

# Biosynthesis and Characterization of ZnO Nanoparticle using *Cassia auriculata* Flower Extract and their Antibacterial, Photocatalytic Activity.

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**ABSTRACT:** ZnONp synthesized using *Cassia auriculata* flower extract as reducing agent possess medicinal value and pharmacological potential. The ZnONp were characterized by XRD, FTIR, SEM, EDX, DRS analysis. XRD pattern confirms the presence of hexagonal crystalline structure, The optical absorption spectrum were measured by DRS determines the energy band gap of the scattering absorbance is found to have 3.15eV than compared with bulk ZnO type. The FTIR pattern of biosynthesized ZnONp have stretches of C=C, C-O, O-H(COOH), C-Br involved in bioreduction mechanism. Morphology of ZnONp by SEM analysis shows compact arrangement crystallized particle is observed. The confirmatory presence of elemental zinc and oxygen signals were observed by EDX analysis. Antibacterial activity was studied using five different bacterial strain showed using ZnONp has potential antibacterial and inhibitory activity. The energy band gap of ZnONp was found and tested for its photocatalyst property with dye degradation of textile effluent varying time interval under sunlight also been evaluated.

**KEYWORDS:** ZnONp, *Cassia auriculata*, SEM, EDX, FTIR, DRS, Antibacterial, Photocatalyst.

## I. INTRODUCTION

Nanoparticles are nanomaterial commonly used in various application of industries, medicine, optics, cosmetics, sensor environmental etc. nanoparticles have own importance due to vast number of application. Among nanoparticle, metal oxide nanoparticle has diverse properties and functionalities such as catalytic, magnetic, optical and electrical properties. ZnO nanoparticles have been widely studied and reported is one most important material for nanotechnology research used in application of luminescence, cell imaging, antibacterial, drug delivery, optical, piezoelectric, mechanical, thermodynamic, electrodynamic, electromagnetic and gas sensing. ZnO NPs associated with eco friendly nature it had been explored as powerful catalyst for several organic transformations. ZnO has multifunctional properties has high band gap of ~3.8eV and large exciton binding energy of 60meV at temperature. Due to this ZnO is known to be important photocatalyst and its unique advantage of degrading various pollutant. It is inexpensive non-toxic catalyst and has large environmental applications.

Green synthesis nanoparticle has been achieved using environmentally acceptable plant extract and eco friendly reducing and capping agents. Green synthesis of nanoparticles is kind of a bottom up approach where main reaction occurring are reduction or oxidation. plant phytochemicals are usually responsible for reduction of metal compound with antioxidant and reducing properties into the irrespective nanoparticle. *Cassia auriculata* commonly known as Tanners senna. It is widely used in traditional medicine the flower and leaf extract of *cassia auriculata* used for the treatment for rheumatism, conjunctivitis, anthelmintic, astringent and diabetes, ulcers, liver disease and also antidiabetic, hypolipidemic, antioxidant and hepatoprotective, antipyretic activity also been reported. plant and its individual parts can also used for the treatment of toxicity, fungal infection, microbial infection, inflammation and to relieve pain.

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The therapeutic values of plants has enormous traditional uses against various diseases and principle components in various medicine. Various therapeutic values of *Cassia auriculata* was previously reported had significant activity used in Antidiabetic ,hepatoprotective ,Diuretic ,Antiulcer,liver injury,toxicity studies,antioxidant,antibacterial activities.The phytoconstituents of flower includes terpenoids,tannin,flavonoids,saponin,cardiac glycosides and steroids these biomolecules mainly used as reducing agent for synthesis.Hence this study was used to evaluate the efficacy of *Cassia auriculata* for synthesis of ZnO nanoparticle and characterized by,XRD,FT-IR,SEM,EDX,DRS study and also assessing its antimicrobial and photocatalytic activity.

## II. MATERIALS AND METHOD

### 2.1 Materials

All chemicals used were obtained from commercial source and used as received .The glassware was washed and sterilized before use.Zinc nitrate hexahydrate ( $Zn(NO_3)_2 \cdot 6H_2O$ ) was used as precursor.

### 2.2 Collection of Plants :

Fresh flowers were collected from *Cassia auriculata* plant. The flowers were identified and authenticated by Botanical survey of India ,Coimbatore, India. The identified specimen (BSI/RC/5/23/2016/Tech/122) was deposited for preservation in their institution herbarium.

### 2.3 Preparation of Flower Extract

Fresh flowers was collected from *Cassia auriculata* plant. Then washed and rinsed with d.H<sub>2</sub>O after sun dried for few minutes to remove moisture content from it. Dried flowers were taken and weighed (5g) placed in 250ml beaker along with 100ml of distilled water. The mixture was boiled for 60 minutes until the aqueous solution slight watery to yellow .The extract was cooled to room temperature and filtered through whatmann no 1 filter paper. Then extract was refrigerated and stored.

### 2.4 Preparation of ZnO Nanoparticle

For preparation of nanoparticles 50ml of *Cassia auriculata* flower extract was taken and boiled or 60-80° C. when temperature of solution reaches at 60° C , 5g of Zinc nitrate hexahydrate  $Zn(NO_3)_2 \cdot 6H_2O$  was added. The mixture is boiled until it changes to yellow color paste. Then the paste was collected in ceramic crucible and heated at 400° C for 2 hrs. Finally the yellow color powder was grinded with mortar and pestle for fine particle and further used for characterization.

### 2.5 Characterization of ZnO Nanoparticle

The characterization of *Cassia auriculata* and ZnO Nanoparticle was carried out by optical absorbance using DRS spectrophotometry. The reduction of ZnO ions was monitored from 200-800 nm by spectrophotometer. The purity and crystalline size were characterized by X-ray diffractometer. Size and shape of synthesized zinc oxide nanoparticle was examined by SEM analysis. Elemental composition of the particle was obtained from energy dispersive X-Ray diffraction. FT-IR pattern of the synthesized nanoparticle were studied in the scanning range from 4500-500  $cm^{-1}$ .

### 2.6 Antibacterial activity

Method for Antibacterial Activity

Antibacterial activity for ZnO nanoparticle was prepared using agar well diffusion method , cotton swab, nutrient agar ,zinc oxide sample, Tested organism were *E.coli*, *Bacillus Subtilis*, *Klebsiella pneumonia*, *S.aureus*.

Antibacterial activity was determined by agar well diffusion method. Stock cultures were maintained in slant at 4°C. A loopful of colonies were transferred to peptone water till it turn to turbid and incubated for 24 hrs at 37°C. Suspension of bacteria on agar plates were evenly spread using cotton swab. Dimethyl sulphoxide and methanol was used to dissolve the sample. Wells were made on agar surface and different bacterial strain were swabbed on the surface and incubated for 24 hrs at 37°C.

Green synthesized nanoparticle powder was dissolved in various concentration of 40,60,80,100( $\mu g/ml$ ). Along with this 10  $\mu g/ml$  tetracyclin antibiotic was used as positive control and the solvent used to dissolve used as negative

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control. At end of incubation, inhibition zones formed were measured. This study was done in triplicates and the mean value were presented.

### 2.7 Photocatalytic Degradation of Dye

The photocatalytic activity was studied by degradation of textile effluent dye by solar radiation. The dye solution was prepared by diluting 3ml of raw effluent in 100ml of water to make 3% concentration . A known amt of 10mg ZnO nanoparticle was added to dye solution and stirred magnetically for 30 minutes before exposing to sunlight. At every 30 min ,5 ml of the solution was collected and scanned at different wavelength from 200-800nm by using UV spectroscopy to study dye degradation of ZnO nanoparticle. The  $\lambda_{max}$  of textile effluent was found to be 420nm. Further initial study was done to determine the characteristics of raw effluent dye. These experiment was conducted between 10 am to 12pm based on the study the decolourization results obtained from photocatalytic study.

**Table 1** :Characteristics of raw wastewater from textile industry

S.No.	Parameter	Value
1.	pH	7.8
2.	Temperature	32°C
3.	COD	2000ppm
4.	Odour	unpleasant
5.	Colour	purple

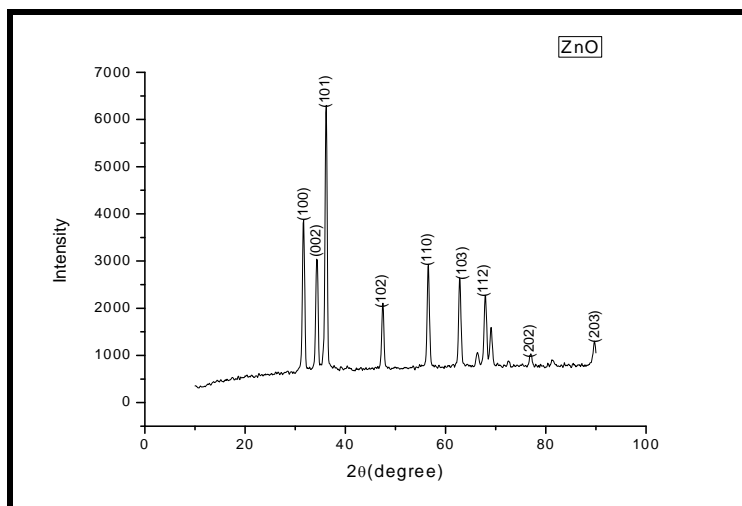
## III.RESULT AND DISCUSSION

### 3.1 X-ray diffraction analysis

XRD pattern for the synthesized from *Cassia auriculata* flower extract ZnO nanoparticle is shown in fig 1.XRD pattern confirms presence of hexagonal wurtzite crystalline structure and mean crystallize size is calculated using Debye-Scherrer formula

$$D = \frac{K\lambda}{\beta \cos\theta} \quad \dots\dots(1)$$

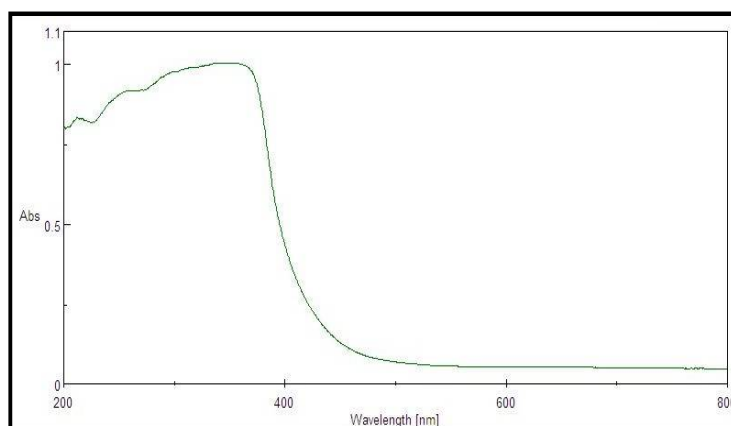
Where  $\beta$  is the full-width at half-maximum (FWHM) value of XRD diffraction lines the wavelength  $\lambda = 0.154056$  nm and  $\theta$  is the half diffraction angle of  $2\theta$ . The particle size is determined by taking the average of the sizes at the peaks and it was found to be average from 30 nm to 60nm. The average grain size that is obtained using this formula is 34.5 nm.The diffraction peaks at  $2\theta$  of 31.63,34.29,36.12,47.44,56.51,67.88,69.02,81.28,89.62 were assigned to (100),(002),(101),(102),(110),(103),(112),(202),(203) of ZnONp ,(hkl) plane values.The peak values confirmed the hexagonal wurtzite structure with data JCPDS card no :01-075-0576.The lattice parameter are  $a=3.253\text{\AA}$ , $c=5.213\text{\AA}$ , with respective diffraction peaks.



**Fig .1.** XRD pattern of ZnO Nps.

### 3.2 DRS(Diffused Reflectance Spectroscopy)

UV-Vis optical absorption spectrum of ZnONp shows absorbance at 393nm .The optical absorbance of ZnO nanoparticle were measured by diffuse reflectance spectroscopy at room temperature.The reflectance peak showed wavelength absorbance from 345 to 400nm.The band gap ( $E_g$ ) of ZnO nanoparticle were calculated by using  $E_g = hc/\lambda$ .  $h$ =plancks constant  $c$  = velocity of light and  $\lambda$ = wavelength.The band gap of corresponding ZnO nanoparticle was found to be 3.2 ev.



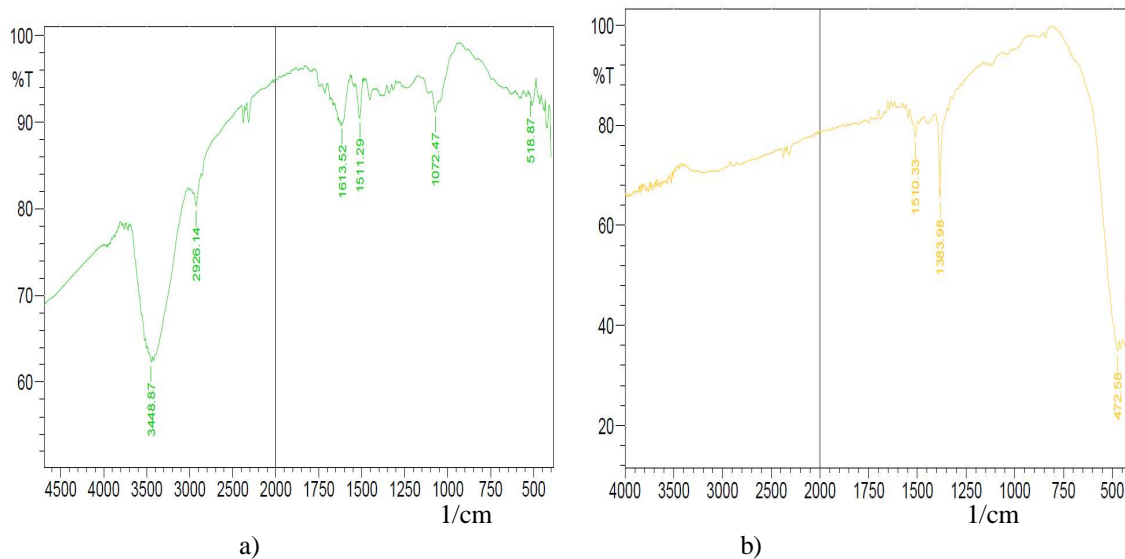
**Fig 2.**UV optical absorbance of biosynthesized ZnO Nps .

### 3.3 Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR pattern of the synthesized nanoparticle were studied(fig 3)it ranges from  $500-4000\text{cm}^{-1}$ .fig 3(a) and 3(b) shown are synthesized nanoparticle and flower extract of *Cassia auriculata* .The possible biomolecules responsible for capping and reduction of ZnO nanoparticle of particular bond vibrational peaks are identified .The absorption peak found around  $3446.87, 2926.14, 1613.62, 1511.29, 1072.47, 518.87\text{cm}^{-1}$  in flower extract of *C.auriculata* and the synthesized ZnONp peaks were  $1510.33, 1303.98, 472.58\text{cm}^{-1}$ .The stretches of ZnO nanoparticle were found around  $472.5\text{cm}^{-1}$  with metal oxygen.Therefore synthesized nanoparticle were surrounded by ( $1511.29, 1613.62, 1510.33\text{cm}^{-1}$ ) C=C

aromatic ring,(1072.47,1303.98  $\text{cm}^{-1}$ ) C-O stretch,(3446.87,2926.14  $\text{cm}^{-1}$ ),O-H(COOH) stretch, (518.87  $\text{cm}^{-1}$ ) C-Br stretch functional group.

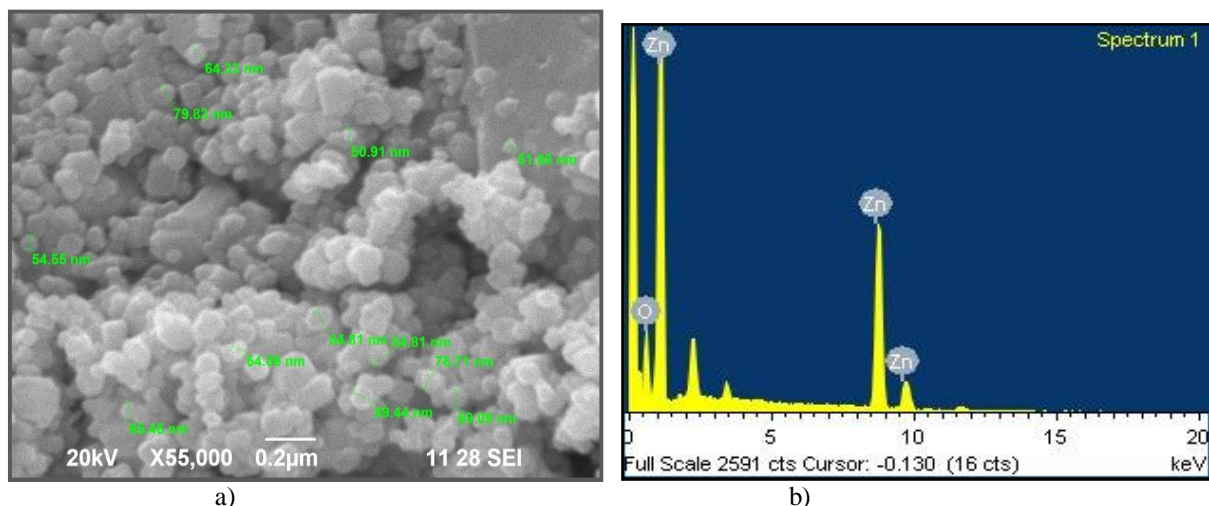
FTIR spectrum indicates carboxyl,alcohols,aromatic and alkyl halide involved in bioreduction mechanism.



**Fig .3.** FTIR Spectra of (a) Flower extract of *Cassia auriculata* (b) Green synthesized ZnO Nps.

### 3.4 SEM WITH EDX ANALYSIS

The size,shape,surface morphology of ZnO nanoparticle were clearly indicated in SEM analysis shown in (fig 4a).ZnO nanoparticle shows compact arrangement uniform particle with regular and well crystallized particles with size around 50-80nm can be observed. The fine particle are aggregate of very small crystallites of spherical shape.Energy dispersive X-ray spectrophotometer analysis is confirmatory presence of elemental zinc and oxygen signals of ZnO NPs.The weight and atomic percentage of Zinc ,oxygen was found to be 52.43,47.57 and 78.76,21.24 these corresponds peaks without impurities to the optical absorption produced by the nanoparticle.



**Fig .4.**(a) Morphology of biosynthesized ZnO Nps by SEM (b)EDX Spectrum.

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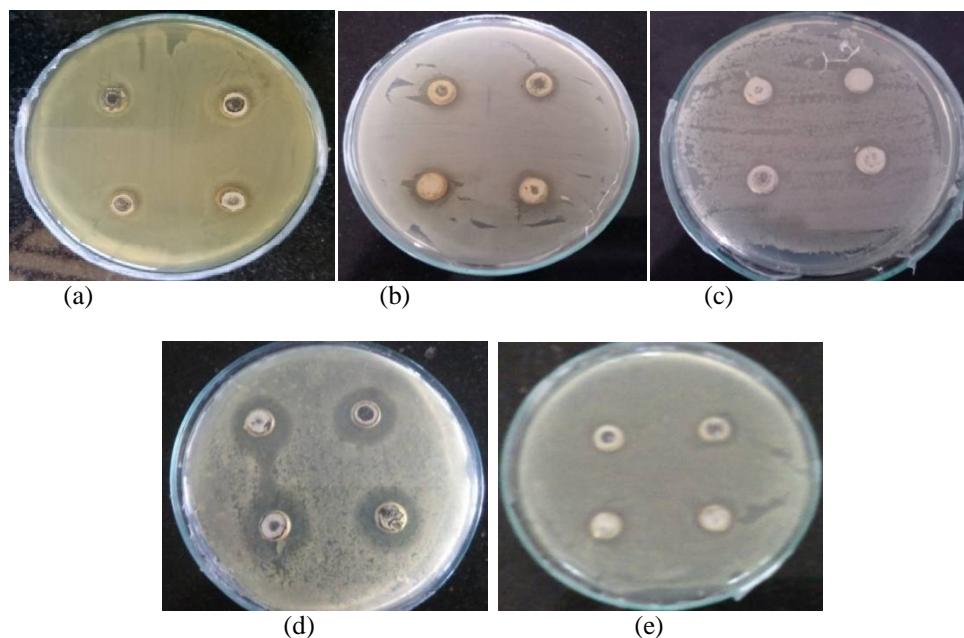
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### 3.5 ANTIBACTERIAL ACTIVITY

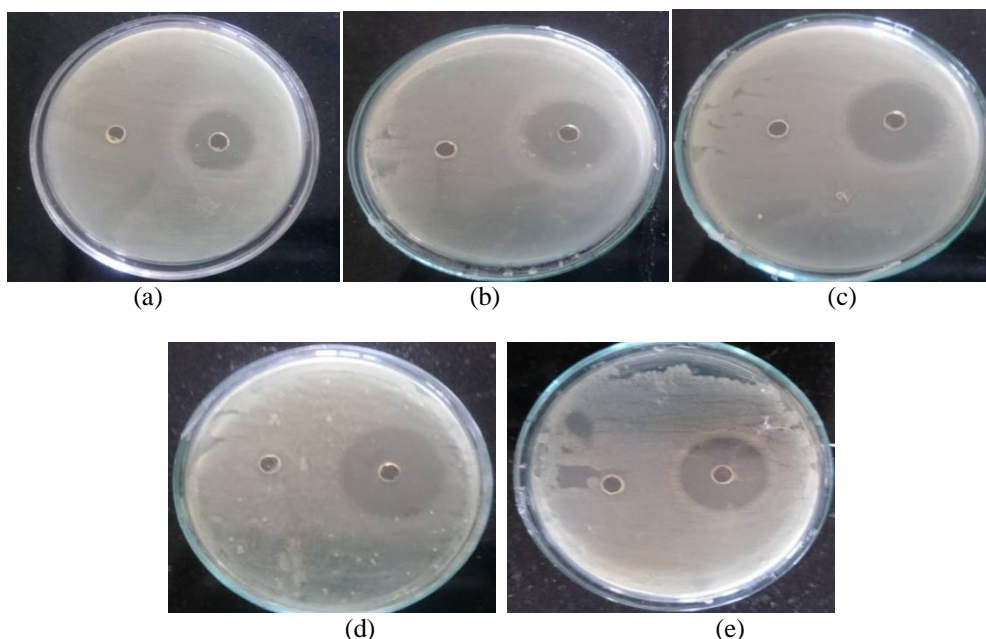
The antibacterial activity was monitored by agar well diffusion method with zone of inhibition measured in mm **Table 2**. Antibacterial activity was checked against five different bacterial strain

MICRO-ORGANISM	Zone of inhibition (mm)		40ug/ml	60ug/ml	80ug/ml	100ug/ml
	DMSO:M (20 ul)	Tetracyclin (10ug/ml)				
<i>E.coli</i>	nil	12	9	10	11	13
<i>S.aureus</i>	nil	19	12	15	17	19
<i>Klebsiella pneumoniae</i>	nil	15	10	12	14	16
<i>Bacillus substilis</i>	nil	16	11	13	15	17
<i>Salmonella typii</i>	nil	13	10	11	12	13

The study revealed that biosynthesized ZnONp has potential inhibitory action. The highest inhibitory activity was seen in *S.aureus* and *Bacillus substilis*, The activity increased concentration increases further inhibitory effect shown to be active component over wide range of organism.



**Fig.5.** Antibacterial activity of ZnO Nps against a) *E.coli* b) *Bacillus substilis* c) *Klebsiella pneumoniae* d) *S.aureus* e) *Salmonella typii*. Varying concentration of 40ug/ml,60ug/ml,80ug/ml,100ug/ml.



**Fig.6.**Antibacterial activity of ZnO Nps a) *E.coli* b) *Bacillus subtilis* c) *Klebsiella pneumoniae* d) *S.aureus* e) *Salmonella typhi* with positive control of 10 ug/ml of tetracycline and ZnO Nps along 10ug/ml negative control.

### 3.6 PHOTOCATLYTIC EFFECT :

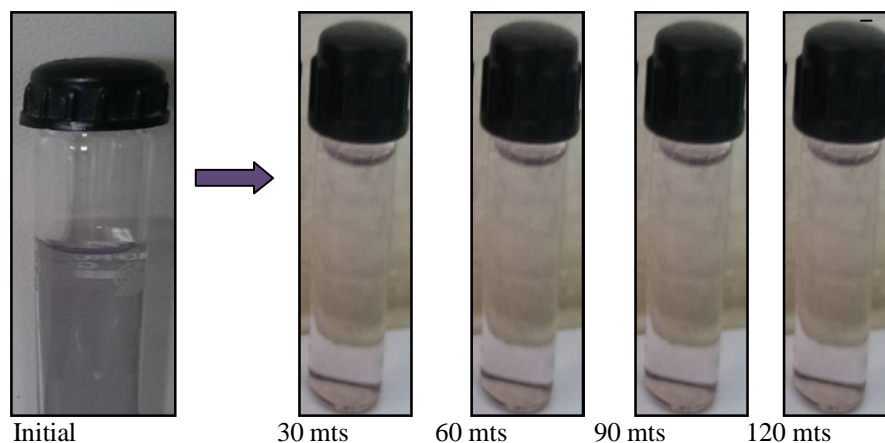
Photocatalytic activity for the synthesized ZnO nanoparticle was observed under solar irradiation .The presence of peak absorption was observed at 420nm for 2 hrs ,decolourization enhanced dye degradation.The absorbance for photocatalytic activity was observed under UV-Spectrophotometer.The decrease in absorbance from initial dye concentration and biosynthesized ZnO Np photodegradation effienicy of decolourization was noted.

The degradation of the synthesized nanoparticle is used to study the effect of photocatalytic activity and decrease in absorbance of peak intensity was calculated using the following equation with varing time period .

The percentage decolourization was calculated using this formula

$$\text{The percentage degradation (\%)} = \frac{(C_0 - C_t)}{C_0} \times 100$$

Where  $C_0$  is initial concentration of the solution,  $C_t$  concentration of dye solution at time interval 't'.



**Fig. 7.**Degradation of dye under sunlight from biosynthesized ZnONp.

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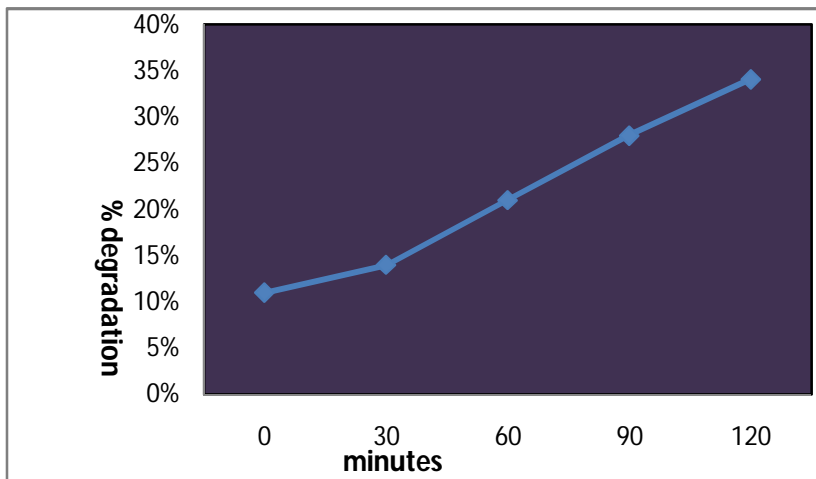


Fig .8.% Degradation of dye with time ‘t’ using biosynthesized ZnONp.

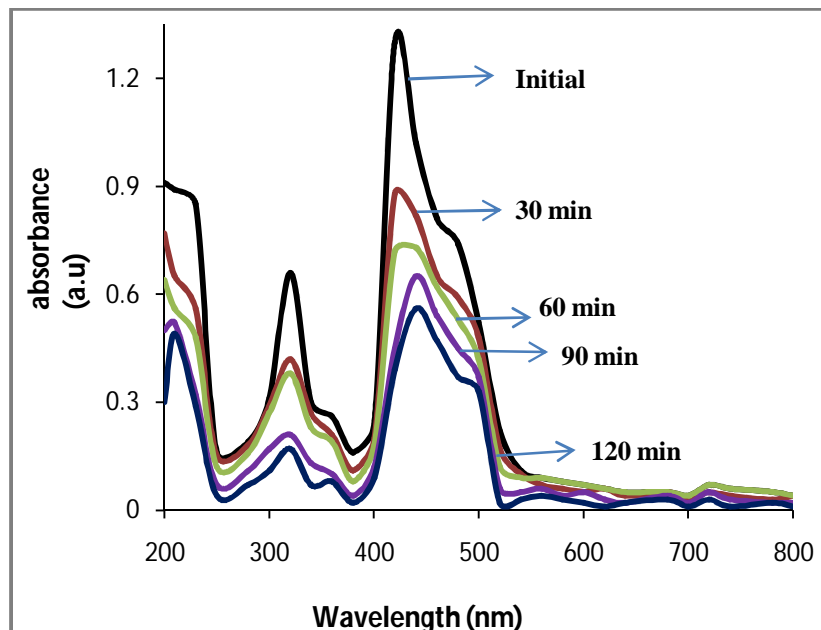


Fig. 9.Photocatalytic degradation using ZnO nanoparticle from c.auriculata.

## IV. CONCLUSION

The synthesized metal oxide nanoparticle is cost effective and ecofriendly method. Synthesized ZnONp from *Cassia auriculata* from flower extract have definite hexagonal shape around 50-80nm in size and optical absorbance of band gap of bulk ZnO nanoparticle to determine photocatalysis is dependent on light intensity. Under sunlight radiation using ZnONp as catalyst degradation takes place rapidly with maximum decolorization of textile effluent in most efficient



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way. Antibacterial activity of the synthesized nanoparticle were tested with both gram negative and gram negative bacteria both act as stabilizing and reducing agent.

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