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BIOGENIC SYNTHESIS OF COBALT NANOPARTICLES FROM ZINGIBER OFFICINALE POWDERED PEEL EXTRACT- ITS CHARACTERISATION AND ANTIMICROBIAL ACTIVITIES

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Abstract: Green synthesis of metallic nanoparticles have attracted the attention of researchers nowadays and it is considered as an alternative method to Physical and Chemical approaches. A novel method has been developed by green route to synthesise Cobalt nano particles through plant extract. In the present study the aqueous extract of the powdered peels of Zingiber officinale (ZO) acted both as reducing and capping agent to synthesise the Cobalt nanoparticles (CoNPs). The new route is eco-friendly, cost effective and natural way to synthesise nanoparticles. The synthesised CoNPs was characterised using Ultra Violet Visible (UV-Vis) Spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy and by scanning Electron microscopy (SEM) technique. The newly synthesised CoNPs was tested for its antimicrobial activities and results showed that it had great potential as antimicrobial agent.

Keywords - Cobalt nanoparticles (CoNP), Zingiber officinale (ZO), UV-Vis, FTIR, SEM, antimicrobial

1. Introduction:

There is a tendency in recent years towards developing and applying new materials with new properties [1, 2] and all the attention has fallen upon nanomaterials and nanoparticles [3-5] due to their unique properties. Plant based nanoparticles (NPs) have gained importance in recent times due to their nontoxic production, environment friendly technology and the safety-by-design concept [6-10]. Plant based NPs are now being regarded as a gold standard among green biological techniques owing to their ease of use and diversity of available plants. Currently, attention is being paid to metal nanoparticles for their developed surface, optical, magnetic, electronic and catalytic properties [11, 12]. Cobalt nanoparticles (CoNPs) are commercially in demand as they are being used for energy storage, catalysts in medicine, in microelectronics, contrast agents and as basis for drug delivery system. CoNPs can be magnetic, opening up new windows of possibilities for using it as a carrier for targeted drug delivery and as sensors to determine various substances [13-19]. Due to the simplicity of obtaining Cobalt nanoparticles and the high applied value in industries increased our interest in synthesising Cobalt nanoparticles by the greener route. In the present study a novel approach for the biosynthesis of Cobalt nanoparticles using the aqueous extract of powdered peel of *Zingiber officinale* at room temperature has been reported which belongs to the Zingiberaceae family. Zingiber genus, has been commonly consumed as a spice and an herbal medicine for a long time. Ginger root is used to attenuate and treat several common diseases, such as headaches, colds, nausea, and emesis. Many bioactive compounds in ginger have been identified, such as phenolic and terpene compounds. Hence, the Bio- synthesis of CoNPs from the aqueous extract of powdered peel of *Zingiber officinale* were found to be stable for a long time, eco-friendly and reproducible. The reduction of Cobalt ion in aqueous solution exhibits colloidal cobalt with particle diameter in nanometer. The biosynthesised CoNPs showed higher antimicrobial activity against different multi drug resistant bacterium isolated from clinical specimen.

2. Experiment:

2.1 Green synthesis of ZO-CoNPs:

All the glassware were washed with deionised water and dried in oven. Cobalt sulphate was obtained from Sigma-Aldrich Chemical Co. The Petri-plates and agar were autoclaved before use. Fresh *Zingiberene,* were bought from local Madurai Market, washed several times with water to remove dust particles and then the peel sun dried to remove the residual moisture and grinded to powdered form. 0.2 g of the peel powder was boiled with 100mL of deionised water for 15 minutes and filtered to obtain the aqueous extract. The extract of *Zingiber officinale* (10 mL) were mixed with 40 mL of 1mM cobalt sulphate ($CoSO_4$) and a colour change was observed indicating the formation of CoNPs.

2.2 Characterisation of CoNPs:

Initial characterisation of the CoNPs was carried out using UV-Vis spectroscopy. The measurement was carried out with a Jasco dual-beam spectrophotometer (model V-760, Japan) having operational range of wavelength between 190 nm and 1100 nm. The biosynthesised CoNPs were characterised by UV-Vis spectroscopy in order to observe rapid reduction of Cobalt nanoparticles by the action of aqueous peel extract of *Zingiber officinale* (ZO) using 1mM $CoSO_4$ solution. The colour change was observed and noted every minute and the reaction mixture changed its colour from light yellow to dark brownish pink in 30 min. The prepared CoNPs were dried at 70 ºC for 5 h and mixed with KBr and converted into a pellet form by using a bench press. The formed pellet was further used for testing functional groups present in the CoNPs by IR spectroscopy. The size and morphological characterisation of the synthesised and lyophilised CoNPs were studied using scanning electron microscope (SEM).

2.3 Study of Antimicrobial activity of CoNPs:

The CoNPs were serial diluted and plated on nutrient agar medium and various microbial colonies were isolated and those organisms are: *Staphylococcus aureus, Klebsiella Oxytoca, Staphylococcus Epidermidis, Escherichia coli and Candida Albicans.* Well Diffusion method was used for checking antimicrobial activity. Sterile Muller Hinton Agar plates were prepared. These plates were swabbed with the respective clinical isolates. Using a sterile cork borer, wells were cut on the Muller Hinton Agar plates. The biosynthesized CoNPs, cobalt sulphate solution and ZO peel powder extract was added in their respective wells. The plates were incubated at 37°C for 24 hours. Control plates with Cobalt sulphate solution and culture broth were kept for incubation along with the test plates. After incubation the zones were examined and the zone diameter was measured. For identifying Zone of Inhibition (ZOI) 100 µL of Cobalt nanoparticles was added.

3. Results and Discussion:

3.1 Effect of reaction time on the formation of CoNPs:

To the aqueous ZO powdered peel extract, cobalt salt solution was added, it resulted into a colour change from pale yellow to dark brownish pink as shown in Fig. 1. The change in colour of the solution happens because of cobalt nanoparticle formation by the reduction of cobalt salt. The reduction of cobalt salt to cobalt ions is due to the presence of reducing agents. ZO extract contains natural reducing agents like terpenoids and flavanones, which are responsible for reducing cobalt salt into CoNPs. The complete colour change of the synthesised CoNPs takes place because of the reduction and the time taken for the colour change was noticed to be 30 min, thereafter no further colour change occured. This indicated complete reduction of Cobalt salt present in the reaction mixture to CoNPs.

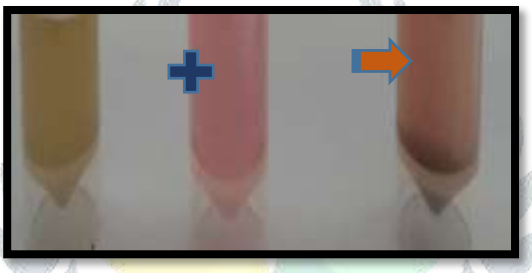


Fig. 1 Change in Colour of the solution with time as Cobalt salt is added to ZO peel extract

3.2 Effect of concentration on the formation of CoNPs:

The formation of CoNPs was confirmed by examining the absorption spectra that originated due to the strong surface plasmon resonance (SPR),i.e., due to resonant absorption of photons by CoNPs. The absorption spectrum as shown in Fig. 2 of CoNPs obtained from the ZO powdered peel aqueous extract and $CoSO_4$ was recorded in the range of 200 – 800 nm. Absorption maximum was observed at 510 nm, indicating a complete reaction and no cobalt salt being left for further reaction. With time increase the intensity of SPR band increased without any shift in peak wavelength. In the present study in accordance with Mie theory, absorption spectra of the synthesised CoNPs exhibited single SPR band, therefore, confirming the formation of spherical CoNPs.

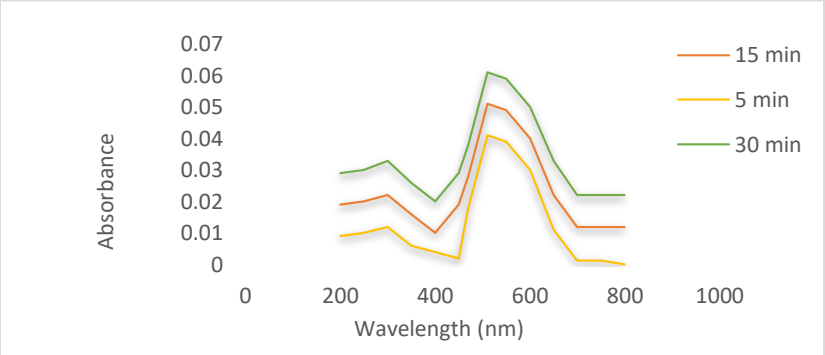


Fig. 2 Absorption spectra of synthesised CoNPs observed at three different reaction times.
Figure shows an increase of absorption intensity as a function of reaction time

3.3 FT-IR Analysis:

The FTIR spectrum of biologically synthesised CoNPs is shown in Fig.3. FTIR measurements when examined for the CoNPs showed transmission peaks at 3379, 2927, 1734, 1640. 1527, 1103 and 1031 cm^{-1}. The peak at 1031 cm^{-1} is due to saturated alkanes and the peak at 1527 cm^{-1} maybe is because of amide presence. The peak at 1103 cm^{-1} represents C-O stretching , bands seen at 1640-1734 cm^{-1} indicates C=C stretching and 3379 cm^{-1} peak shows the presence of O-H stretch. Based on these results the presence of phenolic compound and protein are believed to be responsible for the formation and stabilisation of the biologically synthesised CoNPs.

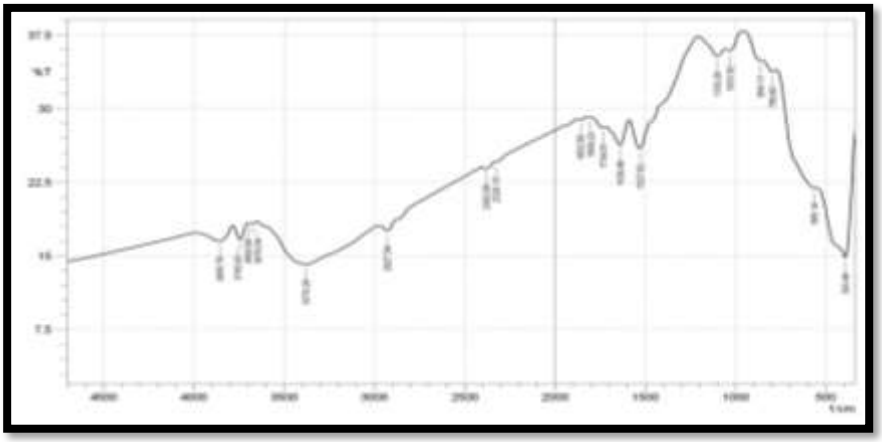


Fig. 3 FTIR Spectra of synthesised CoNPs by ZO powdered peel aqueous extract

3.4 Surface Morphology of CoNPs:

The surface morphology and size of the green synthesised CoNPs was examined using Scanning electron microscopy (SEM) method. Fig. 4 (a) and (b) represent the SEM images of CoNPs synthesised by the of ZO powdered peel aqueous extract at two different resolutions. The electrostatic interaction and hydrogen bond between the bio-organic capping molecules are responsible for the synthesis of CoNPs using ZO extract. It is seen from the images that relatively uniform and slightly spherical shape of CoNPs was confirmed in the range of 60-80 nm. This is due to the reducing ability of the phytochemicals present in ZO.

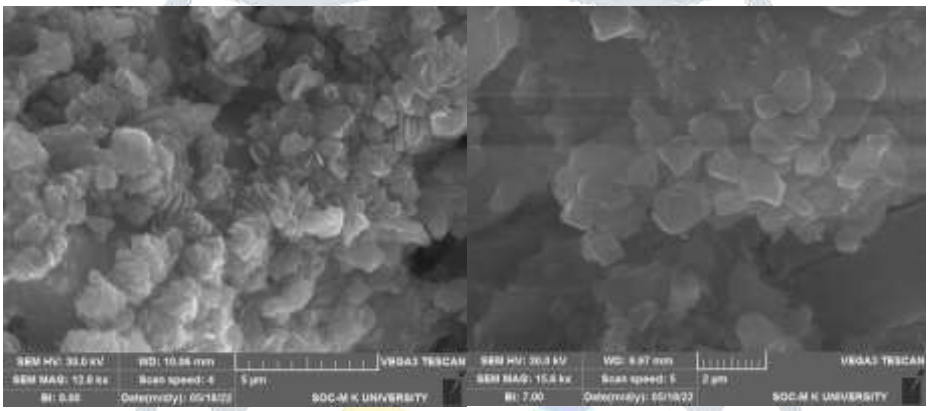
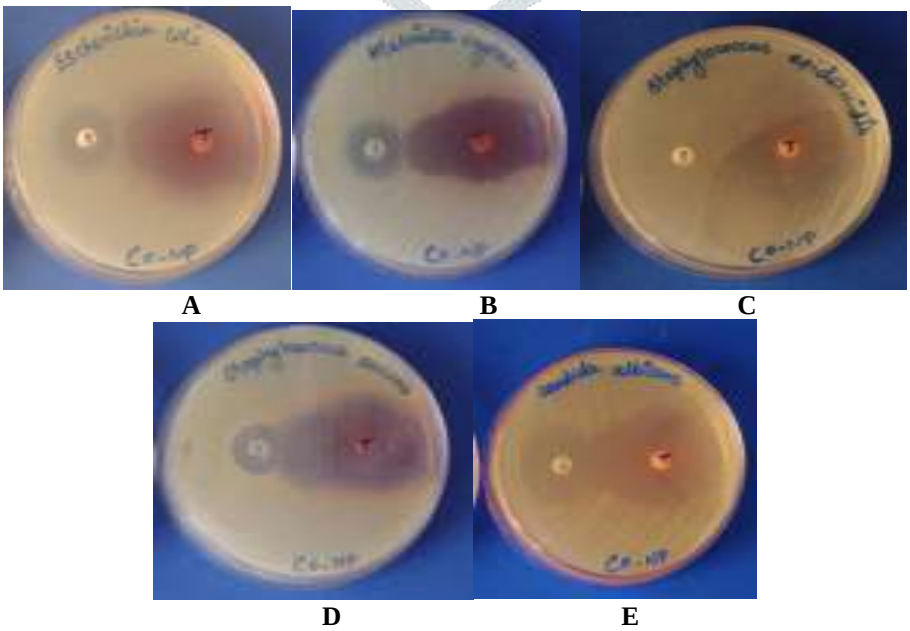


Fig.4 (a) & (b) SEM images of green synthesized CoNPs by ZO Powdered Peel aqueous extract

3.5 Antimicrobial study on ZO-CoNPs:

The antimicrobial study of green synthesised CoNPs were established against two gram negative, two gram positive pathogenic bacteria such as Staphylococcus aureus (Gram positive) and E. coli (gram negative) and a fungi strain using disk diffusion method as shown in Table 1. The diameter of the zone of inhibition (ZOI) in millimetre around each well in a single concentration level of CoNPs were determined as shown in Fig.5. The MIC of cobalt nanoparticles from ZO peel extract showed better inhibition zone (Table1) for 100 µL of CoNPs; *Escherichia Coli* - 32 mm, *Klebsiella Oxytoca* - 29 mm, *Staphylococcus Aureus* - 32 mm, *Staphylococcus Epidermidis* - 30 mm, *Candida Albicans* - 36 mm. The inhibition zone shown by standard drugs Amikacin and Ketoconazole taken for investigation had lower values than the synthesized nanoparticle and that proves the efficacy of the synthesized CoNPs.



A **B** **C**

D **E**

Fig.5 Antimicrobial efficacy of CoNPs against multi drug resistant clinical isolates (A) Escherichia coli (B) Klebsiella oxytoca (C) Staphylococcus epidermidis (D) Staphylococcus aureus (E) Candida albicans

Table 1. Zone of Inhibition of Synthesised CoNPs from aqueous extract of ZO Powdered peel against various human pathogenic microbes

Study No.	Multi drug resistant clinical isolates	Zone of Inhibition (mm)		
		Standard Drugs (100 µL)		CoNPs (100 µL)
1.	Escherichia Coli	Amikacin	18	**32**
2.	Klebsiella Oxytoca	Amikacin	18	29
3.	Staphylococcus Aureus	Amikacin	18	32
4.	Staphylococcus Epidermidis	Amikacin	18	30
5.	Candida Albicans	Ketoconazole	20	36

4. Conclusion:

CoNPs have been synthesised with a well-known herb, Zingiber officinale (ZO). The rapid biological synthesis of Cobalt nanoparticles using Zingiber officinale (ZO) powdered peel aqueous extract provides an environmental friendly, simple, and efficient route to nanoparticle synthesis. The synthesis is found to be efficient in terms of reaction time as well as stability of the CoNPs. The synthesized nanoparticles were of spherical shape and the estimated sizes were 60 - 80 nm. From the UV-Vis, FTIR techniques, it is proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The lower concentrated nanoparticles show spherical shaped appearance. From the technological point of view the green method of synthesis of CoNPs have potential applications in the biomedical field due to its enhanced antimicrobial activity. Furthermore, this greener route opens up more options and windows for the potential application of the CoNPs in various fields such as medical imaging and drug delivery.

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