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Biosynthesis of Zinc oxide nanoparticles using *Royal poinciana* leaf extract and evaluating its biomedical potential against biological agents

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ABSTRACT
The field of nanotechnology is concerned with numerous creations and it has various application of materials and it had a nanoscale spatial dimensioning. It possesses higher surface area to volume ratio and have analytical properties as well. Zinc is considered to be the essential element for living organisms. Several chemical and physical methods are used to prepare zinc oxide nanoparticles. By using precipitation method, the Zinc oxide Nanoparticles had been synthesized from zinc nitrate. ZnO NPs were considered to be inexpensive and relatively less toxic and it exhibits excellent biomedical applications, such as larvicidal activity, anticancer, antibacterial, anti-fungal, & anti-diabetic, properties. This study will help to understand the potential biomedical applications and its activity against biological agents. Finally, the UV- spectroscopy analysis was performed to know its concentration of the synthesized nanoparticle. The synthesized Zinc oxide nanoparticles have high biodegradable and biocompatible factor against therapeutic disease and microorganisms

KEYWORDS: *Nanotechnology, Zinc oxide, Biomedical application, Biodegradable, Biocompatible.*

INTRODUCTION

Nanotechnology is considered to be a proven state-of-the art and technology with numerous branches embedded in industrial fields including chemical, pharmaceutical and food processing industries. Nanotechnology also plays crucial role in the areas of computing, optics, drug delivery, and environmental sciences [1]. Nanoparticles possess the efficient characteristics that are supported within particle size, distribution, and morphology [2]. Zinc is one of the important trace elements that are extensively involved in all body tissues, including the muscle, brain, skin, and bone marrow [2]. Zinc oxide nanoparticle is an inorganic metal oxide that would accomplish the demands. Zinc oxide nanoparticles are eventually of low-cost material. Zinc oxide nanoparticles were less toxic in nature and it exhibits excellent biomedical applications, includes anticancer, antiulcer and antibacterial activities [2]. Zinc oxide nanoparticles have specific characteristics like biodegradable, biocompatible, non-toxicity and eco-friendly. Zinc oxide -NPs absorb and disperse light very efficiently, making them excellent materials for optoelectronics applications and that operates in the ultraviolet and visible spectrum region [3]. So, Zinc oxide-NPs seems to be the most promising choices for many purposes. Zinc oxide -NPs are made in a way that it does not cause any harmful effects to the environment, and it can be used to control harmful microbes [3]. There are two approaches that can be used to synthesize NPs, they include top-down approach and the bottom-up approach. The examples of top-down approaches include Electro explosion, etching, sputtering, and mechanical milling, whereas bottom-up approaches comprise of three basic methods used for producing NPs: chemical, physical, and biological processes [3]. It is possible to produce high pure quality of nanoparticles using

conventional methods. The other synthesis process is of green synthesis that involves the use of plants and herbal extracts. It is considered to be safe and secure for medical purposes. For the green synthesis, the different parts of medicinal plants are used to produce the NPs. The phytochemical properties play a crucial role as a biocatalyst, and organic stabilizer for NPs. It does not require high temperatures, pressures, expensive tools, or toxic chemicals. Therefore, the synthesis of nanoparticles using green synthesis is more cost efficient, toxic and hazardous free [3]. This study highlights the synthesis of Zinc oxide NPs by conventional green synthesis method. It mainly focused on potential biomedical applications which includes larvicidal activity, antifungal activity, anti-diabetic activity, anti-bacterial, antioxidant and MTT-anticancer activity of Zinc oxide particles and its activity against the biological agents.

II.METHODOLOGY

Mosquito larvae culture

The eggs of female Anopheles mosquito were collected from rice field stagnant water using the "O"- type brush. And the eggs were brought to the laboratory and transferred to 18 × 13 × 4cm enamel trays containing 500 mL of water for its hatching. They were maintained at 28°C, 75–85 percent relative humidity and 14:10 h light and dark cycles. Larvae were fed a diet of yeast, dog biscuit, and algae collected from ponds in a ratio of 3:1:1, respectively. The feeding was continued until the larvae transformed into pupa stage.

Larvicidal activity

During the experiment, mosquito larvae were taken in five batches along with the control. The larvae death was counted after 24 hrs exposure of Zinc Oxide nanoparticles sample and the percentage of mortality was noted from the average of five replicates. The percentage of mortality was calculated.

Antibacterial activity of Zinc oxide nanoparticles

Petri plates containing 20 ml of nutrient agar medium were seeded in 24 hrs. culture of bacterial strains and were adjusted to 0.5 OD value according to McFarland standard, Staphylococcus aureus wells were cut and concentration of Zinc oxide nanoparticles sample (500, 250, 100 and 50 µg/ml) was added to the well. The plates were allowed to incubate at 37°C for 24 hours. The antibacterial activity assay was performed by measuring the diameter of the inhibition zone that are formed around the wells. For the positive control Gentamicin was used. Finally, the values were calculated using Graph Pad Prism 6.0 software

Antifungal activity of Zinc oxide nanoparticles

Petri plates containing 20ml potato dextrose agar medium was seeded in the 72hr culture of fungal strain (Aspergillus flavus) wells were cut and different concentration of Zinc oxide nanoparticles samples (500, 250,100 and 50 µg/ml) were added. The plates were then allowed to incubate at 37°C for 48-72 hours. The anti-fungal activity assay was monitored by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B (100 units) was used as a positive control. Finally, the values were calculated using Graph Pad Prism 6.0 software.

Antidiabetic activity of Zinc oxide nanoparticles

The α- amylase inhibitory activity was performed by the various concentration. Broadly (500, 250, 100, 50 and 10 µg/ml) of the eucalyptus test sample of Zinc oxide Nanoparticles were again mixed with 200 µl of α-amylase solution (1.0 µg/ml in phosphate buffer pH 6.9), and were allowed to incubate at 25˚C for 30 min. After pre-incubation period the 400 µl of 0.25 % starch solution in the phosphate buffer (pH 6.9) was added to each of the tube to start the reaction. The reaction was taken out at 37˚C for 5 min and terminated by the addition of 1 ml of the DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The test tubes were kept in a boiling water bath for 10 min and cooled to room temperature. The reaction mixture was then diluted by making the volume to 10 ml with distilled water and absorbent (A) was measured at 540nm. Control incubations shows 100% enzyme activity were conducted in the same way by replacing the extracts with buffers. For unknown incubation (to allow for absorbance produced by the extracts), enzyme solution was replaced by the buffers solution and absorbance were recorded. The α-amylase inhibitory activity was expressed as percent inhibition and was noted as follows:

$$\%\text{Inhibition} = \frac{\text{A control} - (\text{A Test OD})}{\text{Control}} \times 100$$

Where A control, A test represent the absorbance of 100% enzyme activity, test sample with the enzyme and test sample without the enzyme, are taken respectively.

Antioxidant activity of Zinc oxide nanoparticles

Broadly, prepare 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of Sample (Zinc oxide nanoparticles +ethanol) at various concentration (500, 250, 100, 50, and 10 µg/ml). The mixtures are shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance (A) were measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as a control). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The potential of scavenging the DPPH radical can be measured by using the following formula.

DPPH scavenging effect (% inhibition) = [(absorbance of control-absorbance reaction mixture)/absorbance of control *100

Anti-cancer activity of Zinc oxide nanoparticles

Cell culture

Hela cell lines were cultured in liquid medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, are maintained under an atmosphere of 5% CO2 at 37˚C

MTT Assay

The Zinc oxide nanoparticles sample was tested for in vitro cytotoxicity, using Hela cells by the MTT assay. Briefly, the cultured Hela cells were collected by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×105 cells/ml cells/well (200 µL) in the 96-well tissue culture plate and treated with various concentrations of the Zinc oxide nanoparticles sample in a serum free RPMI medium. Each and every sample was triplicated and the cells were allowed to incubate at 37°C in a humidified 5% CO2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml) were added into each well and the cells were incubated for 4 h until purple precipitates were clearly seen under an inverted microscope. Finally, the medium together with MTT (220 µL) were washed with 1X PBS (200 µl). For, to dissolve formazan crystals, DMSO (100 µL) were added and the plate were shaken for 5 min. The absorbance for each well were measured at 570 nm using a micro plate reader and the percentage of cell viability and IC50 value were calculated using Graph Pad Prism 8.0 software

RESULTS

Larvicidal activity

Name of the sample	500 µg/ml		250 µg/ml		100 µg/ml		50 µg/ml		Control	
	D	L	D	L	D	L	D	L	D	L
Zn Nps	1	2	0	3	0	3		3	0	3

Table 1. Various concentrations of larvicidal activity

Percentage of mortality

Name of the sample	500 µg / ml	250 µg/ml	100 µg/ml	50 µg/ml	Control
Zn Nps	33.3	0	0	0	0

Table 2. Mortality rate of *Anopheles subpictus* mosquito

Percentage of viability:

Table 3: Viability rate percentage at various concentration

Name of the sample	500 µg/ ml	250 µg/ ml	100 µg/ ml	50 µg/ ml	Control
Zn Nps	66.6	100	100	100	100

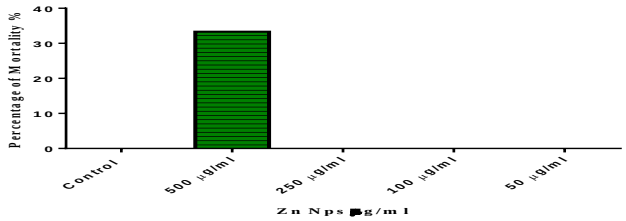

Figure 1. Representation of mortality in concentration 500µg/ml in mortality of mosquito larvae.

Figure 2. Representation of viability percentage

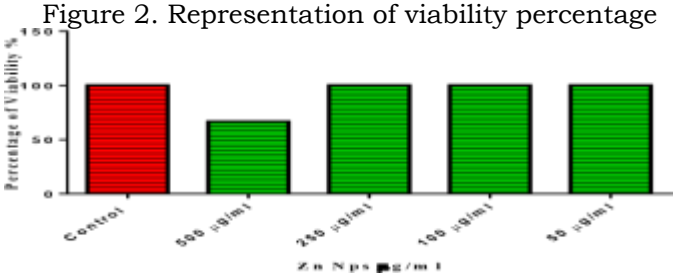

Figure 2. Representation of viability percentage

Antibacterial activity of zinc oxide nanoparticles

Name of the organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
		500 µg/ ml	250 µg/ ml	100 µg/ ml	50 µg/ ml	PC
S. aureus	ZnO Nps	11.5± 0.7	11.5± 3.5	8±0	5±0.35	10± 1.4

SD – Standard Deviation, *Significance - p< 0.05 Table 4. SD± Mean zone of inhibition obtained by synthesized ZnO Nps against *S. aureus*

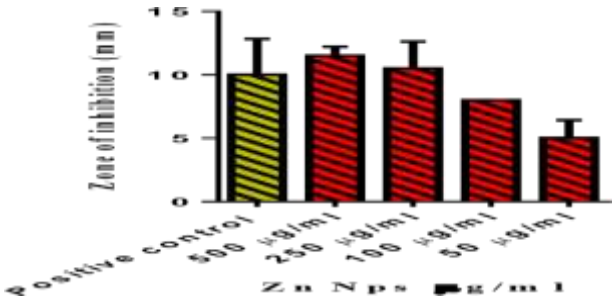

Figure 3. Effect of synthesized ZnO Nps against *S. aureus*.

Antifungal activity of zinc oxide nanoparticles

Name of the organism	**Name of the test sample**	**Zone of inhibition (mm) SD ± mean**				
		500 µg/ml	**250 µg/ml**	**100 µg/ml**	**50 µg/ml**	**PC**
A. flavus	ZnO Nps	5.5±0.7	0	0	0	12±1.4

SD – Standard Deviation, *Significance - p< 0.5

Table 5. SD± Mean of zone of inhibition obtained by synthesized ZnO Nps against *A. flavus*

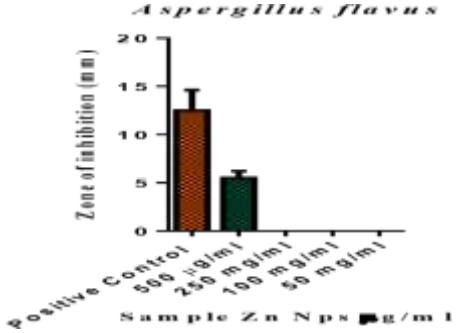

Figure 4. Effect of synthesized ZnO Nps against *A. flavus*.

Anti-diabetic activity of zinc oxide nanoparticles

OD Value at 540 nm

Control Mean OD value: 1.041

S. No	**Concentration of synthesized ZnO Nps (µg/ml)**	**OD Value at 540 nm (In triplicates)**		
1.	Control	0.993	1.107	1.023
2.	500 µg/ml	0.591	0.722	0.734
3.	250 µg/ml	0.805	0.83	0.854
4.	100 µg/ml	1.165	1.257	1
5.	50 µg/ml	1.234	1.044	1.112

6.	10 µg/ml	1.221	0.964	1.186

Table 6. Various concentration of the synthesized ZnO Nps Vs OD value at 540 nm

Percentage of inhibition

S. No	**Concentration of synthesized ZnO Nps (µg/ml)**	**Percentage of inhibition (in triplicates)**			**Mean value** (%)
1.	control	100	100	100	100
2.	500 µg/ml	43.23	30.64	29.49	34.45
3.	250 µg/ml	22.67	20.27	17.96	20.3
4.	100 µg/ml	0	0	3.94	1.31
5.	50 µg/ml	0	0	0	0
6.	10 µg/ml	0	0	0	0

Table 7. Anti-diabetic inhibition of synthesized ZnO Nps

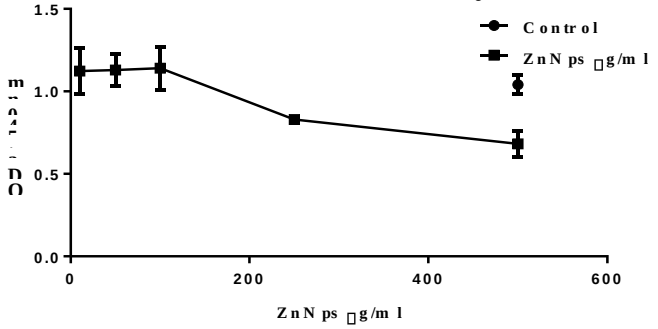

Figure 5. Graphical representation of OD Value at 540 nm

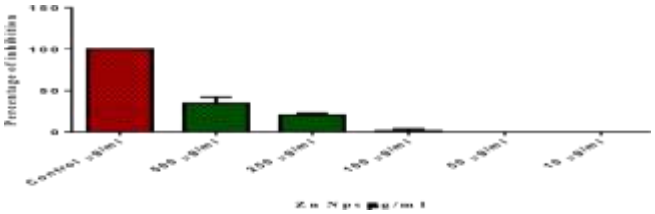

Fig 6: The graph represents the activity of ZnO NPs against anti-diabetic activity

IC50 Value of tested sample: 159.0 µg/ml

Log (inhibitor) vs Normalized response	**Variable slope**
IC50	159.0

Table 8. IC50 value of anti-diabetic activity

Anti-oxidant activity of zinc oxide nanoparticles

OD Value at 517 nm

Control Mean OD value: 0.92

Concentration of synthesized ZnO Nps (µg/ml)	OD Value at 517 nm (in triplicates)			Mean value (%)
Control	0.93	0.90	0.91	0.92
500 µg/ml	0.55	0.62	0.61	0.59
250 µg/ml	0.61	0.67	0.64	0.64
100 µg/ml	0.62	0.78	0.74	1.07
50 µg/ml	0.81	0.76	0.72	0.76
10 µg/ml	0.84	0.82	0.86	0.84
Ascorbic acid	0.56	0.50	0.44	0.50

Table 9. Concentration of synthesized ZnO nanoparticles and its OD value at 517 nm

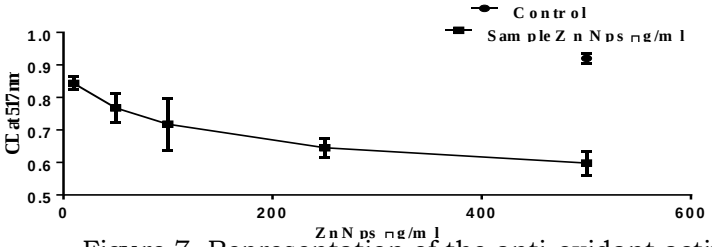

Figure 7. Representation of the anti-oxidant activity of Synthesized Zinc oxide nanoparticles

Percentage of inhibition (%)

S. No	Concentration of synthesized ZnO Nps (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1	Ascorbic acid	39.13	45.5	51.84	45.51
2	500 µg/ml	39.45	31.74	33.69	34.96
3	250 µg/ml	33.15	26.63	29.78	29.85
4	100 µg/ml	31.63	14.78	19.56	21.99
5	50 µg/ml	11.52	17.17	20.97	16.55
6	10 µg/ml	7.82	10.76	6.52	8.37

Table 10: Percentage of inhibition at various concentration of the synthesized ZnO nanoparticles

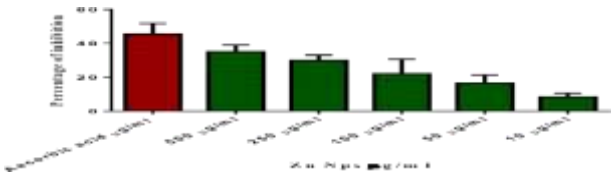

Figure 8. The graph represents the activity of Synthesized Zinc oxide nanoparticles against anti- oxidant activity

IC50 value of tested sample: 62.81 µg/ml

Log (inhibitor) vs Normalized response	Variable slope

IC50	62.81

Table 11. IC50 Value of anti-oxidant activity

Anti-cancer activity of Zinc oxide nanoparticles

OD Value at 570 nm

S. No	**Concentration of synthesized ZnO Nps (µg/ml)**	**OD Value at 570 nm (In triplicates)**		
1.	Control	1.571	1.607	1.458
2.	500µg/ml	0.167	0.171	0.62
3.	400µg/ml	0.226	0.22	0.238
4.	300µg/ml	0.371	0.374	0.38
5.	200µg/ml	0.484	0.466	0.479
6.	100µg/ml	0.549	0.567	0.523
7.	80 µg/ml	0.616	0.571	0.611
8.	60 µg/ml	0.627	0.626	0.63
9.	40µg/ml	0.801	0.78	0.795
10.	20µg/ml	0.891	0.881	0.898
11.	10µg/ml	1.062	0.948	0.997

Table 12. Various concentration of the synthesized ZnO NPs and it OD value at 570 nm

Cell viability percentage

S.NO	**Concentration of synthesized ZnO Nps (µg/ml)**	**Cell viability (%) (in triplicates)**			**Mean Value (%)**
1	Control	100	100	100	100
2	500µg/ml	10.81	11.07	40.13	20.67
3	400µg/ml	14.63	14.24	15.4	14.76
4	300µg/ml	24.01	24.21	24.56	24.27
5	200µg/ml	31.32	30.16	31	30.83
6	100µg/ml	35.53	36.67	33.85	35.36
7	80 µg/ml	39.87	36.96	39.55	38.79
8	60 µg/ml	40.58	40.52	40.77	40.62
9	40µg/ml	51.84	50.48	51.45	51.26
10	20µg/ml	57.67	57.02	58.12	57.6
11	10µg/ml	68.74	61.36	64.53	64.87

Table 13. cell viability percentage at various concentration of synthesized ZnO NPs

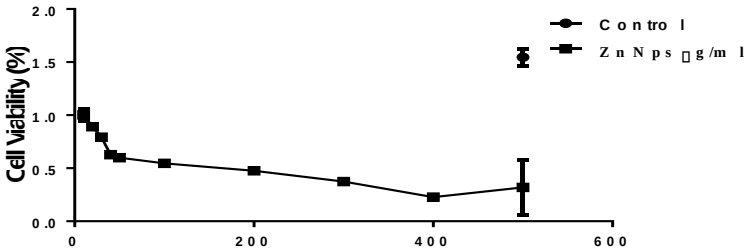

Figure 8. Representation of Cell viability percentage of anti-cancer activity of Synthesized Zinc oxide nanoparticles

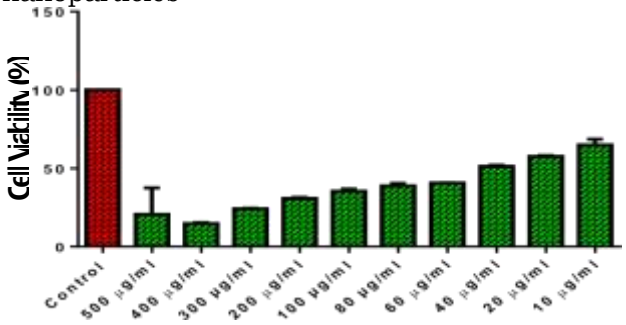

Figure 9. Representation of various concentration and the percentage of cell viability percentage for anti-cancer activity using Synthesized Zinc oxide nanoparticles

IC50 Value of tested sample: 64.13 µg/ml

Log (inhibitor) vs Normalized response	**Variable slope**
IC50	64.13

Table 14. IC50 value of the anti-cancer activity

IV.DISCUSSION

This research gives a detailed description about the synthesis of ZnO Nanoparticles and its various biomedical application including larvicidal activity, anti- fungal, anti-bacterial activity, anti-diabetic activity, anti-oxidant and anti-cancer activity. Using ZnO nanoparticles the larvicidal activity of female *Anopheles* mosquito larvae showed higher mortality rate in the concentration of 500(µg/ml) whereas reported that the *Anopheles stephensi* have a higher mortality rate of 100(µg/ml) using selenium nanoparticles [4]. It was found out that *Anopheles subpictus* have a high mortality in the concentration of 10(µg/ml) using silver nanoparticles [5]. Anti-fungal activity of *A. flavus* was observed by measuring the zone of inhibition where the size of inhibition is 5.5±0.7(mm). It inhibited the growth of fungi with the use of ZnO nanoparticles. Comparing the three fungi *Candida albicans, Fusarium solani* and *Aspergillus Niger* to determine the antifungal activity against the chitosan nanoparticles, proved that the minimal inhibitory concentration of these fungi was analyzed by chitosan nanoparticles. The antibacterial activity of S. *aureus* showed that minimal zone of inhibition against ZnO nanoparticles was 11.5±0.7(mm)[6]. Biosynthesis of silver nanoparticles using *citrus sinensis* peel extract determine the antibacterial activity in E. *coli*, *P. aeruginosa*, and *S. aureus*. Its size was 16.0mm,13.4mm,9.2mm respectively [7]. It results exhibit that the antibacterial activity of *E. coli* and *S. aureus* showed minimal inhibitory concentration in *E. coli* than *S. aureus* when they used different sized silver nanoparticles [8]. Anti-diabetic activity of α- amylase using ZnO nanoparticles showed the percentage of inhibition in the concentration of 500(µg/ml) with acarbose as positive control of IC50 value is 159(µg/ml). The percentage of inhibition of low and high concentration was found to be 34.45% and 1.31%. IC value results of positive control (Acarbose) is 180(µg/ml) in biosynthesized *Pterocarpus marsupium* silver nanoparticles as 700 µg/ml [9]. Anti-oxidant activity of ZnO nanoparticles has the free radical scavenging ability of lowest concentration of (10 µg/ml) was found to be 8.31% and this was increased by 45.5% where its positive control was Ascorbic acid and IC50 value was 62.81. Synthesize of silver nanoparticles using *Elephantopus scaber* leaf extract to determine the scavenging ability of anti-oxidant was found at low concentration of 50 µg/ml and its positive control IC50 value was found to be 126.6±0.06[10]. The Anti-cancer activity of ZnO nanoparticles was performed at different concentration

against the cytotoxic activity of Hela cell lines and its absorbance was measured at 570nm. Its IC50 value was 64.13µg/ml. Anti-cancer activity of silver nanoparticles using a proteinaceous pigment phycocyanin extracted from *Nostoc linckia* exhibited effective cytotoxic activity against MCF-7 cell line [11]. Its inhibitory concentration IC50 value is 27.79± 2.3µg/ ml.

V.CONCLUSION

In this study, Zinc Oxide nanoparticles were synthesized by chemical method. With the help of synthesized nanoparticles, the various experiments has been performed against the biological agents to know its activity and its action against the obtained ZnO nanoparticles. Larvicidal activity helps to predict the mortality and the viability of mosquito larvae (*Anopheles subcutis).* The anti-bacterial activity *(Staphylococcus aureus)* showed the inhibition of bacteria against the ZnO nanoparticles, antifungal activity was performed using fungal species (*Aspergillus flavus)* where ZnO nanoparticles inhibited its growth. Anti-diabetic activity of various concentration of α-amylase were taken to check its action. Anti-oxidant assay used for knowing the scavenging capability of DPPH free radical was calculated by the color change and it is evaluated by UV absorption at 517nm. MTT Assay was performed to check the cell viability at various concentrations and it was determined by UV- spectrophotometer at 570nm. Zinc oxide nanoparticles play a crucial role in the field of environmental science and medical field. Many new researches are required to bring a revolutionary change in the field of Nanotechnology.

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