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Synthesis of Zinc oxide nano-particles using *Anthocephalus cadamba* plant extracts and explores its anti-oxidant, anti-inflammatory and anti-diabetics activity

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Abstract: The scientists have been examining throughout the world to reduce the impacts of hazardous synthetic methods utilized in the nanoparticles formation. The current examination expresses the green methodology for the making of zinc oxide nanoparticles from the aqueous stem extract of *Anthocephalus cadamba*. Stem extract used as the biological reduction agent for the production of zinc oxide nanoparticles from zinc acetate dihydrate. Synthetic conditions optimized to get maximum and narrow size zinc oxide nanoparticles. The formed nanopowder characterized using various analytical techniques, such as UV–Visible spectroscopy, Fourier Transform Infrared spectroscopy, X-ray diffraction and Transmission Electron Microscopy. Nanoparticles tested for their anti-oxidant, anti-inflammatory and anti-diabetics properties.

Keywords: *Anthocephalus cadamba*, zinc oxide nanoparticles, green synthesis, characterization, anti-oxidant, anti-inflammatory and anti-diabetics activity.

1. Introduction:

Anthocephalus cadamba (Family-Rubiaceae) typically called kadamba. It has a blessed position in Ayurveda-an Indian homegrown collection of medication. The height of the tree is 20-40 m and thickness of the trunk around 1.5-2 m with clean tube shaped branches. The tree distributed in the India, Thailand, China, and Malaysia [1]. The bark of the plant accounted to treat the fever and irritation of eyes. The leaves are marginally sweet smelling with unpleasant taste yet the decoction of leaves useful for ulcers, wounds, amenorrhea, diabetics and inflammation [1,2,3]. Nanoscience is emerging area in which the cognizance, use, and control of matter at extents of minute scale, much the same as moving toward atomic levels, with which to make

new substances, instruments, and structures [4]. Nano technology at present utilized as an opportunity to investigate the haziest roads of clinical sciences in a few different ways like imaging [5], sensing [6], directed medication conveyance [7], gene technology and artificial implants [8]. The making of nano particles is in the spotlight in present day of nano field. Nanoparticles synthesized either biologically or chemically. Biosynthesis of nanoparticles by plant extracts is one of the ways in the present synthetic methods [9].The chemical synthetic procedure for the production of nanoparticles is more complicated and causes the hazardous impact on the ecosystem. On the other hand, biosynthesis of nanoparticles is simple, less expensive and more ecofriendly. Therefore, researchers considered the biosynthesis method is suitable for the synthesis of nanoparticles [10]. Zinc oxide (ZnO) one of the best metal oxides used at a nanoscale due to its unique optical, electrical, anti microbial and pharmacological activities [11]. In the present study, the green synthesis of zinc oxide nanoparticles from the *Anthocephalus cadamba* stem extract carried out and characterized by UV-Vis spectra, SEM and FTIR analysis. Nanoparticles tested for their anti-oxidant, anti-inflammatory and anti-diabetics properties.

2. Materials and Methods:

2.1 Materials:

Zinc acetate dihydrate and remaining chemicals obtained from Merck Scientific India Pvt. Ltd., Mumbai.

2.2 Collection of Plant Material:

Fresh plant of *Anthocephalus cadamba* collected in the Godavari river area near Rajahmundry city, Andhra Pradesh, India. The plant stem parts were clean with water absorbent paper (wet filter paper). Then it cut into small pieces and grounded into powder.

2.3 Preparation of Extract:

The plant extract was prepared based on the procedure described by *Behzad Shareghi et al* (2016) [12]. 10 g of plant stem powder taken in 100 ml of distilled water and boiled under continuous stirring using a magnetic stirrer for around 3h. After cooling, the extract was centrifuged twice for 10 min at 4500 rpm and the supernatant was filtered using Whatman filter paper and stored at 4ºC for the green synthesis of ZnO nanoparticles.

2.4 Synthesis of ZnO nanoparticles:

Green synthesis of ZnO nano particles synthesized as per the procedure described by the *Behzad Shareghi et al* (2016) [12] briefly 0.1, 0.01 and 0.001M aqueous solutions of zinc acetate was used as the precursor. The precursor and the plant extract mixed in 9:1 volume ratio were prepared by adding the *Anthocephalus cadamba* extract drop by drop to zinc acetate dihydrate solution, with constant stirring. The colour change of the solution observed at times and the formation of ZnO nano particles determined using UV-Visible spectral studies.

2.5 Characterization of synthesized ZnO nano particles:

2.5.1 UV–Visible spectroscopy:

For UV–Visible spectroscopy, the formed nanopowder dissolved in the sterile demineralised water and spectrum scans using UV–Vis Spectrophotometer Techomp (UV-2301) analytical instrument in the wavelength range of 300–800 nm, and having the path length of 1 cm. The absorption values were analyzed using Hitachi UV solution software version 2.

2.5.2 FT-IR spectroscopy:

Fourier transform infrared (FT-IR) spectroscopy assist to identity different phyto-chemical components caught up in the reduction and stabilization of the nanoparticles. Perkin Elmer FT-IR Spectrophotometer Frontier using the technique of Attenuated Total Reflectance (ATR) in the range of 4000–500 cm^{-1} used to characterize the ZnO nanoparticles.

2.5.3 X- ray diffraction (XRD):

The crystalline nature of the synthesized zinc oxide nano particles studied using XRD study. For the XRD analysis ashed and dried sample of ZnO NPs was used using Bruker-D4 ENDEAVOR (manufacture by Bruker Corporation) at the wavelength of 1.5406 Å. XRD was performed in the 2θ range of 20–80 degrees at 40 kV and 40 mA with a divergence slit of 10 mm in 2θ/θ continuous scanning mode.

2.5.4 Scanning electron microscope:

The morphology and shape of the zinc oxide nanoparticles examined using field emission electron microscopy (LEO 1420 VP Compact variable pressure Digital SEM manufacture by Leo Electron Microscopy Ltd).

2.6 Pharmacological studies:

2.6.1. DPPH Inhibition Activity (anti-oxidant activity):

The scavenging capacity of DPPH evaluated using the procedure of *Mohamed S. Abdel-Aziz et al*. (2013) [13]. Each extract (25-250 µg/ml) in water and ethanol mixed with 1 ml of methanolic solution containing DPPH radicals (0.2 mM). The mixture shaken vigorously and allows settling for 30 min in the dark. Then measure the absorbance at 517 nm against a blank and the results shown in the Table 2. The scavenging capacity measured using the subsequent equation:

$$\% \text{ inhibition} = (A_{blank} - A_{sample}) / A_{blank} \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

2.6.2. Anti-inflammatory activity:

Based on the study of *Happy Agarwal et al* (2019) [14] the anti-inflammatory nature can be examined. 2 mL of 1% Bovine serum albumin (BSA) mixed with 400µL of plant extract in different concentrations (5-35 µg/mL) and the pH of the reaction mixture adjusted to 6.8 with 1N HCl. The reaction fusion was incubate at room temperature for 20 min and then heated to 55 °C for 20 min on the water bath. The mixture was cool to room temperature and the absorbance value recorded at 660 nm and values shown in the Table 4. A BSA mixture with 30% methanol solution used as a control. Diclofenac sodium in different concentrations assumed as the standard. The experiment performed in triplicate.

Percent inhibition calculated using the following formula

$$\% \text{ Inhibition} = \frac{\text{Control O.D.} - \text{Sample O.D.}}{\text{Control O.D.}} \times 100$$

Control O.D = Optical density of control
Sample O.D = Optical density of test sample

2.6.3 α-Amylase inhibition Activity:

According to the procedure of FJ Gella *et al* (1997) [15], the in vitro α-amylase inhibition activity of all extracts was determined based on the spectrophotometric assay using Acarbose as the reference compound. The assays conducted by mixing 80 µL of fixed concentration of

synthesized nano particles, 20 µL of α-amylase solution and 1 mL of 2-Chloro-4-Nitrophenyl-α-D-Maltotrioside. The mixture incubated at 37 °C for 5 min. The absorbance measured at 405 nm spectrophotometrically and it is recorded in the Table 6. Similarly, a control reaction carried out without the plant extract/Acarbose. Percentage inhibition calculated by the expression.

Percentage inhibition = [(Absorbance $_{Control}$- Absorbance $_{Test}$) / Absorbance $_{Control}$] × 100

3. Results and Discussions:

3.1 Synthesis of ZnO nanoparticles:

0.1, 0.01 and 0.001M aqueous solutions of zinc acetate dihydrate used as the precursor. The composition of precursor and the plant extract in 9:1 volume ratio were prepared by adding the *Anthocephalus cadamba* extract drop by drop to zinc acetate solution, with constant stirring. The colour change in the solution measured periodically (**Figure 1**). Based on the yield and time taken for the formation of ZnO nano-particles, 0.01M concentration of metal solution and *Anthocephalus cadamba* as a biological reducing agent selected for further studies. The results are showing in the Table 1.

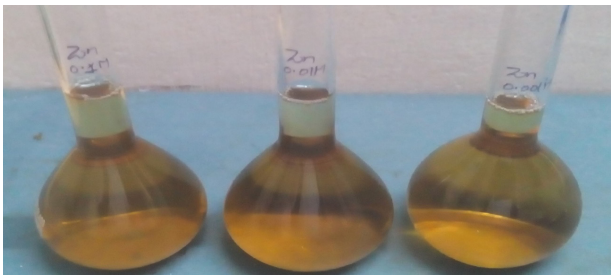


Figure 1: synthesizes of ZnO NPs from the stem extracts of *Anthocephalus cadamba*

Table 1: Yield of Zn Nano particle:

S.No	Dry weight of ZnO NPs obtained after 6 h of time		
	0.1M	**0.01M**	0.001M
1	11mg	**21mg**	8mg

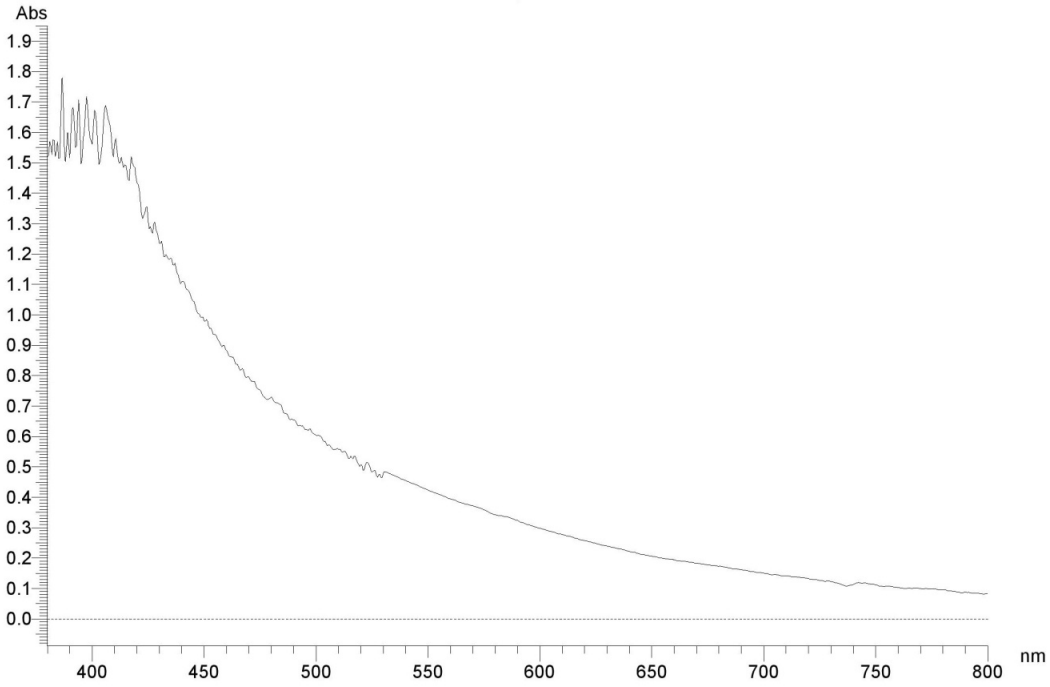


Figure 2: UV-visible scanning spectra of synthesized ZnO NPs

3.2 Characterization of nanoparticles

In the current examination, the *Anthocephalus cadamba* stem extracts used for the reduction of zinc acetate into ZnO nanoparticles determined by the UV-Vis spectroscopy running from 340 to 800 nm. The nano ZnO shows greatest absorption peak at 386.5nm wavelength. The bioreduction of zinc acetate ions in the solution observed by periodical testing of aliquots (0.1 mL) of aqueous component and measuring UV-Vis spectra of the solution. UV-Visible spectra shows no proof of ingestion in the scope of 400–800 nm for the plant extracts and the zinc oxide nano particles show an distinctive absorption peak at around 386.5nm (Figure 2), with the highest absorbance of 1.780. It relates to surface plasmon resonance (SPR) of zinc nanoparticles [13].

FTIR spectra of ZnO NPs (Figure 3) verified an absorption band at 3707 cm^{-1} be evidence for the existence of Amide N-H Stretch or OH group in alcohol. Two medium groups seen at 3426 and 3357 cm^{-1} .The 3707 cm^{-1} affirms essential amine gatherings. Strong bond at 2998 and 2943 cm^{-1} indicates C-H stretching vibrations. Strong bond at 1731 cm^{-1} speaks to C=O stretch vibrations in carbonyls. The 1367 cm^{-1} indicating to C-N stretch in amines in the IR spectra. C=C sweet-smelling stretching was seen at 1464 cm^{-1}. The shift noticed in the FT-IR spectra of ZnO NPs after the bioreduction band demonstrates the support of polyols, terpenoids, and proteins having useful gatherings of amines, alcohols, ketones, and carboxylic acids in bioreduction responses. Terpenoids are ineffectively water-soluble and subsequently may not be among prime moieties engaged with the bioreduction response. Nevertheless, proteins appear to show little significance in the biosynthesis of nanoparticles as announced before [14]. Subsequently, water-soluble phenolic derivatives and flavonoid compounds accepted to assume a significant function in the bioreduction response.

The crystalline size, shape and surface morphology of the ZnO nanoparticles determined by utilizing SEM as appeared in the Figure 4.

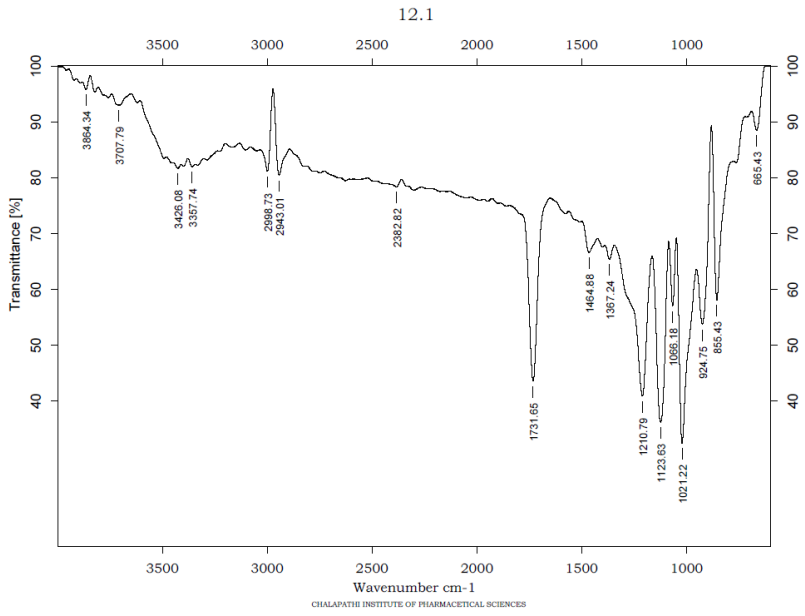
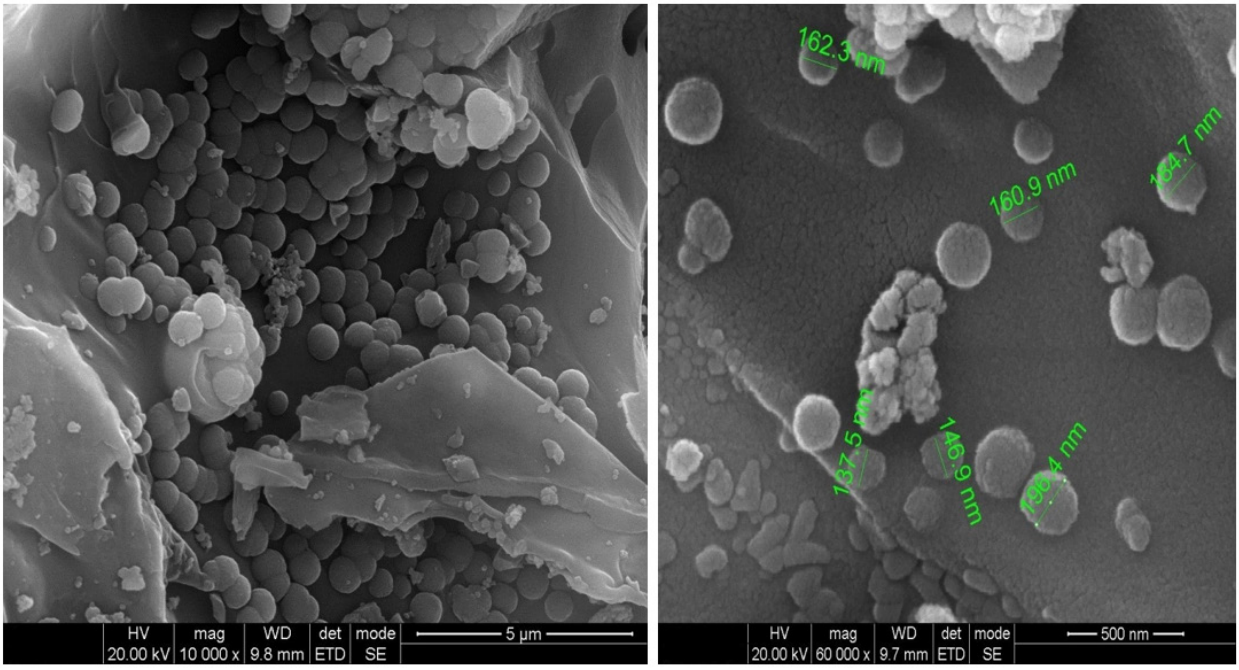
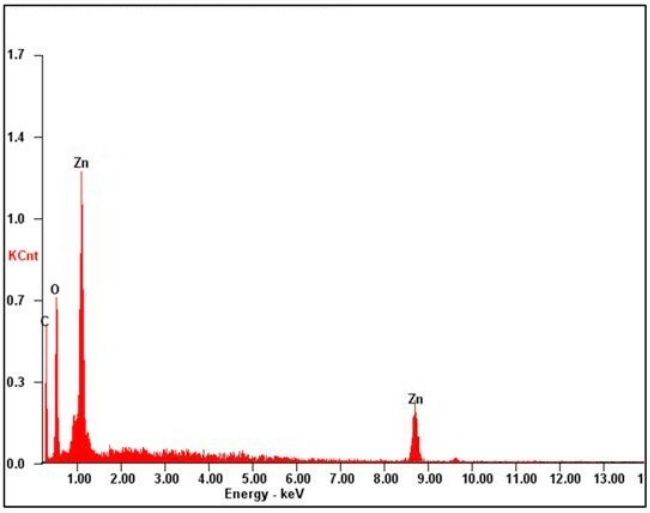


Figure 3: FT-IR spectra of synthesized ZnO NPs



a) SEM image of NPs at 5-µm **b)** SEM image of NPs at 500 nm

Figure 4: SEM images of synthesized ZnO NPs



Element	*Wt%*	*At%*
CK	38.37	53.96
OK	26.34	37.91
ZnK	25.29	8.13
Matrix	Correction	ZAF

Figure 5: EDX results of synthesized ZnO NPs

The micrograph pictures of ZnO nanoparticles demonstrate that they are in nanoscale range and have a uniform appropriation with round shape and less aggregation noticed. The average molecule size discovered to be 167nm. The outcomes portray polycrystalline with permeable nature of Zn-NPs.

The EDX pattern of the ZnO nanoparticles appeared in Figure 5. The C signal observed in EDX spectra is from the carbon film coated on the support formvar. A distinctive signal corresponding to Zn metal was observed in the spectra. No other signals spotted within the exposure confines of EDS, which authenticate the purity of the ZnO nanoparticles. The percentage of Zn content in the crystalline particle found to be 25.29%.

3.3. Study of antioxidant capacity

Antioxidant agents including enzymatic and non-enzymatic substances manage the free radical development. Free radicals causing cell harm including cerebrum harm, atherosclerosis disease. These are forming by reactive oxygen species (ROS, for example, superoxide dismutase, hydrogen peroxide). Biomolecules, for example, proteins, glycoprotein, lipids, unsaturated fats, phenolics, flavonoids, and sugars emphatically controlled the free radical arrangement. The scavenging intensity of enzymatic and non-enzymatic cell is useful for the management of various chronic diseases such as diabetes, cancer, AIDS, nephritis, metabolic disorders and neurodegenerative. In this study, the ZnO nanoparticles anti oxidant nature explore by DPPH inhibition activity test.

3.3.1 DPPH Inhibition Activity

An inspection of Table 2, Table 3 and Figure 6 disclose that the total antioxidant activity measured by DPPH method, ranged from 25 to 250 µg/ml, which is compare to standard ascorbic acid. The results from the antioxidant assay shown that stem extract of plant could scavenge the radical to a certain extent. Therefore, in the current work, *Anthocephalus cadamba* tested for its antioxidant potential and radical scavenging activity by DPPH. The radical scavenging reaction of ascorbic acid with DPPH was effectively instantaneous. The reaction of DPPH with *Anthocephalus cadamba* was also quick however slower contrast with ascorbic acid. The discoloration of methanolic extract of plant samples from purple to yellow observed. DPPH usually used to estimate the free radical scavenging ability of antioxidant due to its low cost [16]

DPPH Inhibition Activity:

Table 2: Concentration Vs Absorbance

S No	Concentration in µg/ml	Absorbance observed		
		Ascorbic acid	Aqueous Plant Extract	ZnO NPs
1	25	0.846	0.904	0.888
2	50	0.803	0.882	0.834
3	75	0.737	0.834	0.747
4	100	0.599	0.753	0.686
5	125	0.407	0.602	0.565
6	150	0.259	0.539	0.363
7	200	0.11	0.413	0.208
8	250	0.034	0.276	0.087

Table 3: % DPPH Activity**:**

S No	Concentration in µg/ml	Absorbance observed		
		Ascorbic acid	Aqueous Plant Extract	ZnO NPs
1	25	11.414	5.3403	7.0157
2	50	15.916	7.644	12.670
3	75	22.827	12.670	21.780
4	100	37.277	21.152	28.167
5	125	57.382	36.963	40.838
6	150	72.879	43.560	61.989
7	200	88.482	56.754	78.220
8	**250**	**96.440**	**71.099**	**90.890**
IC 50 values		**121.11 µg/ml**	**157.87 µg/ml**	**122.62 µg/ml**

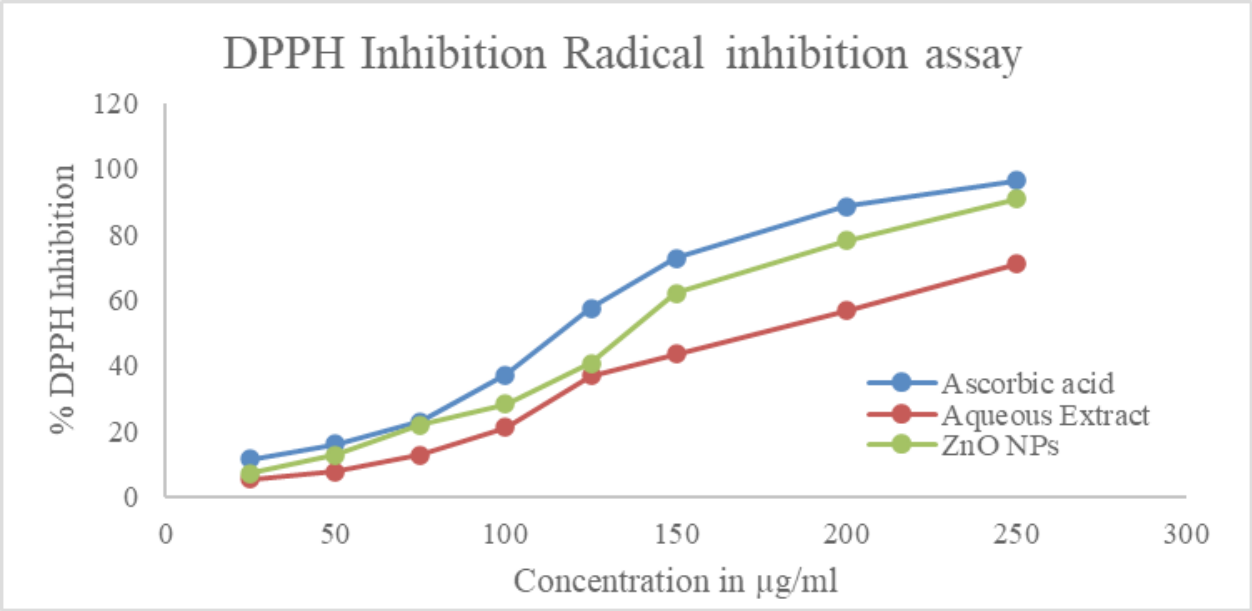


Figure 6: DPPH Inhibition Radical inhibition assay of ZnO NPs and aqueous plant extract

3.4 Anti-inflammatory activity

Protein denaturation is one of the ways for the inflammation the reaction of cells. BSA used as a chemical tool for the assay [17]. The 60% of entire protein content in animal serum composed from BSA alone and usually used in cell culture, predominantly when protein supplementation is obligatory and the supplementary constituents of serum are unwanted. BSA go through denaturation when expose to heat, and states antigens allied with Type III hypersensitive reaction, which are related to diseases such as rheumatoid arthritis, systemic lupus erythematosus, and serum sickness [18]. The results of Table 4, Table 5 and Figure 7 point out that the nanoparticles and plant extracts characterizes dose-dependent inhibition of BSA denaturation. The IC_{50} values of plant extracts and nano ZnO was determined to be **25.04µg/mL** and **21.23µg/mL** respectively. The IC_{50} value attained for the standard drug was **22.07µg/mL.**

Table 4: Concentration Vs Absorbance

S No	Concentration in µg/ml	Absorbance observed		
		Diclofenac sodium	Aqueous Plant Extract	ZnO NPs
1	5	0.821	0.854	0.845
2	10	0.783	0.839	0.831
3	15	0.705	0.824	0.806
4	20	0.528	0.728	0.692
5	25	0.365	0.637	0.558
6	30	0.165	0.556	0.419
7	35	0.090	0.32	0.283

Table 5: Albumin Denaturation Inhibition Assay:

S No	Concentration in µg/ml	% Albumin Denaturation Inhibition		
		Diclofenac sodium	Aqueous Plant Extract	ZnO NPs
1	5	6.278	2.511	3.539
2	10	10.616	4.224	5.137
3	15	19.520	5.936	7.991
4	20	39.726	16.895	21.004
5	25	58.333	27.283	36.301
6	30	81.164	36.530	52.169
7	**35**	**89.726**	**63.470**	**67.694**
IC 50 values		**22.07 µg/mL**	**25.04 µg/mL**	**21.23 µg/mL**

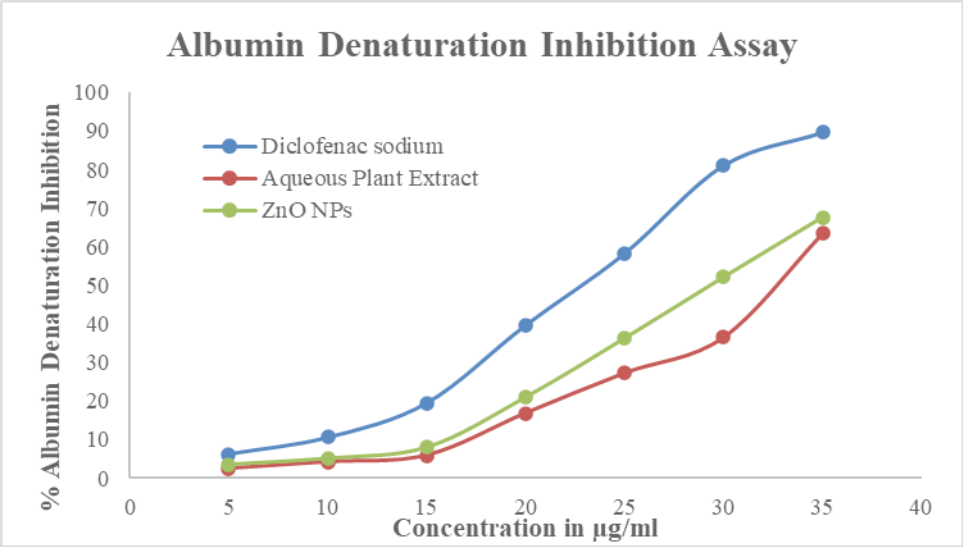


Figure 7: percentage of Albumin Denaturation Inhibition Assay of ZnO NPs and Plant extracts

3.5 α-Amylase inhibition Activity

The absorbance of nanoparticles, plant extracts with different concentrations shown in the table 6. These values compared with absorbance of Acarbose. The absorbance of NPs close to the absorbance of Acarbose. ZnO NPs (at a concentration 400 µg/mL) illustrated 76.777% inhibitory property on the α-amylase activity with an IC_{50} value 283.73 µg/ml described in Table 7 and Figure 8. Several herbal extracts reported to have antidiabetic activities and used in Ayurveda for the treatment of diabetes. It is mainly α-amylase inhibitory components are present in ethanolic extract of S.amaranthoides [19]. Similarly, the synthesized nanoparticles act as powerful therapeutic agent to control diabetes with few side effects.

Table 6: Concentration Vs Absorbance

S No	Concentration in µg/ml	Absorbance observed		
		Acarbose	Aqueous Plant extract	ZnO NPs
1	50	0.706	0.762	0.724
2	100	0.602	0.741	0.68
3	150	0.506	0.705	0.604
4	200	0.423	0.662	0.528
5	250	0.372	0.603	0.471
6	300	0.257	0.528	0.395
7	350	0.179	0.42	0.287
8	400	0.064	0.309	0.183

Table 7: **α-amylase inhibition activity:**

S No	Concentration in µg/ml	α-amylase inhibition activity		
		Acarbose	Aqueous Plant extract	ZnO NPs
1	50	10.406	3.299	8.122
2	100	23.604	5.964	13.706
3	150	35.787	10.533	23.350
4	200	46.320	15.990	32.995
5	250	52.792	23.477	40.228
6	300	67.386	32.995	49.873
7	350	77.284	46.700	63.579
8	**400**	**91.878**	**60.787**	**76.777**

4. Conclusion:

Green synthesis of crystalline ZnO NPs of about 167nm has been achieved using stem extract of *Anthocephalus cadamba*, thus bringing into light yet another use of the plant besides its usual utilities. The water-soluble phenolic acid and flavonoid compounds to play a major role in bioreduction reaction of zinc acetate confirmed by FT-IR. UV spectrum identified peaks were located in the range of the blue-violet spectrum had maximum absorption of 386.5nm. The ZnO

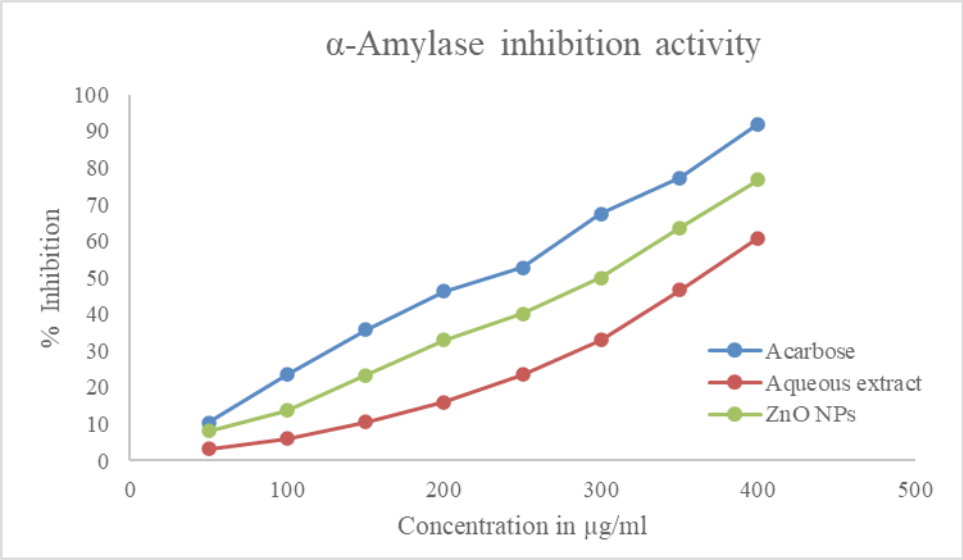


Figure 8: α-Amylase inhibition Activity

NPs show significance anti oxidant activity (DPPH inhibition nature) against the Ascorbic acid. However, the synthesis process for making of nanoparticles stands out eco-friendly and shuts down the demerits of conventional physical and chemical methods.

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