

## GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLE OF *THYMUS VULGARIS* L. LEAVES AND ITS ANTIBACTERIAL ACTIVITY

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Received-02.10.2018, Revised-23.10.2018

**Abstract:** In the present study, the green method was used for the synthesis of zinc oxide nanoparticle from the dried leaves of *Thymus vulgaris* L. The synthesized ZnO NPs was characterized by UV-Vis spectrophotometer, XR Diffractometer, Scanning Electron Microscopy (SEM) and EDX (Energy Dispersive X-ray) spectrophotometer. The result suggests that the synthesized nanoparticles are crystalline in nature and in the nanorange. The average sizes of nanoparticle are 13.06 nm. The synthesized ZnO NPs was screened for the antibacterial activity against six pathogenic bacteria. Out of six bacterial strains tested, the ZnO NPs was found active against *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus cereus* but it does not have shown activity against *E. coli* and *Enterococcus spp.*

**Keywords:** Thymus vulgaris L., ZnO NPs, Antibacterial activity

### INTRODUCTION

The development of resistance in microorganism is matter of great concern for the medical science that the bacterial species which is sensitive to the basic antimicrobial drugs now become ineffective. This property of resistance is the result of mutation in gene of the bacteria, horizontal transfer of resistance gene to bacterial progeny (Srivastava *et al.*, 2016) and other reason was irrational use of antimicrobial agents by medical practitioner. In most of the cases of diseases people hesitate to visit clinician due to the high cost of consultancy and expensive drugs prescribed by them. So, people consult their disease to the pharmacist or local drug distributor and take drugs without knowing the full course of the drug and after 2-3 dose he/she feel better and discontinue the drug. After sometime they again infected with same bacteria and the use of the same drug become ineffective due to development of resistance in bacterial pathogen. This problem was not only faced by India but it is global problem. The bacterial species resist or evade the mechanism of drug action by doing certain modification in itself and become resistant strain (Chandra *et al.* 2017). The researcher now in search of some alternate medicine or substance which have significant activity against these pathogenic bacterial species.

*Thymus vulgaris* L is aromatic plant and it is belongs to the family Lamiaceae (Labiatae) in which most of members are herbs and shrubs. It is small perennial herbs attain height upto 40 cm. The leaves of thyme are small ranges from 2.5 to 4.5 mm long and have oval to rectangular shape. Its aerial part is used for the production of essential oil. This plant does not need too much irrigation. The excessive water will make plant prone to rot disease. It has worldwide

distribution and it is native to Mediterranean and cultivated in many parts of Africa such as Egypt, Morocco, Algeria, Tunisia, Libya, South Africa, France, Svizzera, Spain, Italy, Bulgaria, and Portuguese Republic. Thyme plant is known for its great medicinal value. It is used for the treatment of various ailments like intestinal infection of bacterial as well as worm's infestation. The essential oil has been known for its antimicrobial activity and its property is due to the presence of phenolic compounds (Boruga *et al.*, 2014)

### METHODOLOGY

#### Chemical and Reagent

All chemicals and media used in the study were of analytical grade and procured from HiMedia Laboratories Ltd. The dried plant sample of Thyme was purchased from the Human India, Srikot, Srinagar, Uttarakhand.

#### Synthesis of ZnO Nanoparticle

The synthesis of ZnO Nanoparticle was done as per the method described by Patel *et al* (2017) in which dried leaves of thyme was boiled in double distilled water for 10 min and the extract after cooling filtered and stored in refrigerator for further processing. 50 mL of leaf extract was heated at 65°C with addition of zinc acetate and sodium hydroxide till the formation of color. The solution after cooling centrifuged and precipitate was collected and stored in vial after oven drying

#### Characterization of Synthesized ZnO NPs

The synthesized nanoparticles were characterized by using UV-Vis spectrophotometer, X-Ray Diffraction, Scanning Electron Microscopy and Energy Dispersive X-ray spectroscopy. All the characterization was done in the Centralized facility

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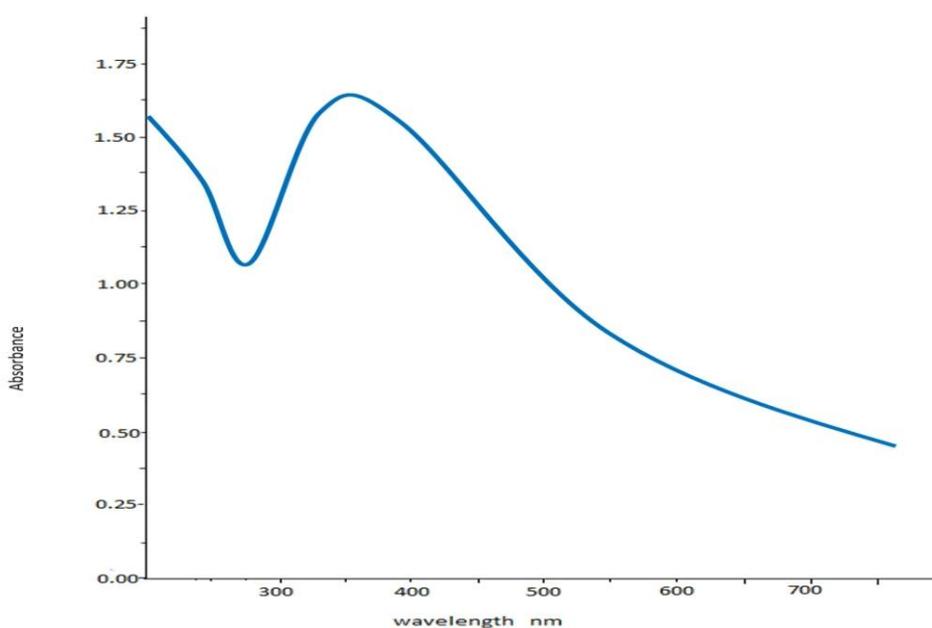
of USIC (University Science Instrumentation Centre), H. N. B. Garhwal University.

#### Antibacterial activity of synthesized nanoparticles against pathogenic bacteria

Antibacterial activity of synthesized ZnO NPs was tested according to the Agar well diffusion method described by Chandra *et al* (2016) in which appropriate well was created with help of cork borer in Mueller Hinton Agar medium which was previously inoculated with test bacteria and then well was filled with 100 mg/mL concentration of ZnO NPs. The seeded plate with ZnO NPs was incubated at 37°C for 18-24 h. The zone of inhibition was recorded in mm. The MIC of synthesized ZnO NPs was also evaluated by method described by Chandra *et al.* (2016).

## RESULTS AND DISCUSSION

The Fig 1 showed the UV absorption spectra of synthesized ZnO NPs and it was observed that the maximum absorption at 345 nm (Fig.1). The UV absorption of ZnO NPs of *Thyme* was in agreement of finding reported by Sutradhar and Saha (2015) and Azizi *et al.* (2013). The almost similar finding was also reported in earlier report of our group in which the synthesized zinc oxide nanoparticles from *Morinda citrifolia* have maximum absorption at 340 nm (Joshi *et al.* 2018). The other researcher also reported the absorption of ZnO NPs in the range of 340 -380 nm (Sutradhar and Saha, 2016; Saputra and Yulizar. 2017; Safawoa *et al.*, 2018).



**Fig.1.** UV-Vis spectra of synthesized ZnO NPs

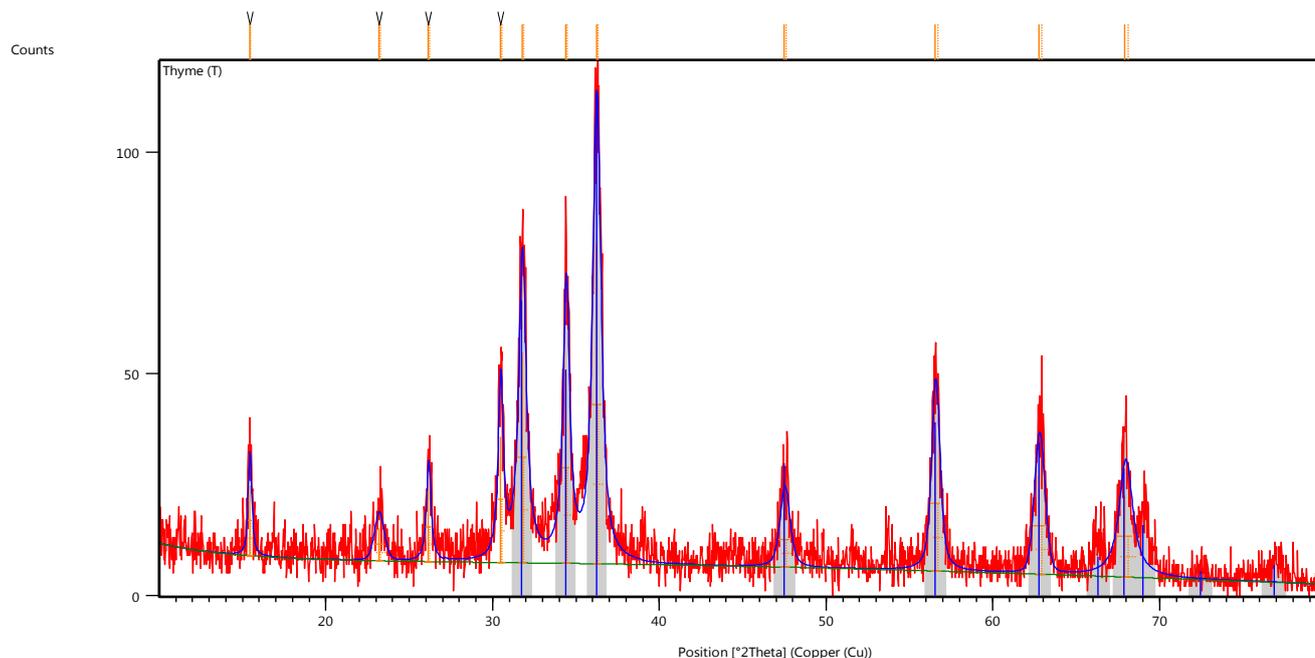
#### XRD analysis

Table showed the crystalline size of synthesized zinc oxide nanoparticles. The size of nanoparticle was calculated and found to have in the range of 11.2 to

14.7 nm. The average size of nanoparticle was 13.06 nm (Table 1). The XRD spectra of ZnO NPs were presented in Fig. 2

**Table 1.** Crystalline size of synthesized ZnO NPs synthesized using *T. vulgaris* L.

	2θ value (degree)	d- spacing (Å)	FWHM (degree)	Crystallite Size in nm
Thyme leaf extract	31.77	2.81	0.52	12.4
	34.39	2.60	0.45	14.7
	47.50	1.9	0.69	11.2
	56.54	1.6	0.57	13.8
	62.76	1.4	0.72	13.2

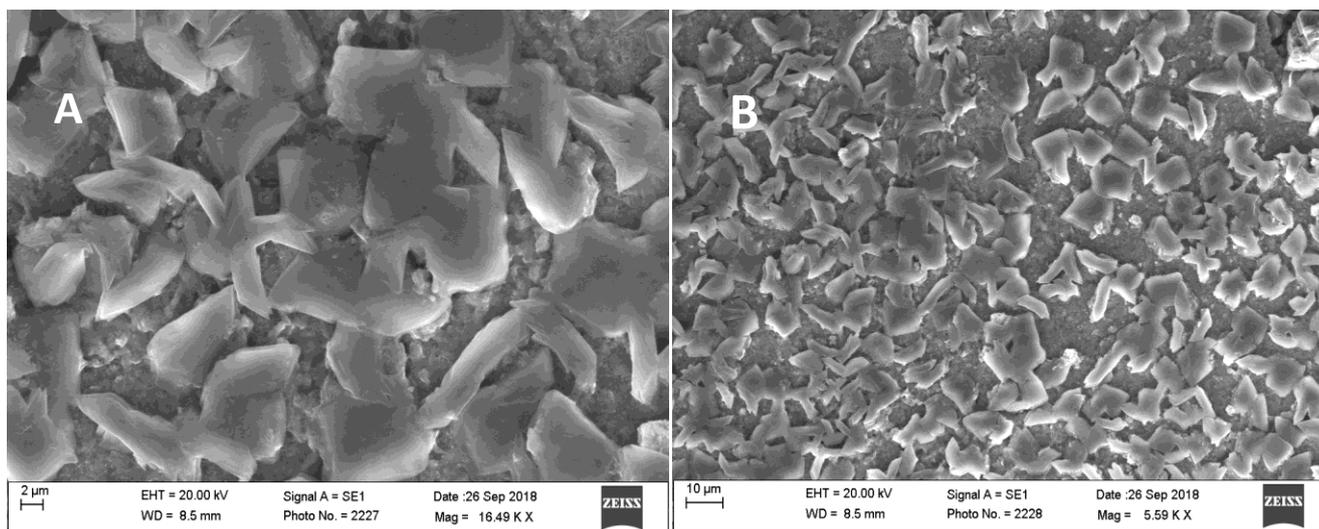


**Fig. 2** XRD spectra of ZnO NPs synthesized from *T. vulgaris*

The peak shown in the Fig. confirmed the crystalline nature of synthesized nanoparticle. The peak obtained at  $2\theta$  angle value ranges from 31.77, 34.39, 47.50, 56.54 and 62.56 which clearly suggestive of formation of crystalline nanoparticles. The results obtained are in agreement with the finding of Suresh *et al.* (2018)

**SEM analysis**

The SEM image of synthesized ZnO nanoparticle was presented in Fig.3. The SEM image was taken at two different magnification i.e. 2  $\mu\text{m}$  and 10  $\mu\text{m}$ . The irregular and rectangular shape nanoparticles are clearly seen in Fig.3.



**Fig. 3.** SEM image of ZnO NPs at different magnification A.) 2  $\mu\text{m}$  B.) 10  $\mu\text{m}$ .

**EDX**

The Energy dispersive X-ray Spectroscopy generally done to know the elemental composition of synthesized nanoparticle. The Fig 3 confirmed that the synthesized nanoparticles were composed of zinc

and oxide. There was appearance of major peak of O and Zn at 0.5 keV and 1 KeV respectively. This is in agreement of our previous reports (Joshi *et al.*, 2018) and Patel *et al.*, 2017)

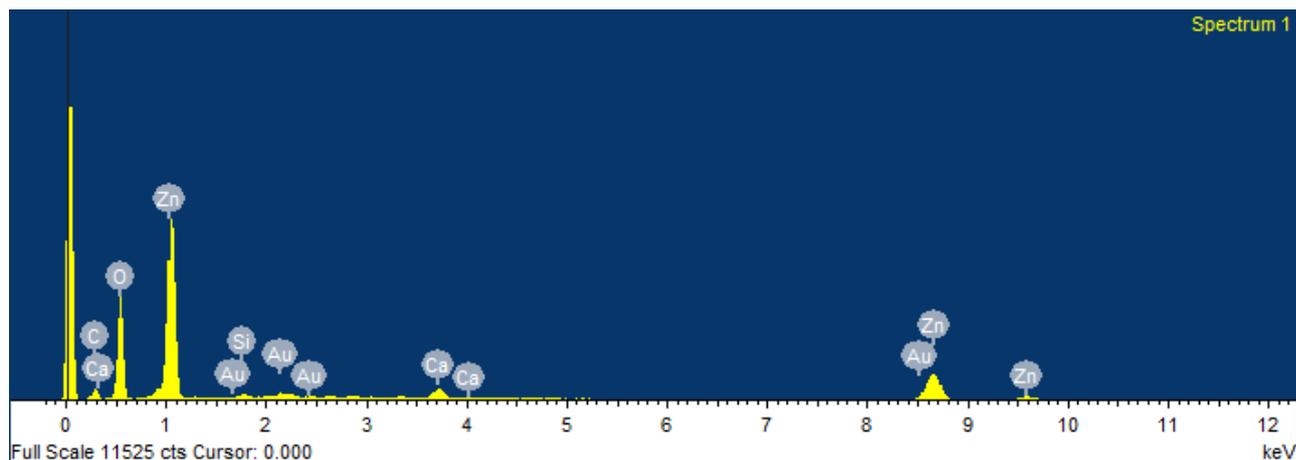


Fig. 4. EDX spectra of Synthesized ZnO NPs from *T. vulgaris*.

#### Antibacterial activity of synthesized Nanoparticle

The synthesized ZnO Nanoparticle was evaluated against five uropathogens i.e. *S.typhi*, *Enterococcus* spp., *Klebsiella pneumoniae*, *E. coli*, *S. aureus* and sixth bacteria isolated from other source, *B. cereus*. The Table showed the antibacterial activity of ZnO NPs synthesized from Thyme. Out of six bacterial

species tested no activity was reported against *E. coli* and *Enterococcus* spp. However, highest antibacterial activity was seen against *Staphylococcus aureus* ( $20.0 \pm 0.0$ mm) followed by *B. cereus* ( $16.0 \pm 0.5$  mm) and moderate activity was seen in case of *S.typhi* ( $9.0 \pm 0.0$  mm) and *K. pneumoniae* ( $13.0 \pm 0.2$  mm).

S.No.	Name of Microorganism	Gram Reaction	Zone of inhibition in mm			
			DMSO	Chloramphenicol	ZnO NPs	MIC $\mu$ g/ml
1	<i>Salmonella typhi</i>	GNB	NA	16.0 $\pm$ 0.2	9.0 $\pm$ 0.0	512
2	<i>Klebsiella pneumoniae</i>	GNB	NA	21.0 $\pm$ 1.1	13.0 $\pm$ 0.2	256
3	<i>Escherchia coli</i>	GNB	NA	26.0 $\pm$ 0.5	NA	NA
4	<i>Enterococcus spp.</i>	GPB	NA	19.2 $\pm$ 0.2	NA	NA
5	<i>Staphylococcus aureus</i>	GPB	NA	23.5 $\pm$ 1.1	20.0 $\pm$ 0.0	128
6	<i>Bacillus cereus</i>	GPB	NA	20.0 $\pm$ 0.2	16.0 $\pm$ 0.5	256

The antibacterial activity of synthesized ZnO NPs from *Morinda citrifolia* against uropathogens was reported by our group (Joshi *et al.*, 2018).

The antimicrobial property of photosynthesized nanoparticle may be attributed due to the presence of potential phytochemicals present in the Thyme. The essential oil of thyme was reported to have significant activity against both Gram positive and Gram negative bacteria (Prasanth *et al.*, 2014; Saleh *et al.*, 2015). The synthesized nanoparticle shown excellent activity against *S. aureus* and almost shown similar inhibition zone as compared to standard antibiotic i.e. Chloramphenicol. This antibiotic is broad spectrum antibiotic which is quite effective against both Gram positive and Gram negative bacteria. The *S. aureus* is known for causing boils, cellulitis and also responsible for causing septicemia. So, our synthesized ZnO NPs can be utilized for the preparation of medicated skin creams

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