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Green Synthesis of Zinc Oxide Nanoparticles via Murrayakoenigii Leaf Extract: A Sustainable Approach

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ABSTRACT–
A common medicinal herb, Murrayakoenigii (M. koenigii) is a member of the Rutaceae family. Carbazole alkaloids, which have strong biological and pharmacological effects, are abundant in the leaves, roots, and bark of this plant. These consist of neuroprotective, anti-inflammatory, anticancer, antidiabetic, and antioxidant properties. Due to their special qualities, zinc nanoparticles have found extensive application as gas sensors, semiconductors, magnetic materials, electroluminescent materials, and ingredients in cosmetics.

Keywords: Fourier transform infrared,1, 1 Diphenyl 2- PicrylHydrazyl, ultraviolet

I. INTRODUCTION

The "miracle of modern medicine" is the term used to describe nanomaterials. Because of their optical, magnetic, electrical, and catalytic properties, metal nanoparticles and metal oxides are of great interest for characterization. Using plants to create nanoparticles is a new approach that provides an economical and sustainable substitute for physical and chemical synthesis. Nanoparticles exhibit a greater surface area to volume ratio with reduced particle size, dispersion and shape.

For use in optical devices, sensors, biotechnology, DNA labelling, drug delivery, medical sensors, chemistry and biology, and as catalysts, zinc oxide nanoparticles are presently the subject of extensive research. Because nanosizedZnO is so effective at absorbing UV rays and transmitting visible light, it is employed in paints and sunscreen coatings.

The goal of green nanotechnology is to create nanomaterials and products that don't negatively impact the environment or human health, as well as nanoproducts that address environmental issues. It produces non-toxic nanomaterials and nanoproducts at low temperatures with minimal energy usage and renewable raw materials using conventional green chemistry concepts and environmental technology.

II. MATERIALS AND METHODS

The supplier of zinc nitratehexahydrate (ZnNO3)2.6H2O is Siddhivinayak, Islampur, and the glassware was removed from KIP Karad's laboratory. Every piece of glassware was thoroughly cleaned and dried.

Figure 1 Leaf Extract Preparation

Murrayakoenigii leaves were gathered. The Department of Botany at YashwantraoChavan College of Science in Karad, Maharashtra, India, has identified and authenticated the leaves. After being repeatedly cleaned with water to get rid of

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dust, the leaves are let to dry in a sunny area. The dried leaves are turned into a powder after being crushed coarsely. 20 g of cleaned, dried, and finely crushed leaves were added to a 250 ml beaker with 100 ml of double-distilled water to create the extract that was utilised to convert zinc ions (Zn2) to zinc nanoparticles (ZnO). A magnetic stirrer was then used to boil the mixture for 60 minutes, or until the aqueous solution's colour changed from light yellow. After cooling to room temperature, the extract was filtered using filter paper.

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III. SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING MURRAYAKOENIGII LEAF EXTRACT

Secondary distilled water is used to sterilise the collected leaves after they have been carefully cleaned two or three times under running tap water. Twenty grammes of leaf samples were obtained for synthesis after they were dried at room temperature (32°C). After being weighed, 20 g of leaves were cooked for 20 minutes at 60°C in 100 ml of double-distilled water. A vivid yellow solution forms during cooking and then cools to room temperature. The filter paper was cleaned (Whatmann No. 1) after the yellow extract was filtered, and it was kept in the refrigerator until it was needed again.

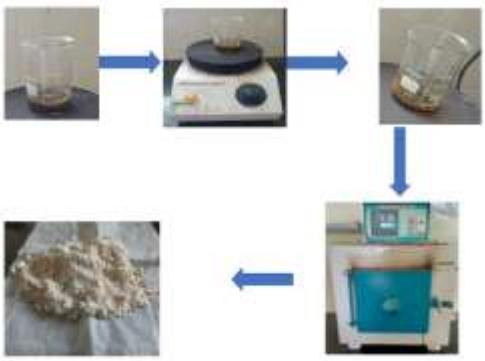

Figure 2 Synthesis of Zinc oxide Nanoparticles

Furthermore, 20 millilitres of Murrayakoenigii leaf extract was extracted from the mother liquor, which was kept in a refrigerator, and cooked at 60 to 80 degrees Celsius. Two grammes of crystals of zinc nitrate hexahydrate (Zn(NO3)2.6H2O) were added to the solution when it had reached 60°C. The mixture should be cooked until it turns into a golden brown paste. After being moved into a ceramic crucible, the paste was heated for two hours at 400° C in a muffle furnace. The white powder that is produced is utilised in optical and structural research.

IV. THE CHARACTERIZATION TECHNIQUES

4.4.1 UV- visible spectroscopy

The absorbance spectra of a substance in solution or as a solid are obtained using ultraviolet-visible spectroscopy. Green's synthesisedZnO nanoparticles' UV-visible spectrum, which spans the 200–900 nm range, was acquired with SHIMADZU UV-1800. An ultraviolet visible spectrometer was used to observe the ZnNPs.

4.5 FTIR

The most popular type of infrared spectroscopy is called "Fourier transform infrared" or FTIR. It measures how much infrared radiation a sample can absorb. The functional groups that are present in the chemical can then be determined using the resulting spectrum.It can be used to determine a compound's identity, purity, and physical and chemical characteristics, as well as to identify novel compounds or validate the identities of known compounds.

4.6 Antioxidant activity

Since antioxidants are used to treat a variety of illnesses, the produced ZnO nanoparticles' antioxidant capacity was investigated using conventional in vitro techniques.

These techniques rely on measurements of the inhibition of free radicals, which differ substantially depending on the radical's production, repeatability, and end point.

4.3.1 DPPH assay

The antioxidant activity of synthesized zinc oxide nanoparticles was tested by using the DPPH assay method.

1, 1 Diphenyl 2- PicrylHydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (HA) can be written as, DPPH-H + (A)◊(DPPH) + (H-A) Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Antioxidant activity in the sample

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compounds was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals. 100µL of test compounds water were taken in the micro titer plate. 100µL of 0.1% methanolicDPPH was added over the samples at different concentration (1000µg/ml) and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490nm Radical scavenging activity was calculated by the following equation:
[(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100

4.2 Antidiabetic activity

In humans, the digestion of starch involves several stages. Initially, partial digestion by the salivary amylase results in the degradation of polymeric substrates into shorter oligomers. Later on in the gut these are further hydrolyzed by pancreatic alpha amylases into maltose, maltotriose and small malto-oligosaccharides. The digestive enzyme (alpha-amylase) is responsible for hydrolyzing dietary starch (maltose), which breaks down into glucose prior to absorption. Inhibition of alpha amylase can lead to reduction in post prandial hyperglycemia in diabetic conditions.

4.5.1 Alpha amylase inhibitory activity

Treatment of diabetes include improvement of the activity of insulin at the objective tissues, with the utilization of sensitizers (biguanides, thiozolidinediones); incitement of endogenous insulin discharge with the utilization of sulfonylureas (glibenclamide, glimepiride), and decrease of the interest in insulin utilizing particular enzyme inhibitors (acarbose, miglitol).

In vitro amylase inhibition was studied by the method of Bernfeld. In brief, 200 µL to 1000µL of the test compound was allowed to react with 500 µL of 0.1M phosphate buffer pH 6.9 containing α-amylase enzyme (fungal diastage (0.5%)) After 10-minute incubation at 250C, 500 µL of 1% starch solution in 0.1M phosphate buffer pH6.8 was added. Again, incubated at 250c for 10 min. The same was performed for the controls where 500 µL of the enzyme was replaced by buffer. After incubation, 1000 µL of dinitrosalicylic acid reagent was added to both control and test. They were kept in boiling water bath for 10 min and cooled. The absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula.

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (extract)} * 100}{\text{Abs 540(control)}}$$

Suitable reagent blank and inhibitor controls were simultaneously carried out.

V. RESULTS

5.1 UV results

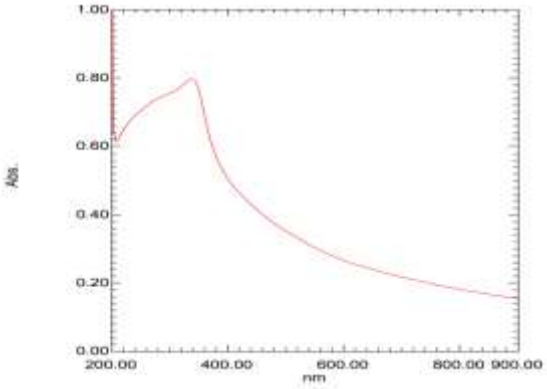

Figure 3 UV–vis spectroscopy of ZnNPsusingMurrayakoenigii leaf extract

The first stage of synthesised nanoparticle production was verified using UV-vis spectroscopy. The 200–900 nm wavelength region is where the ZnO nanoparticles' UV–vis absorption spectra is measured. The UV-visible absorption spectrum of ZnO nanoparticles is depicted in the figure. SynthesisedZnO NPs are shown by the spectrum's peak at 368 nm.TheZnNPs' absorption peaks are located between 360 and 370 nm. Since there were no additional peaks in the UV spectrum, the synthesised product is verified to contain ZnO NPs.

5.2 FTIRresults

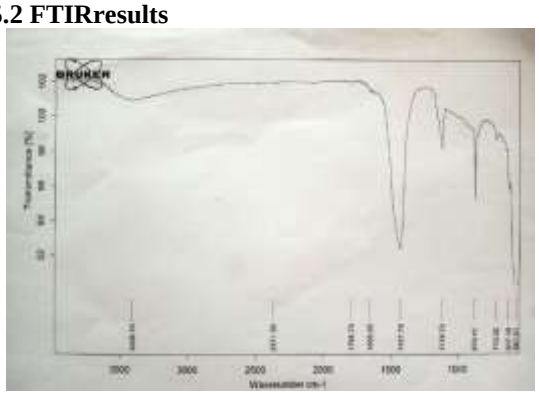

Figure 5 FTIR Results of ZnNPs using Murrayakoenigii leaf extract

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Synthesized zinc nanoparticles were subjected to FT-IR analysis to detect the various characteristic functional group associated with the synthesized nanoparticles. The peaks indicate the characteristics functional group present in the synthesized zinc oxide nanoparticles. It is inferred that the samples have absorption peaks in the range of 3408.76 cm-1, 2371.36 cm^{-1}, 1794.70 cm^{-1}, 1655.05cm^{-1}, 1427.79cm^{-1}, 1118.72 cm^{-1}, 876.40 cm^{-1}, 713.02 cm^{-1}, 617.19 cm^{-1} and561.51 cm^{-1}.

The absorption peak at 561.51 cm^{-1},617 cm^{-1} and 876.40 cm^{-1} corresponds to metal-oxygen (ZnO stretching vibrations) vibration mode. It confirms the presence of Zn-O in the sample. The peak at 1118.72cm^{-1}corresponds to the stretching vibration of the C-N bond of the amine or the stretching vibration of the C-O bond of the secondary alcohol.The absorption peak at 1427.79 cm^{-1} corresponds to C=C stretching. The peak at 1655.5 cm^{-1} and 1794.70cm^{-1} corresponds to the vibration modes of aromatic nitro compounds and alkyl.The presence of C-O is indicated by a peak at 2371.36 cm^{-1}. The peaks at 3408.76 cm^{-1} correspond to the stretching vibration of hydroxyl compounds. [5,6]

5.3 DPPH activity results

		Antioxidant activity by DPPH (96 well method)		
Sample code	Concentration	Absorbance	Mean	% inhibition
Control	-	2.101 1.912 1.813	1.942	
Standard Ascorbic acid	1000 µg	0.150 0.106 0.120	0.125	93.56
Sample - ZN2	1000 µg	0.788 0.750 0.749	0.762	60.76

Table No. 1 DPPH Activity Results

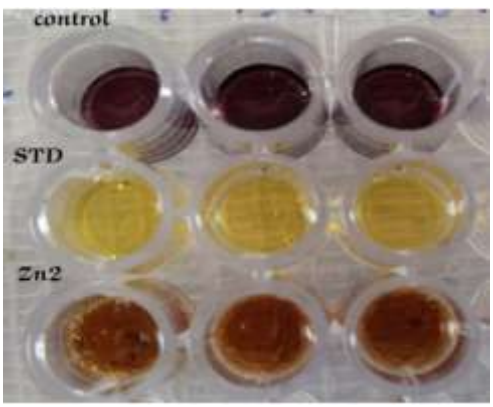

Figure 6 DPPH Activity Testing

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The deep violet hue, which is characterised by an absorption band in ethanol solution with a centre at roughly 517 nm, is similarly caused by the delocalisation of electrons. The reduced form, which loses its violet hue, is created when a DPPH solution is combined with one of a substrate that may donate a hydrogen atom. Sample-ZN2 at a concentration of 1000µg/ml shown good activity when the direct scavenging activity of the samples was assessed against DPPH scavenging assays.

4.7 Alpha amylase inhibitory activity results

		α-amylase enzyme inhibition assay	
Sample code	Concentration	Absorbance at 540nm	% inhibition
Control		1.38	
Standard Acarbose	1mg/ml	0.31	77.53
ZN1	1mg/ml	0.58	57.97

Table No. 2 α-amylase enzyme inhibition assay results

Figure 7 Alpha Amylase Inhibitory Activity Testing

5.5 Molecular docking results

Ligand	Docking Score	RMSD	Receptor Interaction	Distance Angstrom	E (Kcal/mol)
1	-7.3	Lower-0.033 Upper-4.311	PPAR/2GOH	2.30	344.7
1	-5.9	Lower-1.429 Upper-2.125	PPAR/2YFE	2	344.7
1	-6.5	Lower-0.012 Upper-4.311	BRCA1/4Y2G	2.50	344.7
1	-6.3	Lower-0.065 Upper-4.312	PPAR GAMMA-LBD/3B1M	1.60	344.7
1	-6.6	Lower-1.192 Upper-4.315	LRH1/4DOS	2	344.7
1	-6.5	Lower-0.03 Upper-4.311	GluRIIB/4WXI	2	344.7

Table No. 3 Molecular Docking Results

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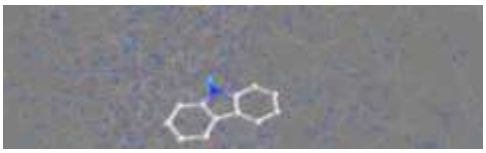
1) 2GOH



2)2YFE

3)4Y2G

4)3B1M

5)4DOS

6)4WXJ

VI. CONCLUSION

This review discusses the synthesis of ZnO nanoparticles, which have various applications in various fields. The green synthesis of zinc nanoparticles using green plants is cost-effective, safe, non-toxic, and eco-friendly. The enhanced bioactivity of ZnO nanoparticles is due to the higher surface area-to-volume ratio. Curry leaf was used for the synthesis, mediated by Zinc nitrate hexahydrate (ZnNO3)2.6H2O. Murrayakoenigi demonstrated excellent temperature-optimization for synthesizing ZnNPs. The NPs were characterized using UV spectroscopy and FTIR, and their anti-diabetic and antioxidant activities were observed. The phenolic compounds and amino acids in Murrayakoenigi plant extracts were found to be more effective than other phytochemical constituents.

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