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Sunlight induced rapid and efficient biogenic synthesis and characterization of silver nanoparticles from leaf and dye extract of *Bixa orellana* L. A natural food dye plant

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Abstract
Nanotechnology is emerging as an important field in modern scientific research for the purpose of synthesizing new materials at the nanoscale level. It deals with the synthesis and application of materials under the dimensions of nanometers. (<100 nm). Nanotechnology is gaining tremendous attention in the present century due to its expected impact on many important areas such as biomedical services, cosmetics, drug gene delivery, environmental health, food, health care, catalysis, mechanics, non linear optical devices, optics, photo-electrochemical application, single electron transistors and space industries. Conventionally, the chemical synthesis of nanoparticles is the most popular approach. This approach has its drawbacks with respect to medical applications since chemical synthesis leads to production of toxic substances. There have been many reports on the synthesis of silver nanoparticles using microorganisms including bacteria, fungi and plants with good surface and size characteristics which confer a range of biological properties namely antimicrobial, anticancer, anti-biofueling, antimalarial, antiparasitic, antioxidant, etc. Of all the methods employed, the use of plants extracts is the most sought after. Development of green protocols for the synthesis of AgNPs has evolved as a novel field of science and is referred as Green nanotechnology. Green nanotechnology is also known as photobiological approach which utilizes plants or their extracts as reducing and capping agents in the synthesis of AgNPs. Hence, the biosynthesis of NPs is a reliable, cost effective, eco-friendly and important aspect of green chemistry approach that intersects biotechnology and nanotechnology. With the literature available, there have been no reports on the biosynthesis of silver nanoparticles from *Bixa orellana* L leaf and the dye Nor-bixin under sunlight. Hence, our study is the first of its kind in this regard to report a simple, efficient and one-step protocol for the rapid synthesis of AgNPs from *Bixa orellana* L leaf and seed dye extract (Nor-Bixin) under direct sunlight and the characterisation of AgNPs by TEM Studies.

Keywords: Nanotechnology, *Bixa orellana* L., Nor-Bixin, sunlight and silver nanoparticles and TEM

Introduction

Nanotechnology has become a popular and necessary technology in recent years. The field of nanoscience has blossomed over the last twenty years and the need for nanotechnology will only increase as miniaturization becomes more important in areas such as computing, sensors and biomedical applications. Advances in this field largely depend on the ability to synthesize nanoparticles of various materials, sizes, and shapes, as well as to efficiently assemble them into complex architectures [1]. With the advancements in nanotechnology, it has become possible to engineer the properties of nanoparticles and hence utilizing them in various areas of research [2]. Nanomaterials that can act as biological mimetics, ‘‘nanomachines”, biomaterials for tissue engineering, shape-memory polymers as molecular switches, biosensors, laboratory diagnostics and nanoscale devices for drug release, are just a few of the applications being explored.

Nanotechnology provides the ability to engineer the properties of materials by controlling their size, and this has driven research towards a multitude of potential uses for nanomaterials. Nanoparticle synthesis and the study of their size and properties is of fundamental importance in the advancement of recent research. It is found that the optical, electronic, magnetic, and catalytic properties of metal nanoparticles depend on their size, shape and chemical surroundings. As nanotechnology is an innovative and scientific growth area with an exponential production, more information is needed concerning the impacts of these nanomaterials (NMs) in the environment and particularly in plants performance. So, research on NPs as emerging contaminants is therefore a new field in environmental health [3]. There is an array of metals which are used in the production of nanoparticles but silver nanoparticles

are gaining most interest in the field of Nanotechnology. Silver nanoparticles are one of the promising products in the nanotechnology industry. The development of consistent processes for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research [4]. The extensive use of silver nanoparticles is mainly due to its effective antimicrobial activity against bacteria, viruses and other eukaryotic microorganisms as compared to other metals [5]. A small evergreen tree, *Bixa orellana* (Annatto) of family Bixaceae is a native plant of Northern South America but is widely cultivated in the tropical countries for its dye yielding seeds which are used as food colourants and is also of medicinal value. Annatto is obtained from oily arils of the seeds is the world's second most important natural colorant after caramel [6]. It yields non toxic food dye called Bixin and Nor Bixin. Bixin is oil soluble and typically used to colour higher fat dairy products, cosmetics and leather while Norbixin is water soluble and used for colouring cheese. Annatto (E160 b) is one of the 13 basic food pigments derived from natural sources that are currently permitted for food colouring by the US-FDA and there is ever increasing demand. With reference to the synthesis of Silver nanoparticles, in the literature available, the biosynthesis of silver nanoparticles using leaf extract of plant *Bixa orellana* L. has been reported by 1mM silver nitrate solution in dark for 72 hrs. These silver nanoparticles synthesized have been characterised using UV-Visible spectroscopy and by SEM studies [7]. But there have been no studies conducted on the biosynthesis of silver nanoparticles from *Bixa orellana* L leaf and dye extract (Nor-bixin) using sunlight with 5mM silver nitrate solution and the characterisation of silver nanoparticles by TEM. Hence, our study is aimed to be the first of its kind in this regard.

Materials and Methods

Materials

A. Biological samples: A healthy plant of *Bixa orellana* L. accession of White flower and green capsule was identified which was cultivated in the Department of Life Sciences, Bangalore University Campus, Bangalore and the leaves and dye yielding seeds were collected (Plate 1).

Bacterial strains: *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) were procured from the Department of Biotechnology, BUB.

Plate 1: Habit of *Bixa orellana* L. a natural food dye yielding plant

B. Chemicals: Analytical grade $AgNO_3$, KOH, Nutrient Agar, Ampicillin (Antibiotic).

C. Instruments: Elico Colorimeter (Model no.CL-223), UV visible Spectrophotometer (Model no. RS 232 C), JEOL Transmission Electron Microscope (TEM) Model No: JEM, 2100HR.

Methods

A (a). Preparation of the *Bixa orellana* L Leaf extract

10g of leaf sample was weighed and washed thoroughly with distilled water and macerated in mortar and pestle with distilled water and volume was made upto 100ml with the same. The filtrate was then centrifuged at 10,000 rpm for 20 minutes; supernatant was collected and stored at 4℃.

(b). Preparation of the *Bixa orellana* L Dye Extract (Nor-Bixin)

0.1g of the seed sample was weighed and macerated in 15 ml 0.5% KOH, the filtrate was centrifuged at 10,000 rpm for 20 minutes, the supernatant was collected and volume made upto 100ml with distilled water and stored at 4℃.

(c). Preparation of Diluted Dye Extract (Nor- Bixin)

2ml of the concentrated dye sample was measured and diluted up to the final volume of 100ml and stored at 4℃.

B. Biogenic Synthesis of Silver Nanoparticles (AgNPs) from leaf and Dye Extract

5 ml of a) leaf extract, b) Concentrated Dye extract and c) Diluted dye extract were transferred into three conical flasks and 45 ml of 5 mM $AgNO_3$ solution was added to each and the resulting solutions were kept under direct sunlight and gradual colour change was noted in each case. The solution thus obtained was centrifuged at 15,000 rpm for 20 minutes and the pellet after being washed twice was stored.

C. Characterization of Silver Nanoparticles Synthesized from leaf and dye extract of *Bixa orellana* L.

1. **UV-Visible Spectroscopy:** To observe the optical property of biosynthesized silver nanoparticles, samples (both of the supernatant and pellet of the leaf and the dye extract) were analyzed with spectrophotometer and colorimeter between 230- 640 nm range.
2. **Transmission Electron Microscopic Studies (TEM):** TEM technique was used to visualize the morphology-size and shape of the silver nanoparticles. 200kV Ultra-high resolution transmission electron microscope (JOEL, Model No: JEM, 2100HR). Prior to analysis, AgNPs were sonicated for 5 min, and a drop of diluted sample was placed on carbon -coated copper grid. The liquid fraction was allowed to evaporate at room temperature.

D. Antibacterial Assay of AgNPs from Leaf and dye Extract of *Bixa orellana* L. by Agar Well Diffusion Method

The AgNPs synthesized from the leaf and dye extracts were tested for their antimicrobial activity against *Eischeria Coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) bacteria. Each strain was spread uniformly on a nutrient agar plate and a well of 7mm was made using a gel puncture. 80 µl of the leaf and dye samples (supernatant and pellets) were poured into the wells and incubated at 35℃ overnight and the zone of inhibition was measured. Ampicillin antibiotic of 80 µl was used as a reference drug.

Results

A. Biosynthesis of AgNPs from $AgNO_3$: In the presence of the leaf and dye extracts of *Bixa orellana L.* the biosynthesis of AgNPs was observed by the resultant reddish brown

colour. This colour change indicates that silver ion in reaction mixture have been converted into elemental silver (Plate 2).

a. Biosynthesis of Silver Nanoparticles with leaf extract- The time taken for the reaction to take place under direct sunlight was 1 minute 20 seconds.

Plate 2: Leaf extract of *Bixa orellana* L. before and after reaction with silver nitrate solution.

b. Biosynthesis of silver nanoparticles from Dye extract The time taken for the reaction to take place under sunlight was 1 minute 45 seconds and 2 minutes 10 seconds for concentrated and diluted dye extracts respectively (Plate 3&4).

Plate 3, 4: Concentrated Dye extract and the diluted dye extract of *Bixa orellana* L. before and after reaction with silver nitrate solution

B. Characterization of Silver Nanoparticles Synthesized from leaf and dye extract of *Bixa orellana* L.

1. Characterization of AgNPs by UV-Visible spectroscopy and colorimeter: To observe the optical property of biosynthesized silver nanoparticles, samples were analysed with spectrophotometer and colorimeter (Fig. 1,2 &3).

a. Absorbance spectrum of *Bixa orellana* L. leaf extract: The spectrophotometer and colorimeter peak for the leaf extract was found to be 260 nm and 470 nm respectively.

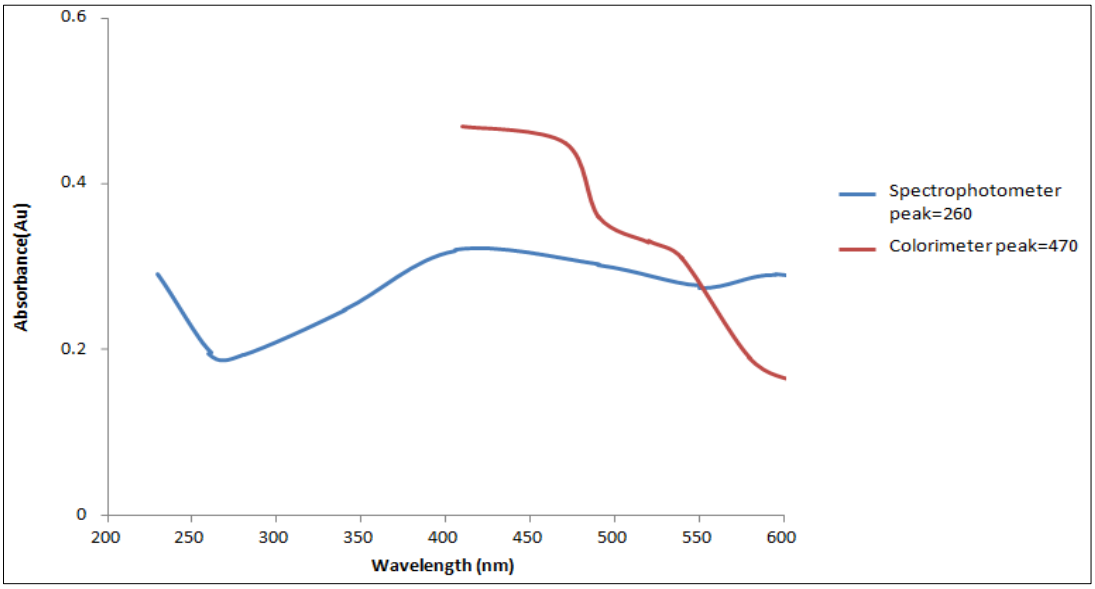

Fig 1: UV –Visible spectrum of Leaf Extract of *Bixa orellana* L.

b. Absorbance spectrum of Silver Nanoparticles synthesized from *Bixa orellana* L. leaf extract

The spectrophotometer and colorimeter peak for the Silver Nanoparticles synthesized from leaf extract was found to be 405 nm and 470 nm respectively, for both the supernatant and the pellet.

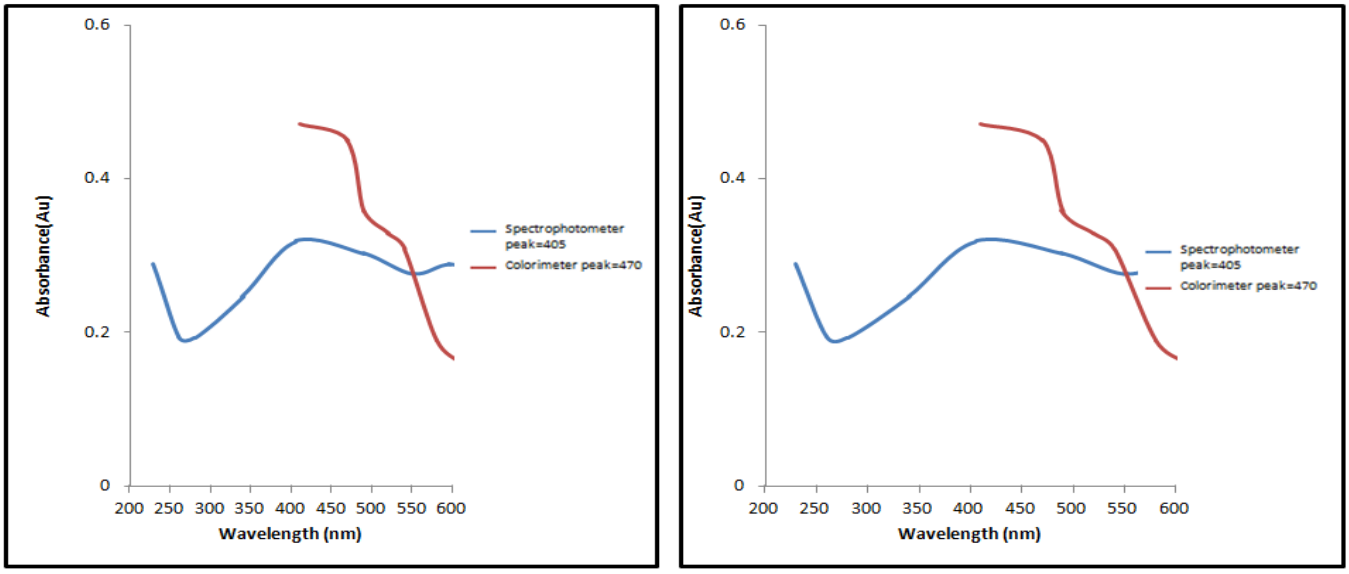

Fig 2, 3: UV spectrum of AgNPs synthesized from *Bixa orellana* L. leaf extract (Supernatant and Pellet)

c. Absorbance spectrum of concentrated dye extract (Nor bixin) of *Bixa orellana* L: The spectrophotometer and colorimeter peak for the concentrated dye extract (nor-bixin) was found to be 250 nm and 470 nm respectively (Fig. 4, 5 & 6).

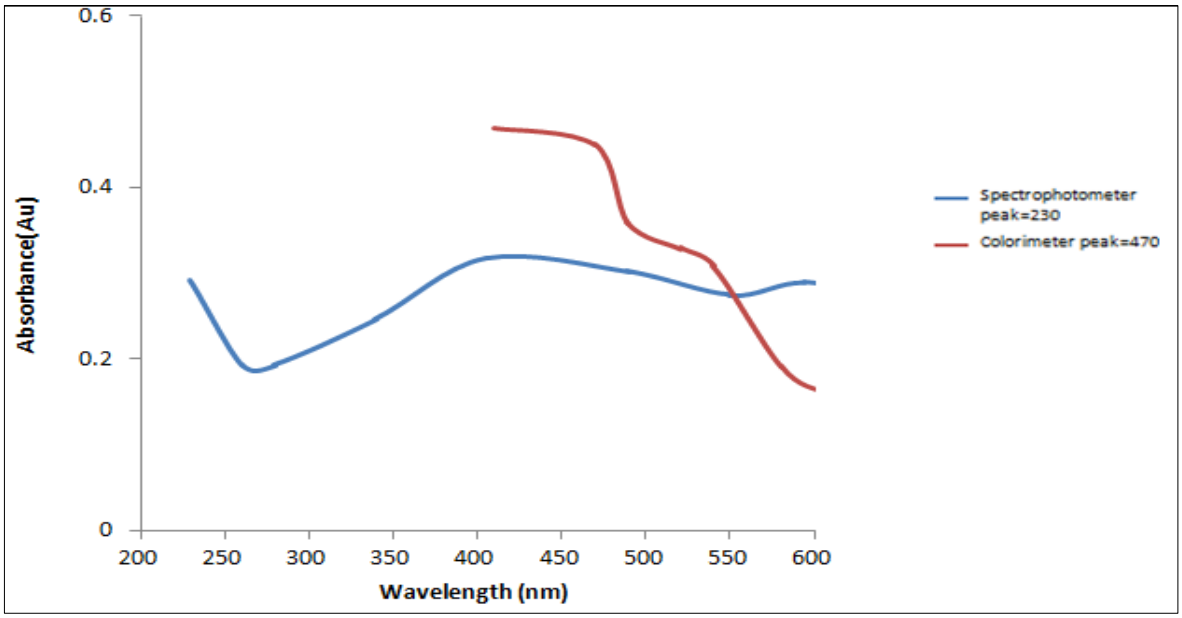

Fig 4: UV spectrum of Concentrated Dye extract (Nor-Bixin) of *Bixa orellana* L

d. Absorbance spectrum of Silver Nanoparticles synthesized from *Bixa orellana* L. Concentrated Dye Extract (Nor-Bixin)

The spectrophotometer and colorimeter peak for the Silver Nanoparticles synthesized from concentrated dye extract was found to be 405 nm and 470 nm respectively, for both the supernatant and the pellet.

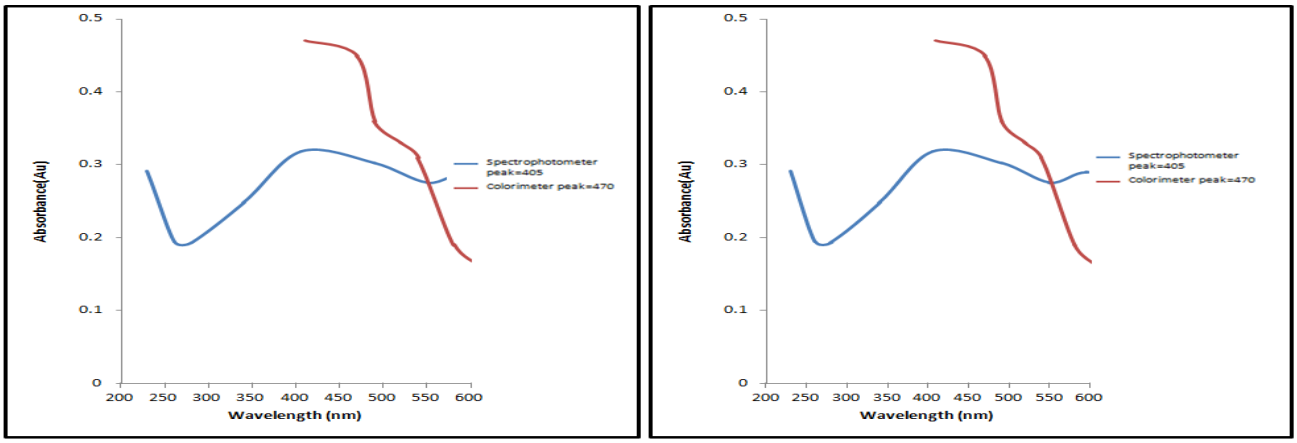

Fig 5, 6: UV spectrum of AgNPs synthesized from the Concentrated Dye extract (Nor-Bixin) Supernatant and Pellet.

e. Absorbance spectrum of *Bixa orellana* L. diluted dye extract (Nor-Bixin): The spectrophotometer and colorimeter peak for the Silver Nanoparticles synthesized from diluted dye extract was found to be 230 nm and 470 nm respectively (Fig. 7, 8 & 9).

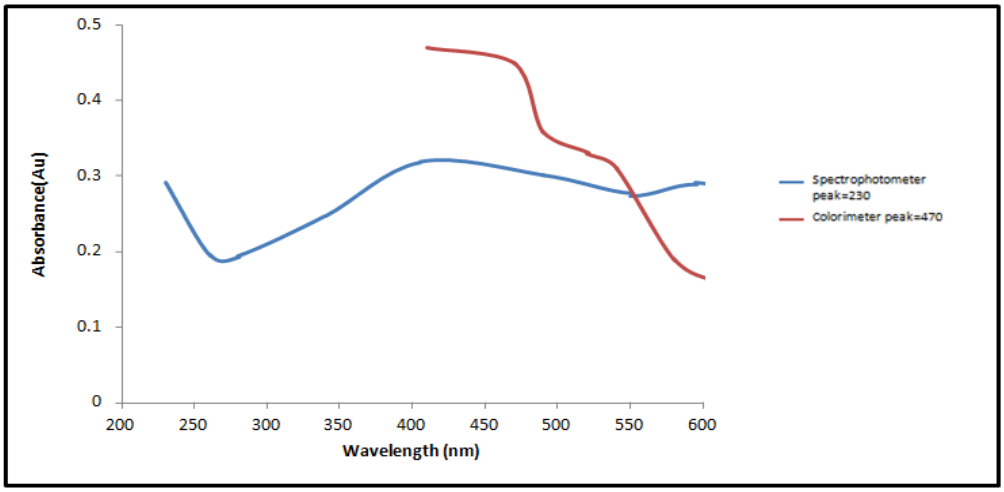

Fig 7: UV Spectrum of Diluted Dye Extract (nor-bixin)

f. Absorbance spectrum of Silver Nanoparticles synthesized from *Bixa orellana* L.
Diluted Dye Extract (Nor-Bixin): The spectrophotometer and colorimeter peak for the Silver Nanoparticles synthesized from Diluted dye extract was found to be 405 nm and 470 nm respectively, for both the supernatant and the pellet.

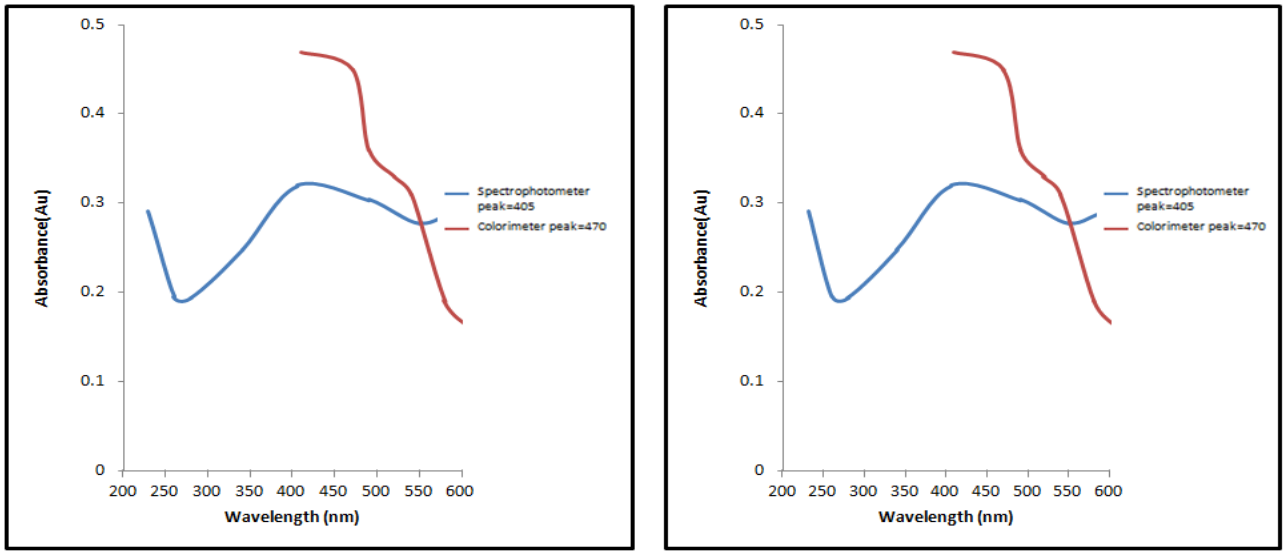

Fig 8, 9: UV Spectrum of AgNPs synthesized from diluted Dye extract (Supernatant) and Pellet

C. Characterization of AgNPs by Transmission Electron Microscopy (TEM) -The shape and size of AgNPs were elaborated by TEM Analysis.

a. Leaf Extract- The TEM image of the scale ranging from10nm-100nm confirmed the size to be ranging from 30nm-100nm and were spherical in shape (Plate 5).

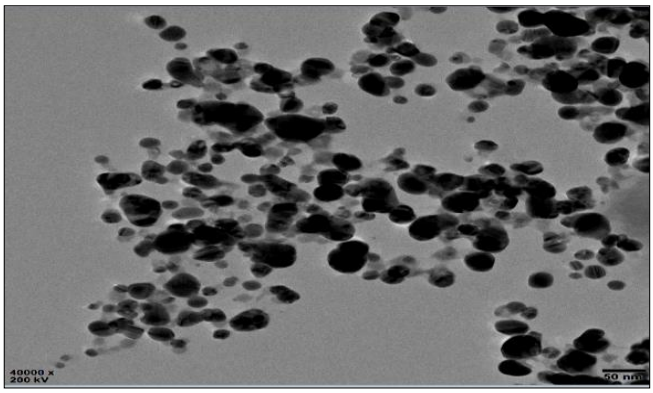

Plate 5: TEM Image of biosynthesized silver nanoparticles using *Bixa orellana* L. leaf extract

b. Concentrated Dye extract (Nor-Bixin): The TEM image of the scale ranging from10nm-100nm confirmed the size to be ranging from 10nm-80nm and were spherical in shape (Plate 6).

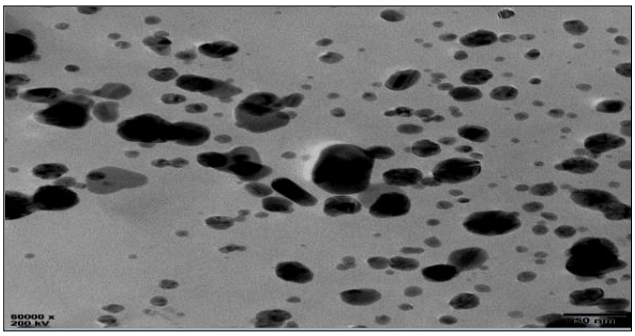

Plate 6: TEM Image of biosynthesized silver nanoparticles from *Bixa orellana* L. Dye extract

C. Antibacterial Assay of AgNPs from Leaf and dye Extract of *Bixa orellana* L. by Agar Well Diffusion Method:

a. Antibacterial activity of *Bixa orellana* Leaf extract-

1) The zone of inhibition of AgNPs in the supernatant and pellet of *Bixa orellana* L. leaf extract against *S. aureus* was 8 mm and 9.6 mm respectively (Plate 7 & Table 1)).

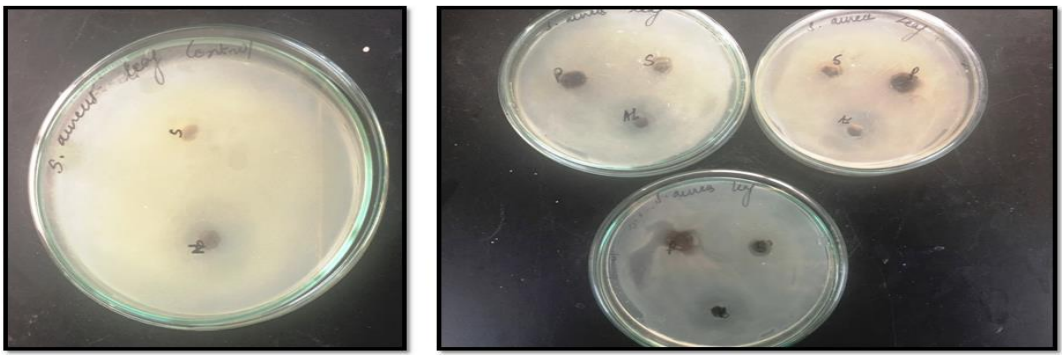

Plate 7: Antibacterial activity of AgNPs synthsized from *Bixa orellana* L. leaf extract against *S. aureus*

2) The zone of inhibition of AgNPs in the leaf supernatant and pellet of *Bixa orellana* L. against *E.coli* was 7.6 mm and 6.3mm respectively (Plate 8).

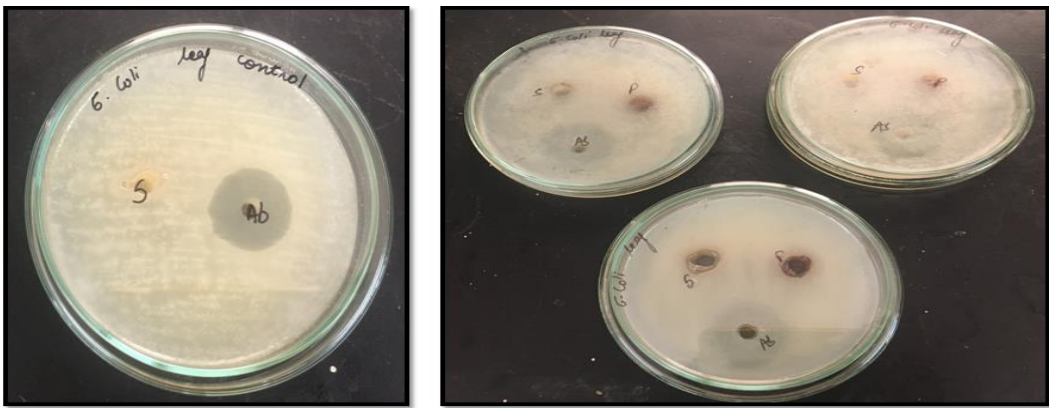

Plate 8: Antibacterial Activity of AgNPs synthesized from *Bixa orellana* L. leaf extract against *E.coli*

b. Antibacterial Activity of *Bixa orellana* L. dye (Concentrated and diluted) extract

1) The zone of inhibition Of Ag NPs in the supernatant of *Bixa orellana* L. dye (Concentrated and diluted) extract against *S. aureus* was 9mm and 9.6 respectively (Plate 9).

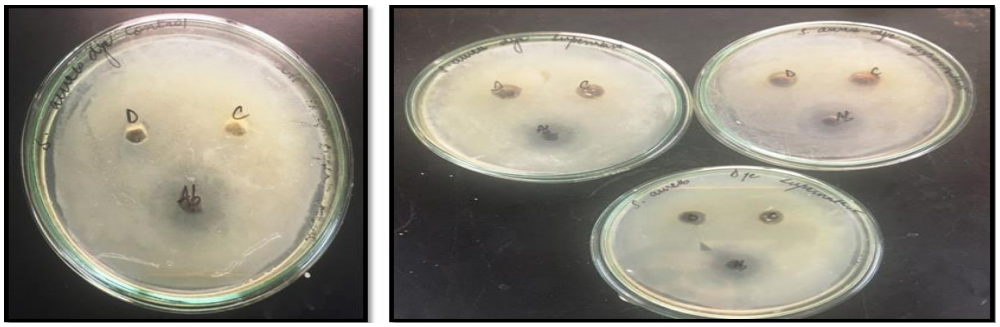

Plate 9: Antibacterial activities of AgNPs synthesized from *Bixa orellana* L. dye extract supernatant against *S. aureus*

2) The zone of Inhibition of AgNPs synthsised in the pellet of *Bixa orellana* L. dye (Concentrated and diluted) extract against *S. aurues* was 8.6mm and 8.3mm respectively(Plate 10).

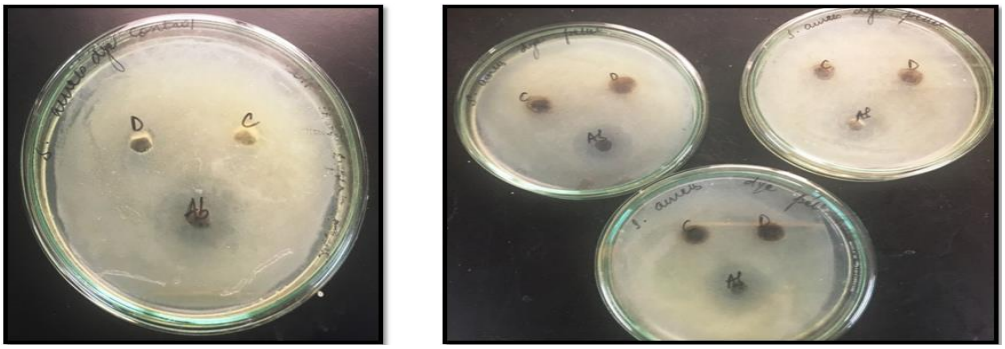

Plate 10: Antibacterial Activities of AgNPs synthesized from *Bixa orellana* L. pellet against *S. aureus*

3) The zone of inhibition Of AgNPs synthesized in the supernatant *Of Bixa orellana* L. dye (concentrated and diluted) dye against *E. coli* was 9mm and 8.3 mm respectively (Plate 11).

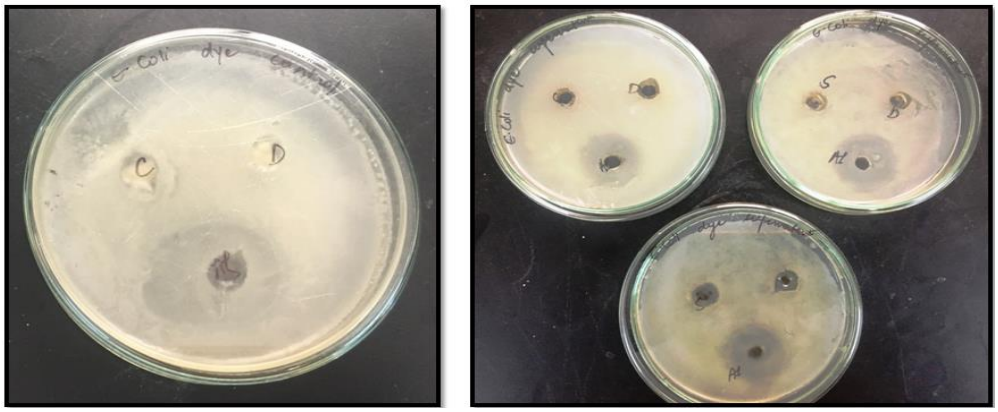

Plate 11: Antibacterial activities of AgNPs synthesized from *Bixa orellana* dye extract supernatant against *E.coli*

4) The zone of inhibition Of AgNPs synthesized in the pellet Of *Bixa orellana* L. dye (concentrated and diluted) dye against *E. coli* was 9.6 mm and 9.6 mm respectively (Plate 12).

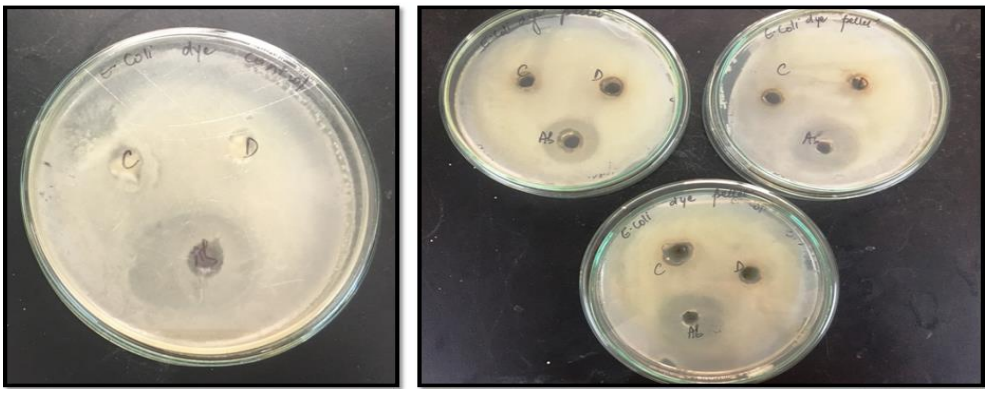

Plate 12: Antibacterial activities of AgNPs synthesized from *Bixa orellana* L. dye extract pellet against *E.coli*

Table 1: Antibacterial activity of AgNPs as a measure of zone of inhibition

Test Organism	Zone Of Inhibition (mm)									
	AB	**LC**	**LS**	**LP**	**DCn**	**0 Cn S**	**DCn P**	**DDI**	**D DI S**	**D DI P**
S.OUMUS	31	2	8	9.6	1	9	8.6	1	9.6	8.3
E. coli	31	2	7.6	6.3	1	9	9.6	1	8.3	9.6

Zone Of Inhibition (mm), mean value of three replicates
(AB: Antibiotic (Ampicillin)
(LC: Leaf Extract, LS: AgNPs in leaf supernatant, LP: AgNPs in Leaf Pellet, DCn: Concentrated dye extract DCnS: AgNPs in concentrated dye supernatant, DCnS: AgNPs in concentarted dye pellet, DDl: Diluted dye Extract, DDlS: AgNPs in diluted dye supernatant and DDlP: AgNPs in diluted dye pellet)

Discussion

Our studies is the first of its kind for efficient and rapid biosynthesis of silver nanoparticles whch has been carried out in the leaf and the dye extract (nor-bixin) extract of *Bixa orellana* L. using sunlight.

The characterization of the silver nanoparticles have been successfully carried out by using UV- visible spectrophotometer and Transmission Electron Microscopic Studies (TEM).

With respect to UV-Visible spectroscopic studies, the peak absorption of silver nanoparticles of *Bixa orellana* L. leaf and dye extract was found to be 405 nm in spectrophotometer and 475nm in colorimeter respectively.

With respect to TEM studies, the leaf extract confirmed the presence of silver nanoparticles ranging from 30-100nm and dye extract with 10-80nm in size respectively and was found to be spherical in shape.

The AgNPs synthesized have shown promising antibacterial activity against both gram positive and gram negative bacteria – *Staphylococcus aureus* and *Escherichia coli.*

The Biosynthesis of Metal nanoparticles using the plant derivates is extremely studied in the last two decades. The plant metabolites induce the production of metallic silver nanoparticles in eco-friendly manner. As a future prospect, the eco friendly synthesis of nanoparticles using plant crude extracts and purified metabolites is novel substrates for large scale production. In future, the plants have wide perspective for the synthesis of metallic nanoparticles in healthcare, commercial products, pharmaceuticals and therapeutics etc.

Conclusion

Our studies reported an economical, efficient, eco- friendly and rapid route for the green synthesis of silver nanoparticles from *Bixa orellana* L leaf and the seed dye- a non toxic Food colourant (nor-bixin) and its characterization by TEM for the first time ever in the literature.

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