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Green synthesis of ZnO Nanoparticles using *Phyllanthus embilica* Stem extract and their Antibacterial activity

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ABSTRACT

In this paper, a green synthesis of ZnO nanoparticles using Phyllanthus embilica stem extract as a reducing/capping agent. The prepared ZnO nanoparticles were characterized using fourier transform infrared spectroscopy (FTIR), UV-visible diffuse reflectance spectroscopy (UV-vis-DRS), X-Ray diffraction (XRD) and Scanning Electron Microscopy (SEM). The synthesized ZnO nanoparticles are wurtzite hexagonal structure with an average average crystallite size of ZnO prepared using Phyllanthus embilica stem extract was smaller (25.96 nm) when compared to the same ZnO prepared using a chemical method (36.73 nm). FT-IR spectra revealed the functional groups and the presence of protein as the stabilizing agent for surrounding the ZnO nanoparticles. The antibacterial activity of the ZnO was tested against gram negative bacteria Salmonella typhi and Klebsiella phnemonea by disc diffusion method. ZnO nanoparticles were subjected to antimicrobial studies and significant results were obtained.

Keywords: Metal Oxide, Nanomaterial, XRD, Antibacterial activity

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INTRODUCTION

ZnO is abundant in nature and environmentally friendly. These characteristics make this material attractive for many applications [1, 2]. The bacteriostatic and fungistatic behaviour of ZnO is well studied and utilized in personal care products. Zinc oxide is a material with many important and diverse applications. Approximately, 45% of the world year production of ZnO is used in the rubber industry to control the vulcanization process and as additive [3]. In the methanol synthetic process ZnO is part of the Cu, ZnO, Al_2O_3 catalyst [4]. In the pharmaceutical industry ZnO is applied in ointments because of its antiseptic properties [4]. The optical properties make ZnO also suitable for many applications, like as a pigment in paints, as a UV filter in products for sun protection and for the production of LEDs and TFTs [5]. In this wide range of applications ZnO is used often in the form of particles and the size of the particles plays an important role.

A number of synthetic routes have been employed to synthesize ZnO nanoparticles such as sol-gel processing, homogeneous precipitation [6], mechanical milling [7], organometallic synthesis [8], microwave method [9], spray pyrolysis [10], thermal evaporation [11] and mechano-chemical synthesis [12]. These methods used in organic solvents and toxic reducing agent majority of which are highly reactive and are unsafe to the environment, to avoid such implications and for sustainable synthesis of ZnO nanoparticles by biological approaches. Biosynthesis of nanoparticles is a bottom up approach where in the main reaction occurring is reduction/oxidation. Among the various biosynthetic approaches, the use of plant extracts has advantages such as easy availability, safe to metabolites. The plant extract has been used as a reducing and capping agent for the synthesis of nanoparticles which could be advantageous over chemical methods. Microbial contamination is a serious issue in healthcare. Hence, the developments of antimicrobial agents have attracted increasing attention in recent times [13]. The developments of nanoparticles with antimicrobial properties are of considerable interest now. ZnO is an antimicrobial agent and the particles are effective to inhibit both gram positive and gram negative bacteria [14, 15].

This study, therefore, is aimed to evaluate the toxicity of biological and chemically synthesized ZnO nanoparticles along with bulk formulations against plant and human pathogens under laboratory conditions.

MATERIALS AND METHODS

2.1 Preparation of aqueous *Phyllanthus embilica* stem extract:

The collected Fresh stem of *Phyllanthus embilica* was washed thrice with tap water and twice with distilled water to remove the adhering salts and other associated contaminants and they were cut into small pieces. 10 g of the stem was taken and boiled with 100 ml of double distilled water at 100 ˚C for half an hour. During the procedure of boiling, a light brown coloured solution was formed and which was cool at room temperature. After that, the boiled extract was filtered through Whatman No.1 and was stored in the refrigerator at 4 ˚C for further studies.

2.2 Synthesis of ZnO nanoparticles by chemical precipitation method:

ZnO nanoparticles were prepared by simple precipitation method. Zinc acetate used as the precipitator 0.1 M Zinc acetate and 0.52 M of potassium hydroxide were dissolved separately in 50 ml distilled water in two glass beakers with magnetic stirring, Potassium hydroxide was added to Zinc acetate solution dropwise with constant stirring which led to the rapid formation of white precipitation, it was stirred for 2 h to form a homogeneous precipitate. After filtering, the precipitate was washed with distilled water and dried at 80˚C for 5 h. The obtained nanoparticle was calcinated at 500 ˚C for 1 h.

1.3 Synthesis of ZnO nanoparticles by green method:

For the synthesis of ZnO nanoparticles, 50 mL of extract was taken and boiled at 60^{o}C. Then, 5.5g of zinc acetate was added to the solution. This mixture was then boiled and stirred by using magnetic stirrer until it becomes brown coloured paste. Then it transferred to a ceramic crucible cup and calcinated at 500 ˚C for 3h. Finally, obtained brown coloured powder. The material was powdered using a mortar and pestle so, that got a fine powder.

2.4 Characterization of ZnO nanoparticles

The optical properties were investigated using a UV-Vis-DRS were recorded in air at room temperature in the wave length range of 200-800 nm using Shimadzu UV - 2450 spectrophotometer. Surface structure was characterized by a Fourier-transform infra red (FT-IR) spectrophotometer (JASCO FT-IR 460 plus). The crystalline structure of the nanoparticles was studied by an X-ray diffractometer (XRD; XPERT PRO X-RAY) with Cu Kα radiation at 25 ºC and the structural assignments were made with reference to the JCPDS powder diffraction files. The surface morphology was examined using scanning electron microscopy (SEM) (JSM 6701F - 6701) in both secondary and backscattered electron modes and the elemental analysis was also detected.

2.5 Measurement of antimicrobial activity

ZnO nanoparticles in sterilized distilled water were tested for their antimicrobial activity by the agar diffusion method. Two microbial strains, Salomonella and Klebsiella were used for this analysis. The samples ZnO(chemical) and ZnO(Green) are dissolved in dimethyl sulfoxide (DMSO) and their concentrations are fixed at 200 mg/mL (minimum inhibitory concentration) and Ketoconazole was used as a reference drug. A lawn of test organism was made on the agar plate using a sterile cotton swab and then the antimicrobial discs (Whatman No.1. filter disc with samples at 200 mg/mL) were placed on the agar plate. All the plates were incubated at 37 ^{0}C for 24 h. The zone of inhibition was measured and expressed as millimetre in diameter [16].

RESULTS AND DISCUSSION

3.1 UV –Visible spectroscopy

Optical properties of ZnO nanoparticle become important as the size of particle is reduced to nanoscale. Fig(1) shows the UV-visible absorption spectra of ZnO nanoparticle Synthesis by chemical method and green method. The absorption coefficient (λ) where calculated an plotted for direct transition $(\lambda h\nu)^{1/2}$ Verses hν of the sample the value of the band energy (Eg) of ZnO NPs synthesized by chemical method was an increase in band gap of ZnO NPs synthesized by green method. The band gap energy of samples is determined by the formula $\alpha h\nu = Ed(h\nu - Eg)^2$ where ,α is absorption coefficient, hν is the energy of photon ,Eg is the direct band gap and Ed is the constant. By plot of $(\alpha h\nu)^2$ vs hν and extra plotting the linear region of the curve to absorption equal to zero as shown in the Fig(2a) and Fig(2b) gives the value of direct band gap (Eg). The unique exciton absorption and bang gap energy of ZnO (chemical) and green ZnO are shown in Table 1

Fig.1 UV-vis-DRS of ZnO (chemical) and ZnO (green)

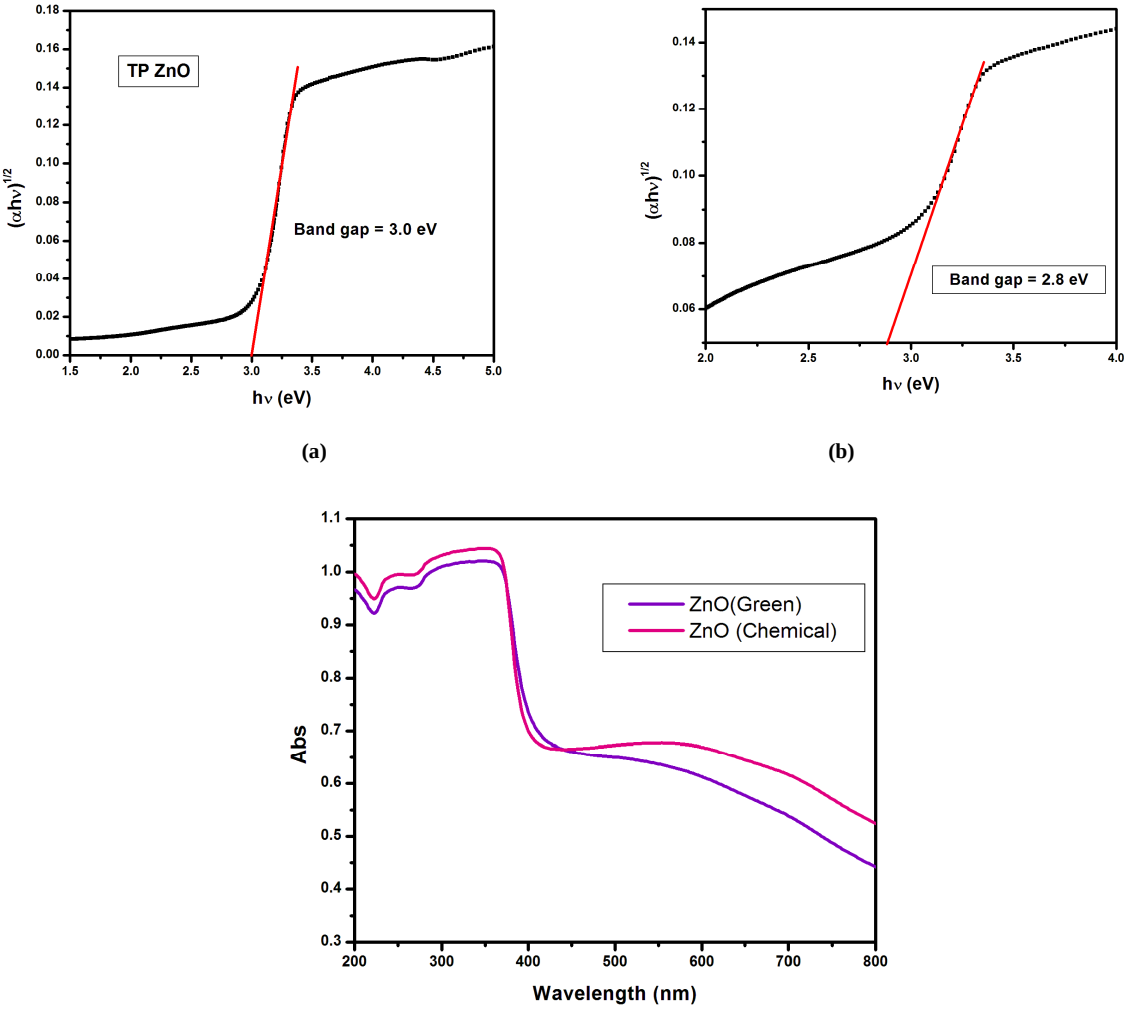


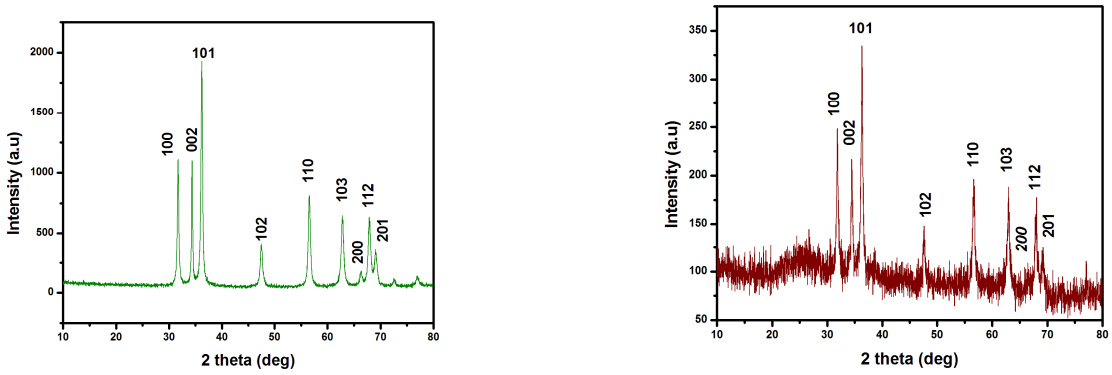
Fig.2 Tauc plots of (a) ZnO (chemical) (b) ZnO (green)

Table 1: UV –Visible absorption intensity and band gap energies ZnO nanoparticles synthesized by chemical and green method

SAMPLES	ABSORPTION INTENSITY (λ)	BAND GAP(eV)
ZnO (Chemical)	413nm	3.0 eV
ZnO (Green)	443nm	2.8 eV

3.2 Powder XRD studies

The end product in the chemical synthesized ZnO nanoparticles was pale white powder where in green synthesized ZnO is brown powder. XRD is used to investigate the changes of phase structure and crystallite size of the synthesized nanoparticles before and after addition of plant extract. Fig. 3 Shows the XRD pattern of chemical ZnO and green ZnO nanoparticles. From the diffractogram of XRD are very well matched with the hexagonal wurtzite structure by comparison with the data from JCPDS card No. 89-1397.



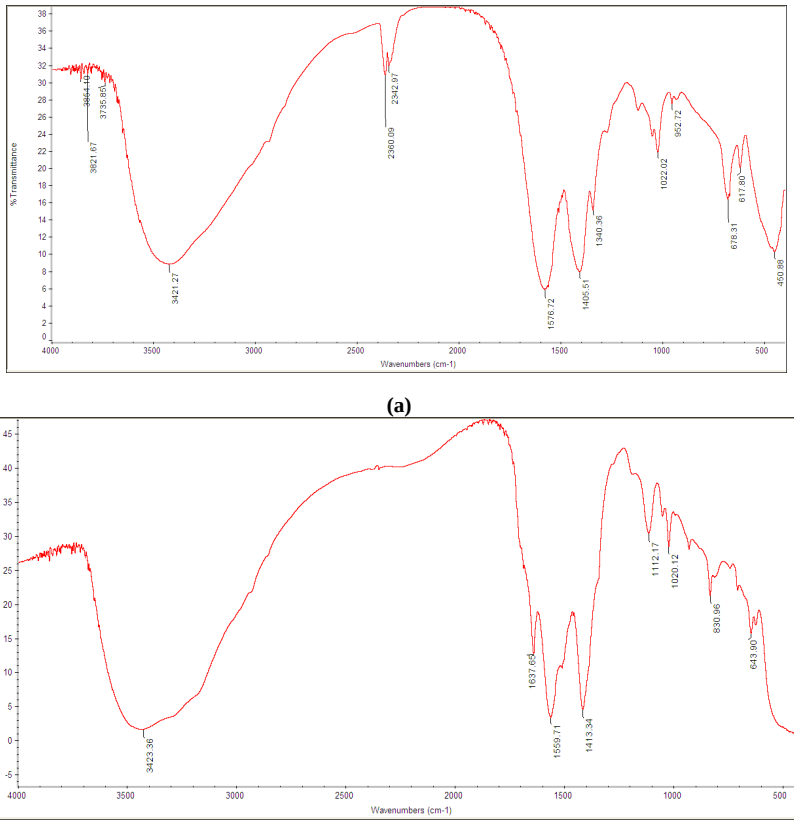
(a) **(b)**

Fig.3 XRD patterns of a) ZnO (chemical) b) ZnO (green)

These peaks are broad, suggesting that the crystallites have sizes in the nanometer range and the diameter D was calculated using Debye-Scherrer formula[17] $D = K\lambda/(\beta\cos\theta)$, where K is the scherrer constant, λ the X-ray wavelength, β the peak width of half maximum, and θ is the Bragg diffraction angle. The broad diffraction peaks of the sample after adding plant extract shown in Fig.3b confirmed that the crystal structure of ZnO nanoparticles was not altered during the plant extract. The XRD peaks give the diameter of about 25 nm for green synthesized ZnO and about 36 nm for chemical synthesized ZnO nanoparticles.

3.3 FT-IR spectral studies

The FT-IR spectra showed the presence of bonds due to O-H stretching around 3423 cm^{-1}. Peak at 1405 cm^{-1} may be assigned to symmetric stretching of the carbonyl side groups in the amino acid residues of the protein molecules [18]. The band at 1022 cm^{-1} corresponding to C-N stretching vibration of amine [19]. The peak at around 1340 cm^{-1} present in green ZnO signified amide III band of the random coil of protein [20].



(a)

(b)

Fig.4 FT-IR spectrum of a) ZnO (chemical) b) ZnO (green)

3.4 Morphological studies:

The SEM images of ZnO (chemical) and ZnO (green) are shown in Fig. 5a and 5b which shows that the chemically synthesized ZnO nanoparticles are spherical shaped with particle size of 25 - 35 nm which is in line with the results from XRD while the Green synthesized ZnO nanoparticles were obtained as nanospheres with particle size of 15 -25 nm which proves the role of *Phyllanthus embilica* extract to change in the size and morphology of ZnO nanoparticles.

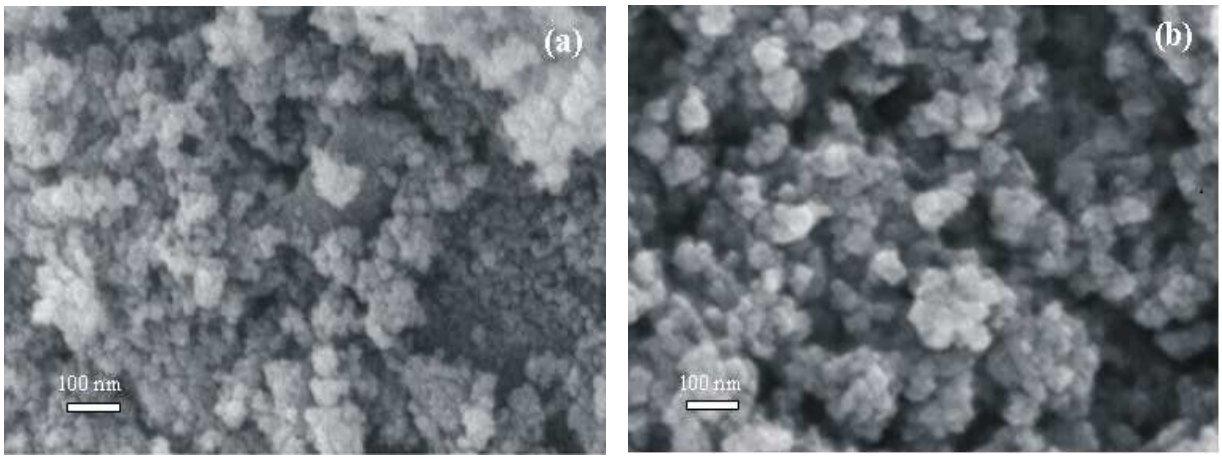


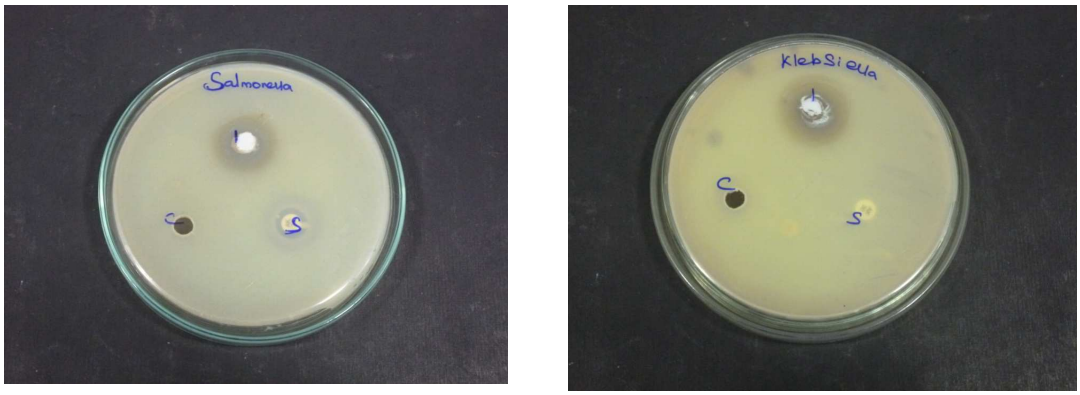
Fig. 5 SEM images of a) ZnO (chemical) b) ZnO (green)

3.5 Antibacterial activity

The antimicrobial activity of the ZnO nanoparticles was tested against gram negative bacteria S*almonella typhi* and *Klebsiella phnemonea*. The results for the antimicrobial activity of ZnO (chemical) and green ZnO are shown in Table 2 and it is observed that green ZnO showed excellent anti-bacterial activity against *Salmonella typhi* and *Klebsiella phenomena.* The remarkable antimicrobial activities of green ZnO nanoparticles are due to the generation of surface oxygen species which leads to the killing of the pathogens [20].

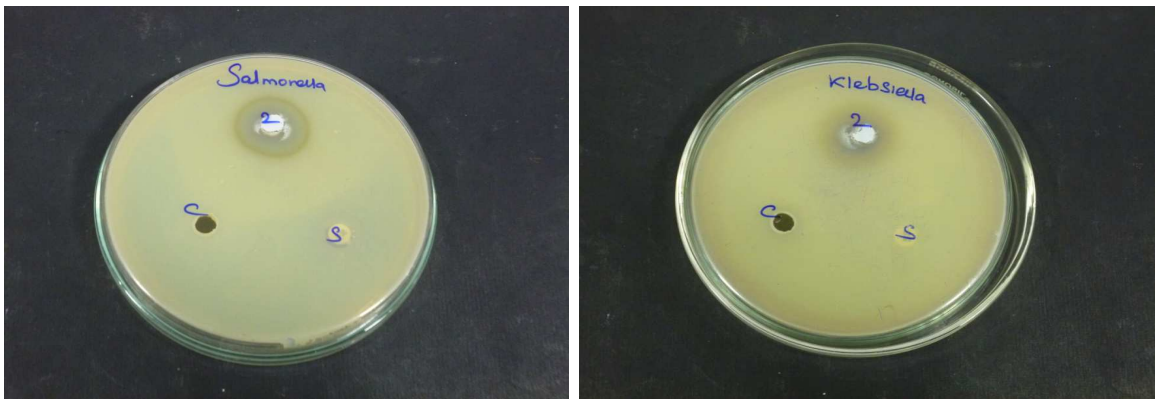
Table 2: Antimicrobial activity of ZnO nanoparticles synthesized by chemical and green methods

Sample	*Salmonella typhi*	*Klebsiella phnemoniea*
ZnO(Chemical)	10	11
ZnO(Green)	12	12
Control	R	R
Standard	06	R



(a) **(b)**

Fig. 6 The zone of inhibition of ZnO (chemical) against Salmonella typhi and Klebsiella pneumonia



(a) **(b)**

Fig. 7 The zone of inhibition of ZnO (green) against Salmonella typhi and Klebsiella pneumonia

CONCLUSION

ZnO nanoparticles are successfully synthesized via gree method using *Phyllanthus embilica* plant extract. The structure, morphology and size of the prepared ZnO nanoparticles were characterized by XRD, FT-IR and SEM analysis. UV-vis DRS studies confirmed the indirect band gap 2.8 eV. The average grain size lies between 25 to 35nm were found from XRD. The FT-IR spectra revealed the functional groups of stretching bands for ZnO Nps were found around 800 – 400 cm^{-1}. The synthesized ZnO Nps was studied for antibacterial activities against diseases pathogenic bacteria like *Salmonella typhi* and *Klebsiella phenomena.* Finally, the present study is so helpful and useful to the human and animals. In addition, the green synthesized ZnO Nps are inexpensive, stable and eco-friendly without side effect of human being.

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REFERENCES

[1] J.C. Pivin, G. Socol, I. Mihailescu, P. Berthet, F. Singh, M.K. Patel, *Thin Solid Films.*, **2008,** 517, 916.
[2] K.Sato and H. Katayama-Yoshida, *Jpn. J. Appl .Phys.*, **2000**, 39, 555.
[3] H.W. Engels, H.J. Weidenhaupt, M. Abele, M. Pieroth and W. Hofmann, in: *Ullmann's Encyclopedia of Industrial Chemistry*, sixth ed., **2001**.
[4] H. Heine, H.G. Vo lz, J. Kischkewitz, P. Woditsch, A. Westhaus, W.D. Griebler, M. de Liederkerke, in: *Ullmann's Encyclopedia of Industrial Chemistry*, sixth ed., **2001**.
[5] C.H. Yan, J. Zhang, L.D. Sun, in: H.S. Nalwa (Ed.), *Encyclopedia of Nanoscience and Nanotechnology*, **2004**, 10, 767.
[6] J.H.Kim, W.C. Choi, H.Y. Kim, Y. Kang and Y.K. Park, *Powder Technol.*, **2005,** 153, 16
[7] L.C. Damonte, L.A.Memdoza Zelis, B. Mari Soucase, M.A. Hernandez Fenollosa, *Powder Technol.*, **2004**, 148**,** 15.
[8] M.L. Kahn and M. Monge, *Adv. Funct. Mater.*, **2005**, 3**,** 458.
[9] S. Daniel Abraham, S. Theodore David, R. Biju Bennie, C. Joel, D. Sanjay Kumar, *J. Mol. Struct.*, **2016,** 1113, 174.
[10] X.Y. Zhao, B.C. Zheng, C.Z. Li, H.C. Gu, *Powder Technol.*, **1998**, 100, 20.
[11] T. Tani, L. Madler, S.E. Pratsinis, *J. Nanopart. Res.*, **2002**, 4**,** 337.
[12] Z.R. Dai, Z.W. Pan, Z.L. Wang, *Adv. Funct. Mater.*, **2003**, 13, 9.
[13] K.H. Tam, A.B. Djurisic, C.M.N. Chan, Y.Y. Xi, C.W. Tse, Y.H. Leung, W.K. Chan, F.C.C. Leung, D.W.T. Au, *Thin Solid Films*, **2008**, 516, 6167.
[14] C. Karunakaran, P. Gomathisankar, G. Manikandan, *Material Chemistry and Physics*, **2010**, 123, 585.
[15] Zarrindokht Emami-Karvani, Pegah Chehrazi, *African Journal of Microbiology Research*, **2011**, 5, 1368.
[16] Venubabu Thati, Aashis.S.Roy, M.V.N.Ambika Prasad, C.T. Shivannavar, S.M.Gaddad.. J Biosci Tech, **2010**, 1, 64.
[17] B.D. Cullity, *Acta Crystallographica section A*, **1979**, 35, 255.
[18] S. K Das, A .R. Das, A. K. Guha, *Langmuir*, **2009**, 25, 8192.
[19] N. Islam Bhuiyan, J.Begum, M. Sultana, *J. Bangladesh. Pharmacol. Soc.*, **2009**, 4, 66.
[20] S. Cai, B. R. Sing, *Biochemistry*, **2004**, 43, 2541.