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Biosynthesis of zinc oxide nanoparticles using *Cassia siamea* leaves extracts and their efficacy evaluation as potential antimicrobial agent

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Abstract
The present study is focused at evaluation of antimicrobial activity of bio synthesized zinc oxide nanoparticles with special reference to *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes, Proteus mirabilis. Cassia siamea* (Kassod) leaf extract was used for biosynthesis of zinc oxide nanoparticles. The synthesized Zinc oxide nanoparticles were characterized using UV, FTIR and SEM. Further these particles were subjected for evaluation of their antimicrobial activity. The FTIR studies showed the presence of several chemical functional groups, SEM results obtained showed that the synthesized nanoparticles were in the range below 100nm and shape of the particles was slightly ellipsoidal/ spherical. The synthesized nanoparticles have showed significant antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes, Proteus mirabilis.* The present study will provide new directions for safer and effective therapies.

Keywords: Biosynthesis, nanoparticles, characterization, antimicrobial activity

Introduction
There are various methods available for nano particles synthesis such as Physical, chemical and biological method [1]. Biological method however has some added advantage over physical and chemical method as they are eco friendly, economically viable and less toxic [2]. These properties of biosynthesized nanoparticles make them more suitable to use in various biomedical and clinical applications [3-4].
Impaired wound healing in diabetes is a major complication. The elevated infection may interfere in the proper healing process. If left untreated there is a risk of developing gangrene, sepsis or bone infection [5]. The control over the infectious load by proper medication, diet and maintaining aseptic conditions may improve Healing [6]. Many antimicrobial agents have limitations in clinical applications, because of their many complications and resistance towards pathogenic microorganisms [6]. Silver nanoparticles and copper nanoparticles have been commercially synthesized nowadays as an antimicrobial agent [8-11].
Colonization, contamination and infections by the pathogenic flora over wound may also results in delayed wound healing [12]. Previous studies have showed that wound associated pathogens have developed resistance toward pre-existing drug [13]. Whereas, some cases are reported where few metal nanoparticles are known to have potential antimicrobial and consequential wound rejuvenating activity, but they also have some disadvantages related to the toxicity level [14]. It was thoughtful worthwhile to synthesize nanoparticles by bio-assisted pathway to reduce their toxicity effect and then after particles were used to provide better wound healing therapy to avoid the resistance related problem. Thus this study may provide insight to methodize nanoparticles synthesis with high efficiency towards antimicrobial activity against wound associated pathogens. As this area is less explored and thus there is a requirement of new drugs with high potential and less side effects, which can be provided with the help of metal NPs based formulations.

Materials and methods
Materials
Zinc sulphate, Leaf extract (*Cassia siamea*).

Microbial strains collected from MTCC
Pseudomonas aeruginosa (MTCC No. 3542), *Staphylococcus saprophyticus* (MTCC N0. 6155), *Streptococcus pyogenes* (MTCC No. 5969), *Proteus mirabilis* (MTCC No. 3310).

Methods

Preparation of *Plant* leaf extract
Fresh leaves of *Cassia siamea* were washed with distilled water. The leaves were air dried and are subjected to extract preparation. The prepared leaf extract was stored at 4 °C for further use [15].

Synthesis of Zinc oxide Nanoparticles
1mM Zinc sulphate solution was kept in a conical flask. By keeping the conical flask (containing metal salt solution) on magnetic stirrer, the prepared leaf extract was added drop wise. Slight change in the colour indicates the formation of zinc oxide nanoparicles. Zinc oxide nanoparticles were further allowed to centrifuge. To remove impurities organic solvents were used for washing of nanoparticles. Nano particles were then dried, collected and stored [16].

Characterization

UV–Visible spectroscopy analysis
The optical properties can be determined by absorbance spectroscopy. A beam of Light is allowed to pass through the sample solution. With the change in wavelength, absorbance is measured. The amount of light which is absorbed when passed through the sample is measured. The concentration of solution can be measured by analyzing the amount of absorbed light, by Beer-Lamberts Law. UV–vis spectroscopy works on the principle of surface plasmon resonance of the particles thus can be to analyze the size and shape of the prepared nanoparticles present in aqueous medium [17].

FTIR analysis
FTIR spectroscope is an instrument used to measure infrared intensity v/s wavelength along with the possible functional groups, bonding interaction and vibration characteristics of different chemical functional groups. With the infrared light interacts with matter, stretching, contraction and bending frequencies are observed, due to adsorption [18].

SEM analysis
Size, shape along with morphologies of synthesized nanoparticles can be characterized by scanning electron microscopy. It provides high-resolution images of nanoparticles surface and it works on the principle of optical microscope. It measures the electrons accelerated by an electric potential scattered from the sample rather than photon. Scanning electron microscope provides high resolution pictures, which make it useful to identify shape and size of the particles [19].

Collection of microbial strains from MTCC
For *in-vitro* evaluation of synthesized nanoparticles against four different wound associated bacterial strains were procured from MTCC.

Antimicrobial activity evaluation of the nanoparticle against micro-organisms
The *in-vitro* activity of the synthesized nanoparticles was evaluated by Kirby Bauer well diffusion method was used method against wound associated bacterial strains collected from MTCC, Chandigarh (Punjab). Cell suspension of micro-organisms equivalent to a 0.5 McFarland standard is used [20].

Minimum inhibitor concentration (MIC) studies by tube dilution method
Minimum inhibitory concentration of synthesized nanoparticles was then calculated at different concentrations ranging 0.1mg/ml, 0.3mg/ml, 0.5mg/ml, 0.7mg/ml and 0.9mg/ml against *P. mirabilis*, *S. saprophyticus*, *S. pyogenes*, and *P. aeroginosa* by broth dilution method in nutrient broth. The concentration of culture was adjusted to 0.2 at 568 nm (1×10^8 CFU/ ml, 0.5 McFarland's standard). Positive and negative control was used as standard. Minimum inhibitory concentration was denoted by analysing the turbidity of the tubes. Small aliquot of the sample (approx 50 μl) from the culture tubes showing least or no turbidity was taken and poured in agar plate for 24 h at optimum temperature for bacterial growth and was observed for growth if any. Experiment was performed in triplicates [21-24].

Results and discussion
Nature created several functional materials. With an urge to synthesize non-hazardous materials, bio fabrication of metal nanoparticles came into light employing environment-friendly, economically feasible, energy efficient as well as cost effective synthesis techniques with the aim of protecting human health and environment. Plants are beneficial than other biological entities for metal nanoparticles synthesis, as less time is required and no stringent procedures are needed. The green synthesis of nanoparticles have an added advantage as it does not need artificial reducing agents and stabilizing agents, also it is easy to deal with. The particles thus synthesized are stable at room temperature and do not show agglomeration. The detailed study was conducted as per given in materials and methods section. The results obtained from the study and their detailed discussion is mentioned here below as:

Visible Observation
After addition of plant extract or chemical reducing agent in metal salt solution, the colour of the solution changes. In the reduction mechanism, when metal salt dissolved in distilled water it soon get converted into its ionic form. It may be due to the addition of reducing agent into the ionic solution the chemical functional group of the reducing agent interacts with metal ions (monovalent or bivalent) and causes its reduction into zerovalent state or metallic nanoparticles of smaller size leaving rest of the components as by-product.

UV–Visible spectral analysis
In UV-Vis spectroscopy the applied electromagnetic field causes the excitation on periphery of nanoparticles which leads to occurrence of the phenomena called Surface Plasmon Resonance. The characteristic peaks obtained from the UV-Vis analysis of the synthesized nanoparticles is at 385 nm.

FTIR Analysis
The results obtained from FTIR spectroscopic studies on biosynthesized zinc oxide nanoparticles are as given below (Figure 1):

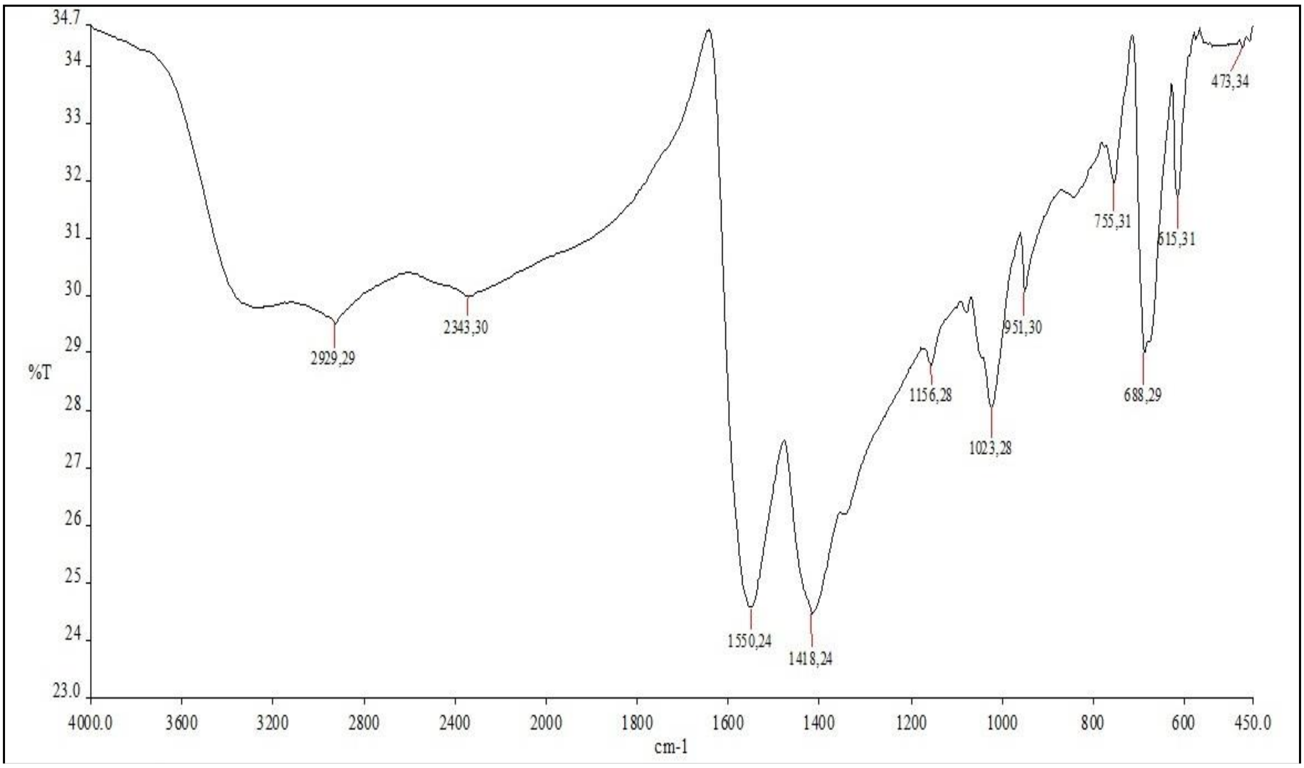


Fig 1: FTIR analysis of biosynthesized zinc oxide nanoparticles

The FTIR results obtained from biosynthesized zinc oxide nanoparticles gave characteristic peaks. The peak at thus obtained corresponds to O-H stretch vibration of carboxylic acids, N-O asymmetric stretch vibration of nitro compounds, –C-H- bend vibration of alkanes, C-N stretch vibration of aliphatic amines, O-H bend of carboxylic acids, C-Cl stretch vibration of alkyl halides and C-H bend of alkynes.

Scanning Electron Microscopy Analysis:

SEM analysis clearly depicts the ellipsoidal shape of the synthesized nanoparticles. The result obtained is shown below as (Figure 2):

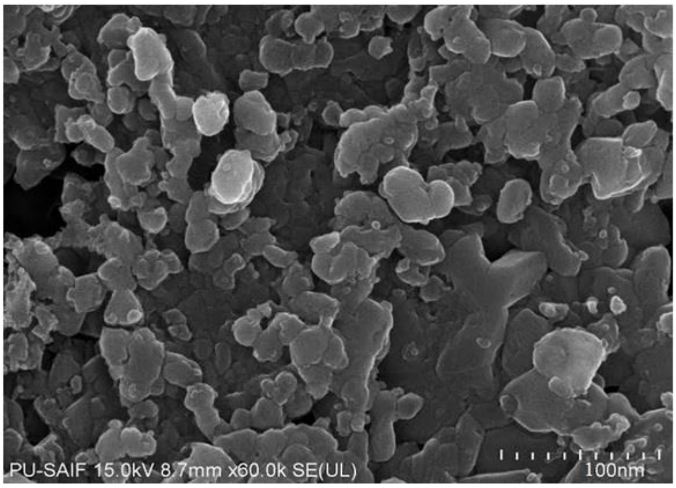
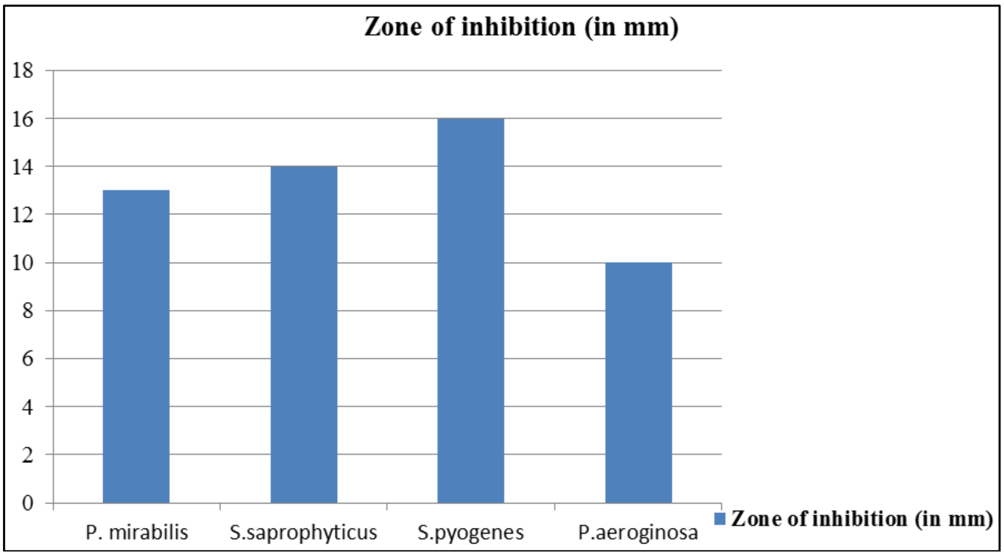


Fig 2: SEM analysis of biosynthesized zinc oxide nanoparticles

Antimicrobial activity of synthesized zinc oxide nanoparticles

Antimicrobial activity of synthesized nanoparticles against wound associated pathogens revealed that biosynthesized zinc oxide nanoparticles exhibited significant antibacterial activity against *P. mirabilis, S. saprophyticus, S. pyogenes, and P. aeroginosa* i.e. 13mm, 14mm, 16mm and 10mm (Zone of inhibition) respectively. (Graph 1)



Graph 1: Showing zone of inhibition of biosynthesized zinc oxide nanoparticles against wound associated pathogens

Minimum inhibitory concentrations of zinc oxide nanoparticles

Culture tubes containing zinc oxide nanoparticles ranging from 0.1mg/ml to 0.5mg/ml showed bacterial growth, whereas no growth was seen in culture tubes containing nanoparticles ranging from 0.7mg/ml to 0.9mg/ml. Thus it can be concluded that both MIC of zinc oxide nanoparticles is effective at concentration of 0.7mg/ml. The results obtained are shown below in Table 1.

Table 1: Minimum inhibitory concentrations of zinc oxide nanoparticles

Dilution for same bacterial concentration	Sets	Bacterial growth at different concentrations Biosynthesized zinc oxide nanoparticles				
		0.9mg/ml	0.7mg/ml	0.5mg/ml	0.3mg/ml	0.1mg/ml
P. mirabilis	1	-	-	-	+	+
	2	-	-	-	+	+
	3	-	-	-	+	+
S. saprophyticus	1	-	-	+	+	+
	2	-	-	-	+	+
	3	-	-	-	+	+
S. pyogenes	1	-	-	-	+	+
	2	-	-	-	+	+
	3	-	-	+	+	+
P. aeroginosa	1	-	-	-	+	+
	2	-	-	+	+	+
	3	-	-	+	+	+

+ = Turbidity due to microbial growth; - = No turbidity

Conclusion

The present study clearly depicts the efficiency of chosen leaf extract i.e. *Cassia siamea* in synthesis of metal nanoparticles. The plant extract used for the metal nanoparticles synthesis itself act as a capping agent. The synthesized particles were stable at room temperature and do not showed agglomeration. The nanoparticles synthesized by the plant extract had proven to be an effective tool for synthesizing nanoparticles in the size range below 100 nm as confirmed by the results obtained from SEM analysis. The FTIR results obtained confirms the availability of different functional groups present within the phyto-chemical constituent of plant extract. The following studies justified the synthesis of stable nanoparticles, which could be due to the presence of capping and stabilizing materials such as flavonoids, phenols and terpenoids present within the plant extract. Moreover, the results obtained from *in-vitro* analysis clearly shows that the biologically synthesized zinc oxide nanoparticles have potential antibacterial activity and could be used as a better therapeutic agent against infectious microbial flora.

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References

1. Pantidos N, Horsfall LE. Biological Synthesis of Metallic Nanoparticles by Bacteria, Fungi and Plants. J Nanomed Nanotechnol. 2014; 5:233.

2. Capolupo L, Faraco V. Green methods of lignocellulose pretreatment for biorefinery development. Appl Microbiol Biotechnol. 2016; 100(22):9451–9467.
3. Wang EC, Wang AZ. Nanoparticles and Their Applications in Cell and Molecular Biology. Integr Biol (Camb). 2014; 6(1):9-26.
4. Shrivastava V, Chauhan PS, Tomar RS. Bio-Fabrication of metal nanoparticles: A review. International Journal of Current Research in Life Sciences. 2018; 7(04):1927-1932.
5. Jiang JL, Tseng LW, Chang HR. Symmetrical peripheral gangrene in sepsis after treatment with inotropes. Ci Ji Yi Xue Za Zhi. 2017; 29(2):121-124.
6. Tacconelli E, Müller NF, Lemmen S, Mutters NT, Hagel S, Meyer E. Infection Risk in Sterile Operative Procedures A Systematic Review and Meta-analysis. Dtsch Arztebl Int. 2016; 113(16):271-278.
7. Özgenç O. Methodology in improving antibiotic implementation policies. World J Methodol. 2016; 6(2):143-153.
8. Arya A, Gupta K, Chundawat TS, Vaya D. Biogenic Synthesis of Copper and Silver Nanoparticles Using Green Alga *Botryococcus braunii* and Its Antimicrobial Activity. Bioinorg Chem Appl. 2018; 2018:7879403.
9. Chauhan PS, Tomar RS, Shrivastava V. Bio fabrication of Copper Nanoparticles: A Next Generation Antibacterial Agent against Wound Associated Pathogens. Turkish Journal of Pharmaceutical Sciences, 2018, 15(3).
10. Chauhan PS, Shrivastava V, Prasad GBKS, Tomar RS. Effect of Silver Nanoparticle-Mediated Wound Therapy on Biochemical, Hematological, and Histological Parameters. APCR. 2018; 11(3):251-258.
11. Shrivastava V, Chauhan PS, Tomar RS. A Biomimetic Approach for Synthesis of Silver Nanoparticles using Murraya paniculata Leaf Extract with Reference to Antimicrobial Activity. J Pharm. Sci. & Res. 2016; 8(4):247-250.
12. Bigliardi PL, Alsagoff SAL, El-Kafrawi HY, Pyon JK, Wa CTC, Villa MA. Povidone iodine in wound healing: A review of current concepts and practices. International Journal of Surgery. 2017; 44:260-268.
13. Miró-Canturri A, Ayerbe-Algaba R, Smani Y. Drug Repurposing for the Treatment of Bacterial and Fungal Infections. Front Microbiol. 2019; 10:41.
14. Hoseinzadeh E, Makhdoumi P, Taha P, Hossini H, Stelling J, Kamal MA *et al*. A Review on Nano-Antimicrobials: Metal Nanoparticles, Methods and Mechanisms. Curr Drug Metab. 2017; 18(2):120-128.
15. Umaru IJ, Badruddin FA, Umaru HA. Phytochemical Screening of Essential Oils and Antibacterial Activity and Antioxidant Properties of *Barringtonia asiatica* (L) Leaf Extract. Biochem Res Int. 2019; 2019:7143989.
16. Yu C, Tang J, Liu X, Ren X, Zhen M, Wang L. Green Biosynthesis of Silver Nanoparticles Using *Eriobotrya japonica* (Thunb.) Leaf Extract for Reductive Catalysis. Materials (Basel). 2019; 12(1):189.
17. Thanh NTK, Maclean N, Mahiddine S. Mechanisms of Nucleation and Growth of Nanoparticles in Solution. Chem. Rev. 2014; 114(15):7610-7630.
18. Ojeda JJ, Dittrich M. Fourier transform infrared spectroscopy for molecular analysis of microbial cells. Methods Mol Biol. 2012; 881:187-211.
19. Antunović V, Ilić M, Baošić R, Jelić D, Lolić A. Synthesis of MnCo2O4 nanoparticles as modifiers for simultaneous determination of Pb(II) and Cd(II). PLoS One. 2019; 14(2):e0210904.
20. St O. MIC and MBC of Honey and Gold Nanoparticles against methicillin-resistant (MRSA) and vancomycin-resistant (VRSA) coagulase-positive S. aureus isolated from contagious bovine clinical mastitis. Journal of Genetic Engineering and Biotechnology 2017; 15(1):219-230.
21. Krishnan R, Arumugam V, Vasaviah SK. The MIC and MBC of Silver Nanoparticles against Enterococcus faecalis - A Facultative Anaerobe. J Nanomed Nanotechnol. 2015; 6:285.
22. Chauhan PS, Shrivastava V, Tomar RS. Phytomediated Synthesis of Silver Nanoparticles and Evaluation of Its Antibacterial Activity against Bacillus Subtilis and Staphylococcus Aureus. International Journal of Pharma and Bio Sciences, 2016, 184-195.
23. Shrivastava V, Chauhan PS, Tomar RS. Nanobiotechnology: A Potential Tool for Biomedics World Journal of Pharmacy and Pharmaceutical Sciences. 2015; 4(5):1929-1943.
24. Tomar RS, Chauhan PS, Shrivastava V. A critical review on nanoparticles synthesis: physicochemical v/s biological approach. World journal of pharmaceutical research. 2014; 4(1):595-620.