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Study of microbial activity of synthetically prepared zinc- oxide (ZnO) nanoparticle utilizing *Rivea -Hypocrateriformis* extract against chosen micro- organisms

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Abstract

Due to its multifunctional efficiency and application in nano-cosmeceuticals and nano-pharmaceuticals, despite its fabrication manipulate, zinc oxide (ZnO) is currently employed superficially. Globally recognised for their ability to achieve stable, reliable, and toxic-free synthesis, bio-reducers. Consequently, the purpose of the present study is to create ZnO NPs in a more environmentally friendly method employing *Rivea hypocrateriformis* plant extract and to assess their antibacterial and antifungal activity. Hyperchromic shift were observed at 305nm. By using Fourier-transform Infrared spectroscopy, it was possible to gather proof that proteins serve as reducing and capping agents. The lattice planes and discovered peak positions from the X-Ray diffraction experiments supported the hexagonal close-packed crystalline structure of ZnO- NPs. The biosynthesized ZnO -NPs had star shapes with mean diameters of around 20–100 nm, according to surface topographical analyses. The synthesized ZnO-NPs have been characterized by various techniques and anti-microbial and anti-fungal activities are evaluated against Staphylococcus epidermidis and Candida vulgaris.

Keywords: Zinc oxide; *Rivea hypocrateriformis;* Surface functionalization anti-bacterial; Anti-fungal

1. Introduction

The nano technology provides a perspective chance across multiple fields like biomedical, Drug delivery, Electronics and Energy applications. Numerous physical and chemical characteristics are used in nano technology. We can advance new material with diameter less than 100nm through nano technology. The use of these nanomaterial in field including nanomedicine, nanoelectronics and the development of energy [1-9]. The utilization of biological micro-organisms like bacteria, fungus and plant extract in biosynthetic system has shown to be efficient. Because it is ecologically sound, biodegradable and harmless. The environmentally friendly production of metal oxide nanoparticle using plant has captured the attention of many scientists and researcher system of production [1-4]. Plant based nanoparticles are produced from specific plant source and are widely employed in a variety of application. Discrete highly active phenolic compound plays a vital part in redox process that occur during the formulation of the nano particles [15]. Alkaloids, Terpenoids and Quinoline, which stimulate the creation of nanoparticle are only a few of the numeracies advantages that plant- based nanoparticles offer [16-17]. In present work, the synthesized zinc oxide [ZnO] nanoparticles showed good anti-bacterial and anti-fungal activity [11]. The primary objective for researchers is the environmentally friendly, safe and biodegradable synthesis Zinc oxide [ZnO] nanoparticles, for the development of pharmaceuticals and device application. Recent research has focused on the bio synthesis of Zinc Oxide [ZnO] nano particles and their control using microorganisms [11]. Plant source [12] for the synthesis of zinc oxide nano particles, plant extract of *Rivea hypocrateriformis* [RH] is used. *Rivea hypocrateriformis* is commonly used *Choisy* is a woody climbing, shrub belonging to the *Convolvulaceae* family and is essentially distributed in India, Nepal, Sri Lanka, Myanmar and Thailand. Its stem, leaves and bark are herbily used to cure range of animals such as malva cancer, mental disorders analgesia [9].

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According to the literature survey a plant extract of *Rivea hypocrateriformis* has not been used for the synthesis of Zinc oxide [ZnO] nanoparticles. The synthesized Zinc Oxide nanoparticle [ZnO] nanoparticles have been characterized by many techniques and antimicrobial activities are evaluated against gram Negative bacterial strains Pseudomonas aeruginosa and Klebsiella pneumonia, gram two positive bacterial strains Staphylococcus aureus and Staphylococcus epidermidis and Fungal studies with *Candida albicans, candida vulgaris, Aspergillus niger* and *Aspergillus flavus*.

2. Material and methods

2.1. Material

The chemicals Zinc sulphate heptahydrate [$ZnSo_4$ $7H_2O$], Sodium Hydroxide [NaOH], Ethanol was purchased from Merck. The plant was collected from Rasipuram, Namakkal District ,Tamil Nadu

2.2. Preparation of plant extract

The *Rivea hypocrateriformis* plant were first washed with tap water followed by distilled water and dried under air room temperature for 15 days. Then these dried plants were blended to powder. The powdered plant 50 gram were taken in a Soxhlet apparatus and Ethanol was added and the process is allowed to run around 12 hours. Then the extract was collected and stored in refrigerated for future use.

2.3. Synthesis of zinc oxide (ZnO) nano particles

The zinc oxide nano particles were synthesized by mediated method in which distinct functional group exist in plant acts as a reducing agent and result in the formation of nano particles. The 60 ml of aqueous plant extract was taken in beaker, and 100 ml of 0.1M aqueous solution [$ZnSo_4.7H_2O$] was added to it then placed in a magnetic stirrer for 1 hour, NaOH was added drop by drop until the pH attained. The resultant extract solution was centrifuged at10000 rpm for 15 mins, the supernatant was discarded and the pellet containing precipitate of zinc oxide (ZnO) nano particles was collected and washed with ethanol to remove impurities and transfer into China dish. The precipitate was kept in an oven at 80°C for drying. The dried fine powder is used for the characterization.

2.4. Characterization

The zinc oxide (ZnO) nano particles were characterized by many techniques. The absorbance spectra of the zinc oxide (ZnO) nano particles were studied using UV visible spectroscopy. The surface factualisation of *Rivea hypocrateriformis* plant extract on the surface of Zinc Oxide nanoparticles were studied by FT-IR (SCHIMADZU IR affinity model is double beam spectrometer) with the wave number range of 4000 cm^{-1} to 400 cm^{-1}. The crystalline nature and phase identification were studied by XRD, [Empyrean Malveren analytical] method CuK α [λ=1.54Å] radiation. Surface morphology and partial spectra of prepared nanoparticle were analyzed by Scanning electron microscopic technique [Carl zeiss (USA)] model – sigma with Gemini column, EDAX performed to know about the estimation of the elemental composition of Zinc Oxide (ZnO) nanoparticles.

3. Results and discussion

3.1. UV – visible spectroscopy

The plant extract of *Rivea hypocrateriformis* 1(a) shows an absorbance band around 200 nm-800 nm which corresponds to the presence of alkaloids, flavonoids, phenolic compounds, amino acids and terpenoids. The UV visible spectroscopy of ZnO-NPs revealed a strong absorbance in visible region around 300 nm-360 nm.

The ZnO – NPs synthesized from plant extract 1 (b) shows strong absorbance band at 305 nm conformed the formation of ZnO nanoparticles and the particle were stable.[15]

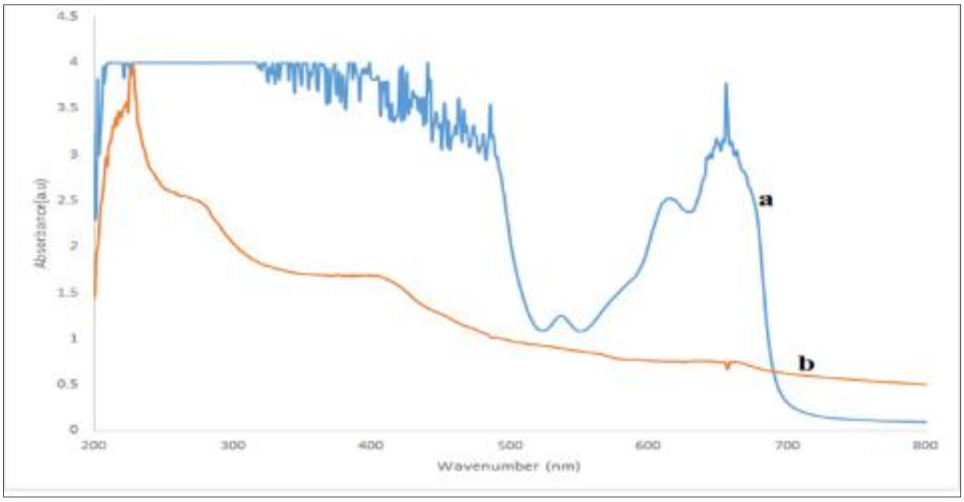

Figure 1 UV- visible spectroscopy of *Rivea hypocrateriformis* plant extract 1 (a) and ZnO- NPs synthesized with *Rivea hypocrateriformis* plant extract 1(b)

3.2. FT-IR

The FT-IR transmission spectra of *Rivea hypocrateriformis* plant extract and ZnO-NPs synthesized from the plant extract were shown in Fig 2.

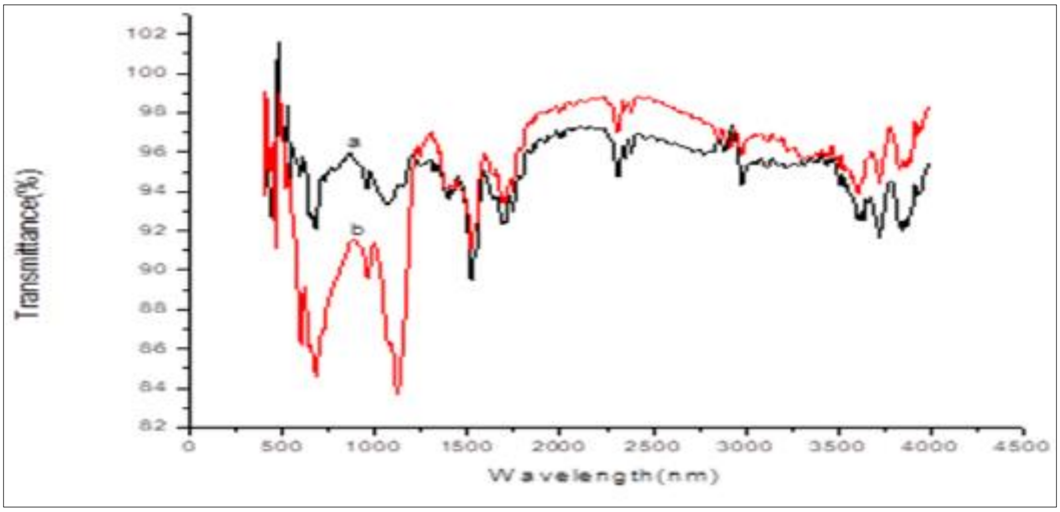

Figure 2 FT-IR Spectra of *Rivea hypocrateriformis* plant extract 2 (a) and ZnO -NPs synthesized from the plant extract of *Rivea hypocrateriformis* 2(b)

The FT-IR spectra of plant extract shows the peak at 3655cm^{-1}, 2980cm^{-1},1509cm^{-1} and 693cm^{1}. These absorption bands assigned to O-H, C-H stretching vibration, O-H, C-OH and N-H amines bonds respectively. The spectrum was examined for the biomolecules responsible for the stabilization of ZnO-NPs. The bands with low intensity at 1736cm^{-1} and 1386 cm^{-1} denote C-O stretching vibration (carboxylic acid). Absorption bands from FT-IR indicates phenolic acid, flavonoids and protein compounds rich in R.H plant extract. Whereas FT-IR spectra of R.H-ZnO-NPs exhibits the bands at 596cm$^{-1,}$ 1053cm$^{-1,}$ 1684cm$^{-1,}$ 2306cm^{-1}, 2986cm^{-1} and 3601cm^{-1}, as shown in 2(b). The peak between 400cm^{-1} and 600cm^{-1} is originated from (Metal oxide) Zn-O [16]. Plant extract also matched other bands at various places that could be attributed to phytochemical components required for the production and stabilization of ZnO NPs.

3.3. XRD

The XRD analysis was performed to confirm the synthesis of ZnO NPs. To know the crystalline structure XRD analysis was carried out and major peaks were observed at Bragg's angle and 24.09°,32.4°,36.6°,44.7°,58.3° and 64.7°

corresponded to the lattice planes of (110), (210), (002), (112), (320) and (222) respectively that could be indexes to the hexagonal phase of ZnO NPs in the previously reported data [18].

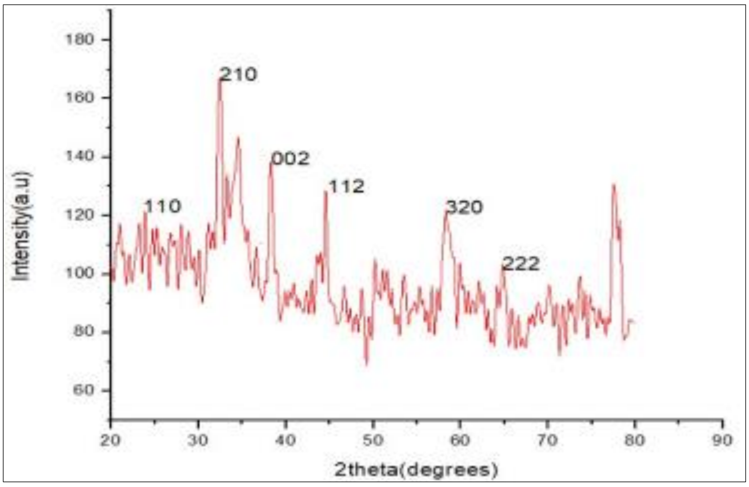

Figure 3 XRD image of ZnO-NPs synthesized from plant extract of *Rivea hypocrateriformis*

3.4. SEM

The morphology of ZnO-NPs was determined using scanning electron microscope. The SEM images of ZnO-NPs shows star shaped nanoparticles with diameter range from 100nm.

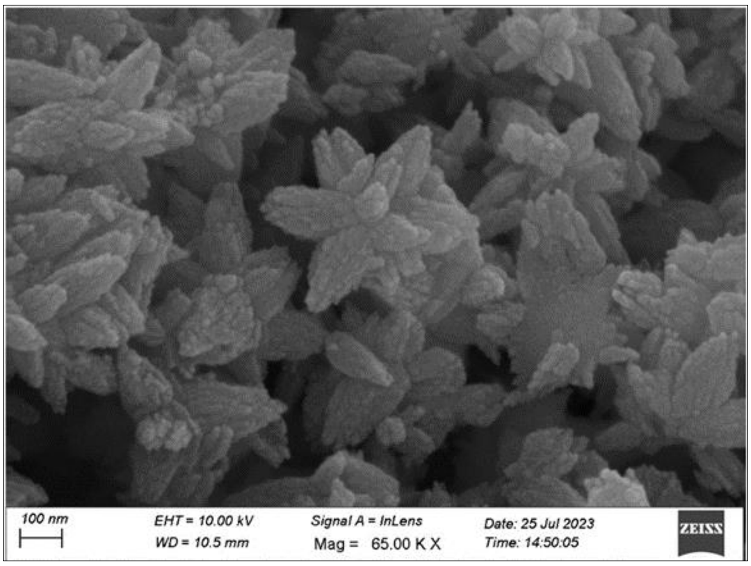

Figure 4 SEM image of ZnO-NPs synthesized from the plant extract of *Rivea hypocrateriformis*

The surface of synthesized NPs is very smooth which arise act better contact with bacterial cell wall and hence increases the bacterial killing ability of nanoparticles such a behaviour of smooth surfaced nanoparticles has already been established in the literature.

3.5. EDAX

The elemental composition synthesized ZnO-NPs from the plant extract of *Rivea hypocrateriformis* was studied from EDAX analysis in Fig 5.

The peaks of the elements zinc and oxygen, with atomic percents of 66.82% and 33.18%, respectively, were visible in ZnO -NPs. The EDS spectra and chemical composition both show that ZnO NPs are largely impure-free [18].

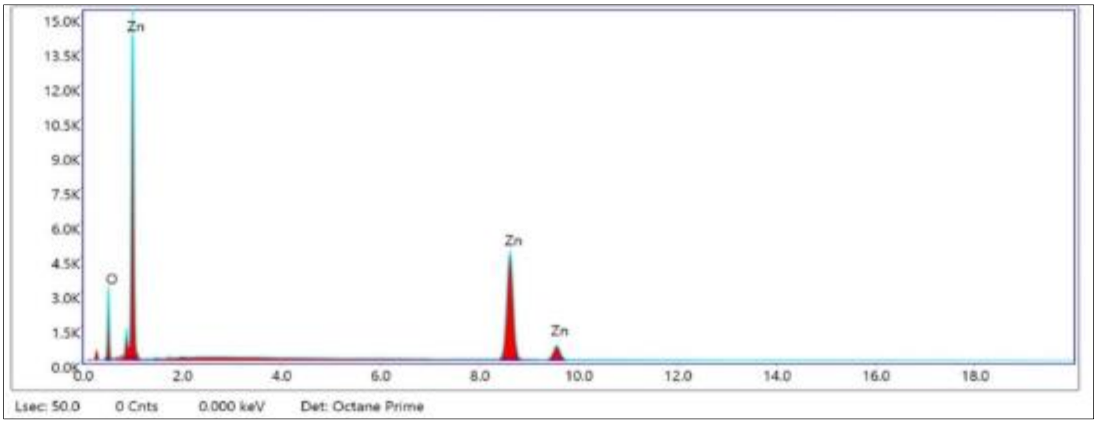

Figure 5 EDAX image of ZnO-NPs synthesized from the plant extract of *Rivea hypocrateriformis*

3.6. Anti-bacterial activity

By using the Agar well diffusion method, the antibacterial effectiveness of *Rivea hypocrateriformis* mediated ZnO- NPs and additive potency with topical antibiotic were evaluated, against both the bacterial gramme strains**.** ZnO nanoparticles produced good antibacterial activity against Staphylococcus epidermidis. The gram-positive bacteria Staphylococcus epidermidis showed maximum zone of inhibition around 11mm in Fig 6

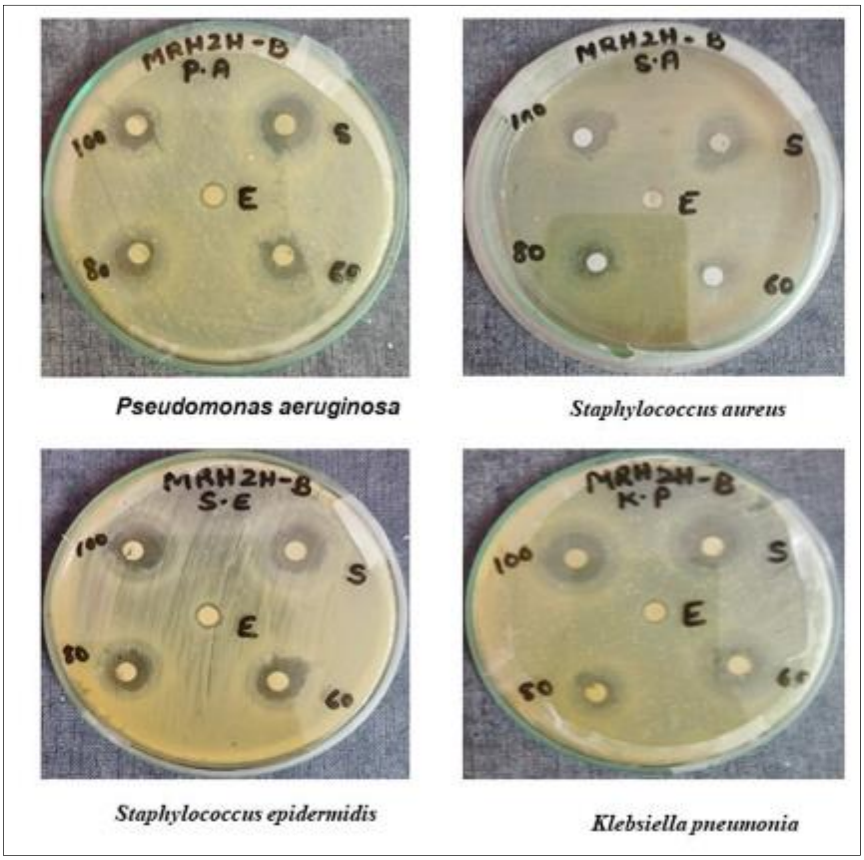

Figure 6 Effect of sample ZnO – NPs synthesized from the plant extract of *Rivea hypocrateriformis* against the bacterial

The Anti-bacterial of ZnO nanoparticles are presented in Table-1.

Table 1 *In Vitro* Antibacterial activity

Samples	Concentrations (µg/ml)	Organisms/Zone of inhibition (mm)			
		Pseudomonas aeruginosa	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Klebsiella pneumonia***
Samples	60	3	2	4	2
	80	4	3	5	3
	100	6	4	7	5
Standard (Std) (Amoxicillin)	10 µl/disc	9	7	11	10

3.7. Anti-fungal activity

ZnO-NPs produced good antifungal activity against Candida vulgaris, the maximum zone of inhibition showed by the Fungi around 12mm in Fig 7.

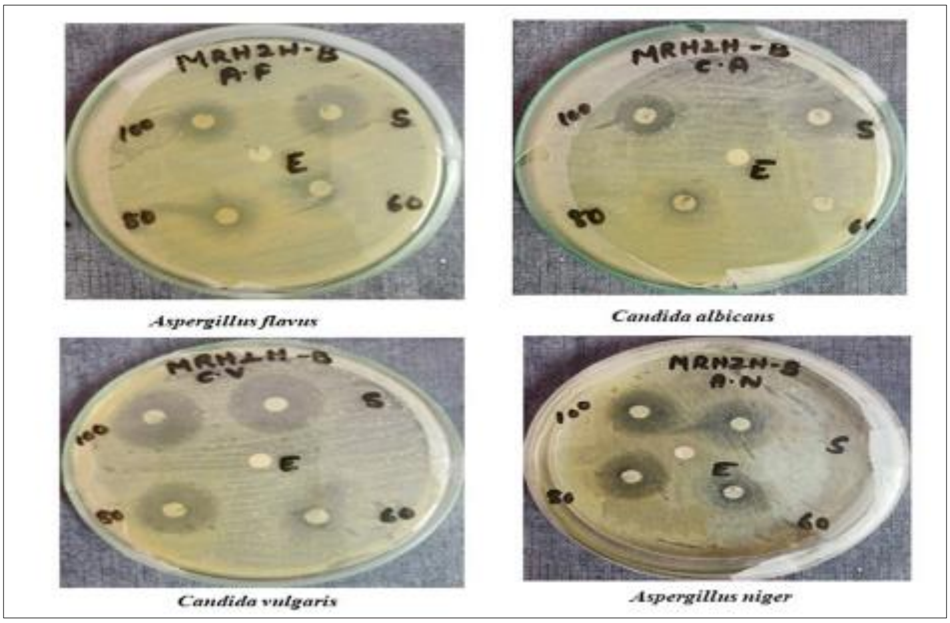

Figure 7 Effect of sample ZnO – NPs synthesized from the plant extract of *Rivea hypocrateriformis* against the Fungal

Anti-fungal activity of ZnO NPs is presented in Table-2.

Table 2 *In Vitro* Antifungal activity

Samples	Concentrations (µg/ml)	Organisms/Zone of inhibition (mm)			
		Aspergillus flavus	***Candida albicans***	***Candida vulgaris***	***Aspergillus flavus***
Samples	60	3	0	2	2
	80	5	2	6	3
	100	7	4	9	5
Standard (Std) (Amoxicillin)	10 µl/disc	10	9	12	8

4. Conclusion

Zinc oxide nanoparticles were synthesized by cost effective, bio-degradable and ecological friendly green method by using plant extract of *Rivea hypocrateriformis.* The prepared nanoparticles were analyzed by UV-Visible spectroscopy, FT-IR, XRD and SEM techniques. These techniques showed successful synthesis of Zinc oxide nanoparticles. Anti-bacterial activity of synthesized Zinc oxide nanoparticles was analyzed against gram-positive bacteria Staphylococcus epidermidis. The Anti-fungal activity of synthesized Zinc oxide nanoparticles was analyzed against Candida vulgaris. It was found that by increasing concentration of zinc oxide nanoparticles, Anti-bacterial activity and Anti-fungal activity was increased.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no Competing interests.

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