SYNTHESIS OF NANOPARTICLES AND ANTIHELMENTIC ACTIVITY ON *Ficus dalhousie*

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ABSTRACT :

In this work, zinc oxide nanoparticles were readily synthesized by the aqueous method, and antihelmintic activity was performed by using *ficus dalhousie* leaf extract. The morphology of synthesized ZnO nanoparticles was observed using X-ray diffraction(XRD), FTIR analysis, and scanning electron microscopy (SEM), and their optical properties were characterized using UV-visible spectroscopy. XRD reveals that the prepared ZnO is highly amorphous. FTIR spectra peak at 344.4cm indicated absorption bands ZnO nanoparticles. The U-V-visible spectrum showed 360nm of absorption. The SEM image shows that the ZnO nanoparticles prepared are spherical in shape. The anthelmintic activity evaluated using Indian earthworm showed the best results at 100µg/ml concentration of the ZnONPs using Ficus dalhousie leaf extract.

KEYWORDS: nanoparticles, SEM, XRD, ZnO

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INTRODUCTION:

Nanotechnology is the field of science that deals with the synthesis, manipulation, and use of particles ranging in size from 1 to 100 nm. Such particles are termed nanoparticles. Nanoparticles show unique and significantly modified physical, chemical, and biological properties, as compared to their macroscale counterparts, which makes them of particular interest. Day-by day increasing incidence of microbial challenges, multiple drug resistance (MDR) microorganisms, dietary intake, and serious health hazardous drugs call on new sites for researchers to work on prominent microbial active metabolites with good antioxidant activity to boost the metabolism of an individual and overcome the problem of clinically significant microorganisms including MDR microorganisms. (Jasmeet Kaur etl , 2019)

Recent studies show the significance of green synthesis of metal oxide nanoparticles where oxides of metals like zinc, gold, copper, silver, nickel, etc., are gaining importance. Our point of interest is to synthesize nanoparticles that can be utilized in the realization of optical biosensors, which favors materials with good optical properties like optical absorption, optical emission, photoluminescence, chemical luminescence, etc. Of the various metal oxides, ZnO nanoparticles exhibit electron mobility, large exciton binding energy, wide bandgap, and high optical transmittance. These optical properties of zinc oxide are utilized in sensor fabrication (Singh etl, 2018).

Extensive literature on the *ficus* genus reveals its importance in (Traditional, Ayurvedic, Unani, Siddha, and Homeopathic) systems of medicine.

Zinc oxide has high biocompatibility and fast electric transfer kinetics, such phenomena encourage the use of this material as a biomimic membrane to immobilize and modify the biomolecules. (Sirelkatim A etl, 2019) In many works of literature, it can be learned that nano ZnO offers better performance compared to that bulk size. (Radyum I et al , 2012) Zinc is a necessary element of our health and ZnO nanoparticles also have good biocompatibility with human cells. (Zhang Y et al , 2013) Recently ZnO is listed as generally documented as safe material by FDA(food and drug administration,(USA) (Emamifar A et al, 2010). Ceramic powders like MgO, CaO, $TiO_{2,}$ and ZnO were found to inhibit strongly bacterial growth. (7). Many methods have been used to prepare ZnO nanoparticles like the sol-gel method (Ranvir S. P et al 2009), thermal decomposition, chemical vapor decomposition (CVD), and alloy evaporation - deposition (Tonto P et al , 2008).

Most of the scientific research work and extensive literature has been reported on either the whole plant or different parts (i.e, Roots, Stem, Bark, Leaves, and fruits). Further Other species of *ficus* belonging to the family Moraceae. But Antihelmentic activity has not been reported yet.(Alziro de Amorin et al., 1999)

Hence, in the present investigation, an initiative has been taken to carry out preliminary phytochemical screening and Anti helminthic activity of nanoparticles of *ficus dalhousie* leaves aqueous extract. Moreover, from the phytochemical screening, it is evident that the plant is bestowed with several phytoconstituents like Flavonoids, Tannins Saponins. etc. in the leaf fraction, keeping in view all the above aspects we have made an effort to establish anti-helminthic properties of the extract leaf plant. (Rahman Gul et al , 2017).

Materials and Methods:

Synthesis of zinc oxide nanoparticles and *ficus dalhousie* aqueous extract:

20ml of the plant extract was heated at 50° Cfor 10 min and 50 ml 0f 91mM of zinc acetate solution (1g of zinc acetate was dissolved in 50 ml of distilled water) was added dropwise to it by continuous stirring. The reaction mixture becomes yellowish and a cream-colored precipitate of zinc hydroxide will be formed. The reaction was left for 30 min for the complete reduction of zinc hydroxide. Then the precipitate is collected by centrifugation at 16000rpm for 10 min at 4° C. The precipitate is vacuum dried at 30° C and the sample (PNZ30) was stored for further studies. Two other samples were prepared by heating (PZN30) for 4 hrs at 60° C (PZN60) and 100 C (PZN100) (Jayachandran A et al , 2021).

Anthelmintic activity:

For the antihelmintic activity of leaves of *F.dalhousie*, the animals were divided into five groups containing six earthworms in each group. All the extracts and standard drug solutions were freshly prepared before starting the experiment. Extracts and the standard drug solution of different concentrations were poured into different Petri dishes. All the earthworms were washed in normal saline before they were released into 10ml of respective formulations as follows: Vehicle (5%DMF in normal saline), albendazole (100mg/ 5ml) of *Ficus dalhousie* used as standard. The selected amount of extracts was weighed and taken into test tubes containing normal saline. The extracts were aqueous extract (50& 100mg/ml), Methanol extract (50 &100 mg/ml) .(Kokate C.K et al 2002)

Observations were made for the time taken to paralyze (Paralysis is when the worm did not even have normal saline) and Death(death is when the worms lost their motility followed by their body colors fading away) the results of each petri dish were recorded.(Mohammed Idrees Hussain et al , 2018)

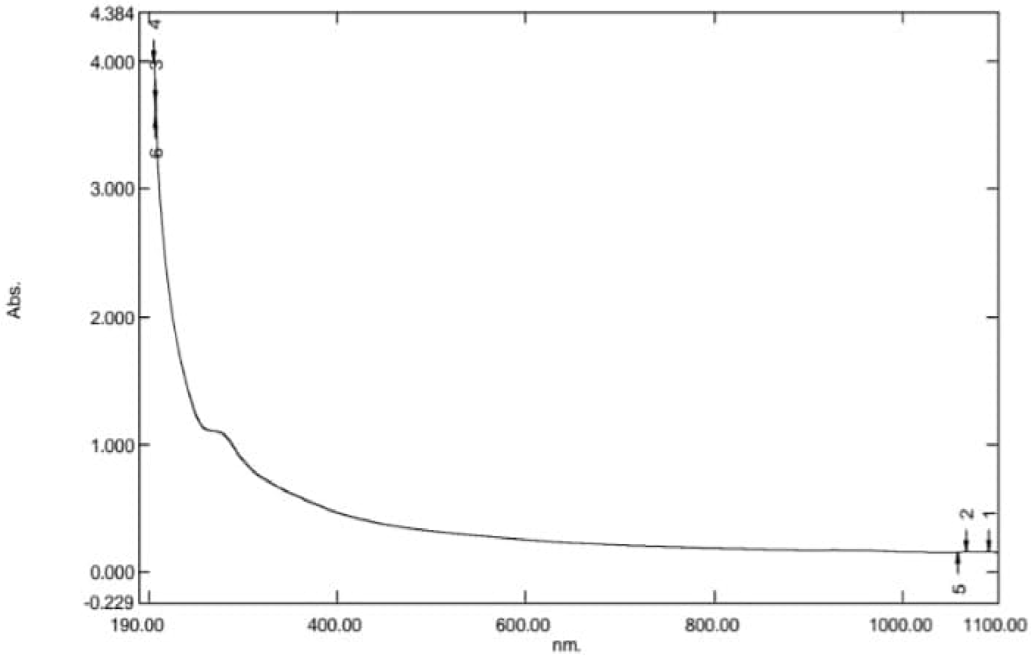
RESULTS AND DISCUSSION:

UV VISIBLE ANALYSIS:

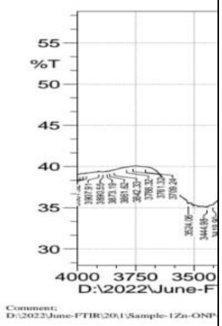
The leaf extract of ficus dalhousie has secondary metabolites like alkaloids, flavonoids, glycosides, phenols, saponins, sterols, etc which are responsible for the formation of ZnO nanoparticles. The UV spectra indicate absorption at nearly 360 indicating the presence of FD ZnoPs. The ZnO nanoparticles show maximum excitation and absorption because of their greater excitation binding at room temperature and which shows that zinc ions are reduced by leaf extract. Visually there is a color change in plant extract after the magnetic stirring. ZnoNP.

FTIR SPECTRA ANALYSIS :

The IR spectrum analysis of the ZnO nanoaggregates was determined at a spectral range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} as illustrated in the Figure below. The detailed analysis of the FTIR spectra reveals the presence of various functional groups and compounds as listed in the table below.



S.no	Frequency	Group	Compound class
1	1548.89	N-O stretching	Nitro compound
2	1535.39	N-O stretching	Nitro compound
3	1629.9	N-H bending	Amine
4	669.32	C-Br stretching	Halo compounds
5	601.81	C-Br stretching	Halo compounds
6	565.16	C-Br stretching	Halo compounds
7	543.94	C-Br stretching	Halo compounds
8	526.58	C-Br stretching	Halo compounds
9	503.44	C-I stretching	Halo compounds
10	3419.9	O-H stretching	Alcohol
11	3444.96	O-H stretching	Alcohol
12	2000.25	C=C=N stretching	ketenimine

 13	2017.61	N=C=S stretching	Isothiocyanate
14	2042.68	N=C=S stretching	Isothiocyanate
15	2065.83	N=C=S stretching	Isothiocyanate
16	2088.98	N=C=S stretching	Isothiocyanate
17	2102.48	N=C=S stretching	Isothiocyanate
18	2114.05	N=C=S stretching	Isothiocyanate
19	2198.92	C=C stretching	Alkyne
20	2216.28	C=C stretching	Alkyne

SEMSPECTRAL ANALYSIS:

The shape and size of the FdZNONPS were analyzed by using SEM. SEM images were seen in different magnifications ranging from 5nm–160 nm which demonstrated the presence of spherical-shaped nanoparticles.

XRD Analysis:

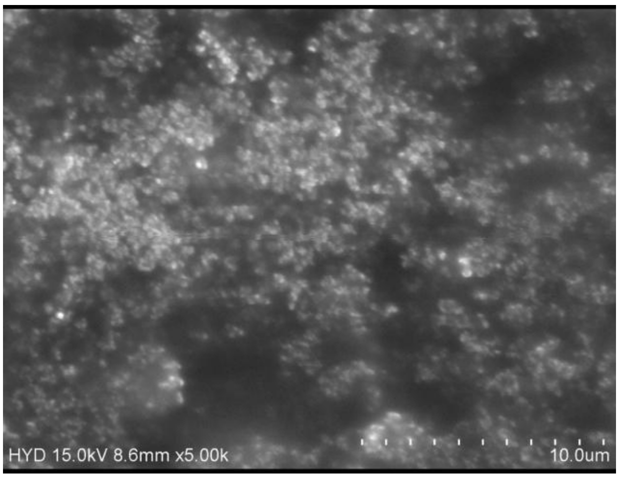
The surface morphology and crystal size can be determined by using an X-ray diffractometer. The observed diffraction pattern confirms the formation of nanoparticles. From XRD data, the Debye-Scherrer equation is applied to determine the particle size.

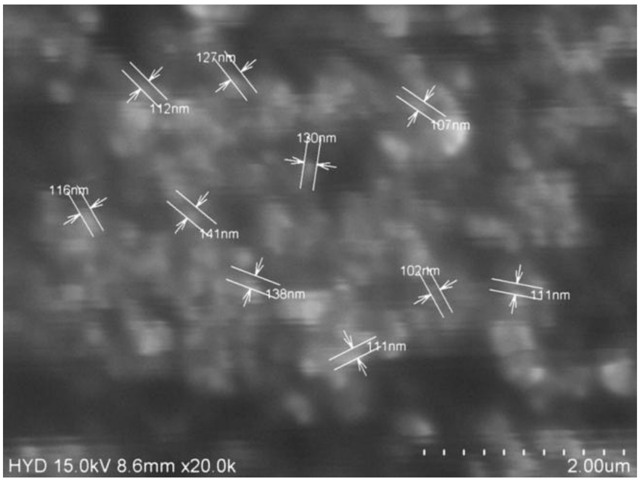
Scherrer equation:

$D=K\lambda/(\beta \cos \theta)$

Where D is the particle size(nm), K is the Scherrer's constant, λ is the wavelength, β is full width half maximum and θ is the diffraction angle.

The presence of nanoparticles and examination of their structural properties were confirmed by XRD. *Ficus dalhousie* associated with ZnONPs showed peaks with 2 θ=19.37,41.35,75.80 with sizes 14.03nm, 12.67nm, and 20.93nm respectively. From the spectra bandwidth, we can suggest the amorphous nature of the nanoparticles.



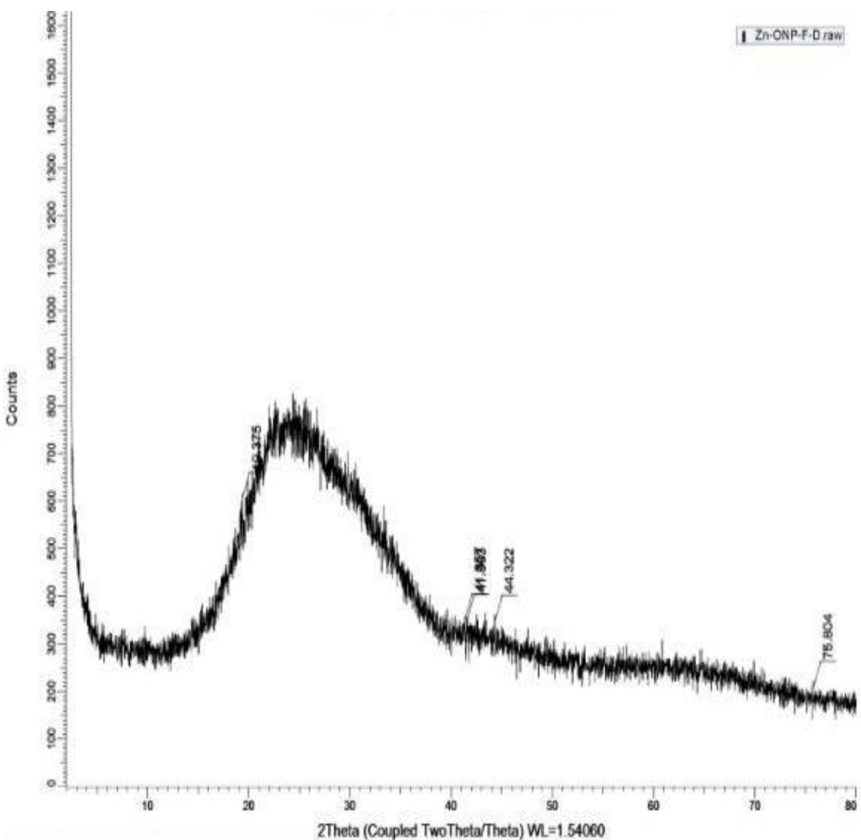


Evaluation of Results of anthelmintic activity:

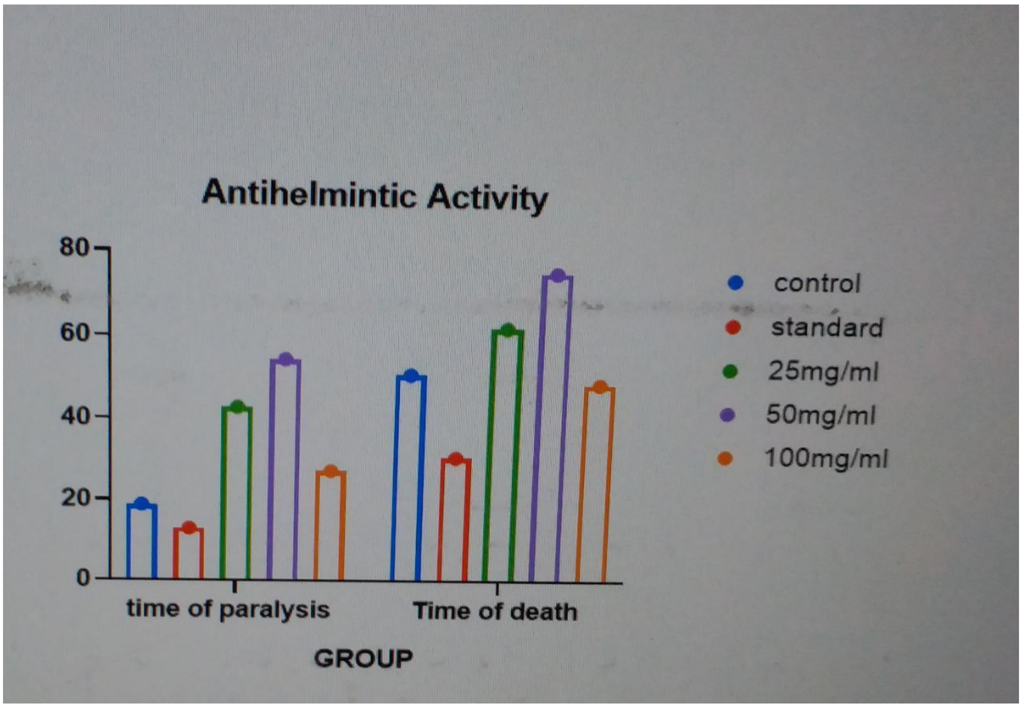
The nanoparticles showed different degrees of anthelmintic activity against *P. Posthuma.* FDZnONPs at,100 µg/ml showed the highest anthelmintic activity compared to all other prepared ZnO NPs when compared with Albendazole which is a familiar medicine for parasitic worm infection. It may be proposed that the ZnONps of Ficus dalhousie may work as potential alternatives in biomedical applications like human Parasite Cleanse. The in-vitro anthelmintic evaluation is shown in the table below. It is concluded that the ROS (reactive oxygen species)mechanism is a suggestive mechanism responsible for the anthelmintic activity

of the ZnO NPs *via* interaction with the cell membrane and consequent damage to DNA and the cell walls. The phytochemicals like alkaloids, flavonoids, polyphenols, and tannins may react with the cell wall of earthworms resulting in precipitation and other reactions resulting in the death of the worms.

s.no	Test/ concentrations(mg/ml)	Time of paralysis (min)	Time of death (min)
1	Control	18.8±3.2	51.3±3.3
2	Standard	13±1.5	31±1.7
3	FDA (25 mg/mL)	43.1±2.9	62.6±3
4	(50 mg/mL)	54.8±2.7	75.8±3.7
5	(100 mg/mL)	27.6±3.6	49.1±4.4



Index	Angle	d Value	Net Intensity	Gross Intensity	Rel. Intensity
1	19.37481	4.577688	84.44659	607.8366	1
2	41.35703	2.181386	51.40521	351.6522	0.60873
3	41.5634	2.171031	55.42752	350.1341	0.656362
4	44.32233	2.042081	46.66125	348.45	0.552553
5	75.80386	1.253924	35.76251	210.9447	0.423493



Statistical analysis :

All the data were expressed as the mean ± SEM. The statistical analysis was conducted with graph pad instant and graph pad prism software. Data were subjected to one-way ANOVA followed by the Dunnett test

Conclusion:

It is evident from the present study that the biogenic synthesis of ZnO nanoparticles is eco-friendly, simple, and efficient. The characterization of ZnO nanoparticles with SEM showed a spherical shape with an average size of 7 to 150nm confirming the ability of ficus dalhousie extract to synthesize the ZnO nanoparticle. FTIR peaks confirmed the presence of chemical bonds that showed the involvement of ficus leaf extract in the production of the nanoparticles. The mechanism by which these ficus leaf extracts are responsible for the synthesis of nanoparticles was not clear. Hence it is justifiable to conclude from the earlier reference that the synthesis is due to the presence of the polyphenolic compound, flavonoids that mediate the synthesis of ZnO nanoparticles. Thus the ZnO nanoparticles produced from Ficus dalhousie are expected to have extensive applications in various industries. The anthelmintic activity evaluated using Indian earthworm showed the best results at 100µg/ml concentration of the ZnONPs using Ficus dalhousie leaf extract.

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