Exploring the potency of Green Synthesized Zinc oxide Nanoparticle for Antibacterial activity.

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ABSTRACT:- Phyllanthus niruri Linnaus (Euphorbiaceae) also known as Chanka piedra is widely grown and used throughout the tropical and Subtropical countries of the world. Because of their importance in numerous Scientific Fields, Scientists have increasingly focused their attention on the nanostructures that are Produced by the green Synthesis process. An overview of the production of Zinc oxide (ZnO) nanosheets (NS_2) utilising extract from the Phyllanthus emblica plant (PEP). The antibacterial efficacy of ZnO-Nps that were biosynthesized was assessed when applied to both Gram-positive (S.aureus) and gram- negative (E.coli) bacterial Strains, ZnO-Nps demonstrate Potent antibacterial action. The result of FTIR analysis of green synthesized ZnO Nps revealed the presence Functional group such as carboxylic, hydroxyl, Aromatic diketons, alkyl amine. The result of antibacterial activity against two bacterial Species showed that green Synthesized Zno nanoparticle was found to be efficient in inhibiting the growth of the bacterial isolates when it came to Staphylococcus aureus.Keywords:-Anti-bacterial activity, Zinc oxide nanoparticle, Pathogen.

INTRODUCTION

In the past, humans used plants to cure infectious diseases and scientific research has proven the therapeutic efficiency of plants over the time. The existence of the plant is very important for the livelihood of humans. Plant products play a very important role in the lives of humans.In the 16th century, plants were the source of treatment and prophylaxis. In the Indian holy book, the Vedas, plants arementioned as being used to treat disease. Several chemical compounds produced by medicinal plants show their medicinal actions towards sick people by resolving a wide range of diseases and disorders. According to the International Union for Conservation of Nature and the World Wildlife Fund, the number of flowering plants used for medicinal purposes ranges from 50,000 to 80,000. According to the National Medicinal Plant Board of India, there are 17,000 to 18,000 flowering plants in India, of which 6000 to 7000 are known for their medicinal properties in traditional medicinal systems like Ayurveda,Siddha, Unani, and Homeopathy (Gusain et al., 2021). Pathogens are typically conceived of as hostile invaders that harm our bodies,but like any other organism, a pathogen or parasite isjust attempting to survive and reproduce. Every living thing on earth may be the victim of some form of infection or parasitism, since it is a very alluring technique to liveat the expense of a host organism. A nutrient-rich, warm, wet environment that maintains steady temperature and is continually renewing itself is the human host. It is not shocking that several bacteria have developed the capacity to endure and multiply in this favourable habitat. Bacteria are metabolically active, single-celled microorganisms that divide by binary fission and lack a nuclear membrane. In terms of medicine, they are a major cause of disease. On the surface, bacteria seem to be very simple life forms, but they are actually highly intelligent and adaptive. An organism that infects its host with disease is referred to as a pathogen, and the severity of the disease symptoms is referred to as virulence. Pathogens include viruses,bacteria, unicellular and multicellular eukaryotes, as well as other taxonomically diverse organisms.

Phyllanthus niruri

Of all themedicinal properties the extract of Phyllanthus niruri contains the antibacterial propertyagainst many pathogenic Phyllanthus niruri Linn. Euphorbiaceae is sporadically found in all tropical and subtropical nations. This is an annual herb that is common along India's coast. It has been employed in Indian ayurvedic systems for more than 2000 years, despite having an extremely short lifespan. Field weed P. niruri belongs to the Phyllanthus genus, which has 600–700 species with only a few minor differences. The Phyllanthus niruri plant extract is used as medication in the Indian ayurvedic system and is suggested for bronchitis, anaemia, leprosy, asthma, urinary diseases, etc. P. niruri is mentioned in the Chraka Samhita literature as a good cure for asthma, as well as forstimulating the liver, easing digestion, boosting appetite, and having laxative effects (Narendra et al., 2012). There has been a lot of research looking at the varied therapeutic potential of this plant species because of the substantial scientific interest in this herb. Since Ottow originally extracted the lignan phyllanthin from this plant in 1861, phytochemical investigations have been conducted on this plant. This plant is rich in tannins, flavonoids, alkaloids, terpenes, coumarins, lignans, and phenylpropanoids, which are responsible for the pharmacological activity of P. niruri, according to research going back to the isolation of the putative anti-HBV phytochemicals nirtetralin and niranthin. Although it has a wide variety of ethnomedicinal uses, most of these possible therapeutic uses have not been the subject of research that has advanced to the point of clinical trials. Despite bacteria.Zinc oxide nanoparticle and its green synthesisNobel laureate Richard P. Feynman introduced the concept of "nanotechnology" in his well-known 1959 lecture "There's Plenty of Room at the Bottom", the area of nanotechnology hasundergone numerous breakthrough advancements. The nanoparticles (NPs) are abroadcategory of materials that include particulate compounds with at least one dimension smallerthan 100 nm. These materials can be 0D, 1D, 2D, or 3D depending on the overall shape.Numerous researchers and scientists have shown a keen interest in the unique properties ofnanoparticles and have found that, although having outstanding applications in manyindustries, many nanoparticle materials have displayed toxicity at the nanoscale size.Currently, a variety of physico-chemical processes are used to make nanoparticles (NPs).

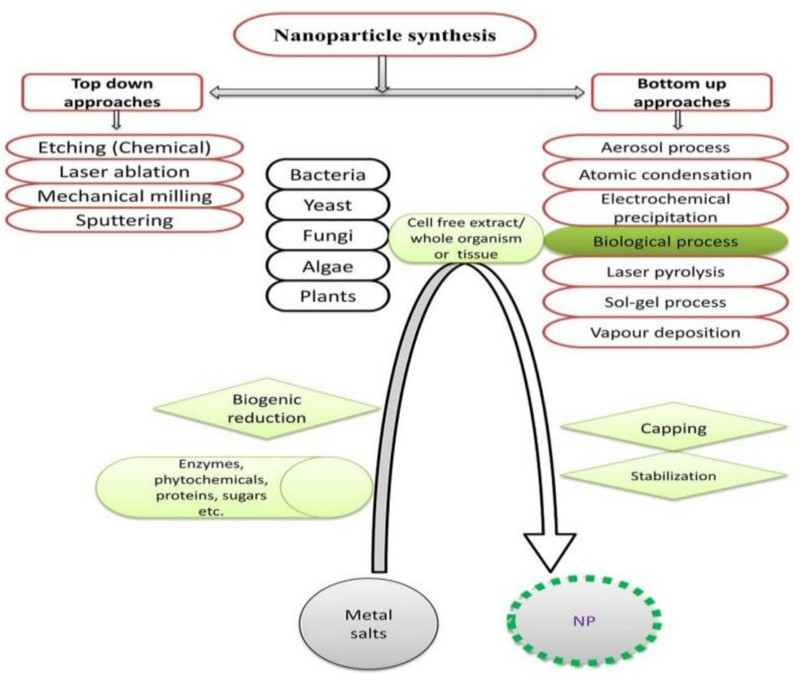

Fig.1: Generalized flow chart of various physico–chemical approaches of nanoparticles synthesis with highlighting of biological synthesis (Hussain et al., 2016).

Antibacterial activity

One of the most serious infectious disorders is bacterial infection. As a result, substantial research has been conducted for more than 50 years to develop new antimicrobial drugs that have been extracted from various sources. Despite advancements in antibacterial agent development, multidrug-resistant bacteria have created a unique demand for novel antibacterial medicines. Since their invention, antibiotics have significantly improved human health and are one of our most effective weapons against bacterial infections. However, in recent decades, these health advantages have come under danger because of the development of drug-resistant bacteria and the fact that many regularly used antibiotics are no longer as efficient at treating certain infections as they once were (Bhalodia et al., 2011). A wide variety of complex and structurally diverse chemicals are available from plants and other sources. The exploration of plant and microbial extracts, essential oils, pure secondary metabolites, and newly synthesized compounds as possible antibacterial agents has recently drawn the attention of many researchers. A compound's ability to block the growth of a microorganisms at its lowest concentration is known as its minimum inhibitory concentration (MIC), which is a common way to define a compound's antimicrobial utility. The minimum inhibitoryconcentrations (MICS) of antimicrobial drugs are determined using dilution procedures, which are also the standard methodology for antimicrobial susceptibility testing against which alternative methods, including disc diffusion, are calibrated.Zinc nanoparticles possess the antibacterial activity. Bacteria are killed by ZnO NPs inseveral ways. The cell wall may be pierced, or DNA may also be attacked. The bacterial cellwall is harmed by the Zn2+ ions produced from ZnO NPs. The bacterial cell membrane'slayer of carbohydrates, proteins, and lipids is also broken down by this process. Bacteria maypotentially perish as a result of the disruption of DNA caused by Zn2+ ions attacking it. Theirattack disrupts DNA, preventing the development of bacteria to cause bacteria to perish. Thematerial's surface area is increased by the nanosize, creating an antibacterial activity hasincreased. Gram-positive bacteria have a thicker cell wall (20–80 nm) and a singlecytoplasmic membrane with many layers of peptidoglycan polymer. In contrast, the wall ofgram-negative bacteria is made up of two cell membranes: an outer membrane and a plasmamembrane with a thin layer of peptidoglycan that is 7-8 nm thick. NPs of a size in theseranges can easily pass through the peptidoglycan and are hence quite vulnerableto harm.

Materials and Methods

1. **Collection of plant material**

The plant Phyllanthus niruri, commonly known as Bhoomi Amla, was obtained from thecampus of the School of Studies in Biotechnology, PRSU, Raipur (C.G.). It was washedthoroughly with tap water and distilled water. After washing, it was kept in a sun-light andhot-air oven for drying. The dried sample was ground into a fine powder and kept in anairtight container for further use.

2. **Microbial culture**

The gram-positive bacteria Staphylococcus aureus and the gram-negative bacteriaEscherichia coli were procured from the School of Studies in Biotechnology, PRSU Raipur.

3. **Preparation of aqueous extract of Phyllanthus niruri**

20g of crushed, dried leaves of Phyllanthus niruri, were mixed with 300 ml of distilledwater. It was boiled for 10 minutes and kept for 1-2 hours in a shaking incubator. Then itwas centrifuged for 10 minutes at 1000 rpm. The supernatant was used for the synthesis ofzinc oxide nanoparticles.

4. **Green synthesis of zinc oxide nanoparticles**

For the biosynthesis of ZnO NPs, an aqueous solution of 0.01M zinc acetate dehydrate with6.0 g of zinc acetate dehydrate salt was added to 100 mL of plant extract, which was thenstirred for two hours at 60°C using a magnetic stirrer. It was centrifuged at 10,000 rpm for10 minutes at 25 °C. The residual pellet was rinsed three times with sterile water and keptfor drying in an oven at 80°C. To remove impurities, the dried material was powdered andheated to 500°C for two hours. The obtained powder was placed in a sealed sample vial,tagged, and kept to be later used for physical characterization and biological applications.

5. **Antibacterial activity of green synthesized zinc nanoparticle**

The antibacterial potential of ZnO NPs was determined against the gram-positive bacteriaStaphylococcus aureus and the gram-negative bacteria Escherichia coli by the agar-welldiffusion method. Nutrient agar plates are prepared and swabbed using a sterile L-shapedglass rod with the broth culture of individual bacterial strains. The well is made by using asterile cork borer; 6-mm wells are created into each Petri plate. Various concentrations of12 ZnO nanoparticles were used to assess the activity of the compound. The antibioticgentamycin was kept as a positive control. Then the plates are incubated at 37 °C for 24 h.After the incubation period, the zone of inhibition of each well was measured, and thevalues were noted.

6. Characterization of zinc nanoparticles

ZnO NPs were characterized by several techniques viz., UV-Vis. spectroscopy and FTIR which provide important information for better understanding of the role of different physicochemical features.

RESULTS AND DISCUSSION

Antibacterial activity of green synthesized ZnO nanoparticles

The results of the antibacterial activity of aqueous extract green synthesized ZnO Nanoparticle against the gram positive and gram-negative bacteria are shown in Table 1 &2.The Results of the study showed that ZnO nanoparticle formed a zone size ranged from0.25mg/ml (15 mm) to 10mg/ml (35 mm) in diameter. The maximum zone of 9 mg/ml (37mm) was exhibited toward Staphylococcus aureus bacteria. It was found that ZnONanoparticle was efficient in inhibiting the growth of the bacterial isolates.Amalorpavamary et al (2019) also used the plant Phyllanthus niruri for the green synthesis ofsilver nanoparticles and studies their antibacterial properties. Gupta et al (2018) worked onthe green synthesis of ZnO by the plant extract of Catheranthus roseus and studied theantibacterial activity on gram positive and negative bacteria. The synthesized ZnO NPs haveshown antibacterial efficacy against both Gram-positive and Gram-negative pathogens.Synergistic effects of ZnO NPs and streptomycin showed increased efficacy as indicated bythe increased zone of clearance in comparison to their individual effects (either ZnO NPs orstreptomycin). Jacob and Rajiv (2019) studied the antibacterial effect of green synthesizednanoparticles with plant extract of Curcuma longa.

Characterization of ZnO NP by FTIR

The use of FTIR is technology is important for analyzing the numerous bonds in preparedmaterial and the chemical purity of the system. The graph displays the FT-IR spectrum ofgreen synthesized ZnO NPs in the range from 4000 to 1000 cm-1. O-H stretching vibration isresponsible for a strong and wide absorption with a centre at 3216.08 cm-1, while H-O-Hbending vibration is responsible for a sharp absorption at 1558.84 cm1. It has beendetermined that the asymmetric and symmetric stretching of a carboxyl (C = O) group is whatcauses the shoulder absorption of the H-O-H bending vibration at 1402 cm-1. The stretchingvibrations of the C-O groups are responsible for the absorption maxima at 1020.5 cm-1.Strong hydroxyl and carboxyl group-corresponding absorptions show that the synthetic(Ramesh et al. 2022;Nandiyanto et al. 2019).

Tables:

Table 1: Antibacterial activity of green synthesized zinc oxide nanoparticles againstgram-positive bacteria.

S.NO.	Concentration of plant extract (mg)	Zone of inhibition(mm)
1.	0.25	15
2.	0.5	22
3.	1	26
4.	2	27
5.	3	31
6.	4	31.5
7.	5	32
8.	6	32
9.	7	35
10.	8	37
11.	9	37
12.	10	35

Table 2: Antibacterial activity of green synthesized zinc oxide nanoparticles against gram-negative bacteria.

S.NO	Concentration of plant extract (mg)	Zone of inhibition (mm)
1.	0.25	13
2.	0.5	13.75
3.	1	15
4.	2	16.5
5.	3	18
6.	4	22
7.	5	22
8.	6	23
9.	7	24
10.	8	27
11.	9	28
12.	10	23

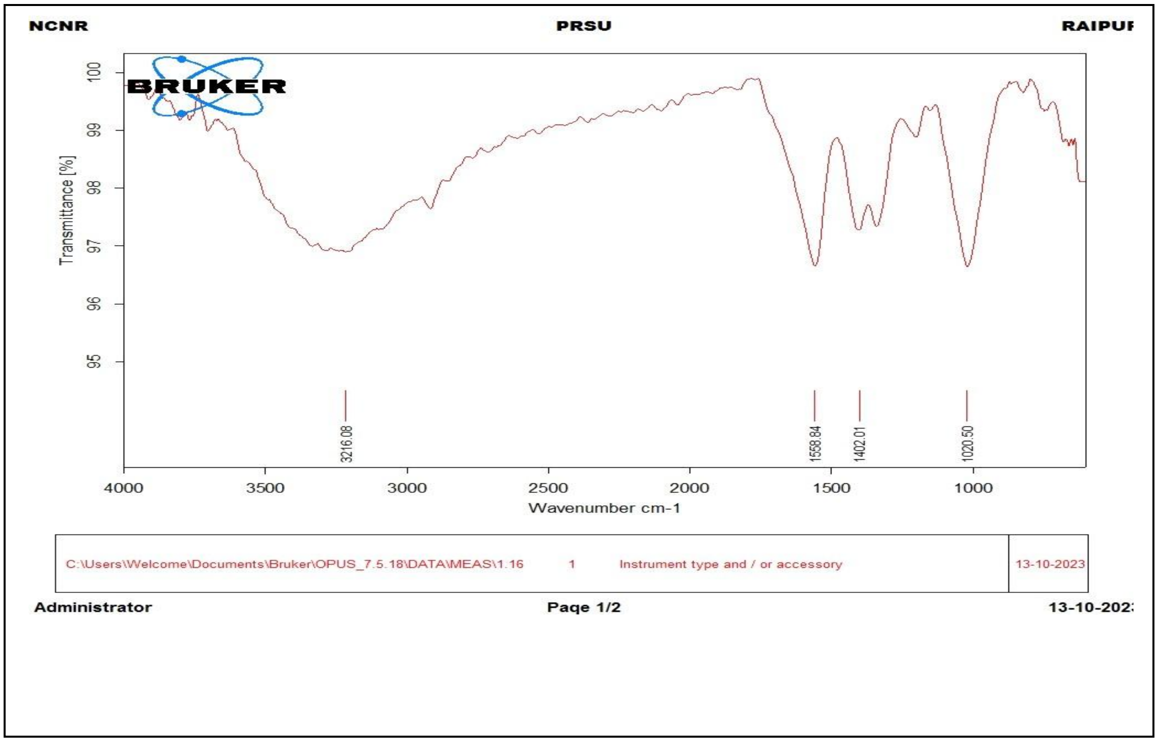

Fig 1: FTIR Characterization of green synthesized zinc oxide nanoparticles

PHOTOGRAPHS:

Fig.2: Phyllanthus niruri

Fig.3: Leaf extract (powder)

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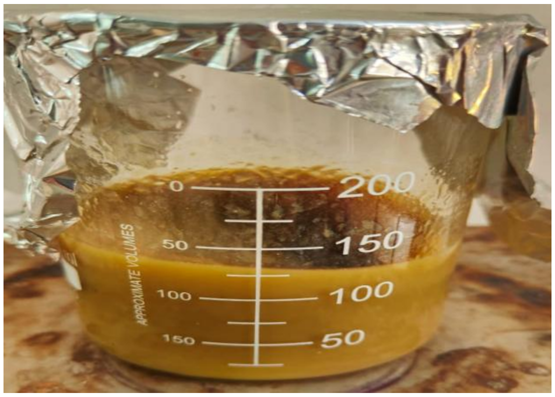

Fig.4: Green-synthesized extract

Fig.5: Positive control

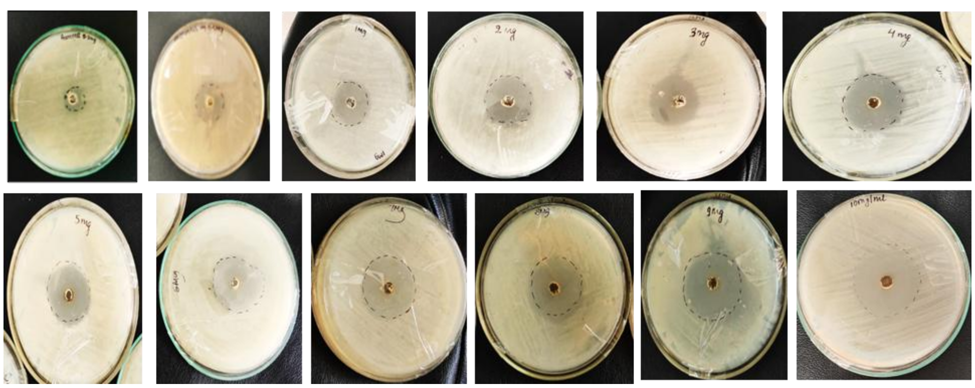

Fig.6: Antibacterial activity of green synthesized zinc oxide nanoparticles (0.3mg/ml-10mg/ml) using gram-positive bacteria (S. aureus)

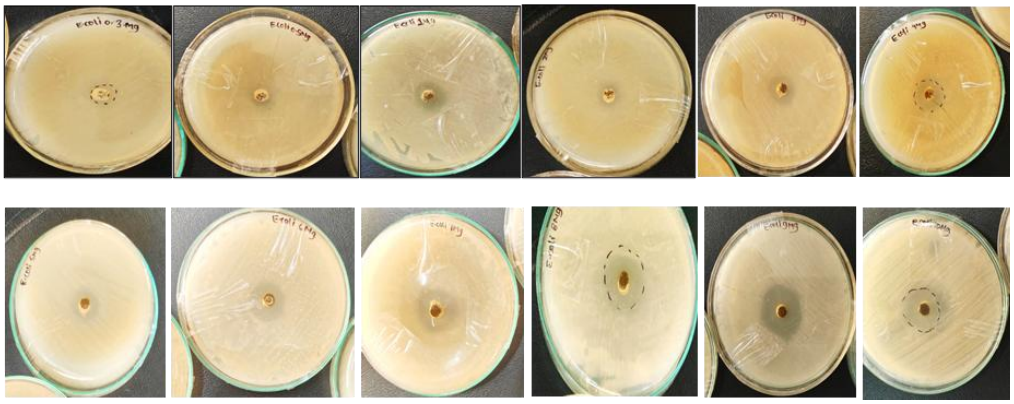

Fig.7: Antibacterial activity of green synthesized zinc oxide nanoparticles (0.3mg/ml-10mg/ml) using gram-negative bacteria (E.coli)

CONCLUSION

In conclusion, the environmentally friendly synthesis of zinc nanoparticles utilizingPhyllanthus niruri plant extract has shown to be a viable path for a variety of uses. Asdemonstrated by their strong antibacterial action against gram-positive and gram-negative bacteria, the produced nanoparticles have the potential to be useful tools inthe fight against a variety of bacterial infections. The combination of the environmentally acceptable greensynthesis method with the intrinsic qualities of Phyllanthus niruri renders thesenanoparticles especially appealing for a range of biomedical and environmentalapplications.

As we look to the future, further exploration and refinement of these green-synthesized zinc nanoparticles may unlock additional nuances in their biologicalactivities. Continued research in this field holds the potential to yield innovativesolutions for addressing challenges in healthcare, environmental sustainability, andbeyond.

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