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'Biosynthesis Of Nano Particles Using Malabar Spinach Fruit Extract With In Vitro Study'

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Abstract: Herbal drugs synthesis based on Nano technology offers a practicable and feasible way to improve the effects of herbal medicine with chemical treatment for varying their properties which help to enhance their biological activity. In current study fruit of Malabar spinach was take out using water extract. The bioactive compounds present in the fruit facilitated the reduction of silver ions (Ag^{+}) to silver nanoparticles (Ag^{0}) under controlled conditions. zinc and copper oxides (ZnO and CuO) were synthesised using a wet chemical reduction method. The synthesized Ag, Zno, CuO nanoparticles were characterized using X-ray diffraction (XRD), and Field Emission Scanning Electron Microscopy (FESEM) to determine their optical, structural, and morphological properties. The antimicrobial activity of the synthesized AgNPs, ZnONPs, CuONPs was evaluated against pathogenic microorganisms, revealing significant antibacterial and antifungal potential. The results highlight the efficacy of Basella rubra fruit extract as a natural and sustainable source for nano material synthesis, providing a promising alternative for biomedical and environmental applications.

Keywords: nanoparticles, Basella rubra, FESEM, XRD

Introduction: Medicinal plants have been used from ancient time to treat diseases. In modern era developing medicines from plants has equally important. In this regard basella rubra plant has many phytochemicals which are responsible to cure various diseases. This paper deals with the synthesis of silver, Zinc and copper nano partials using *Basella rubra* fruit. A Red-violet colour of basella rubra fruit can be also used as an indicator. *Basella rubra* belongs to the family Basellaceae, and commonly known as Malabar spinach. In Ayurveda, the plant has shown immense potential in androgenic, antiulcer, antioxidant, cytotoxic, antibacterial activity, ant inflammatory, central nervous system (CNS) depressant activity, nephroprotective and wound healing properties etc.

Silver nanoparticles have attracted intensive research interest because of their important application as antimicrobial, catalytic, and surface-enhanced Raman scattering effect [1]. Silver has been used as an antimicrobial agent for centuries [2]. Copper and zinc oxides are considered as suitable alternatives to organic based antimicrobials. Their antibacterial effect is dependent on a number of factors which are mostly determined by the method of synthesis. [3] The development of cost efficient and ecologically benign methods of synthesis of nanomaterials still remains a scientific challenge as metal nanoparticles are of use in various catalytic applications, viz electronics, biology and biomedical applications, material science, physics, environmental remediation fields.[4]

Material and Methodology:

Plant authentication: Authentication done by Dr. S. N. Patode at Shri Shivaji Arts, Commerce and Science College, Akot, Maharashtra.

Preparation of the extract: Dried fruits powder was weighed 10gm and it is mixed with 90ml of water. The extract was heated about 60^0 C with stirring. The solution was filtered through Whatman filter paper No. 41. The filtered sample was collected in beaker.

Synthesis of silver nano particles: 90 ml of the silver nitrate solution was taken in conical flask. To this added 10ml of the fruit extract. This Solution was kept for stirring about 2 to 3 hours at 60^0C. The red-violet colour of solution changes to dark brown indicated formation of silver nano particles.

Synthesis of copper oxide nanoparticles: 0.1 M copper nitrate was dissolved in 100ml of water. The solution was continuously stirred at 500 rpm on a magnetic stirrer hot plate at 60^0C. To this added 2 M NaOH solution and 2ml of fruit extract. A green suspension with precipitates was formed, which is indicative of the formation of copper oxides nano particles. The precipitates were separated by centrifugation and particles obtained were dried overnight.

Synthesis of zinc oxide nanoparticles: 0.1 M zinc nitrate was dissolved in 100ml of water. A homogeneous solution was obtained after keeping the solution at 500 rpm on a hot magnetic stirrer plate 60^0C. 2 M NaOH was added to the homogeneous solution and 2ml of fruit extract. A white precipitate was formed. The synthesis was allowed to continue for another 60 min. The precipitates were separated by centrifugation and were dried.

Result and discussion:

Change in colour of solution from red-violet to dark brown indicates formation of silver nano particles.

XRD: Crystalline size and structure of the silver nanoparticles were carried out by XRD. The XRD (X-ray Diffraction) pattern of silver nanoparticles reveals their crystalline structure (Fig. 1. The peaks at approximately 38°, 42°, 62°, and 77° align with the (111), (200), (220), and (311) planes of face centered cubic (FCC), confirming their structure is typical for silver in this form. The most intense peak at around 38° (111 plane) suggests a preferred orientation, which is common for silver nanoparticles and indicates stability along this plane. Fig.2 illustrates the patterns for zinc oxide. The diffraction peaks of zinc oxide correctly matched the hexagonal wurtzite structure of zinc oxide. XRD patterns obtained for copper oxide are in exact agreement with the monoclinic phase of copper oxide shows the XRD spectra pattern for copper oxide. (Fig.3)

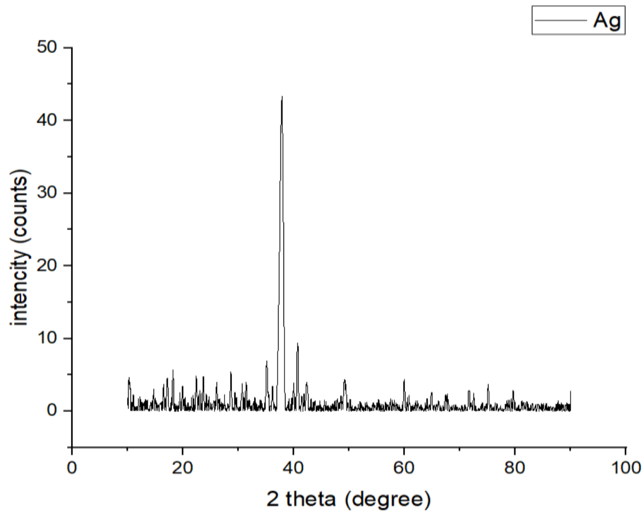

Fig.1 XRD of Silver

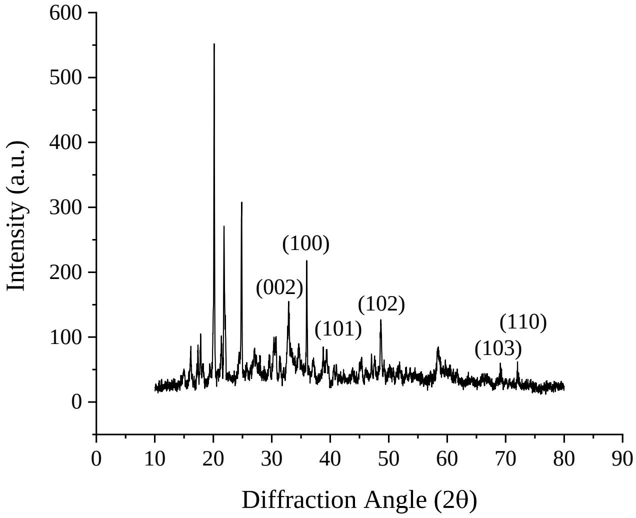

Fig.2 XRD of Zinc oxide

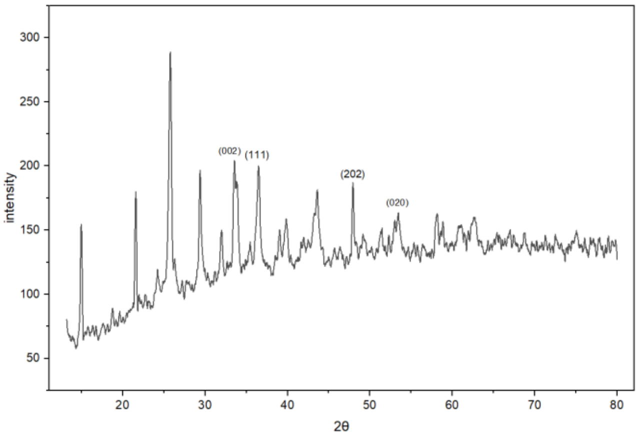

Fig.3 XRD of Copper oxide

FESEM

Field emission scanning electron Spectron microscopy further provides information about morphology and size details of silver nanoparticles. Experimental results showed that the diameter of prepared nanoparticles in the solution was about 65-105 nm. Figure shows confirmation of AgNps.

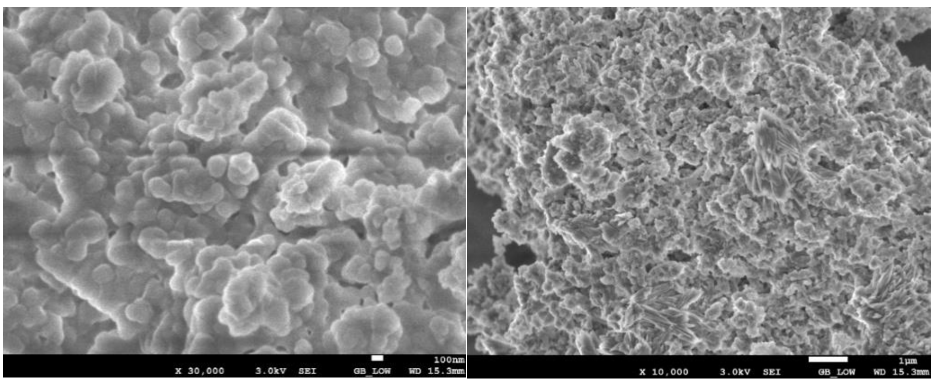

Fig. 4 SEM analysis of silver nano particles

SEM image had shown individual ZnO nanoparticles Fig.5 illustrates the particles are predominantly spherical in shape and aggregates into larger particles with no well-defined morphology. The SEM image shows the size of the ZnO nanoparticles ranging from 50-70 nm. The surface morphology of the prepared

CuO nano particles was revealed through the SEM image shown. It shows a homogeneous distribution of spherical particles of the of the prepared CuO nanoparticles with size 20-50nm.

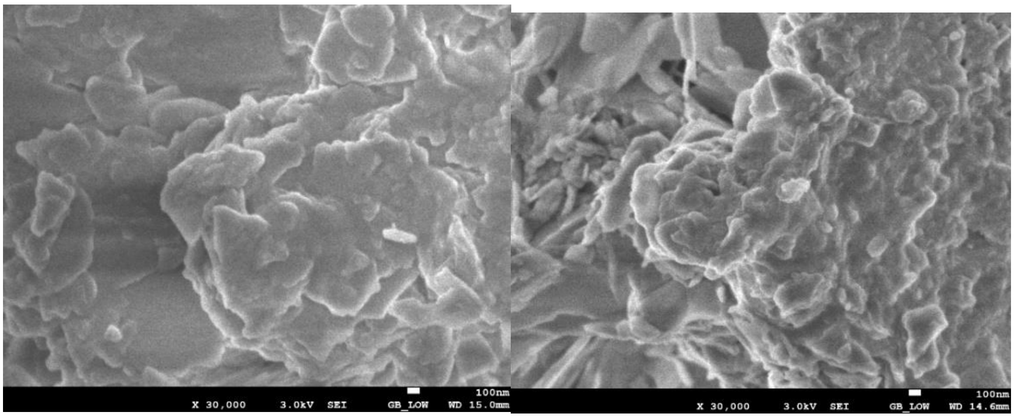

Fig.5 SEM analysis of ZnO nano particles & CuO nano particles

Anti-microbial Activity:

Anti-bacterial activity: The antibacterial activity of nanoparticles was studied against pathogenic bacterial strains of gram-negative E. coli, pseudomonas fluorescens and gram-positive Staphylococcus aureus, Streptococcus pneumonia and pseudomonas fluorescens using the Disc diffusion method (Fig.7). Standard antibiotic used for bacterial sensitivity is Ofloxacin. Broth medium was used to subculture bacteria and was incubated at 37 °C for 24 h, afterwards, overnight cultures were taken and spread on the agar plates to cultivate a uniform microbial growth plate. In order to evaluate the antibacterial activity of the synthesized nanoparticle, the diameter of the inhibition zone was measured and compared with the control groups. **(table 1)**

Fig.6 showing the zone of inhibition of synthesized AgNPs & (A) E. coli & Staphylococcus aureus (B) Streptococcus pneumonia and pseudomonas fluorescens (AgNPs: silver nanoparticle; Rf: reference sample)

Fig. 7 Antibacterial activity of zinc oxide a) gram negative bacteria Escherichia coli b) gram positive bacteria Staphylococcus aureus.

Anti-fungal activity: The Anti-fungal activity of Silver nano particles done with Candida albicans and Trichophyton rubrum. ZiO & CuO nano particles showing zone with fugus Aspergillus niger. The Standard antifungal antibiotic used is Fluconazole.

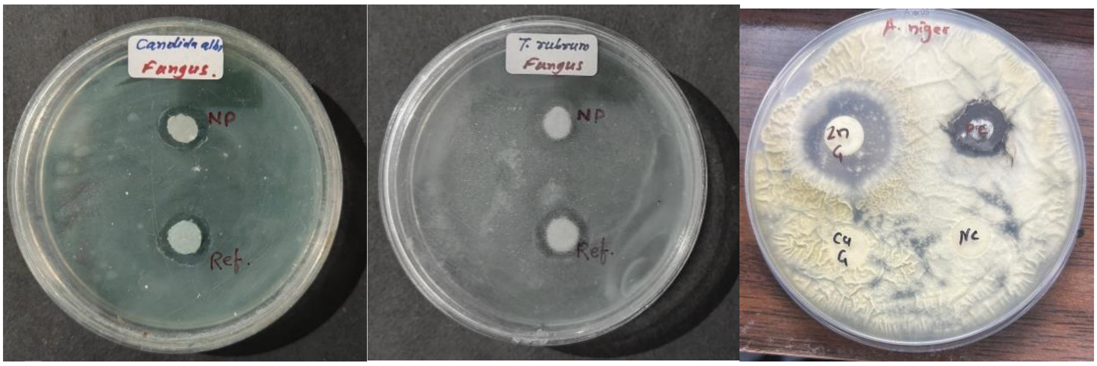

Fig. 8 showing the zone of inhibition of synthesized AgNPs a) Candida albicans, Trichophyton rubrum b) anti-fungal activity of Zinc & Copper oxides with Aspergillus niger

Observation: Table showing result of zone in mm of anti-microbial activity against nano materials & reference sample

Test compound	**Antimicrobial Sensitivity test against bacteria & Fungus**					
	GRAM -ve bacteria		GRAM +ve bacteria		Fungus	
	Escherichia coli NCBI-01578	**Pseudomonas fluorescens ATCC-13525**	**Staphylococcus Aureus MTCC-1430**	**Streptococcus Pneumonia MTCC-109**	**Candida albicans SC5314/ Aspergillus Niger**	**Trichophyton rubrum NUBS21011**
Ag Nano Particle zone	21mm	13mm	14mm	14mm	13mm	14mm
CuO Nano Particle zone	-	-	18mm	-	-	-
ZnO Nano Particle zone	13mm	-	16mm	-	17mm	-
Reference (Bacteria) Ofloxacin (10mcg) & Fluconazole for fungus	27mm	32mm	35mm	30mm	15mm	14mm

Conclusion: Present study showed that biosynthesized Silver, Zinc oxide and Copper oxide nano particles are crystalline and confirmed by XRD. Particle size was confirmed by FESEM analysis. Silver nanoparticles prepared by green route found to have both antibacterial and Antifungal activities and can very well applied in biological system. Zinc oxide nano particles synthesised from Basella rubra fruits also had antibacterial and antifungal activity. While copper oxide nano particles had less microbial activity.

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