ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING THE EXTRACTS OF *CALOTROPIS GIGANTEA, FOENICULUM VULGARE* AND *MURRAYA KOENIGII* AND THEIR ANTIMICROBIAL PROPERTIES

Kumari Subham[1], Jaskirat Kaur[1], Mouli Chakroborty[1], Suba G. A. Manuel*[1], Pradeep N[2]

[1]*Department of Life Science, Mount Carmel College, Bengaluru, India*

[2]*Department of Nano Science and Technology, Mount Carmel College, Bengaluru, India*

**Corresponding author: subaabraham@rediffmail.com*

ABSTRACT

The concept of green chemistry is becoming increasingly popular due to the environment-friendly approach. In the present study, we report green chemistry for the synthesis of zinc oxide nanoparticles (ZnO NPs) using the extracts of *Calotropis gigantea, Foeniculum vulgare* and *Murraya koenigii* and their antimicrobial properties. The technique followed is very simple, rapid, economic and eco- friendly. The plant extracts act as reducing agents, which are responsible for the bio-reduction of zinc ions. The synthesized nanoparticles (both by chemical and green synthesis) were structurally characterized by UV-Vis spectrophotometry, SEM and XRD. Further, the antibacterial activity of the prepared plant extracts and the nanoparticles was screened against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*. From the comparative antimicrobial analysis, it was inferred that the nanoparticles prepared by green synthesis were more effective than their chemical counterparts and, in some cases, the plant extracts were even better than the nanoparticles. Green synthesis can prove to be a better alternative to different physical and chemical procedures. The synthesized nanoparticles can be utilized for the preparation of various pharmaceutical products as well as applied in the field of agriculture to improve the health and well-being of both plants as well as animals.

Keywords: *Calotropis gigantea, Foeniculum vulgare*, *Murraya koenigii, Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus,* nanoparticles, green synthesis, Antimicrobial

1. INTRODUCTION

The authentic knowledge of the uses of medicinal plants passed on from one generation to another, after refining and additions [1].For thousands of years, medicinal plants have played an important role throughout the world in treating and preventing a variety of diseases and knowledge about various medicinal plants makes us aware of their potential to cure cough, cold, fever, headache, poisonous bites and some simple ailments [2]. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine [3], essential oils [4] or isolated compounds such as alkaloids [5], flavonoids [6] or naphthoquinones [7].

On the other hand, research and development in the field of nanotechnology are growing across the world. Nanoparticles possess an array of novel or improved characteristics as compared to their larger counterparts. Nanomaterials are a way to resolve problems pertaining to technological and environmental challenges [8]. Due to the novel properties possessed by the nanoparticles (NPs), they are used in various fields, namely, health, food, feed, space, chemical, cosmetic industries, and in agriculture [9-11].

Various nanoparticles, like gold, silver, zinc oxide, magnesium, copper can be synthesized in vitro for which various physical, chemical, and biological methods are employed. Although physical and chemical methods might successfully produce pure and well-defined nanoparticles, these methods are very expensive and potentially hazardous to the environment [12, 13]. Biological approaches using microorganisms and plants or their extracts for the synthesis of metal nanoparticles have been suggested as valuable and better alternatives to physical and chemical methods [14]. Various biological systems apart from plants such as bacteria, fungi and yeast have been used in synthesis of nanoparticles [15]. The problem lies in the fact that the formation of nanoparticles using microorganisms includes an elaborate

and lengthy process of maintaining the cell cultures, intracellular synthesis, and purification steps. For various reasons, plants are the best possible biological systems which can be used for the synthesis of nanoparticles. The process wherein plant extracts are used instead of chemicals, for the synthesis of nanoparticles is called 'Green Synthesis of Nanoparticles'.

The ability of organic compounds to reduce the specific metal ions and stabilize them into nanoparticles (NPs) forms the basis of green synthesis. It is believed that the green-synthesized NPs (GNPs) contain the conjugation of sugars, secondary metabolites, and proteins, which are derived from the plant extracts which are used for the synthesis of the nanoparticles [16]. Though very fewer studies have compared the activity of chemically synthesized NPs and the GNPs, few comparisons have indicated that GNPs possess better bioactivities than NPs synthesized by chemical or other methods [17, 18], which might be observed due to the presence of plant-derived compounds in these NPs. The plant extract mediated GNPs have gained much popularity due to the simplicity, low cost, eco-friendly nature and easy scaleup efficiency [19]. Various plant extracts have been used for the green synthesis of NPs.

In the present study, extracts of *Calotropis gigantea, Foeniculum vulgare* and *Murraya koenigii* have been prepared using methanol as the solvent. Further, zinc oxide nanoparticles were prepared by chemical method and green synthesis. A comparative evaluation of antimicrobial properties of plant extract, chemically synthesized nanoparticles and the nanoparticles prepared by green synthesis was assessed against *E. coli,S. aureus, and B. cereus.* The synthesized nanoparticles were characterized using UV-Visible Spectroscopy, SEM and XRD.

2. MATERIAL AND METHODS

2.1. Collection of plant material

Leaves of *M. koenigii* and *C. gigantea* plants and seeds of *F. vulgare* were selected for the study. Collected plant material was thoroughly washed with Milli-Q water and finely chopped. The leaves and seeds were kept in a hot air oven for drying at 60°C for 3-4 days; the dried material was finely powdered and stored in an airtight container at room temperature for further use.

A **B** **C**

Fig. 1: A) *Calotropis gigantea*, B) *Murraya koenigii*, C) *Foeniculum vulgare*

2.2. Preparation of leaf extract

Dried leaf powder (50g) was extracted with methanol (100 mL) at room temperature. The solution was kept at 60-70°C for overnight, with continuous stirring. The filtrate was centrifuged, filtered using Whatman filter paper and the filtrate obtained was stored at 4°C for further analysis [21].

2.3. Chemical synthesis of ZnO NPs

After complete dissolution of zinc nitrate (0.5M), aqueous solution of sodium hydroxide (0.9M) was added under high-speed constant stirring, drop by drop (slowly for 45 min) touching the walls of the vessel. The reaction mixture was kept undisturbed for 2 hours. The solution was allowed to settle overnight and the supernatant was separated carefully. The remaining portion was centrifuged for 10 min, and the precipitate was taken. The precipitated ZnO NPs were cleaned three times with deionized water and ethanol and then dried in air atmosphere at about 60°C [20].

2.4. Green synthesis of ZnO NPs

Crude leaf extract (15 ml) was added to zinc acetate dihydrate (200 mM) solution. The reaction mixture was kept on a magnetic stirrer for 6 hours. Sodium hydroxide (2 M) was added to the solution and placed in an

incubator at 60°C with overnight stirring. The mixture was centrifuged, the precipitate was washed with alcohol and distilled water and it was dried in an incubator at 40-50°C. The fine powder was prepared with pestle and mortar and stored at 4°C. The fine powder was used for characterization by UV-Vis, XRD, SEM, EDX [21].

2.5. *In vitro* analysis of Antimicrobial Activity (Well-diffusion method)

Loopful of pure cultures of *E. coli, S. aureus, and B. cereus* was suspended in MH broth and incubated overnight at 37°C. The incubated bacterial culture was swabbed uniformly using a sterile cotton swab on MH agar plate. Crude plant extracts and synthesized ZnO NPs was added into respective wells. They were incubated at 37°C for 18 hours [22]. After incubation, the zone of inhibition was measured. Ampicillin was used as a positive control and methanol was used as a negative control.

2.6. Characterization of biosynthesized ZnO NPs

2.6.1. UV-Vis spectrophotometric analysis

Small aliquots of the reaction mixture were diluted with Milli-Q water and transferred to the cuvette, and analysis was done using UV-Vis spectrophotometer. The absorption range for ZnO NPs was kept 250-550nm.

2.6.2. X-Ray Diffraction (XRD) Analysis

The powdered sample was used for XRD. (CeNSE lab, IISc, Bangalore). By using Scherrer's equation, the average crystallite size was determined:

$$D = K\lambda/\beta \cos\theta$$

2.6.3. Scanning Electron Microscope (SEM) and Energy Dispersive X-Ray (EDX) Analysis

The SEM and EDX analysis were done at CeNSE lab, IISc, Bangalore.

3. RESULTS AND DISCUSSIONS

3.1. *In vitro* analysis of Antimicrobial Activity

Methanolic crude plant extracts and ZnO NPs obtained through chemical synthesis and green synthesis using *C. gigantea*, *M. koenigii* and *F. vulgare* plant extracts were evaluated for their antibacterial activity and compared. Green synthesis showed the better inhibition as compared to chemical synthesis and crude plant extract against *E. coli* but in case of *M. koenigii* plant extract showed better zone of inhibition (Table 1).

Table 1: Comparative analysis of zone of inhibition against *E. coli* for various plant extract and ZnO NPs obtained though chemical and green synthesis

Concentration (%)	Zone of Inhibition (mm)						
	E. coli						
	Chemical synthesis	*C. gigantea*		*F. vulgare*		*M. koenigii*	
		Plant extract	Green synthesis	Plant extract	Green synthesis	Plant extract	Green synthesis
*Positive control	10.0±0.5	10.0±0.5	10.0±0.5	10.0±0.5	10.0±0.5	10.0±0.5	10.0±0.5
100	5.5±0.5	10.5±2.5	11.0±1.0	10.0±1.0	11.0±0.6	15.0±0.7	12.0±0.5

**Positive control: Ampicillin (5%); n=3*

Table 2: Comparative analysis of zone of inhibition against *S. aureus* for various plant extract and ZnO NPs obtained though chemical and green synthesis

Concentration (%)	Zone of Inhibition (mm)						
	S. aureus						
	Chemical synthesis	*C. gigantea*		*F. vulgare*		*M. koenigii*	
		Plant extract	Green synthesis	Plant extract	Green synthesis	Plant extract	Green synthesis
*Positive control	13.0±1.0	13.0±1.0	13.0±1.0	13.0±1.0	13.0±1.0	13.0±1.0	13.0±1.0
100	3.0±0.5	6.0±0.2	9.0±0.7	nil	7.0±0.4	nil	6.0±1.0

**Positive control: Ampicillin (5%); n=3*

When the same assay was conducted against *S. aureus*, in all the cases ZnO NPs obtained by green synthesis showed maximum zone of inhibition (Table 2). Plant extract showed maximum zone of inhibition against *B. cereus* when compared to ZnO NPs obtained by chemical and green synthesis (Table 3). Altogether green synthesis was proved to be better than chemical synthesis against all three bacterial species.

Table 3: Comparative analysis of zone of inhibition against *B. cereus* for various plant extract and ZnO NPs obtained though chemical and green synthesis

Concentration (%)	Zone of Inhibition (mm)						
	B. cereus						
	Chemical synthesis	*C. gigantea*		*F. vulgare*		*M. koenigii*	
		Plant extract	Green synthesis	Plant extract	Green synthesis	Plant extract	Green synthesis
*Positive control	11.0±0.5	11.0±0.5	11.0±0.5	11.0±0.5	11.0±0.5	11.0±0.5	11.0±0.5
100	6.0±0.6	15.0±0.8	10.0±0.5	20.0±0.4	6.5±1.0	20.0±1.5	9.0±0.7

***Positive control:** Ampicillin (5%); **n**=3*

3.2. *Characterization of biosynthesized ZnO NPs*

3.2.1. UV-Vis spectrophotometric analysis

The optical properties of ZnO nanoparticles obtained through chemical synthesis and green synthesis were characterized using UV-Vis spectroscopy. ZnO nanoparticles obtained through green synthesis with *C. gigantea, F. vulgare and M. koenigii* showed absorption maxima around 370-372 nm (Table 4). This result correlates previous literature, in which absorption peak was observed in the spectrum range of 360-380 nm which is a characteristic band for the pure ZnO [23].

Table 4: UV-Vis spectrophotometric analysis of ZnO NPs obtained using chemical synthesis and green synthesis

	ZnO chemical synthesis	*C. gigantea*	*F. vulgare*	*M. koenigii*
Abscis. (nm)	374.5	370.0	370.0	372.0 309.0
Absorbance	0.4536	0.0746	0.0950	0.3392 1.0625

3.2.2. X-Ray Diffraction (XRD) Analysis

For XRD analysis dried powder of the sample is used for confirming the size of the nanoparticles. The intensity of peak (101) was observed to be very strong in comparison with other peaks in the multi-plot shown in Fig.2.

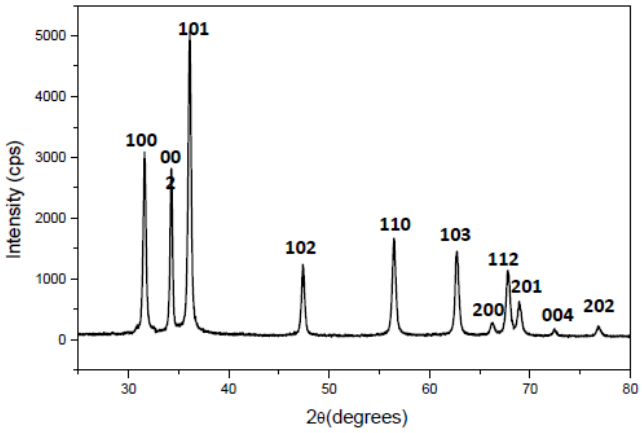

Fig. 2: XRD pattern of ZnO NPs nanoparticles

The various orientations indicate that it is polycrystalline in nature. The location of the peaks was compared to literature values and the presence of zinc oxide particles was confirmed. The average crystallite size was calculated using Debye-Scherrer's formula (Table 5).

Table 5: Average crystallite size of ZnO NPs

Method		Crystallite size
Chemical synthesis		20.73 nm
Green synthesis	*C. gigantea*	21.42 nm
	F. vulgare	20.73 nm
	M. koenigii	17.05 nm

3.2.3. Scanning Electron Microscope (SEM) Analysis

The SEM image of ZnO powder samples obtained by chemical and green synthesis (Fig. 3) showed nanoflakes structure. The Nanoflakes length ranges from 50-200 nm and thickness ranges from 10-40 nm. The evenly

distributed nanoflakes were observed in sample obtained through chemical synthesis whereas aggregated flakes were seen in the samples obtained through green synthesis. All the nanoflakes are of uniform shape with a diameter range of 11-25 nm.

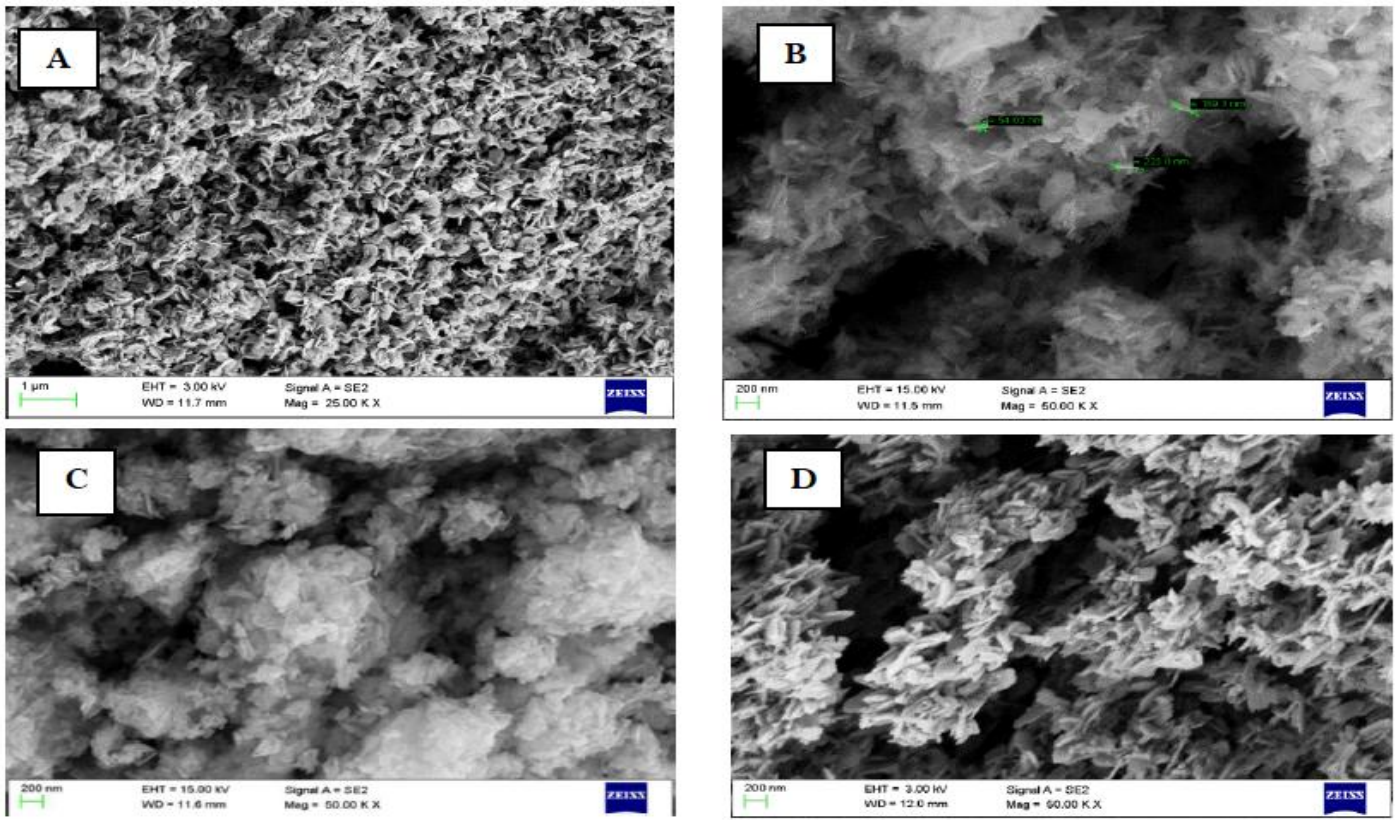

Fig. 3: SEM images of ZnO NPs obtained by (A) chemical synthesis and (B) green synthesis *C. gigantea*, (C) *F. vulgare*, (D) *M. koenigii*

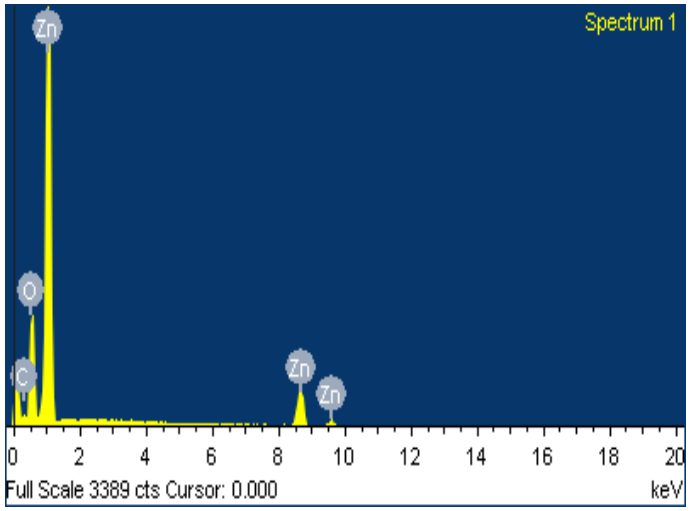

Fig. 4: EDS spectrum of synthesized ZnO NPs

3.2.4. Energy Dispersive X-Ray analysis

EDX analysis was done to determine the elemental composition and stereochemistry of the synthesized ZnO NPs by chemical and green synthesis (using *C. gigantea*, *F. vulgare* and *M. koenigii*). Zinc and oxygen signals detected that the synthesized nanoflakes are in pure state. Three single peaks of Zn were observed, one between 0-2 and two between 8-10 whereas one peak of O was found between 0-2. These results correlate with the already reported results in which similar peaks have been observed in ZnO NP synthesis using *C. gigantea* leaf extract [21]. Presence of carbon can be due to carbon tape used during analysis (Fig.4).

4. CONCLUSION

In the present study, the extracts of *C. gigantea*, *F. vulgare* and *M. koenigii* were used for the synthesis of zinc oxide nanoparticles. On analyzing the antimicrobial activity of these nanoparticles which were prepared by green synthesis, against *E. coli*, *S. aureus*, and *B. cereus* and comparing it with the antimicrobial activity of their synthetic counterpart, it was found that the nanoparticles prepared using the plant extracts had better ability to inhibit all the three bacteria. In fact, the crude plant

extracts themselves have antimicrobial activity and, in many cases, proved to be better than the nanoparticles. It was observed that the crude plant extract of *F. vulgare* and *M. koenigii* did not inhibit the growth of *S. aureus* but when the same plant extracts were used for the synthesis of zinc oxide nanoparticles, the nanoparticles showed antimicrobial activity.

Green-synthesized nanoparticles have a potential role in the form of Nano fertilizers. In the current scenario, this work may help in the future development of Nano nutrients for plant growth and development. In the Thar Desert, Rajasthan *Calotropis* plants are available throughout the year and they can easily survive in high temperature without any irrigation. So, the bioresources of Thar Desert can effectively be used for the biosynthesis of nanoparticles and can further be used as nano-nutrients [24, 25]. The colloidal solution of zinc oxide nanoparticles can be used as fertilizer for fast growth and development in tree seedlings. One of the advantages of nano fertilizers is that they can be used in very small amounts as compared to the synthetic fertilizers. Nanopowders can be used as fertilizers and pesticides as well for increased agricultural crop productivity.

Further, various changes in the green synthesis process, such as the incubation time, temperature, shaking velocity, centrifugation speed can be altered and its effect on the shape and size of the nanoparticles can be studied and consequently, the variation in the antimicrobial activity of the nanoparticles thus synthesized can be analyzed.

Nanoparticles prepared by green synthesis can be used in various fields as an alternative to chemically synthesized nanoparticles as they are more efficient and eco-friendly.

5. REFERENCES

1. Qureshi R, Bhatti GR, Memon RA. *Pak. J. Bot.,* 2010; **42(2)**:839-851.
2. Ayyanar M, Ignacimuthu S. *Journal of Ethnopharmacology*, 2011; **134(3)**:851-864.
3. Ngwendson JN, Bedir E, Efange SMN, Okunji CO, Iwu MM, Schuster BG, Khan IA. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, 2003; **58(8):**587-589.
4. Alma MH, Mavi A, Yildirim A, Digrak M, Hirata T. *Biological & Pharmaceutical Bulletin,* 2003; **26(12)**:1725-1729.
5. Klausmeyer P, Chmurny GN, McCloud TG, Tucker KD, Shoemaker RH. *Journal of Natural Products*, 2004; **67(10)**:1732-1735.
6. Sohn HY, Son KH, Kwon CS, Kwon GS, Kang SS. *Phytomedicine*, 2004; **11(7-8)**:666-672.
7. Machado TB, Pinto AV, Pinto MCFR, Leal IC, Silva MG, Amaral ACF, Kuster RM Nettodos Santos KR. *International Journal of Antimicrobial Agents*, 2003; **21(3)**:279-284.
8. Kasture MB, Patel P, Prabhune AA, Ramana CV, Kulkarni AA, Prasad BL. *Journal of Chemical Sciences*, 2008; **120(6)**:515-520.
9. Agarwal H, Kumar SV, Rajeshkumar S. *Resource-Efficient Technologies*, 2017; **3(4)**:406-413.
10. Prasad R, Bhattacharyya A, Nguyen QD. *Frontiers in Microbiology*,2017; **8**: 1014
11. Dimkpa CO, Bindraban PS. *Journal of Agricultural and Food Chemistry*, 2018; **66(26)**:6462-6473.
12. Song JY, Kim BS. *Bioprocess and Biosystems Engineering*, 2008; **32(1)**:79-84.
13. Meltzer S, Resch R, Koel BE, Thompson ME, Madhukar A, Requicha AA, Will P. *Langmuir*, 2001; **17(5)**:1713-1718.
14. Vidya C, Hiremath S, Chandraprabha MN, Antonyraj ML, Gopal IV, Jain A, Bansal K.*Int J CurrEngTechnol*, 2013; **1(1)**:118-120.
15. Alagumuthu G, Kirubha R. *International Journal of Nanomaterials and Biostructures*, 2012; **2(3):**30-33.
16. Dias ACP, Marslin G, Selvakesavan RK, Franklin G, Sarmento B. *International Journal of Nanomedicine,* 2015; **10**:5955-5963.
17. Sudhasree S, Shakila Banu A, Brindha P, Kurian GA. *Journal Toxicological and environmental chemistry*, 2014; **96(5)**:743-754.
18. Shaik MR, Albalawi GH, Khan ST, Khan M, Adil SF, Kuniyil M, et al. *Molecules,* 2016; **21(11):**1478.
19. Kumar V, Yadav SK. *Journal of Chemical Technology & Biotechnology*, 2009; **84(2)**:151-157.
20. Talam S, Karumuri SR, Gunnam N. *ISRN Nanotechnology*, 2012; 1-6.