BIOSYNTHESIS OF ZINC OXIDE NANOPARTICLES USING FRUITS AND LEAVES EXTRACTS OF *TERMINALIA CHEBULA* RETZ.

Sandar Myint[1], Ni Ni Sein[2], Thida Win[3]

Abstract

Green synthesis of metal oxide nanoparticles using plant extract is a promising alternative to traditional method of chemical synthesis. The biosynthesis of zinc oxide nanoparticles using fruits and leaves extracts of *Terminalia chebula* Retz. has been reported in this study. It is simple, cost-effective, rapid and eco-friendly way to synthesize zinc oxide nanoparticles. The ZnO nanoparticles were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), ultraviolet-visible (UV-Vis) and Fourier transform infra-red (FT IR). The structure of ZnO nanoparticles was indexed as hexagonal structure. The crystallite sizes of ZnO nanoparticles from fruits and leaves extracts of *T. chebula* were calculated by Scherrer formula as 17.0 nm and 23.4 nm, respectively. Morphology of nanocrystals was observed to be aggregated spherical particles. UV absorption spectra of ZnO nanoparticles using fruits and leaves extracts of *T. chebula* indicated the absorption maxima at 376 and 375 nm, respectively. This new eco-friendly approach of synthesis is a novel, cheap, and convenient technique suitable for large scale commercial production.

Keywords: *Terminalia chebula* Retz., green synthesis, ZnO nanoparticles, *T. chebula* , hexagonal structure

Introduction

Nanoparticles are particulate materials with at least one dimension of less than 100 nanometers (nm). Metal nanoparticles have been of great interest due to their distinctive features such as catalytic, optical, magnetic and electrical properties (Garima *et al*., 2011). These nanoscale sized particles possess a larger surface area as compared to microsized particles leading to enhanced properties (Tripathi *et al*., 2014). Nanoparticles present a higher surface area to volume ratio with decrease in the size, distribution and morphology of the particles (Awwad *et al*., 2012).

Researchers are using green methods for the synthesis of various metal oxide nanoparticles because these methods are considered safe and ecologically sound the nanomaterials fabrication as an alternative to conventional methods (Liu and Lin, 2004; Yu, 2007). Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly byproducts. Biological approaches using microorganisms and plant or plant extracts for nanoparticles have been suggested as valuable alternatives to chemical methods. Several biological systems including bacteria, fungi, and yeast have been used in synthesis of nanoparticles (Alagummuthu and Kirubha, 2012). Using microorganisms for the synthesis of nanoparticles were found to be more tedious and required more steps in maintaining cell culture, intracellular synthesis with more purification steps. The use of plants known as ‘Green synthesis’ or ‘Biogenic synthesis’ shows better advancement over chemical and physical methods as it is lesser toxic, cost effective, environmental friendly (Vidya *et al*., 2013) and also involves proteins as capping agents (Sangeetha *et al*., 2011).

The biosynthesis of Zinc oxide (ZnO) nanoparticles gains significant credit with reference to their cost-effective, nontoxic, self-aggregation, antibacterial and gas sensing properties. ZnO nanoparticles play vital role in the synthesis of biological pigments, photocatalytic issues, production of piezoelectric devices, chemical sensors, drug carriers in targeted drug delivery

[1] Lecturer, Department of Chemistry, Nationalities Youth Resources Development Degree College, Sagaing
[2] Dr, Professor (Retd.), Department of Chemistry, University of Yangon
[3] Rector, Dr, University of Mandalay

mechanisms, and the production of cosmetics such as sunscreen lotions etc. Zinc oxide efficiently protects broader UV range than other molecular UV-absorbers. Zinc oxide nanoparticles are employed in various biological and pharmacological applications due to its non-toxic nature.

Recently various parts of plants proved to be the potential reducing agents for production of nanoparticles. Leaves, stem, fruits, flowers, seeds, barks and gums were employed for the synthesis of eco-friendly nontoxic nanoparticles with valuable biomedical properties. Plant based zinc oxide nanoparticles are widely accepted in cosmetic industry and agriculture (Manokari *et al*., 2016). So, this study is aimed to synthesize ZnO nanoparticles with a simple, rapid, cost-effective and environmentally synthesis method of using fruits and leaves extracts of *Terminalia chebula* .

Materials and Methods

Sample Collection

Fruits and leaves of *Terminalia chebula* (Phanga) were collected from Phayapyo village, Magway Township in Magway Region, Myanmar. The botanical name was identified at Department of Botany, Magway University.

Preparation of Aqueous Extract of *Terminalia chebula*

Fresh fruits and leaves of *T. chebula* were washed several times with deionized water to remove dust particles and then air dried to remove the residual moisture for 1 day. The seed from the *T. chebula* fruit were removed and the remaining part was crushed by using an electronic blender. The leaves of *T. chebula* were cut into small pieces and then crushed by using an electronic blender. Fruits extract and leaves extract of *T. chebula* were prepared by mixing 50 g of plant sample with 500 mL of deionized water in a 1000 mL glass beaker. The mixture was heated for 2 h at 80 °C by using a magnetic stirrer. Then, the extract was cooled to room temperature and filtered with Whatman No. 1. filter paper and used for further experiments.

Synthesis of ZnO NPs

Zinc nitrate ($Zn(NO_3)_2.6H_2O$) (29.739 g) was accurately weighed and it was dissolved in deionized water and the volume was made up to 100 mL with deionized water to obtain 1 M solution. For the volume ratio of 1:2 of $Zn(NO_3)_2$ and plant extracts, 100 mL of $Zn(NO_3)_2$ solution was transferred to a 1000 mL beaker and heated for 15 min at 70 °C by using a magnetic stirrer. Then, 200 mL of plant extracts was added drop-wise and the resulting mixture was heated for 2 h at 70 °C using a stirrer-heater. The mixture was then dried in oven at temperature 100 ± 5 °C for 1 h and pale brown powder was obtained. It was washed several times with deionized water followed by washed with EtOH in 3 times. Then, pale yellow powder was calcined in a muffle furnace for 2 h at 400 °C and finally white powder ZnO was obtained. This powder was carefully collected and packed for characterization purposes. Similarly 300 mL and 400 mL of plant extracts were used to get volume ratios of 1:3 and 1:4, respectively and the experiment was carried out according to above procedure.

Characterization ZnO Nanoparticles

X-ray diffraction analysis

ZnO nanoparticles were examined by X-ray diffractometer (Rigaku Co., Tokyo, Japan) equipped with Cu K_α radiation of 1.54056 Å wavelength at Universities' Research Center, Yangon. All XRD data were collected under the same experimental conditions, in the angular range of 2θ 10° to 70° at an accelerating voltage of 40 kV.

Scanning Electron Microscopy and Transmission Electron Microscopy

A scanning electron microscope (ZEISS) operating at an accelerating voltage of 10 kV was used to observe the morphology of zinc oxide sample at West Yangon University and transmission electron microscope (TEM, JEOL TEM-3010) with an accelerating voltage of 100 kV (State Key Laboratory, College of Science , Beijing University of Chemical Technology, China) was used to investigate the size and morphology. The crystallite sizes of ZnO NPs were calculated by using Image J software programme.

Fourier transform infrared spectroscopy (FT IR)

FT IR for ZnO nanoparticles was obtained in the range 4000–400 cm^{-1} with FT IR spectrometer (FT IR-8400 SHIMADZU, Japan).

UV-vis Spectroscopy

The optical property of biosynthesized ZnO nanoparticles samples were measured at room temperature by UV-vis spectrophotometer (Shimadzu UV-1800)operated at a resolution of 1 nm between 200 and 400 nm. The ZnO nanoparticles were separately dispersed in distilled water and ethanol with concentration of 0.1 % each and then the solution were subjected for UV–visible measurements.

Results and Discussion

Effect of Volume Ratio of $Zn(NO_3)_2$ and Fruits and Leaves Extracts of *T. chebula*

In the biosynthesis of ZnO nanoparticles, three different volume ratios of $Zn(NO_3)_2.6H_2O$ and fruits and leaves extracts of *T. chebula* (1:2, 1:3 and 1:4) were investigated. After heating at 400 °C, white precipitate of ZnO samples were obtained. As the volume of the extract of fruits and leaves of *T. chebula* increased from 200 mL to 400 mL, the amount of ZnO increased. Higher amounts of ZnO were obtained using 400 mL of fruits and leaves extracts of *T. chebula* i.e. (7.1037 g) and (7.0943 g) respectively (Table 1). However, the amount of ZnO obtained using 400 mL of the extracts was not much different from that using 300 mL of the extracts. Yield percent of ZnO were 87.2798 % and 87.1643 % prepared by using 1:4 volume ratio of $Zn(NO_3)_2$ and fruits and leaves extracts of *T. chebula.*

Table 1 Amount of ZnO and Yield % of ZnO Prepared by Using Fruits and Leaves Extracts of *T. chebula*

No.	Volume of 1M $Zn(NO_3)_2$ (mL)	Volume of extract (mL)	Amount of ZnO (g)		Yield of ZnO(%)	
			Fruits extract	Leaves extract	Fruits extract	Leaves extract
1	100	200	6.0276	5.7112	74.0582	70.1708
2	100	300	6.9298	6.7592	85.1431	83.0471
3	100	400	7.1037	7.0943	87.2798	87.1643

Characterization by X-ray Diffraction Method

X-ray Powder Diffraction (XRD) studies were carried out to confirm the phase purity. Figures 1 and 2 show the XRD patterns of ZnO nanoparticles synthesized using leaves and fruits extracts of *T. chebula*. All the peaks of (100), (002), (101), (102),(110), (103) and (112) in both XRD patterns were found to be well-resolved. The presence of (100),(002) and(101) planes in XRD patterns indicates the formation of high purity of the ZnO nanoparticles. All peaks are in good agreement with standard diffraction data (80-0075 > ZnO). This clearly confirms that ZnO nanoparticles has been successfully synthesized by green synthesis route. Furthermore, no impurity peaks were observed. Strong intensity and narrow width of ZnO diffraction peaks indicated that the resulting product was highly crystalline in nature.

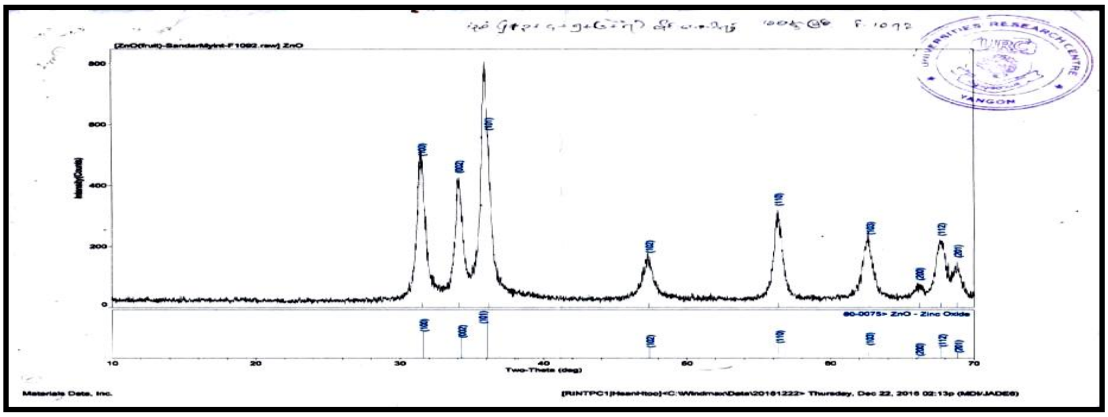


Figure 1 XRD pattern of ZnO prepared by using *T. chebula* fruits extract

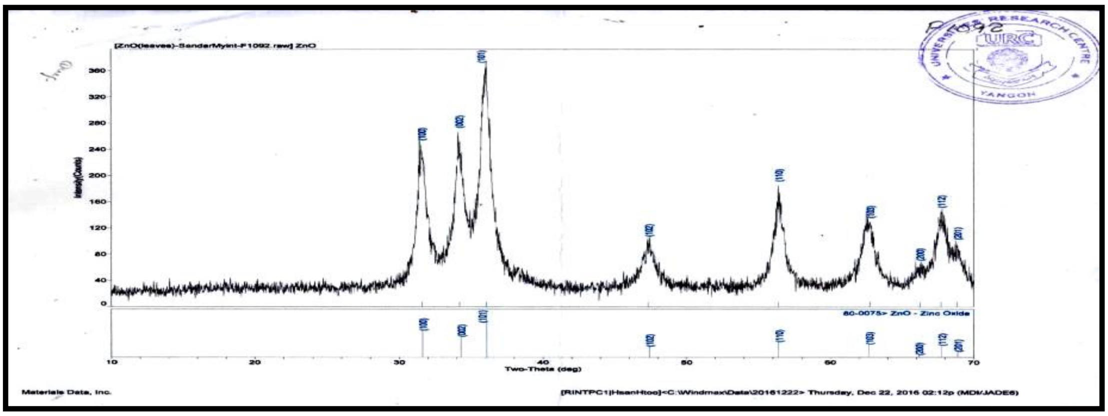


Figure 2 XRD pattern of ZnO prepared by using *T. chebula* leaves extract

The average crystallite sizes of green synthesized zinc oxide particles were calculated by using Debye Scherrer equation, $\tau = \frac{0.9\lambda}{\beta\cos\theta}$, where τ is the crystallite size, λ is the wavelength of the X-ray used, β is the full width at half maximum (FWHM) of the peak in radians and θ is the diffraction angle or the Bragg angle of the peak (Jenkins and Snyder, 1996).

Tables 2 and 3 show the calculated crystallite sizes of ZnO prepared from fruits and leaves extracts of *T. chebula* and those obtained by peak search report. Average crystallite sizes of ZnO nanoparticles prepared using fruits and leaves extracts were 17.0 nm and 23.4 nm respectively. Calculated values were not much different from those given by peak search report from XRD data. Crystallite size of ZnO from fruits extract was found to be smaller than that from leaves extract. ZnO obtained from both extracts were in the nano range.

Table 2 Crystallite Size of ZnO Prepared by Using *T.chebula* Fruits Extract

No.	Diffraction Angle '2θ' (°)	Full Width at Half Maximum 'β' (°)	Full Width at Half Maximum 'β' (radian)	Calculated Crystallite Size (nm)	Crystallite Size from XRD Data (nm)
1	31.499	0.515	0.0090	16.0	16.3
2	34.137	0.481	0.0084	17.3	17.7
3	35.975	0.555	0.0097	15.1	15.3
4	47.274	0.602	0.0105	14.4	14.6
5	56.358	0.553	0.0097	16.3	16.6
6	62.516	0.650	0.0113	14.3	14.5
7	66.080	0.350	0.0061	27.1	28.3
8	67.691	0.684	0.0119	14.0	14.1
9	68.853	0.520	0.0091	18.5	18.9
Average crystallize size				17.0	17.4

Table 3 Crystallite Size of ZnO Prepared by Using *T.chebula* Leaves Extract

No.	Diffraction Angle '2θ' (°)	Full Width at Half Maximum 'β' (°)	Full Width at Half Maximum 'β' (radian)	Calculated Crystallite Size (nm)	Crystallite Size from XRD Data (nm)
1	31.577	0.618	0.0108	13.4	13.5
2	34.190	0.537	0.0094	15.5	15.8
3	36.084	0.621	0.0108	13.4	13.6
4	47.318	0.657	0.0115	13.2	13.4
5	56.400	0.551	0.0096	16.4	16.7
6	62.540	0.566	0.0099	16.4	16.7
7	65.948	0.147	0.0026	64.4	87.7
8	67.704	0.490	0.0086	19.5	20.0
9	68.828	0.252	0.0044	38.2	41.7
Average crystallize size				23.4	26.6

Tables 4 and 5 show phase identification by X-ray diffraction analysis. It was observed that only single phase of ZnO with no other phase was found. It indicates the purity of the ZnO sample.

Table 4 Phase Identification of ZnO Prepared by Using Fruits Extract of *T.chebula*

No.	Diffraction Angle '2θ' (°)	Interplanar Spacing 'd' (Å)	Miller Indices (hkl)	Phase ID
1	31.499	2.8378	100	ZnO
2	34.137	2.6243	002	ZnO
3	35.975	2.4944	101	ZnO
4	47.274	1.9212	102	ZnO
5	56.358	1.6311	110	ZnO
6	62.516	1.4845	103	ZnO
7	66.080	1.4128	200	ZnO
8	67.691	1.3830	112	ZnO
9	68.853	1.3625	201	ZnO

Table 5 Phase Identification of ZnO Prepared by Using Leaves Extract of *T.chebula*

No.	Diffraction Angle '2θ' (°)	Interplanar Spacing 'd' (Å)	Miller Indices (hkl)	Phase ID
1	31.577	2.8310	100	ZnO
2	34.190	2.6204	002	ZnO
3	36.084	2.4871	101	ZnO
4	47.318	1.9195	102	ZnO
5	56.400	1.6300	110	ZnO
6	62.540	1.4840	103	ZnO
7	65.948	1.4153	200	ZnO
8	67.704	1.3828	112	ZnO
9	68.828	1.3629	201	ZnO

Lattice constants and crystal structure of ZnO nanoparticles obtained by using fruits and leaves extracts of *T.chebula* are shown in Table 6. By XRD analysis ZnO obtained from fruits and leaves extracts were indexed to the hexagonal wurtzite structure with equal length of ´a´ and ´b´ (3.2739 Å and 3.2672 Å respectively) and longer ´c´ (5.2313 Å and 5.2243 Å respectively).

Table 6 Lattice Constants and Crystal Structure for ZnO Prepared by Using Fruits and Leaves Extracts of *T.chebula*

No.	Sample	Lattice Constant (Å)			Crystal structure
		a-Axis	b-Axis	c-Axis	
1	ZnO (fruits extract)	3.2739	3.2739	5.2313	Hexagonal
2	ZnO (leaves extract)	3.2672	3.2672	5.2243	Hexagonal

Surface Morphology of Zinc Oxide Nanoparticles Prepared by Using Fruits and Leaves Extracts of *T.chebula* Retz.

The scanning electron microscopy was used for the morphological study of zinc oxide nanoparticles from fruits and leaves of *T.chebula*. Agglomeration of ZnO nanoparticles were observed in ZnO nanoparticles prepared by using fruits and leaves extracts of *T.chebula* (Figure 3). SEM image of ZnO nanoparticles prepared by using *Trifolium pratense* flower extract also showed agglomerated particles of ZnO (Dobrucka and Dugaszewska, 2016; Suresh, *et al*., 2015).

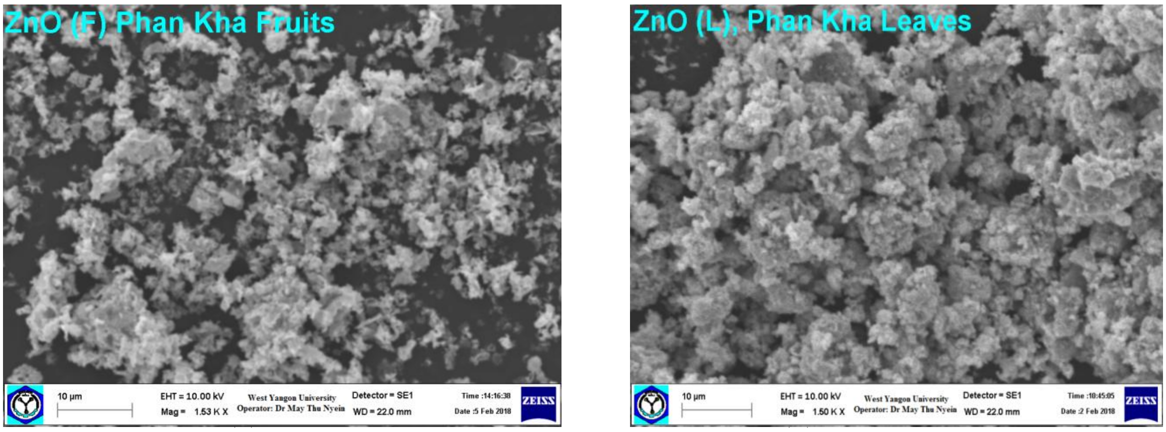
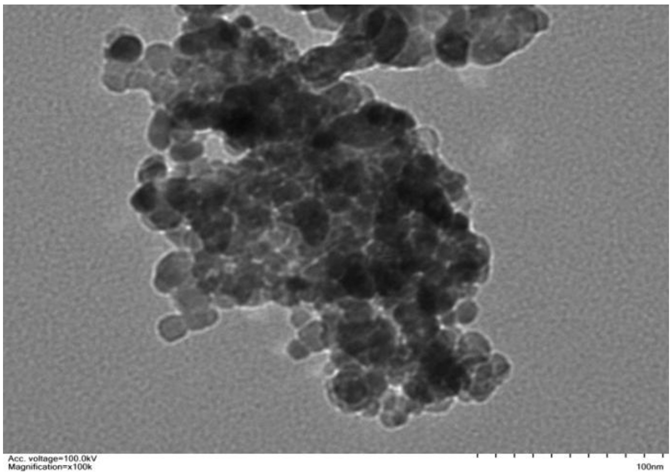
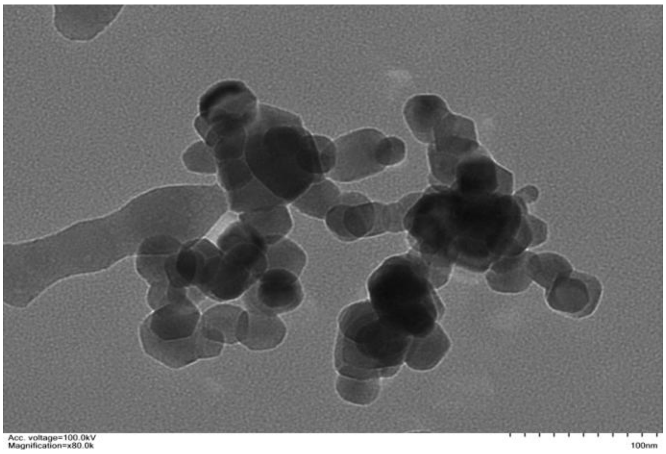


Figure 3 SEM images of ZnO nanoparticles prepared by using *T. chebula* (a) fruits extract and (b) leaves extract

Figure 4 shows the TEM images of ZnO nanoparticles prepared by using *T. chebula* leaves and fruits extracts. Crystallite sizes of ZnO nanoparticles obtained by using fruits and leaves extracts were 20.5 nm and 25.1 nm by TEM analysis which were not much different from those obtained by XRD analysis, 17.0 nm and 23.4 nm, respectively.



(a)



(b)

Figure 4 TEM images of ZnO nanoparticles of (a) fruits and (b) leaves extracts of *T. Chebula* (magnification X 80.0 K)

Characterization by FT IR Spectroscopy

Green synthesized zinc oxide nanoparticles by using fruits and leaves extracts of *T. chebula* were characterized by FT IR technique. Figures 5 and 6 show the FT IR spectra of green synthesized zinc oxide nanoparticles of fruits and leaves extracts of *T. chebula*. FT IR analysis showed the characteristic absorption bands of ZnO from fruits extract of *T. chebula* at 437 cm^{-1} and ZnO from leaves extract at 439 cm^{-1} (Table 7) . The peak in the region between 400 and 600 cm^{-1} was assigned to ZnO region (Yuvakkumar *et al*., 2015).The absorption band at 3435 cm^{-1} was assigned to stretching vibration of O-H stretching vibration of physically absorbed water molecules (Karthikeyan *et al*., 2016).

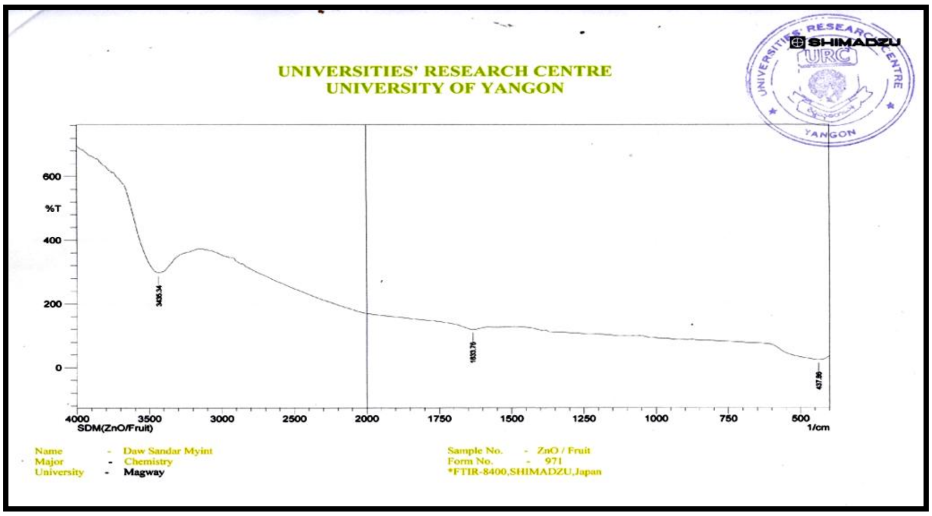


Figure 5 FT IR spectrum of ZnO prepared by using fruits extract of *T. chebula*

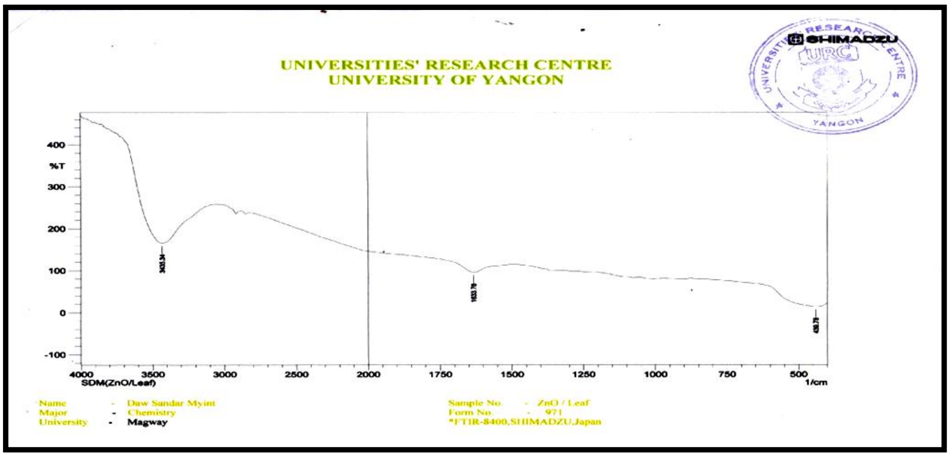


Figure 6 FT IR spectrum of ZnO prepared by using leaves extract of *T. chebula*

Table. 7 FT IR Spectral Data for ZnO Nanoparticles Prepared by Using Fruits and Leaves Extracts of *T.chebula*

No.	Wavenumber (cm^{-1})		Assignment*
	Fruits	Leaves	
1	3435	3435	O – H stretching vibration
2	437	439	Zn – O stretching vibration

* Karthikeyan *et al*., 2016; Yuvakkumar *et al*., 2015

UV Spectrum of ZnO Nanoparticles

Green synthesized zinc oxide nanoparticles of fruits and leaves of *T. chebula* were characterized by UV-visible spectroscopy. Figures 7 and 8 show the absorption spectra of ZnO nanoparticles synthesized using leaves and fruits extracts of *T.chebula*. The spectra revealed characteristic absorption peaks of ZnO prepared using leaves extract and fruits extract at wavelength of 375 nm and 376 nm, respectively. The absorption of ZnO was due to the excitation of valence electrons from the valence band to the conduction band (O2p–Zn3d) (Azarang *et al*., 2015). This implies that the ZnO nanoparticles absorb light in the ultra-violet region (Huang *et al*., 2006). The good absorption of the ZnO-NPs in the UV region proves the applicability of this product in such medical application such as sunscreen protectors or as antiseptic ointments (Gupta, 2014). ZnO nanoparticles using aqueous *Cassia fistula* plant extract showed the absorption band at 370 nm (Suresh *et al*., 2015). Ramasami *et al*. (2017) also reported the ZnO absorption peak at 373 nm. Lakshmi *et al*. (2017) reported the absorption maximum of ZnO synthesized from spinach leaves extract at 375.4 nm and Awwad *et al.* (2012) with 374 nm. Thus, prepared ZnO nanoparticles were also confirmed by UV-visible absorption spectroscopy.

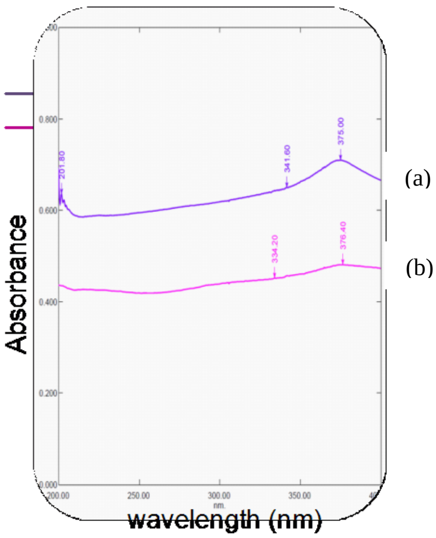


Figure 7 UV absorption spectra of ZnO prepared by using *T. chebula* (a) leaves and (b) fruits extracts (in distilled water)

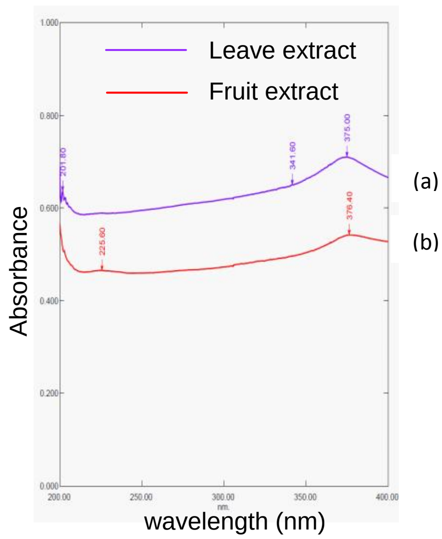


Figure 8 UV absorption spectra of ZnO prepared by using *T. chebula* (a) leaves and (b) fruits extracts (in ethanol)

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Conclusion

ZnO nanoparticles were successfully prepared by using fruits and leaves extracts of *Terminalia chebula* Retz.via green route. It is simple, cost-effective, rapid and eco-friendly way to synthesize zinc oxide nanoparticles. The structure of nanoparticles was indexed as hexagonal using XRD analysis. The crystallite sizes of ZnO nanoparticles from fruits and leaves extracts of *T. chebula* were 17.0 nm and 23.4 nm, respectively. Morphology of ZnO nanoparticles was investigated using the SEM and observed as spherically aggregates particles. The crystallite size by TEM analysis was 20.5 nm for ZnO nanoparticles using fruits extract and 25.1 nm for leaves extract. FT IR absorption spectra showed the characteristic absorption bands of ZnO at 437 and 439 cm^{-1}, respectively, for ZnO nanoparticles using fruits and leaves extracts of *T. chebula*. The optical properties of particles were studied with UV-Vis spectroscopy and both ZnO nanoparticles showed almost the same absorption maxima. Thus, from this present study it was concluded that fruits and leaves extracts of *T. chebula* can be effectively used for synthesizing the zinc oxide nanoparticles. This approach offers environmentally advantageous alternatives to more hazardous chemicals and processes and promotes pollution prevention by the production of nanoparticles in their natural surroundings.

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