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Green Synthesis and Biological efficacy of Zinc Oxide Nanoparticles from *Ocimum sanctum* leaf extract.

K.Rama Sai, Sai Aishwarya Robby, Manju Devi.S and A.Sai Padma*
Department of Biochemistry, Bhavan's Vivekananda College of Science, Humanities and Commerce, Sainikpuri, Secunderabad-94, Telangana, India.
*Corresponding Author:
Email id: saipadmabhavans@yahoo.co.in

ABSTRACT
The biological materials, especially plant extracts, are increasingly employed in the green synthesis of nanoparticles, representing a significant development in the realm of nanotechnology. The current study details about synthesis of Zinc oxide nanoparticles (ZnO-NP) using aqueous extracts of Ocimum sanctum as a reducing and capping agent. Different concentrations of zinc nitrate (0.05M and 1M) were used with aqueous extracts of O. sanctum along with control samples. The nanoparticles produced were analyzed by UV–Visible spectroscopy, XRD, and FTIR. A characteristic absorption peak at 363 nm in the UV–Vis spectra, indicated the formation of ZnO nanoparticles. XRD analysis was done to determine the particle size through the Scherrer equation. The average sizes of nanoparticles from 0.05M zinc nitrate (control, without leaf extract), 0.05M zinc nitrate, and 1M zinc nitrate (test, using leaf extract) were found to be 26 nm, 23 nm, and 33 nm, respectively. This study clearly demonstrates that the proper concentration of zinc nitrate solution, with O. sanctum leaf extract, led to a reduction in nanoparticle size, an increase in surface area, along with a biosafe capping layer. Further, biological efficacy of the synthesised nanoparticles showed significant antibacterial potential against staphylococcus aureus, a gram-positive bacterium. The ZnO-NP demonstrated significant antioxidant potential also, as assessed by DPPH and ABTS assay. The anti-inflammatory activity analysis showed that the synthesized ZnO-NPs have potential as anti-inflammatory agents. This study demonstrated a rapid, affordable, and environmentally friendly approach for synthesizing ZnO nanoparticles, highlighting their potential use as antimicrobial, therapeutic, and environmental agents.
Keywords: Capping agent, *Ocimum sanctum,* UV-VIS spectroscopy, XRD-analysis, Zinc Oxide nanoparticles, biological efficacy.

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INTRODUCTION

Nanoparticles are an emerging field that attracts large attention for physical and fundamental studies in physical sciences and biomedical sciences. Zinc oxide nanoparticles are considered as multifaceted functional substances, and their classic properties find current and potential applications in semiconductors, cosmetics, transducers, textile and water treatment [1].ZnO nanoparticles hold great promise in various biological applications, including as antibacterial, antidiabetic, antioxidant, antiparasitic, and larvicidal agents, as well as in gene delivery, drug delivery, bioimaging, and nanomedicine. [2-5]. Recently, the eco-friendly synthesis of nanoparticles has gained popularity among researcher sowing to its economic viability, ambient conditions, non-toxicity, and environmental compatibility. This method is also advantageous because the resulting nanoparticles are highly water-soluble, and free from toxic stabilizers. Plant extracts have emerged as a highly effective tools for the facile green synthesis method [6-8].

Reaction: $Zn(NO_3)_2.6H_2O + 2\ NaOH \longrightarrow ZnO + 2\ Na(NO_3) + 7\ H_2O$

The synthesis of ZnO -NP can be achieved using various methods, including the sol-gel method, co-precipitation, hydrothermal processes, and spray pyrolysis. [9-11]. Based on chemical and physical

properties such as size, shape, dispersity, surface state, crystal structure, organization onto a support and dispensability options are varied. Owing to their structural versatility, ZnO-NPs, exhibit various nanostructures which are said to be safe, nontoxic, and biocompatible. And these are used in various industrial sectors like rubber manufacturing, optoelectronic and medicinal industry. These chemical synthesis methods often require high energy inputs and involve the use of hazardous and toxic substances, which can pose significant global risks. In contrast, green synthesis and biological methods often considered as safe and cost-effective, compared to physiochemical methods. Biological compounds take part in synthesizing nanoparticles which act as a caping agent to stabilize them.

Plant extracts of *Cassia fistula, Carica Papaya, Ocimum basilicum,* were used for ZnO-NP green synthesis by green method [12-14]. The plant used in this study was *Ocimum sanctum* which has a wide range of pharmacological and medicinal properties [15]. The aqueous leaf extract of *O. sanctum* was analyzed for its phytochemical content and found to contain alkaloids, flavonoids, and tannins. Additionally, the extract's organoleptic properties were also observed. [16].

Characterisation of nanoparticles were done by methods such as UV-Visible spectroscopy, FTIR and X-ray diffraction. The X-ray diffraction method was analysed and derived by using Scherrer equation. A comparative analysis of the size of ZnO-NP produced through green and chemical methods were done in this study. Further, biological efficacy of the green synthesised ZnO-NP was also analysed.

MATERIA AND METHODS

Zinc nitrate, NaOH, Deionized water, *Ocimum sanctum* leaves, UV-VIS spectroscopy, X-ray diffraction technique, Origin software for XRD analysis, Ultra-Sonicator, Heater-stirrer, Hot air oven, high-speed centrifuge.

Ocimum sanctum leaf extract preparation

Fresh Tulsi leaves were collected and cleaned with deionized water. The leaves were dried and made into a fine powder and dissolved in deionized water. The mixture was heated and filtered to remove impurities. The resulting clear solution of Tulsi leaf extract was used for nanoparticles synthesis [17].

ZnO-NP synthesis

The preparation of ZnO-NP by green method was done by co-precipitation method with some changes. Chemical synthesis of ZnO-NP excludes addition of leaf extract solution. In green method, *O. sanctum* leaf extracts were used for reduction of the Zinc nitrate. A comparison in size was made between ZnO-NP synthesized using *O. sanctum* leaf extract and chemical method.

Synthesis and purification of 0.05M ZnO-N Pusing *O. sanctum* leaf extract mixture.

25mL of 0.05M $Zn(NO_3)_2$and 4mL of *O. sanctum* leaf extract solution were mixed together and stirred for 2h. Then post stirring of the mixture, 0.1M sodium hydroxide was added drop by drop into the mixture till it reached 12pH. Then the resultant mixture with 12pH was again placed on magnetic stirrer for 4 hours. During the process of stirring, pale-yellow colour precipitate was formed. After the formation of pale-yellow colour precipitate the mixture was sonicated for 15 minutes. Then the precipitate was purified by rinsing with deionized water. The purified precipitate was then dried at 120°C in an oven. The dried sample was in powdered form which was used for analysis of ZnO-NP[11].

Chemical synthesize of ZnO-NP

In this process, synthesis was carried out by chemical method without using *O. sanctum* leaf extract[11].

Characterisation of the synthesised ZnO-NP

The synthesized ZnO-NPs were characterised by UV-Visible spectroscopy, FT-IR, and X-ray diffraction. UV-Vis analysis was performed on a sonicated suspension of the purified sample. FTIR was carried out on dried ZnO nanoparticles using the KBr pellet method. XRD was done to analyse the phase structure and composition of the ZnO-NP. [18].

Antimicrobial effect -The antibacterial efficacy of the ZnO- NP was tested against two different bacterial strains- Gram positive (*Staphylococcus aureus*) and Gram negative (*Klebsiella pneumoniae*) bacteria. The disc diffusion method was employed to assess the potential of ZnO- NP extracted from *Thulsi*. Mueller-Hinton agar plates were inoculated with bacterial cultures using Q-tips. After 10 minutes the sterile paper discs were loaded with the nanoparticle sample which was then placed on top of the agar plates. Chloramphenicol was used as the standard [19].

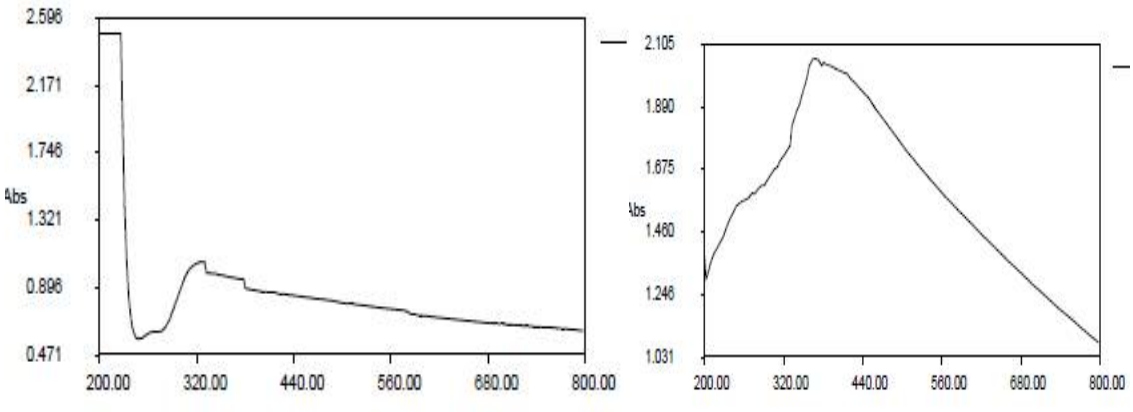
Estimation of free radical scavenging activity was done by both DPPH and ABTS Method. as reported by Manju Devi et al [20].

The anti-inflammatory action of the ZnO- NP samples was studied by albumin denaturation inhibition as reported earlier[21].

RESULTS AND DISCUSSION

UV-Visible Spectroscopy

The formation of zinc oxide nanoparticles wasindicated by UV-vis spectroscopy from 200 nm-800 nm wavelength range. UV–Visible absorption spectrum of 0.05M zinc nitrate (control) synthesized nanoparticles has shown a distinct peak centered around 320 nm and for 0.05M zinc nitrate with *Ocimum sanctum* leaf extract was around 363 nm (Fig.1). According to previous studies zinc oxide nanoparticles exhibit a exhibit a broad absorption peak between 330 and 460 nanometers. The synthesized zinc oxide nanoparticles in this study exhibited a peak at 363 nanometers, confirming their formation from zinc nitrate using leaf extract [22-23].



(A) (B)

Fig 1: UV-Visible absorption spectrum of the ZnO-NPs(A) zinc nitrate by chemical method and (B) Zinc nitrate by green method using *O. sanctum* extract.

The characteristic peak at 350 nm is indicative of zinc oxide nanoparticles due to their strong excitation binding energy at room temperature. The band gap of zinc oxide nanoparticles increases as their particle size decreases, and there is an inverse relationship between band gap and absorption wavelength. The blue shift in absorption observed in the synthesized zinc oxide nanoparticles compared to bulk zinc oxide is likely due to their smaller particle size. Previous studies also specified that the difference in absorption peak is due to differences in particle size[24]

XRD analysis

Results of XRD showed crystalline structure of synthesized ZnO-NP. The sharp diffraction peaks for 0.05M zinc nitrate control were observed at 2θ values 31.28, 33.95, 35.79, 47.10, 56.15, 62.42, and 67.58 degrees (Fig.2). For 0.05M zinc nitrate with leaf extract peaks were at 2θ values 31.44, 34.11, 35.93, 47.23, 56.30, 62.42, 67.57 degrees. For 1M zinc nitrate with leaf extract peaks at 2θ values 31.45, 34.02, 35.94, 47.18, 56.29, 62.63, 67.72 were observed, which specifies the hexagonal wurtzite structure for the nanoparticles. Scherrer equation ($D=0.9\lambda/\beta \cos \theta$) is the formula to analyze the size of the particle resulted from origin software. The size of the zinc oxide nanoparticles was calculated using the Scherrer equation based on XRD. The average size of nanoparticles synthesized from 0.05M zinc nitrate without leaf extract was found to be 26 nanometers and with leaf extract was 23nm(Table 1& 2). For ZnO-NP synthesised with 1M Zn nitrate by green synthesis, the average size was 33nm (data not given).

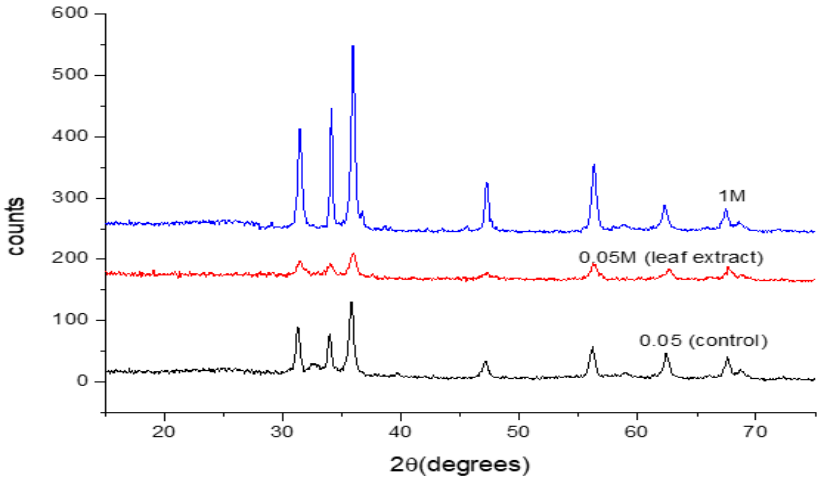


Fig 2: XRD spectra of zinc nitrate, ZnO NP with 0.05M zinc nitrate and O. sanctum extract, ZnO NP with 1M Zinc nitrate with *O.sanctum* leaf extract.

Table.1: 26nm is an average size of sample particle analyzed by using Scherrer equation with 0.05M zinc nitrate synthesised ZnO NPs without leaf extract.

0.05M zinc nitrate synthesised ZnO NPs by chemical method								
2theta	**FWHM**	**/2**	**PI*/180**	**COS**	**K**	**wz**	**Angstrom**	**NM**
31.2825	0.3869	15.6413	0.27285	0.96301	0.9	1.5404	3.45069	34.5069
33.9599	0.3767	16.98	0.29621	0.95645	0.9	1.5404	3.52	35.2
35.7951	0.4557	17.8976	0.31221	0.95166	0.9	1.5404	2.89519	28.9519
47.1063	0.5637	23.5532	0.41087	0.91677	0.9	1.5404	2.25471	22.5471
56.1528	0.53108	28.0764	0.48978	0.88244	0.9	1.5404	2.30356	23.0356
62.4258	0.5354	31.2129	0.54449	0.85539	0.9	1.5404	2.21494	22.1494
67.581	0.5469	33.7905	0.58946	0.83124	0.9	1.5404	2.10715	21.0715

Table.2: 23nm is an average size of sample particle analyzed by using Scherrer equation with 0.05M zinc nitrate synthesised ZnO NPs with *O.sanctum* extract

0.05M zinc nitrate synthesised ZnO NPs with O. *sanctum* leaf extract								
2theta	**FWHM**	**/2**	**PI*/180**	**COS**	**K**	**w**	**Angstrom**	**NM**
31.4558	0.4771	15.7279	0.27436	0.9626	0.9	1.5404	2.79712	27.9712
34.0266	0.5126	17.0133	0.29679	0.95628	0.9	1.5404	2.58632	25.8632
35.9423	0.5455	17.9712	0.3135	0.95126	0.9	1.5404	2.41758	24.1758
47.1845	0.56882	23.5923	0.41155	0.9165	0.9	1.5404	2.23375	22.3375
56.297	0.54	28.1485	0.49104	0.88185	0.9	1.5404	2.26399	22.6399
62.6355	0.6137	31.3178	0.54632	0.85444	0.9	1.5404	1.9302	19.302
67.7237	0.5487	33.8619	0.5907	0.83055	0.9	1.5404	2.09849	20.9849

FTIR analysis- FTIR spectrum of the ZnO-NP showed absorption bands around 3448 cm^{-1} and 1629 cm^{-1}.The absorption at around 3500 cm^{-1} typically indicates the presence of O-H (hydroxyl) or N-H (amine) stretching vibrations. The peak at 1629 cm^{-1} could indicate C=C stretching vibrations in alkenes or aromatic rings. In some cases, particularly in secondary amines, an N-H bending vibration might appear around this region (Fig.3). Previous studies also indicated the same kind of absorption bands for ZnO-NP[25].

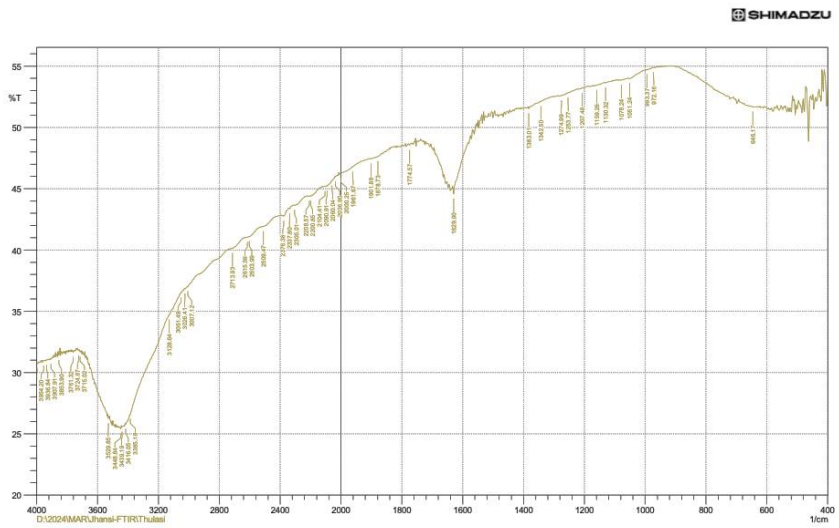


Fig 3: FTIR spectrum of the green synthesised ZnO- NP showing the various functional groups involved.

Antimicrobial activity

The antimicrobial efficacy of the synthesised ZnO-NP was analysed on both gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Klebsiella pneumonia (Fig.4).* The nanoparticles exhibited a significant antibacterial effect against *Staphylococcus aureus* and have shown no significance against *Klebsiella.* Our results align with previous findings, as studies have shown that ZnO nanoparticles are less effective against Gram-negative bacteria [26-27].

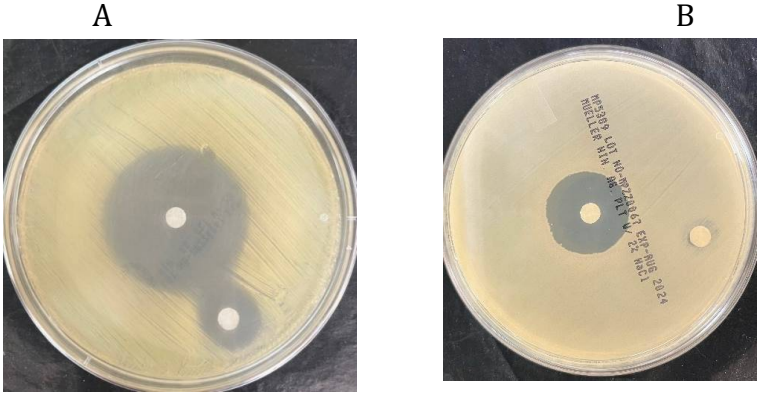


Fig 4: (A) Disc diffusion method against *Staphylococcus aureus* (B) Disc diffusion method against *Klebsiella pneumonia.*

Antioxidant activity

Antioxidant activity analysis using both DPPH and ABTS assay showed that the ZnO -NPs were efficient as antioxidant agents as significant inhibition was observed in samples treated with ZnO-NP (Fig.5).

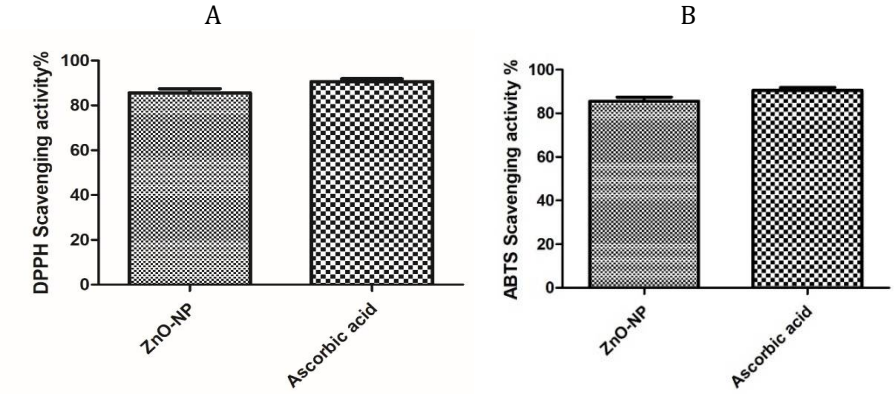


Fig 5: The antioxidant efficacy of zinc oxide nanoparticles was evaluated using the (A)DPPH and (B)ABTS assays

Anti-inflammatory activity

As protein denaturation has been documented as one of the significant causes of inflammation, the anti-inflammatory potential of nanoparticles was analysed by albumin denaturation inhibition. The green synthesised ZnO nanoparticle exhibited significant anti-inflammatory potential which was comparable with standard drug sodium diclofenac (Fig.6). Previous studies also reported antioxidant and anti-inflammatory effects of green synthesised ZnO-NPs [28].

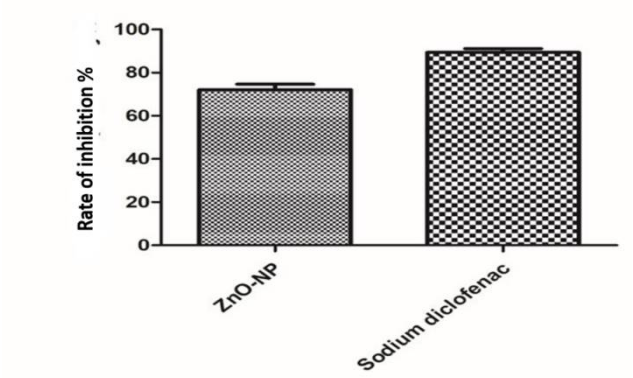


Fig 6**:** Anti-inflammatory analysis of green synthesised ZnO nanoparticles and standard drug sodium diclofenac

CONCLUSION

This study aimed to synthesize zinc oxide nanoparticles using zinc nitrate and reduce their size using a biological capping agent. The caping agent which was used here is *Ocimum sanctum* leaf extract. The resulting nanoparticles were analyzed through XRD, UV-VIS spectroscopy and FTIR. XRD analysis has shown that the size of ZnO-NP formed by using a caping agent (*Ocimum sanctum* leaf extract) were lesser in size compared to zinc oxide nanoparticles formed by using only zinc nitrate through chemical method. The caping agent forming around the nanoparticles stabilizes to control the size and forming agglomerations. Hence the size of nanoparticles was decreased with the presence of leaf extract. FTIR analysis indicated various functional groups involved in the nanoparticle formation. The biological functional efficacy of the green-synthesized ZnO-NP demonstrated that they are effective as antimicrobial, antioxidant, and anti-inflammatory agents.

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