Biogenic Synthesis of ZnO Nanoparticles from *Corollocarpus epigaeus*: Phytochemical Characterization and Antimicrobial Applications

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Abstract
In recent days, nanotechnology incorporates into the medical field that enhances the benefits in drug delivery system. Corallocarpus epigaeus, a tendril, has the number of medicinal properties for diabetes, rheumatism, herpes and for snake bites[23]. In this study, the tuber of this plant was dried and extracted to identify its phytoconstituents qualitatively showing the presence of flavonoids, terpenoids and steroids. The synthesis of nanoparticles using zinc sulphate has widespread use in biomedicine due to its small size. The UV – spectroscopy of ZnONPs showed the peak at 412 nm, 573 nm and 600 nm. and FTIR results explained the peak absorbed at 1637 cm^{-1}, 1412 cm^{-1}, 708 cm^{-1} and 612cm^{-1} indicating the presence of functional groups such as hydroxyl amine, alkenes, alkynes, aliphatic amines, carboxylic acids, alkyl halides and aromatic compounds.

The SEM analysis also showed the clear picture of the presence of ZnONPs in spherical shape. The study was further investigated for antimicrobial properties of E.coli (13 mm), Pseudomonas species (16 mm), Klebsiella species (12 mm) and Staphylococcus species (20 mm) at 80 µg/ml of ZnONPs of Corollocarpus epigaeus. The sensitivity is higher in ZnONPs than in the methanol and aqueous plant extract.

Keywords: *Corallocarpus epigaeus*, Zinc oxide nanoparticles, Antimicrobial properties, Phytoconstiuents, SEM, UV-spectroscopy and FTIR.

Introduction

In most recent scientific disciplines, nanotechnology is concerned with creating materials and nanoparticles with sizes ranging from 1 to 100 nm. The great diffusion, durability and adaptability of nanoparticles and its chemical and biological activities have made them valuable tools for a variety of technological applications. The nanoparticles can be synthesised by physical, chemical and biological methods. These methods can be used to create the nanoparticles, but the green synthesis has a number of benefits over the physical and chemical methods, being more affordable. It just requires one step and is environmentally benign[20]. The plants extracts were used for the synthesis of nanoparticles using many metals such as gold, silver, cobalt, platinum, palladium and zinc. Of all these, the zinc oxide nanomaterials possess various application in sensors, catalysis, fibre paints, targeted drug delivery due to antibacterial and luminescence features. Zinc oxide nanoparticles have the widespread use in biomedicine due to their small size and interaction with biological membranes, receptors, proteins and nucleic acids. There are is extensively used in cosmetic industry and are also used in the preparation of biomedicines against bacteria, fungi, viruses and parasites, they are also used as anticancer drugs and therapeutic agents[2].

The antibacterial activity of ZnONPs was effective by coupling with various factors that impact the antibacterial activity, while comparing to organic-based disinfectants. ZnONPs have exceptional stability and longevity which encourage its usage as an antibacterial agent. ZnONPs are a promising alternative to other metal oxides because of their noble qualities and appealing features, which cause considerable toxicity to living things. It is anticipated that further unique characteristics would increase the uses of ZnONPs in a number of fields, most notably being biomedicine[22].

ZnONPs have demonstrated significant potential in biomedicine, especially in the antibacterial and anticancer domains, due to their potent ability to produce excessive reactive oxygen species (ROS), liberate zinc ions and induce cell death. Zinc also maintains the structural integrity of insulin, which is well known. In light of this, ZnONPs have also been successfully created for the treatment of diabetes[11].

Corollocarpus epigaeus belongs to *Cucurbitaceae* which is a tendril climbing herb that is yellow in colour, bitter in taste with laxative and other pharmaceutical properties. The extract of this tuber has higher inhibitory effects than the leaves and stem used for treating diabetes, rheumatism, herpes and for snake bites. The phytochemical compounds from its characterization showed antibacterial, antifungal and antioxidant properties[23].

Sarvalingam et al[21] had reviewed and listed the curative climbers in South Western Ghats of Tamilnadu, they also listed the roots of *Corallocarpus epigaeus*. Its medicinal properties are widely used for joint pain. Because of its nanosize, optical, chemical, biological and pharmacological qualities, zinc and its compounds can be used in a wide range of biomedical applications[4]. Hence, the aqueous, chloroform and methanol plant extracts were investigated for phytochemical properties and also to investigate

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antibacterial activity of plant extracts and ZnONPs against *Klebsiella sp., E.coli, Pseudomonas sp. and Staphylococcus sp.*

Material and Methods

Preparation of extracts: After being collected, the tubers were cleaned under running water and allowed to dry. Then the tubers were chopped into small bits under shade, they were ground into a fine powder, which was then sieved using a commercial sieve. With minor adjustments of the Jayaseelan et al procedure, the powder was gathered in a zip-lock bag and kept for subsequent extraction.

For solvent extraction, 50 g of *Corollocarpus epigaeus* powder was dissolved in a conical flask containing 250ml of water, methanol and chloroform. The mixture was then kept for overnight. Then the mixture was filtered by Whatmann no.1 filter paper. The extracts were proceeded for phytochemical screening.

Phytochemical screening: The method followed by Joshi et al[12] was used to qualitatively assess the phytoconstituents including alkaloids, phenols, saponins, glycosides, flavonoids and tannins with some minor modifications.

Test for carbohydrates: Add 5 ml of Benedict's reagent to 2 ml of aqueous extract, the liquid is heated to produce a crimson precipitate which signifies the presence of carbohydrates.

Test for saponins: 1 ml of aqueous extract is added to 1 ml of distilled water and shake the mixture vigorously. Appearance of foam indicates the saponins.

Test for Tannins: To 1 ml of extract, 0.1% of $FeCl_3$, was added and mixed well to get the brown precipitate that indicates the presence of tannins.

Test for alkaloids: To 1 ml of extract, add 2-3 drops of Dragendroff's reagent to get the pale yellow mixture that indicates the presence of alkaloids.

Test for flavonoids: To 1 ml of extract, add 1% ammonia solution to get yellow coloration that indicates the presence of flavonoids.

Test for Glycosides: The presence of glycosides is shown by the violet to blue to green precipitate that results from adding 2 ml of extract, 2 ml of chloroform and 2 ml of acetic acid.

Test for Phenols: To 1 ml of aqueous extract, 2 ml of distilled water, 0.5 ml of sodium carbonate and 0.5 ml Folin Ciocalteau's reagent were added for the appearance of blue to green precipitate that indicates the presence of Phenols.

Test for Terpenoids: 2 ml of extract and 3 ml of chloroform are mixed together. To the mixture, add few drops of concentrated H_2So_4 to form deep red colouration, indicating the presence of terpenoids.

Test for Steroids: The presence of steroids is shown by the formation of a brown ring at the interface after adding 1 ml of aqueous plant extract, 2 ml of chloroform and 1 ml of conc. H_2SO_4.

Test for Proteins: To 1 ml of extract, add 1 ml of 40% NaOH and 2 drops of 1% copper sulphate solution. The presence of proteins is shown by the solution turning violet.

Test for Cardiac glycosides: To 2 ml of aqueous plant extract, add 1 ml of acetic acid and few drops of ferric chloride and underlayered with H_2SO_4 to form brown ring indicating the presence of cardiac glycosides.

Preparation of ZnO nanoparticles: The tuber of the *Corollocarpus epigaeus* was obtained and washed in distilled water, the tubers were cut into small pieces and dried under the shade. The shade dried tubers were ground into fine powder and collected in a zip lock cover. 10g of finely powdered tuber was dissolved into 100ml of sterile distilled water and kept in a water bath at 60°C for 2 hours. Then the solution was filtered through a Whatmann no. 1 filter paper, the filtrate was then mixed in an equal volume of 0.2M zinc sulphate solution. The solution was stirred continuously at 50°C for 15 – 20 minutes. The pH of the solution was adjusted to 12 using 2.0M NaOH and kept at overnight incubation.

After incubation, the final product has been centrifuged at 10000 rpm for 20 minutes and the precipitate was kept at oven for 24 hours at 60°C for drying and the dried products were collected.

Characterisation

UV- Visible Spectrophotometer: The Perkin Elmer Lambda 3B UV-vis spectrometer was used to record the UV-Vis spectrum and the UV Shimadzu 3101 PC spectrophotometer was used to measure it.

Fourier Transform Infra-red Spectroscopy (FTIR): FTIR spectroscopy was measured with a Nexus 670 (Nicollet-Madison, WI, USA) FT- IR model. To get FT-IR spectra, the discs were scanned within the 400–4000 cm^{-1} range.

Scanning electron Microscopy (SEM): The morphology and shape of the ZnONPs of *Corollocarpus epigaeus* were assessed by Scanning electron microscope (SEM).

Antibacterial activity: The antibacterial activity of *Corollocarpus epigaeus* zinc-oxide nanoparticles was assessed by a standard protocol followed by Jayaseelan et al. The activity was tested against the organisms isolated in leftover urine samples collected from patients, obtained from a diagnostic laboratory. The ZnO nanoparticles were weighed in a concentration such as 4, 6 and 8 mg mixed in 10% DMSO to obtain 40µg/ml, 60µg/ml and 80µg/ml respectively. The colonies of the isolated organisms were suspended in a peptone water and incubated at 37°C for 2 hours. The suspended organisms were swabbed on to an MHA plate. The agar well was made up to 6mm for different concentrations of zinc nanoparticles. 80- 100µL of each concentration were dropped into the well and gentamicin disc was placed as a control and kept at 37°C for overnight incubation.

Results and Discussion

The phytoconstituents of methanol and chloroform extracts were tabulated in table 1. The results showed that chloroform extract was positive for most of the phytochemical constituents such as carbohydrates, saponins, alkaloids, terpenoids and steroids. Carbohydrates, alkaloids and steroids commonly showed positive in both methanol and chloroform extract.

The chloroform extract of *Corollocarpus epigaeus* tuber has high degree of precipitation in most of the phytochemical constituents. Alkaloids are the heterogenous group of compounds that contain one or more nitrogen atom in acyclic system. The silver nanoparticles have shown the presence of phytochemicals such as flavonoids, saponins, tannins and anthroquinones reported by Chandraker et al[5]. Priyavardhini et al[19] recorded the phytochemical constituents such as alkaloids, flavonoids, phenols, tannins, steroids, saponins, glycosides and terpenoids correlated with the present study.

Subashini et al discussed that *Corollocarpus epigaeus* of chloroform extract was positive. Alkaloids and phytosterols in ethyl acetate extract showed the presence of phenolic compounds and phytoserols, whereas the 85% methanol showed positive for most of the phenolic compounds, carbohydrates, aminoacids and phytosterols and it was also concluded that methanolic extract has the high potential for any biomedical applications. The results were not correlated with the present study, since the report showed that chloroform extract was positive for alkaloids, flavonoids and steroids.

Jena and Mishra[9] reviewed the phytoconstituents of *Cucurbitaceae* family and reported that they possess various phytochemicals such as glycosides, terpenoids, saponins, tannins, steroids, carotenoids and resins. They are also renowned for its anti-bacterial, anti- fungal and antioxidant properties. This research was also well correlated with the present study that showed the presence of tannins, steroids, saponins, glycosides and terpenoids in methanol and chloroform extract of *Corallocapus epigaeus*.

The wide variety of phytochemicals that can function as stabilizing and reducing agents for the creation of nanoparticles, plants are the most popular green source for nanoparticle synthesis among biological materials. Metal ions are reduced to nanoparticle form by a variety of phytochemicals including flavonoids, ketones, aldehydes, amines, amides and organic acids. The stability of produced nanoparticles is guaranteed by various proteins[3].

Characterization

UV – visible spectroscopy: Figure 1 shows the results of UV-spectrophotometer in the range of 200 – 800 nm that showed the peak at 412 nm, 573 nm and 600 nm. The presence of ZnO nanoparticles showed its peak at 412 nm. Fakhari et al[7] reported that the distinct peak is elevated at 350 nm, the bulk ZnO absorption received at 385 nm. The AuNPs showed the surface plasmon resonance absorbance peak at 545 nm by UV – visible spectroscopy reported by Kandhasamy et al. Silver nanoparticle exhibit the absorbance peak in the range of 400 – 500 nm that showed the maximum absorbance at 463 nm reported by Chandraker et al[5].

Papitha and Selvaraj[18] worked on CuO nanoparticles of *Parkia timoriana* bark and CuO – ZnO nanocomposites, they reported that UV – Visible spectrometer showed the peaks at 200 – 400 nm, that is partially correlated with our study. The peaks at 400 nm showed the CuO-ZnO nanocomposites of *Parkia timoriana* bark.

Talam et al[25] studied the spectroscopic properties of ZnONPs, they observed the absorption band at 355 nm. The photoluminescence spectrum shows the two peaks at 392 nm and 520 nm.

Table 1
Qualitative phytochemical analysis of *Corollocarpus epigaeus*

S.N.	Phytochemical constituent	Methanol extract	Chloroform extract
1	Carbohydrates	++	++
2	Saponins	++	-
3	Tannins	-	-
4	Alkaloids	+	+
5	Flavonoids	-	+
6	Glycosides	-	-
7	Phenols	-	-
8	Terpenoids	+++	-
9	Steroids	+	+
10	Proteins	+	+
11	Cardiac glycosides	-	+

FTIR analysis: The FT-IR analysis showed the phytochemicals such as phenols, alcohols, amines and carboxylic acids that can interact with zinc nanoparticles with the peak absorbed at 1637 cm^{-1}, 1412 cm^{-1}, 708 cm^{-1} and 612cm^{-1} as in figure 2. Table 2 corresponds to Zn-O stretching. The results were similar to the results of Fakhari et al[7]. Similarly, the results of Papitha and Selvaraj[18] also documented that 448.34 cm^{-1} peak indicates the stretching vibration of Zn- O bond. Tiwari et al[27] studied the spectroscopic investigation of green synthesis of ZnO- Np. They found that the ZnO nanoparticles showed the FT-IR spectra were recorded in the wavelength range from 4000-500 cm^{-1}. It is also observed that O-H stretch showed the band at 3401 cm^{-1}, the peaks at 2345 cm^{-1} were assigned for C=C and C=N stretching. This observance was well correlated with the present FTIR spectrum that had the peaks at 2928 cm^{-1} and 3396 cm^{-1}.

Karthic et al[15] confirmed that *Corallocarpus epigaeus* extract has some organic functional groups such as alcohols, phenols, amines, amides, alkenes, alkynes, aliphatic amines, carboxylic acids, alkyl halides, aromatics. These findings were well corelated with the present study, proving to be significant in many pharmaceutical industries. According to Woznicka et al, sulfonic derivatives of quercetin were discovered to form compounds with metal ions in aqueous solutions. At pH 5, the metal excess in respect to the ligand causes the NaQSA-8 to precipitate with a solid complex of Fe(II) ions. The 4C=O, 3-OH and 3′-OH, 4′-OH groups of the molecule NaQSA-8 are suggested as the metal's coordination sites.

Kalinowska et al[13] reported that the compound quercetin is a major flavonoid that has significant antioxidant, antibacterial and anticarcinogenic properties when complexed with metal ions. Tan et al[26] also reported that quercetin-zinc complex molecules showed the antitumour activity that intercalate into DNA.

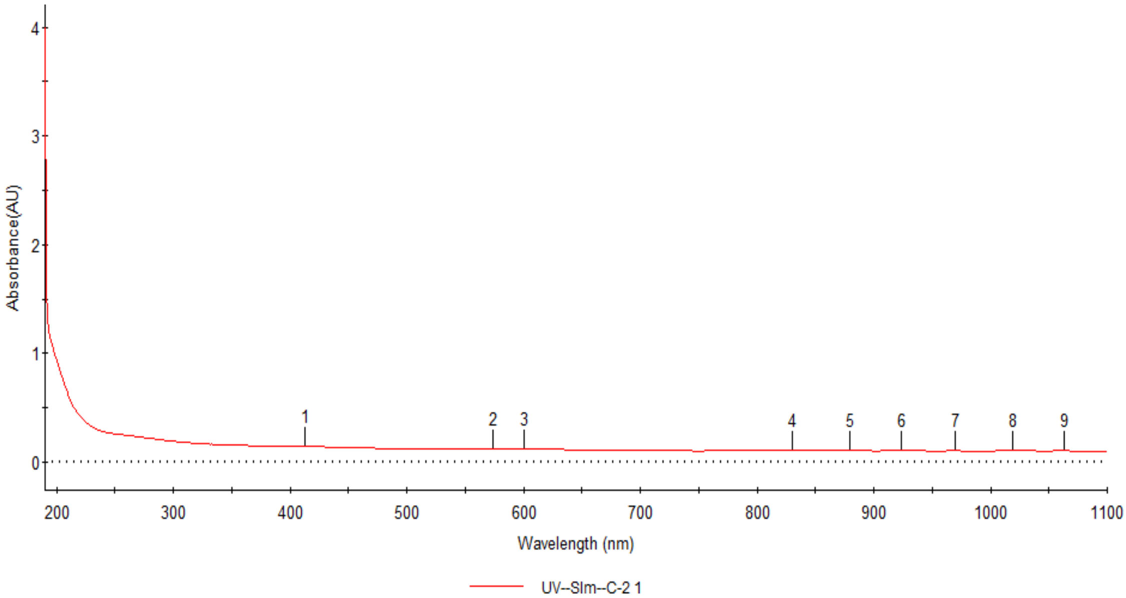


Figure 1: The absorbance peak by UV – Visible spectroscopy

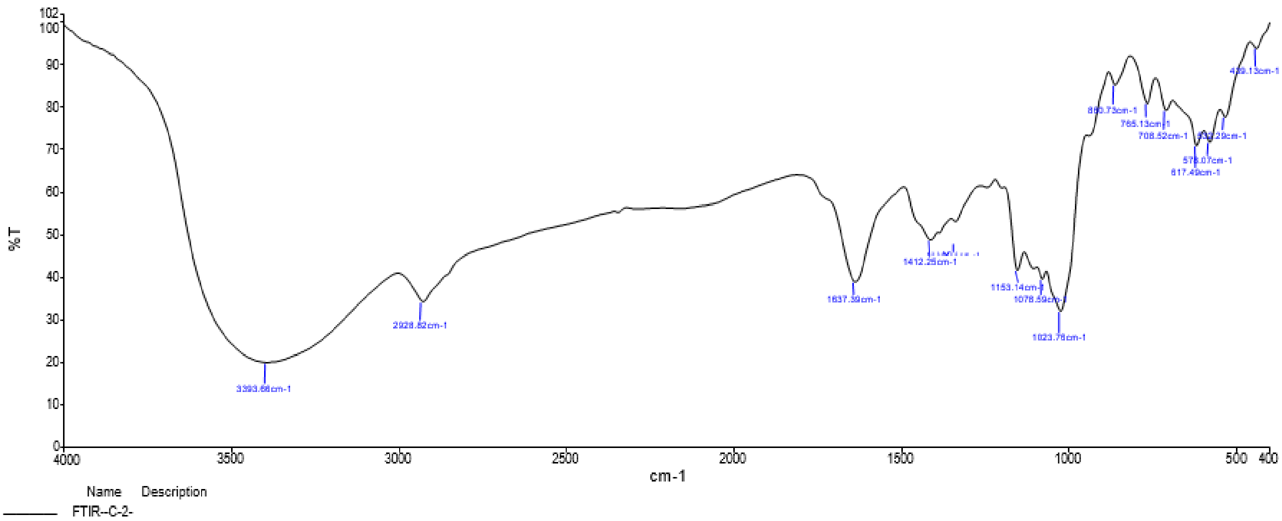


Figure 2: FTIR analysis

Table 2
FTIR Wavelength and its functional group

S.N.	Wavelength	Vibrational modes	Functional group
1	3391.02	Aromatic primary amine, NH stretch Normal ''polymeric'' OH stretch	Primary amine group Alcohol and hydroxy compound
2	2927.02	Methylene C-H asym./sym. Stretch	Saturated Aliphatic (alkene/alkyl)
3	2094.34	Isothiocyanate (-NCS)	Nitrogen multiple and cumulated double bond compound
4	1637.52	Organic nitrates	Simple hetero-oxy compounds
5	1412.71, 935.29	Vinyl C-H in-plane bend	Olefinic (alkene)
6	1384.78	gem-Dimethyl or ''iso''- (doublet)	Methyl (−CH3)
7	1242.57	Aromatic ethers, aryl -O stretch	Ether and oxy compound
8	1106.89	Sulfate ion	Common inorganic ions
9	1153.97	Aliphatic fluoro compounds, C-F stretch	Aliphatic organohalogen compound
10	1080.97, 861.68	Cyclic ethers, large rings, C-O stretch	Ether and oxy compound
13	763.27	Alcohol, OH out-of-plane bend	Alcohol and hydroxy compound
14	708.49, 618.52, 576.29, 529.73	Aliphatic bromo compounds, C-Br stretch	Aliphatic organohalogen compound
18	441.51	Polysulfides (S-S stretch)	Thiols and thio-substituted compounds

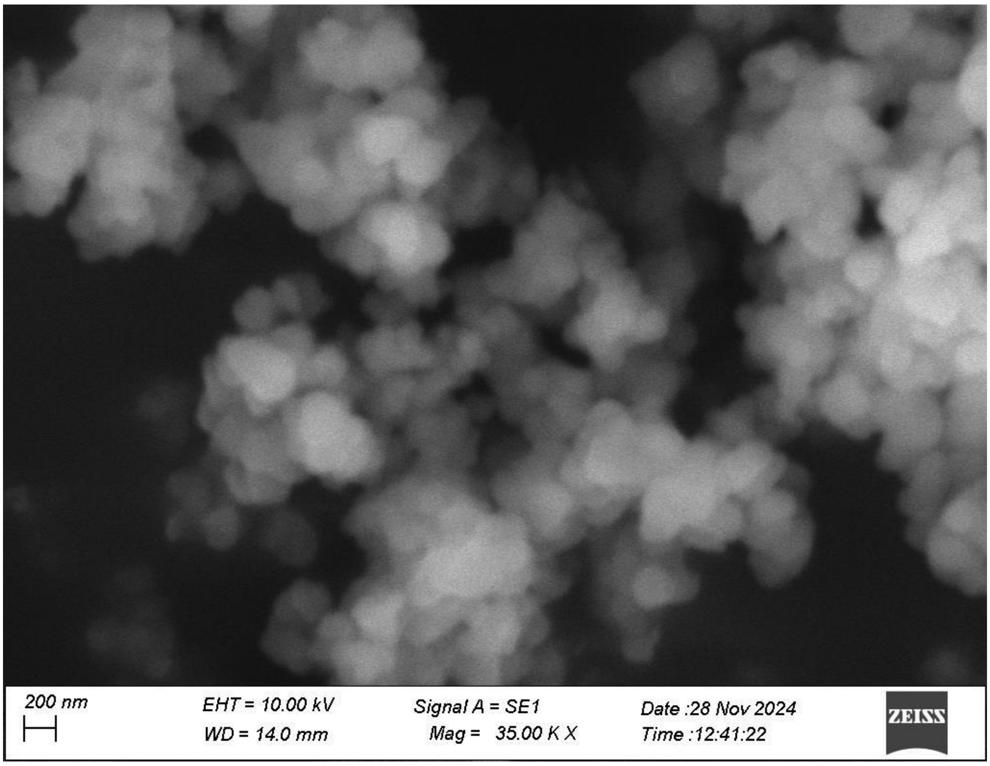


Figure 3: image of SEM

SEM analysis: The shape, structure and size of the synthesized nanoparticles were determined by SEM analysis. Figure 3 showed the SEM picture that showed spherical shape in 85nm by the synthesis of nanoparticles from zinc sulphate that is used as precursor in 200 nm micrographs. Fakhari et al[7] discussed that small spherical particles that accumulate as bullet are formed when zinc acetate is used as precursor and when zinc nitrate is used as precursor, the nanoparticles showed the flower like patterns. In this case, the present study also showed the similar structure as the zinc acetate nanoparticles.

Alhomaidi[1] studied the SEM analysis of pollen of 6 species of Cucurbitaceae family. The results showed that all the species were spheroidal in shape correlating with the present study. The SEM image of Zinc nanoparticles of *Corallocarpus epigaeus* showed the spherical or cylindrical shape.

Kulkarni and Shirsat[16] studied the optical and structural properties of ZnONPs, they concluded and confirmed the spherical granular ZnONPs, also corelated with the present study, showing the *Corallocarpus epigaeus* ZnONPs in spherical shape.

Antimicrobial activities: *E. coli*, *Klebsiella* species, *Pseudomonas* species and *Staphylococcus* species were obtained in urine samples and confirmed with biochemical characterisation. *E.coli* (13 mm) and *Pseudomonas* species (16 mm) showed the zone of inhibition at 60 µg/ml and 80 µg/ml of ZnONPs of *Corollocarpus epigaeus*. *Klebsiella* species (12 mm) showed the sensitivity at only 80 µg/ml and *Staphylococcus* species (20 mm) showed sensitivity at 80 µg/ml (Table 3). Similarly, all the isolates showed the sensitivity at 80 µg/ml of chloroform extract of *Corollocarpus epigaeus*. The study by Dhand et al[6] also showed the similar report in chloroform and acetone extract on *Staphylococcus aureus* and *Pseudomonas* species.

In the same way Priyavardhini et al[19] also documented that nanoparticles synthesized by $AgNo_3$ showing only limited activity on *Bacillus* species and *Staphylococcus* species. Gentamicin was used as a drug control. By this study, the protein inactivation occurs only when nanoparticles react with protein by combining thiol groups playing a very important role in bactericidal activity. Islam et al[8] did research on the synthesis of ZnO-Np of *Allium cepa* for antioxidant and antibacterial study. They observed that ZnO-Np has the extensive anti- bacterial activity than standard drug which was well correlated with the present study.

The produced zinc oxide nanoparticles' pharmacological properties, both *in vitro* and *in vivo*, may offer more convincing proof for their application in the management of medical conditions. Green synthesis is a rapid and low-cost method that prevents any kind of byproduct formation during the nucleation and synthesis of nanoparticles. It produces evenly distributed nanoparticles with controlled size and form[17].

Conclusion

The zinc oxide nanoparticles were synthesised in *Corollocarpus epigaeus* to prove the presence of nanoparticles at 412nm by UV-Visible spectrometry. The FTIR results showed the presence of phytochemicals such as phenols and carboxylic acid with a spherical shape nanoparticle by SEM analysis. The results also concluded that *Corollocarpus epigaeus* nanoparticles have the extensive antimicrobial properties against *E.coli*, *Klebsiella* species, *Pseudomonas* species and *Staphylococcus species.*

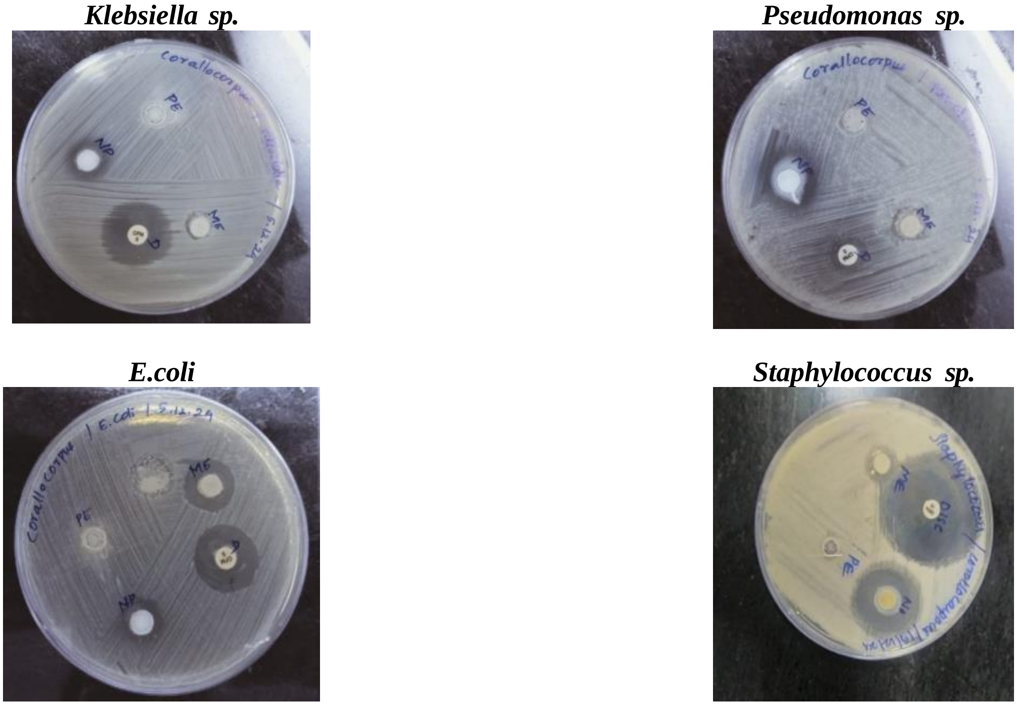
Klebsiella sp. ***Pseudomonas sp.***


Figure 4: Antibacterial activity of ZnO NPs of *Corallocarpus epigaeus*

Table 3
Zone diameter of Microorganisms

S.N.	Organisms	Control	Plant Extract	Methanol extract	ZnONPs
1	*Klebsiella species*	20 mm	Resistant	6 mm	12 mm
2	*Pseudomonas species*	16 mm	Resistant	6 mm	16 mm
3	*Escherichia coli*	20 mm	Resistant	12 mm	13 mm
4	*Staphylococcus species*	26 mm	Resistant	10 mm	20 mm

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