



GREEN SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES FROM *CLADOPHORA GLOMERATA* AND ITS ANTIFUNGAL ACTIVITY AGAINST SOME FUNGAL ISOLATES

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Abstract

The aim of this study is to Green synthesize of ZnO nanoparticles using the algal extract of *Cladophora glomerata* and evaluate its antifungal activity against some fungus isolates. Dried algal biomass was used to prepare the pure algal extract and added with 0.01 mM ZnNO₃.6H₂O, and the color change was noted and recorded by ultraviolet (UV)-vis spectrophotometer. The morphological characteristics were analyzed by scanning electron microscopy (SEM). Crystalline structure was analyzed by EDX. Antifungal activity was performed by evaluated the percentage inhibition growth method against some fungus isolates. white color Formation at 15 minutes indicates the production of ZnO nanoparticles by the extract of alga *Cladophora glomerata*. A film appear bigger in wavelengths than 200nm, which is permitted with the optical properties of ZnO semiconductor in the (UV-Visible). SEM microscopy showing that the ZONES are about 14.39 nm to 37.85 nm in size with a combination of many forms i.n. Nanoparticles of the flowers are obviously noted and predominantly spherical which were well dispersed and the aggregation of the particles could be seen. ZnO nanoparticles show high inhibition activity against *Fusarium oxysporum* and *Rhizoctonia solani*. *Cladophora glomerata* -mediated synthesis of ZnO nanoparticles shows rapid and eco-friendly silver ion reduction process. Therefore, this present study elucidates that algae-mediated Green synthesized Zinc Oxide nanoparticles have antifungal activity against phytopathogenic fungi, so it can be developed as a novel medicine for human welfare in biomedical applications in the near future.

Key words : *Cladophora glomerata*, ZnO nanoparticles, scanning electron microscopy, Antifungal activity, Green synthesis.

Introduction

Synthesis of nanomaterials is one of the most demanding and highest increasing sectors of nanotechnology. Nanotechnology in biological sciences receives the possibility of a global variety of medical uses at molecular and cellular levels Singh *et al.*, (2010). Metal nanoparticles biocompatible play an important role in the biological applications due to their optical properties such as surface plasmon resonance (SPR) and fluorescence, which is used for bioimaging and biosensing Barwal *et al.*, (2011). The NPs are synthesized both inside the living algae and within the sundried biomass, several researchers proposed various mechanistic approaches to understand the hidden pathway behind the green synthesis of ZnO NPs Frattini *et al.*, (2005). Synthesis of nanomaterials by reduced Zn ions are stable and colloidal dispersion in water or organic solvents Tao *et al.*, (2006). Zinc is known

to be safe to human and produces little-to-no allergic reactions when tested for curing various diseases as a natural material. Physical, chemical, and biological methods are available for nanoparticle synthesis. All physical and chemical methods may effectively synthesize pure, well-defined nanoparticles, these methods are expensive and potentially unsafely to the environment. Use of biological mass such as bacteria, fungi, yeast, plant extract or plant biomass, and algae extract or biomass could be an alternative to these methods for the synthesis of nanoparticles in an eco-friendly manner, safely, less time consuming, and low cost Mohanpuria *et al.*, (2008). Green synthesis methods reduce the hazardous waste in the context of global efforts Kaler *et al.*, (2010). Nanoparticles Green synthesized using algae were quite stable in solution Singaravelu *et al.*, (2007), eco-friendly, and safely to a vast extent because the algae are widely distributed, easily available, and safe to handle with a range of metabolites. Moreover, the proposed green

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synthesis method is an advance of bioscience, high yielding, low-cost technology, and non-toxic to vertebrate animals. Algae are the sustainable resources in the water ecosystem which are used as food, feed, and medicine Seenivasan *et al.*, (2012). *Cladophora glomerata* is fresh and marine green algae classified under Chlorophyceae class, isolated from the AL-Zawraa park in Baghdad -Iraq during October 2018. Methanolic extract of *Cladophora glomerata* has potential antimicrobial activity Yousif (2014) and Al-maoula and Dwaish (2018). This present investigation illustrates green synthesis of silver nanoparticles using the extract of *Cladophora glomerata* and characterized by ultraviolet (UV)-vis spectrophotometer. Morphological and elemental analysis was carried out by scanning electron microscopy (SEM), and energy-dispersive X-ray (EDX), then study its antifungal activity against some fungal isolates.

Materials and Methods

Collection and Preparation of Sample

Samplings were carried out from river in AL-Zawraa park in Baghdad-Iraq during October 2018, Samples of *C. glomerata* were collected manually from the rock. The harvested macro algae were stored in plastic bags and transported to the laboratory. Voucher specimen of species were pressed and stored in 5% formalin for identification according to Prescott (1962). Biomass was rinsed with fresh water to eliminate other materials such as sand, shells, etc. The macroalgae were stored in the laboratories and dried at 50°C under ventilation in an oven and then grounded to powder form by the blender.

Preparation of algae extracts

Algae samples were washed with distilled water to remove the adhering particles. They were dried in the shaded place. The dried algae were powdered, weighed and stored in clean containers all algae extraction was done according to Harbone, (1984). The hydro ethanol extraction method was used.

Green synthesis of ZnO nanoparticles

In the typical synthesis of ZnO nanoparticles, Dropwise was added 100 ml of ethanol algal extract heated at 50 C for (5,10 and 15 min) and 500 ml of 0.01, 0, g/ml of zinc nitrate solution (0.297, g of zinc nitrate dissolved in 100 ml of distilled water). This was then put in a PH-controlled magnetic stirrer for 15min. Adding (NaOH 3, 4 and 6ml to change the PH 10). Then the precipitate was formed at 16 000 rpm for 15min.

Purification and characterization of synthesized ZnO nanoparticles

Bio-reduction of zinc nitrate in aqueous solution was

monitored (Color change) by double beam UV-vis spectrophotometer at different wavelength region from 320 to 700 nm. The bio-reduced zinc nitrate was purified for further characterization studies by putting it in centrifugation at 16,000 rpm for 15 minutes. The pellet was collected and washed in sterile double-distilled water to get free of any biological molecule present in the algal extract. The purified silver nanoparticle was morphologically characterized by using the SEM. Elemental analysis and crystalline structure of nanoparticles were examined by EDX Rajeshkumar *et al.*, (2017).

Antifungal assay of ZnO nanoparticles

Two phytopathogenic fungal isolates used in the present study were obtained from department of biology, faculty of science, University of Baghdad-Iraq which were:

Fusarium oxysporum and *Rhizoctonia solani*

Assessment of antifungal

The antifungal activity of the ZnO nanoparticles on various fungal strains was assayed by mix the silver nanoparticles with Potato Dextrose Agar (PDA) medium and the fungal mycelia were inoculated to grow.

Percentage inhibition of mycelial growth in each case was calculated by using following formula:

$$\% \text{ inhibition} = 100 \times (C - T) / C$$

Where

C= Fungal mycelial biomass / dry weight in control.

T= Fungal mycelial biomass / dry weight in control in various test concentration Mondall, *et al.*, (2009).

Results and Discussion

Morphological Structure of algae

Cladophora is a branching, benthic, attached, filamentous green macro alga that forms a moss like structure (Fig. 1). This alga is worldwide distribution in marine as well as freshwater habitats. Filaments are often longer, forming "streamers" which can be in excess of 1-2m length (Fig. 2), Coarse, dark-green to brownish-green, branching, hair-like filaments with cross walls separating segments; each segment has more than one nucleus. *Cladophora* germination and growth usually requires hard substrates for attachment, such as rocks, a further physical requirement for *Cladophora* growth is water motion, that agree with Fish and Fish (1989) and Gibson, *et al.*, (2001).

Visual examination

Green synthesis of ZnO nanoparticles was

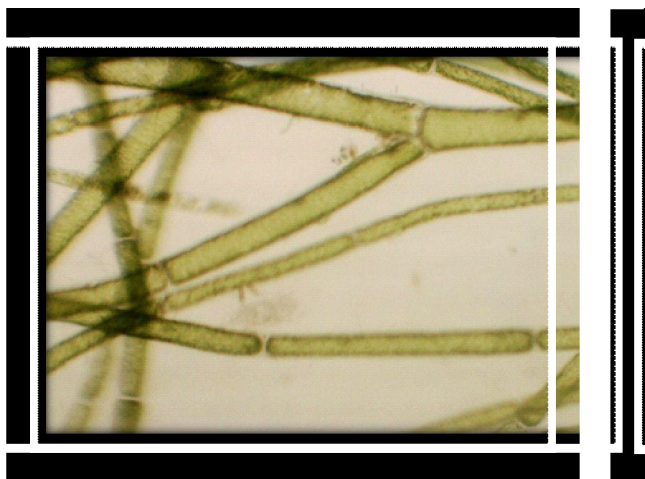


Fig. 1: Filaments of *Cladophora glomerata* showing the branching (40X).



Fig. 2: *Cladophora glomerata* Showing the Hair Shape in Nature.

preliminarily identified by color change during exposure of algal extract into aqueous solution of Zinc ions. Initially, the ZnO nanoparticles formation occurred within 15 minutes which was identified by color change of aqueous solution that exhibited yellowish brown color and changed into white color (Fig. 3). The intensity of color change was directly proportion to the incubation time. After 20 minutes, there is no significant color change, indicating the saturation of the reaction of ZnO nanoparticles formation.

ZnNO₃, 15 minutes

Synthesis of ZnO nanoparticles was visually identified by color change. By adding the algal extract into the Zinc ions solution, color was changed from pale green to brown at the incubation time of 20 minutes due to the excitation of SPR of nanoparticles in the reaction mixture. In this study, we have synthesized silver nanoparticles rapidly at 20 minutes using the extract of *Cladophora glomerata*. Similarly, Kumar *et al.*, (2012).

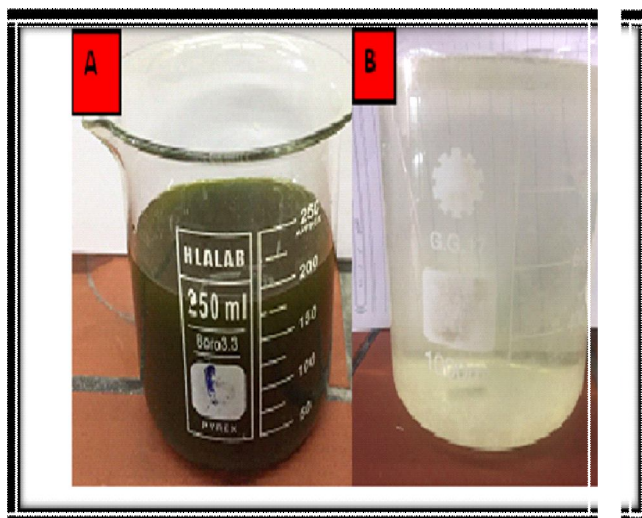


Fig. 3: Visual indication of synthesized ZnO nanoparticles (A) before adding ZnNO₃; (B) after adding ZnNO₃, 15 minutes.

Synthesized silver nanoparticles within 20 minutes by using the extract of *Sargassum tenerrimum* and Sosa *et al.*, (2003), synthesized gold nanoparticles after the 24 hrs incubation time using *Sargassum wightii*.

UV-vis spectrophotometer

UV-vis spectrophotometer analysis ZnO nanoparticles formation in the algal extract and Zinc nitrate solution mixture has been recorded as different functional time. ZnO NPs as-prepared by a hydrothermal method using green alga, the results observed optimum that between from (290-360) as shown in fig. 4

UV-vis spectroscopy analysis it is very important tool for optical and structural characterization of silver

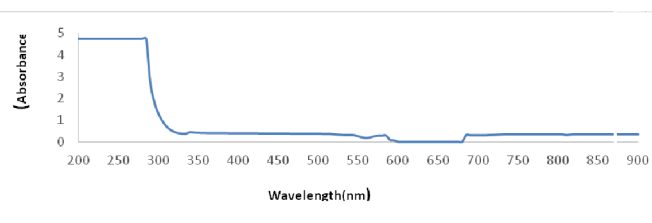


Fig. 4: Ultraviolet-visible spectra recorded the formation of nanoparticles in the reaction mixture of algal extract and ZnNO₃ intervals showing the peak at 290-360 nm.

nanoparticles and it is an indirect method to determine the synthesis of nanoparticles by reduction of zinc nitrate to ZnO nanoparticles in the aqueous solution. The optical property of silver nanoparticles depends mainly on size and shape Sosa *et al.*, (2003). There is no specific peak change in the reaction mixture as shown in UV-vis spectrophotometer. No change in peak due to the excitations of SPR of nanoparticles indicates that nanoparticles are in spherical structure which was further

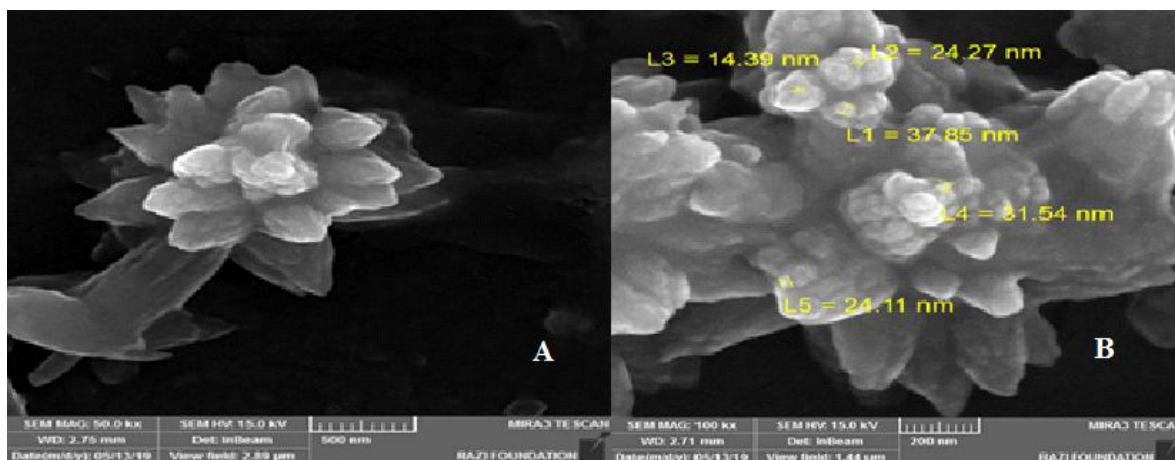


Fig. 5: Scanning electron microscope image of ZnO nanoparticles synthesized from *Cladophora glomerata* extract.

confirmed by SEM image. Similarly, Shankar *et al.*, (2003). reported that geranium leaf-assisted ZnO nanoparticles formed the SPR band at 290-360 nm due to the excitation of longitudinal plasmon vibrations.

SEM and EDX analysis

SEM image refer to the structure of ZnO nanoparticles (Fig. 5). SEM microscopy showing that the ZONES are about 14.39 nm to 37.85 nm in size with a combination of many forms in. Nanoparticles of the flowers are obviously noted and predominantly spherical which were well dispersed and the aggregation of the particles could be seen. SEM images of ZnO NPs as-prepared by a hydrothermal method using Algae green extract under con=0.01 pH=10 at 50°C for 15min, sothat in image (A) with scale bar 500nm, and (B) with scale bar 200nm. Displayed ZnO NPs synthesis by *Cladophora glomerata* extract under optimized physical circumstances.

X-ray diffraction is a powerful way to determine the composition of crystalline materials. XRD provides data on sample parameters for the crystalline phase, orientation, and network. Explore XRD measurements in the Nanotechnology Laboratory College of Education Ibn al-Haytham direction of cultured samples from ZnO. The powder XRD patterns were recorded for the identification of phases exhibited by the synthesized materials. The synthesized ZnO NPs in Fig. 6 showed more diffraction strong peaks at 2θ values of 31.62°, 34.32, 36.12°, 45.63°, 47.46°, 56.84°, 62.78° and 67.86° which correspond to (100), (002), (101), (102), (110), (103) and (112) planes of the face-centered.

Also We thought successfully synthesized ZnO NPS by using procedure the reduction of Zn⁺ using a hydrothermal extract of *Cladophora glomerata* to synthesize ZnO NPs. The chemical compounds present in the methanolic extract of algae and many plant extracts,

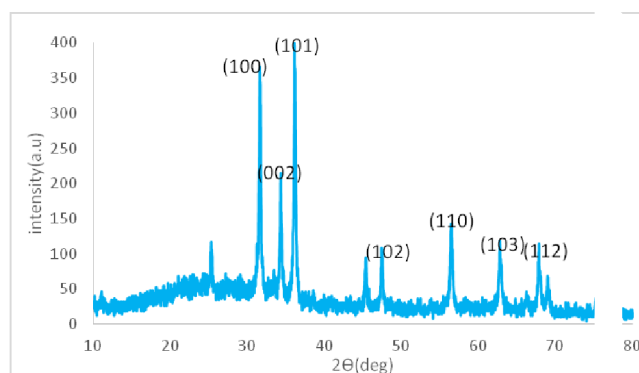


Fig. 6: Energy-dispersive X-ray analysis of *Cladophora glomerata* -mediated synthesized ZnONPS nanoparticles.

such as terpenoids, glycosides, acetylenes and phenols, Engvild (1986) and An *et al.*, (2008) were supposed to be responsible for the reduction reaction to synthesize ZnO NPs in this procedure. The diffractions peaks obtained were in strong agreement with hexagonal geometry of ZnO NPs. The narrow and sharp diffraction peaks demonstrated highly crystalline nature of ZnO NPs. Our results are similar to previous results reported Zak *et al.*, (2011)

Senthilkumar, *et al.*, (2014). The morphological characterization of NPs was done with SEM analysis. The different types of irregular shapes and morphology were noticed, confirming synthesis of NPs. Our results are similar to previous results reported Sangeetha, *et al.*, 2011 and Senthilkumar, *et al.*, (2014).

Antifungal activity

The antifungal activity of ZnO NPs was assayed by validity of experimental results of percentage inhibition obtained against fungi isolated. Table 1 shows the inhibitory of silver nanoparticles against fungi isolated. It shows more high antifungal activity due to their surface area

and size of nanoparticles contacting with environment. Table 1 and Fig. 7 show mean of inhibition growth was caused by ZnO NPs against *Rhizoctonia solani* and *Fusarium oxysporum* with 63.4 and 88.9 respectively, at concentration of ZnO NPs 100 mg/ml.

ZnO nanoparticles have been widely used for many applications. In our study, the antifungal activity of ZnO nanoparticles was evaluated. The activity of ZnO nanoparticles as antimicrobial mainly depends on the size and shape of the nanoparticles. Activity inhibition of ZnO

Table 1: Antifungal activity of ZnO NPs nanoparticles synthesized from algal extract *Cladophora glomerata*.

Algal Extracts	ZnO NPs Concentration (100mg/ml)	Control
<i>Rhizoctonia solani</i>	88.9±0.3	0.00±0
<i>Fusarium oxysporum</i>	63.4±0.5	0.00±0



Fig. 7: The effect of ZnO nanoparticles on *Fusarium oxysporum*.

nanoparticles against fungi was differing from bacteria because the fungal cell wall greatly differs from the bacterial cell wall. The fungal cell wall is not easily affected by antifungal materials. However, the ZnO nanoparticle has great antimicrobial activity. The possible mechanisms of antifungal action of nanoparticles are cell membrane disruption, cell division inhibition, and cell wall formation inhibition. Fungal cell wall is composed of ergosterol, the ZnO nanoparticles disrupt the cell membrane by inhibiting ergosterol synthesis or binding with sterol, forming pits and causing the membrane permeability to become leaky leads to cell death Jain *et al.*, (2009) and Vidya, *et al.*, 2013

ZnO nanoparticles may affect the mitotic spindle cell (fungal cell) division by targeting the microtubule and also inhibit DNA transcription Geoprincy *et al.*, (2011).

The ZnO nanoparticles are positively charged, will interact with negatively charged carboxylic groups and

cell wall of mycelia to inhibit the growth of normal budding fungi Santhoshkumar *et al.*, (2011) and Al-maoula and Dwaish (2018). Thus, it was conclusion that green synthesized ZnO nanoparticles from algae has a great potential activity against fungi.

References

- An, J., M. Zhang, S. Wang and J. Tang (2008). Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles-PVP. *LWT-Food Sci Technol.*, **41**:1100-1107.
- Barwal I, P. Ranjan, S. Kateriya and S.C. Yadav (2011). Cellular oxido-reductive proteins of *Chlamydomonas reinhardtii* control the biosynthesis of silver nanoparticles. *J. Nanobiotechnology*, **9**: 56.
- Engvild, K.C. (1986). *Phytochemistry*, **25**: 781.
- Fish, J.D. and S. Fish (1989). A student's guide to the Seashore. Unwin Hyman Ltd, London.
- Fratini, A., N. Pellegri, D. Nicastro and O. de Sanctis (2005). Preparation of amine coated silver nanoparticles using triethylenetetramine. *Mater Chem. Phys.*, **94**:148-52.
- Geoprincy, G., P. Saravanan, N.N. Gandhi and S. Renganathan (2011). A novel approach for studying the combined antimicrobial effects of silver nanoparticles and antibiotics through agar over layer method and disk diffusion method. *Dig. J. Nanomater Biostruct.*, **6**(4):1557-65.
- Gibson, R., B. Hextall and A. Rogers (2001). Photographic Guide to the Sea and Shore Life of Britain and North-west Europe. Oxford University Press, Oxford.
- Harbone, J.B. (1984). *Phytochemical methods* Chapman and hall. 2nd ed. New York, 288.
- Jain, D., D.H. Kumar, S. Kachhwaha and S.L. Kothari (2009). Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. *Dig. J. Nanomater Biostruct.*, **4**(3): 557-63.
- Kaler, A., N. Patel and U.C. Banerjee (2010). Green synthesis of silver nanoparticles. *Curr. Res. Inf. Pharm. Sci.*, **11**(4): 68-71.
- Kumar, P., S.S. Selvi, A.L. Prabha, K.P. Kumar, R.S. Ganeshkumar and M. Govindaraju (2012). Synthesis of silver nanoparticles from *Sargassum tenerrimum* and screening phytochemicals for its anti-bacterial activity. *Nano Biomed. Eng.*, **4**(1):12-6.
- Maarb, S., Al-maoula and Ahmed, S. Dwaish (2018). Anti-Dermatophytes Activity of some Algal Extracts Isolated From Baghdad City-Iraq. *Research J. Pharm. and Tech.*, **11**(12): December
- Mohanpuria, P., N.K. Rana and S.K. Yadav (2008). Biosynthesis of nanoparticles. Technological concepts and future applications. *J. Nanopart Res.*, **10**(3):507-17.
- Mondall, N.K., A. Mojumdar, S.K. Chatterje, A. Banerjee, J.K. Datta and S. Gupta (2009). Antifungal Activities and

- Chemical Characterization of Neem leaf Extracts on the Growth of Some Selected Fungal Species in vitro Culture Medium. *J. Applied Sci. Environ. Manage.*, **13**: 49-53.
- Prescott, G.W. (1962). algae of the western great lakes area. 2nd ed. W. C. Brown. Co. Dubuque, Iowa 997.
- Rajeshkumar, S., C. Malarkodi and S. Venkat kumar (2017). synthesis and characterization of silver nanoparticles from marine brown seaweed and its antifungal efficiency against clinical fungal pathogens, **10(2)**.
- Sangeetha, G., S. Rajeshwari and R. Venkatesh (2011). Green synthesis of Zinc Oxide nanoparticles by Aloe barbadensis Miller leaf extract: Structure and optical properties. *Mater. Res. Bull.*, **46**: 2560-2566.
- Santhoshkumar, T., A.A. Rahuman, G. Rajakumar, S. Marimuthu, A. Bagavan and C. Jayaseelan (2011). Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. *Parasitol. Res.*, **108(3)**: 693-702.
- Seenivasan, R., M. Rekha, H. Indu and S. Geetha (2012). Antibacterial activity and phytochemical analysis of selected seaweeds from Mandapam coast, India. *J. Appl. Pharm. Sci.*, **2(10)**: 159-69.
- Senthilkumar, S.R. and T. Sivakumar (2014). T. Green tea (*Camellia Sinensis*) mediated synthesis of zinc oxide (ZnO) nanoparticles and studies on their antimicrobial activities. *Int. J. Pharm. Pharm. Sci.*, **6**: 461-465.
- Shankar, S.S., A. Ahmad and M. Sastry (2003). Geranium leaf assisted biosynthesis of silver nanoparticles. *Biotechnol. Prog.*, **19(6)**: 1627-31.
- Singaravelu, G., J.S. Arockiamary, V.G. Kumar and K. Govindaraju (2007). A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii* Greville. *Colloids Surf B Biointerfaces*, **57(1)**: 97-101.
- Singh, M., S. Manikandan and A.K. Kumaraguru Nanoparticles (2010). A new technology with wide applications. *Res. J. Nanosci. Nanotechnol.*, **1(1)**: 1-11.
- Sosa, I.O., C. Noguez and R.G. Barrera (2003). Optical properties of metal nanoparticles with arbitrary shapes. *J. Phys. Chem.*, **107(26)**: 6269-75.
- Tao, A., P. Sinsermuksamol and P. Yang (2006). Polyhedral silver nanocrystals with distinct scattering signatures. *Angew Chem Int Ed Engl*, **45(28)**: 4597-601.
- Vidya, C., S. Hiremath, M.N. Chandraprabha, M.A.L. Antonyraj, I.V. Gopal, A. Jain and K. Bansal (2013). Green synthesis of ZnO nanoparticles by *Calotropis gigantea*. *Int. J. Curr. Eng. Technol.*, **1**: 118-120.
- Yousif.Dina, Y. M. (2014). Activity of Some Macro-algae Extracts Against Phytopathogenic Fungi. PhD. thesis, College of Science, University of Baghdad.
- Zak, A.K., W.H. Abd Majid, M.E. Abrishami and R. Yousefi (2011). X-ray analysis of ZnO nanoparticles by williamson-hall and size-strain plot methods. *Solid State Sci.*, **13**: 251-256.