tin Nanoparticles (SnNPs)

Figure:7 SEM images of Lead Nanoparticles (PbNPs)

The SEM analysis is used to conclude the structure of the reaction products that are formed. Thin films of the sample are equipped on a gold encrusted copper grid by just plummeting a very small amount of the sample on the grid, extra solution was removed by means of a blotting paper and then the film on the SEM grid are sanctioned to dry by putting it under a mercury lamp for 5 min. SEM image has showed individual tin particles as well as a number of aggregates. SEM analysis is completed to dream of shape and size of nanoparticle. The SEM images of synthesized tin nanoparticles are shown in figure 6. The SEM images are seen in different magnification ranges like $10\mu\text{m}$ – $50\mu\text{m}$ which clearly demonstrated the presence of spherical and mountain rock shaped nanoparticles.

The SEM analysis is used to determine the structure of the reaction products that are formed. Thin films of the sample are prepared on a gold coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes. SEM image has showed individual zinc particles as well as a number of aggregates.

SEM analysis is done to visualize shape and size of nanoparticles. The SEM image of synthesized lead nanoparticles is shown in figure 7. The SEM images are seen in different magnification ranges likes 10 μ m– 50 μ m which clearly demonstrated the presence of thick mountain rock shaped nanoparticles.

3.2.5 EDX analysis

Fig 8a: EDX Image of WNPs

Fig 8b; EDX spectrum of WNPs

Fig 9a: EDX Image of SnNPs

Fig 9b; EDX spectrum of SnNPs

Fig 10a: EDX Image of PbNPs

Fig 10b; EDX spectrum of PbNPs

The elemental information of biosynthesized WNPs is analyzed by EDX. As shown in the figure 8a, evident that particle size is 10 μ m. The intensive peak signals of O and W are observed in the spectrum (figure 8b) indicating the composition of W and O. Besides, which could due to the biomolecules on the surface of the biosynthesized WNPs. The elemental information of biosynthesized SnNPs was analyzed by EDX. Figure 9a, reveals that particle size is 10 μ m and the intensive peak signals of P, Cu, Zn and Sn are observed in the spectrum figure

9b, indicating the composition of Sn and O. Besides, the spectrum also exhibits the existence of C, Na and P, which could due to the biomolecules on the surface of the biosynthesized SnNPs. The elemental information of biosynthesized PbNPs is analyzed by EDX and is shown in the figure 10a, reveal that particle size is 10 μ m. The intensive peak signals of P, Al and Pb are observed in the spectrum (figure 10b) indicating the composition of P, Al and Pb. Besides, the spectrum also exhibits the existence of P, Al and Pb, which could due to the biomolecules on the surface of the biosynthesized PbNPs.

4. ANTIMICROBIAL STUDIES

S. aureus

B. subtilis

S. pyogenes

Figure:11. Plate of Antibacterial activities of WNP, SnNP and PbNP against Gram positive Bacterial strains

K. pneumonia *K. oxytoca* *V. parahaemolyticus*

Figure:12. Plate of Antibacterial activities of WNPs, SnNPs and PbNPs against Gram Negative Bacterial strains

The nanoparticles of WNPs, SnNPs and PbNPs mediated by aqueous extract of *cucurbita maxima* (pumpkin) flower are taken. The test solutions are prepared by 100 mg/ml of by dissolving the nanoparticles in pure dimethylsulfoxide (DMSO) (Sigma, USA-Pondy). Test organisms are collected from the Bacteriology Unit of (CAS) Central advanced studies of Marine biology in (Parangipettai) Annamalaiuniversity, Tamil Nadu. The organisms used in this research are three Gram positive (*S. aureus* and *S. pyogenes*, *B.subtilis*) and three Gram negative bacterial strains, (*K. pneumonia* , *K. oxytoca*, *V. parahaemolyticus*). Upon receipt, all isolates are subcultured onto selected culturing media to ensure purity and confirm their identification. The strains are maintained and tested on Mueller Hinton Agar (MHA) (Merck, Germany), which was stored at 4°C. The test organisms are cultured overnight at 37°C before being used in the antibacterial assay described below.

The WNP, SnNP and PbNP are tested same concentrations for antibacterial activity using the disc diffusion method [33]. Sterile commercial blank discs (Oxoid), 6.0 mm diameter, were impregnated with test solutions of WNP, SnNP and PbNP. Discs were stored at -5°C prior to use. During the night broth cultures are adjusted using at urbidometer to yield approximately 1.0×10^8 cfu per ml. Nanoparticles impregnated discs (20 μl) are placed on agar plates and incubated at 37°C for 24 hours. Pure DMSO (20 μl) was used as a negative control, while ciprofloxacin discs (30 μl) are used as a positive control. Antibacterial activities are then determined by measuring the clear zone of inhibition to the nearest millimetre (mm). An *in vitro* test for antibacterial activity revealed that WNP, SnNP and PbNP mediated by the *cucurbita maxima* (Pumpkin) flower. The WNP has shown good antibacterial effect against GPB (Gram positive bacterial) strains *S.aureus*, *B.subtilis* and *S. pyogenes* while SnNP has shown less activity and PbNP has shown moderate antibacterial activity comparing standard ciprofloxacin. Gram negative Bacterial (GNB) strain of *K.pneumoniae* with WNP shows moderate activity, PbNP has shown good activity, poor activity against SnNP. The gram negative bacterial strain of *K. oxytoca* demonstrated poor activity with WNP, PbNP and good activity SnNP. *V.parahaemolyticus* a gram negative antibacterial strain have shown good activity towards SnNP, moderate activity against WNP and PbNP have shown poor activity comparing standard ciprofloxacin. Table.3 shows zone of inhibition values of WNP, SnNP and PbNP against gram positive and gram negative bacterial strains. Figure 11 and 12 expose the images of zone of inhibition plates of gram positive and negative bacterial strain against WNP, SnNP and PbNP, respectively, Figure:13 have shown in the cluster column chart of WNP, SnNP and PbNP against bacterial strains

Figure:13. The Cluster column chart of WNP, SnNP and PbNP against bacterial strains

Table: 3 Zone of inhibition on Antibacterial activities of WNP, SnNPs and PbNPs against Gram positive and Gram negative Bacterial strains

Compound	Zone of Inhibition (mm)					
	Gram positive Bacteria			Gram negative Bacteria		
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>S. pyogenes</i>	<i>K. pneumoniae</i>	<i>K. oxytoca</i>	<i>V. parahaemolyticus</i>
SnNPs	5	10	8	10	8	18
WNPs	13	18	17	14	16	12
PbNPs	11	16	12	16	8	11
ciprofloxacin	22	23	23	24	24	23
Control (DMSO)	-	-	-	-	-	-

5. CONCLUSION

The green synthesis of WNP, SnNPs and PbNPs nanoparticles by *cucurbita maxima* (pumpkin) flower extract was studied. The reduction of the metal charged ions led to the pattern of metal nanoparticles of fairly well-defined dimensions using the extract. Further, antibacterial activity of metal nanoparticles synthesized *cucurbita maxima* (pumpkin) flower extract has been explored. This green chemistry approach towards the synthesis of metal nanoparticles such as WNP, SnNPs and PbNPs has many advantages such as environmental friendly, cost effective and it can be easily scaled up to large scale synthesis. Antibacterial studies of three metal nanoparticles on human being pathogen open a door for a fresh variety of antibacterial agents.

REFERENCE

- [1] H.Agarwal, S.V.Kumar, S. Rajeshkumar., A review on green synthesis of zinc oxide nanoparticles – an eco-friendly approach. *Resour. Effic. Technol.* 3, (2017)406–413.
- [2] M.Fazal-Ur-Rehman, 2018. Novel applications of nanomaterials and nanotechnology in medical sciences-a review. *J. Basic Appl. Sci. Res.* 8 (4) (2018) 11-22.
- [3] S.Taheriniya, Z.Behboodi, Comparing green chemical methods and chemical methods for the synthesis of titanium dioxide nanoparticles. *Int. J. Pharm. Sci. Res.* 7 (12)(2016) 4927–4932.
- [4] V.V.Makarov, A.J.Love, O.V.Sinityna, S.S.Makarova, I.V.Yaminsky, M.E.Taliansky, N.O.Kalinina, Green nanotechnologies: synthesis of metal nanoparticles using plants. *Acta Naturae.*, 6 (1)(2014) 35-44
- [5] H.Mirzaei, M.Darroudi, Zinc oxide nanoparticles: Biological synthesis and biomedical applications. *Ceram. Int.* 43 (1)(2017) 907–914.

- [6] M.Shah, D.Fawcett, S.Sharma, S.Tripathy, G.Poinern,,Green synthesis of metallic nanoparticles via biological entities. *Materials* 8 (11) (2015) 7278–7308.
- [7] S.Hasan, A review on nanoparticles: their synthesis and types biosynthesis: mechanism. *Res. J. Recent. Sci.* 4(2015) 9–11.
- [8] B.Malaikozhundan, Pharmaceutical applications of zinc oxide nanoparticles a review. *Acta Sci. Pharm. Sci.* 2 (1)(2018) 11–12.
- [9] V.Srivastava, D.Gusain, Y.C.Sharma,. Synthesis, characterization and application of zinc oxide nanoparticles (n-ZnO).*Ceram. Int.* 39 (8) (2013) 9803–9808.
- [10] K.S.Siddiqi, A.urRahman, A.Husen, Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale Res Lett.* 13 (1) (2018) 1-13.
- [11] M.Shaik, M.Khan, M.Kuniyil,A.Al-Warthan, H.Alkathlan, M.Siddiqui, S.Adil, Plant-extract-assisted green synthesis of silver nanoparticles using *Origanum vulgare* L. extract and their microbicidal activities. *Sustainability.*, 10 (4) (2018) 913-927.
- [12] A.Stephen, S.Seethalakshmi, Phytochemical synthesis and preliminary characterization of silver nanoparticles using hesperidin. *J Nanosci.*,(2013)1–7
- [13] G.Marslin, K.Siram, Q.Maqqbool, R.Selvakesavan, D.Kruszka, P.Kachlicki, G.Franklin, Secondary metabolites in the green synthesis of metallic nanoparticles. *Materials.* 11 (6), (2018) 940-948.
- [14] S.Mourdikoudis, R.M.Pallares, N.T.Thanh, Characterization techniques fornanoparticles: comparison and complementarity upon studying nanoparticle properties. *Nanoscale*10 (27) (2018) 12871–12934.
- [15] A.M.Fayaz, K.Balaji, M.Girilal, R.Yadav, P.T.Kalaichelvan, R.Venketesan., Biogenic Synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. *Nanomed.Nanotechnol. Biol. Med.*, 6, (2010) 103–109.
- [16] S.Gómez-Graña, M.Perez-Ameneiro, X.Vecino,I.Pastoriza-Santos, J.Perez-Juste, J.Cruz, A.Moldes., Biogenic synthesis of metal nanoparticles using a biosurfactant extracted from corn and their antimicrobial properties. *Nanomaterials*, 7(2017) 139-153.
- [17] K.B.Narayanan, N.Sakthivel, Biological synthesis of metal nanoparticles by microbes. *Adv. Colloid Interface Sci.*, 156(2010) 1–13.
- [18] J.L.Gardea-Torresdey, J.G.Parsons, E.Gomez, J.Peralta-Videa, H.E.Troiani, P.Santiago, M.J.Yacaman,Formation and growth of Au nanoparticles inside live alfalfa plants. *Nano Lett.*, 2(2002) 397–401.
- [19] J.LGardea-Torresdey, E.Gomez, J.R.Peralta-Videa, J.G.Parsons, H.Troiani, M.Jose-Yacaman, A.Alfalfa sprouts: A natural source for the synthesis of silver nanoparticles. *Langmuir*, 19(2003) 1357–1361
- [20] A.T.Harris, R.Bali, On the formation and extent of uptake of silver nanoparticles by live plants. *J. Nanopart. Res.*, 10(2008) 691–695.
- [21] R.G.Haverkamp, A.T.Marshall, The mechanism of metal nanoparticle formation in plants: Limits on accumulation. *J. Nanopart. Res.*, 11(2009) 1453–1463.
- [22] K.H.Roh,B.K.Kwak, J.B.Kim, K.R.Lee, H.U.Kim, S.H.Kim,The influence of silver thiosulfate and thidiazuron on shoot regeneration from cotyledon explants of *Brassica napus*. *J. Plant Biotechnol.*, 39(2012) 133–139.
- [23] R.Kumari, J.S.Singh, D.P.Singh., Biogenic synthesis and spatial distribution of silver nanoparticles in the legume mungbean plant (*Vignaradiata* L.). *Plant Physiol. Biochem.*, 110 (2017) 158–166.

- [24] P.C.Vengaiah, B.Vijayakumari and P.Kiranmayi. Green synthesis Antimicrobial and Antioxidant effects of silver nanoparticle using *CanthiumCoromandelicum* leaves extract. *IAJPR*,**(2015)** 1030-1033.
- [25] E.Wallis,S.B.Gokhate , C.E.Kokate , Dr. Pulok and K.Mukherjee. Nutrients Composition of seed, Chemical Characterization and fatty acid composition of Oil of *Syzygiumaromaticum*. *IJAC*,**6(3)(2010)**363- 366
- [26] J. O. Tijani , O. Ugochukwu, L. A. Fadipe, M. T. Bankole, A. S. Abdulkareem& W. D. Roos., One-step green synthesis of WO₃ nanoparticles using *Spondiasmombin* aqueous extract: effect of solution pH and calcination temperature *Appl. Phys. A.*, 125, 162 **(2019)**. <https://doi.org/10.1007/s00339-019- 2450-y>
- [27] Hu.Junjie, Biosynthesis of SnO₂ Nanoparticles by Fig (*FicusCarica*) Leaf Extract for Electrochemically Determining Hg(II) in Water Samples.,*Int. J. Electrochem. Sci.*, 10 **(2015)** 10668 – 10676
- [28] N. Gandhi, D. Sirisha and SmitaAsthana., Microwave Mediated green synthesis of lead (Pb) nanoparticles and its potential applications, *IJESRT*., 7(1): January, **(2018)** 623-644
- [29] McMurry, Organic Chemistry, 2nd Edn, Pacific Grove, USA **(1988)**.
- [30] J. L. Rios, M. C .Recio, Medicinal Plants and antimicrobial activity. *Jr. of ethnopharmacology*., 100, **(2005)** 80-84.
- [31] M. F. Balandrian, A. F. Kjocke, and E. Wurtele., Natural Plant Chemicals: sources of industrial and mechanical material. *Science*., 228 **(1985)** 1154-1160.
- [32] S.Poompozhi and D.Kumarasamy., studies on phytochemical constituent of some selected mangroves., *JAIR*.,**2(10)(2014)** 590-592
- [33] A.W. Bauer, W. M. M. Kirby, J. C. Sherris and M. Turck, Antibiotic susceptibility Testing by a Standardized Disk Method., *Am. J. Clin. Pathol.*., **45(1966)** 493-496.