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COMPARATIVE ANTIMICROBIAL, PHYTOTOXIC AND HEAMAGLUTINATION POTENTIAL OF *ERIOBOTRYA JAPONICA* LEAF EXTRACT AND ITS ZINC NANO - PARTICLES

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Abstract

The aim of the current study was to synthesize Zinc nanoparticles (ZnNPs) of *Eriobotrya japonica*, their characterization using standard procedures and its screening for various pharmacological activities; antibacterial, antifungal, phytotoxic and heamaglutination in comparison with the methanolic, ethanolicand Ethyl acetate (EtOAc) fractions. The UV-Visible absorption spectra of ZnNPs showed peaks at 455 nm and Fourier Transform Infrared spectroscopy (FTIR) confirmed the presence of various functional groups such as, hydroxyl, amide, carbonyl, aldehyde, and alkynes. The Scanning Electron Microscope (SEM) analysis showed that the particle size was between 30-60 nm with spherical shape. The presence of Zinc along with other elements, such as C, O, S, Si, and K, was confirmed by Energy Dispersive X-ray (EDX), andthe mean size was 33nm as indicated in X-ray diffraction (XRD) spectra. The results of percent antibacterial activity for the methanolic, ethanolic and EtOAc fractions of *E. japonica* against *E. coli* was 62, 59.2, 44.4, *P. aeruginosa* 44.4, 40.7, 37, *S. aureus* 69.2, 46.1, 50, *A. baumannii* 52.1, 43.4, 0, *M. morganii* 59, 40.9, 68, MRSA 73.6, 52.6, 0 and *P. vulgaris* 60, 52, 64. The Zn NPs exhibited significant antibacterial activity against MRSA (94%) and *P. vulgaris* (92%), good against *S. aureus* (73%) and *M. morganii* (63.6%), moderate against *E. coli* (48.1%) and *A. baumannii* (43.4%) and low against *P. aeruginosa* (37%), respectively. The methanolic, ethanolic and EtOAc fractions of *E. japonica* showed low (<24%) and no antifungal activity against the tested fungal pathogens. The antifungal activity of Zn NPs against the test fungal pathogens was; *A. parasiticus* (30%), *P. notatum* and *A. niger* (40%), *P. chrysogenum* (55%), *H. pseudocrispula* (60%) while it was inactive against *V. longisporum*. The methanolic, ethanolic and EtOAc extract of *E. japonica* showed 50, 50 and 60% growth regulation at 1000, 50, 40 and 60% at 100 µg/ml, while at 10 µg/ml, 40, 20 and 50%, respectively, against *Lemna minor*. Phytotoxic activity of ZnNPs against *L.minor* at 1000, 100 and 10µg/ml was 60 50 and 30%, respectively. The results indicated that the selected plants lack phytolectin as no heamaglutination was observed.

Key words: *Eriobotrya japonica*, ZnNPs, UV-VIS, SEM, XRD, EDX, antifungal, antibacterial, phytotoxic, heamaglutination.

Introduction

Pakistan has been blessed with a variety of medicinal plants and enjoys a rich biological diversity (Khalil *et al*., 2014). The country has about 6000 flowering plant species, among these 2000 have been recognized to have therapeutic potential but a large proportion of them are not yet discovered for therapeutic potential on scientific ground (Agar *et al*., 2008; Shinwari *et al*., 2006). These plants possess high contents of biologically active compounds as have been reported in many studies (Shinwari & Nasim, 2014). The world of medicine is reverting back to medicinal plants because of their fewer or no side effects (Xiao, 2015; Shinwari & Gilani, 2003). These medicinal plants are used traditionally for the treatment of various diseases. Some of the examples include; *Achyranthes espera* is used to treat abdominal pain and bowel complaints, *Justicia adahatoda, Adhatoda vasica* has been found effective against diabetes, *Asparagus adescendens*is used as a tonic and to treat diarrhoea and dysentery while *Althea rosea* is active against jaundice and liver problems (El-Abhar & Schaalan, 2014).

The current study is also focusing on one such important medicinal plant *Eriobotrya japonica*, a sub-tropical fruit tree belonging to family *Rosaceae* that is used traditionally for the treatment of various diseases (Chan-Yeung, 2000). *E. japonica*is cultivated mainly in China, Japan, Pakistan, India, Turkey, Spain, United States, Venezuela, Brazil and Australia (Lee *et al*., 2005). The production of *E. japonica* around the world is 549,220 tons, Spain (43,300 tons) and China (460,000 tons) being the major producers and are followed by Japan, India and Pakistan (Lin, 2007, Chan-Yeung, 2000). Fruit and leaves of *E. japonica* are reported to have a range of medicinal applications (Lead & Smith, 2009). *Feitai*, a compound formula, consisting of a number of herbs including *E. japonica* leaves is used for the treatment of pulmonary tuberculosis (Derfus *et al*., 2004). Leaves of *E. japonica*are used traditionally as an expectorant, anti-inflammatory and also to treat skin diseases. *E. japonica* have hypoglycemic and anti-hyperlipidaemic properties due to presence of ursolic and oleanolic acid in the leaves. Leaves of *E. japonica*also have anti-tumor properties (Hoet *et al*., 2004, Kleiner & Hogan, 2003).

Nanotechnology is one of the most attractive and important field of advance material sciences. In the last few years, metal nanoparticles have been studied mostly because of their diverse chemical and physical properties, leading to their extensive applications in biotechnology, electronics and optics (Zeng *et al*., 2007). The reduction of metal salts to nano particles using plant extract has got significant importance in the last 30 years due to the presence of reducing agents in plants. An extract of *Dioscorea bulbifera* tubers has been used to produce silver and gold nanoparticles. These nanoparticles were used in combination with different antibiotics and it was found to have significant antibacterial activity against number bacteria; *Acinetobacter baumannii, Escherichia coli* and

Pseudomonas aeruginosa. Silver nanoparticles of banana peels exhibited good antifungal activity against *Candida lipolytica* and *Candida albicans* (Ghosh *et al*., 2011, Ghosh *et al*., 2012). Keeping in view the traditional medicinal properties of *E. japonica* and nanoparticles, the current study was aimed to synthesize ZnNPs of *E. japonica*, their characterization and its screening for various pharmacological activities; antibacterial, antifungal, phytotoxic and heamaglutination in comparison with the methanolic, ethanolic and EtOAc fractions.

Material and Methods

Extraction: The shade dried leaves of the selected plant were grounded to powder by a grinder. The powder was extracted using methanol for 15 days, twice, with occasional shaking at room temperature. Rotary evaporator was used to concentrate all the filtrates to obtain crude methanolic extract. The same method was followed for ethanolic and EtOAc extracts (Ahmad *et al*., 2009).

Synthesis of Zinc nanoparticles: The dried leaves of *E. japonica* was extracted with 500ml distilled water by heating for half an hour. The solution containing 95 mL of Zn SO_4. $5H_2O$ (1Mm solution) and 5mL of extract was stirred at 75ºC on water bath for 4 hours. The indication for the formation of Zn NPs was the change in color of the solution from dark green to light brown. This process was repeated many times to get enough solution, which upon centrifugation resulted in ZnNPs (Elumalai *et al*., 2015).

Characterization of Synthesized ZnNPs and CuNPs

UV-Vis Spectrophotometer: A dual beam UV–1100 Shimadzu spectrophotometer was used to confirm the formation of ZnNPs. Aqueous extracts of *E. japonica* were diluted with distilled water and were scanned in the wavelength range of 300–600 nm to observe peaks characteristic for ZnNPs.

Fourier transform infrared spectroscopy: The FTIR spectrum of ZnNPs from *E. japonica* were obtained by FTIR Prestige–21 Shimadzu Japan. The scanning was carried out from 3900–500 cm^{-1} at a resolution of 4 cm^{-1} for 10 scans and the obtained data was analyzed by IR solution software.

Scanning electron microscopy: For sample preparation, a minute quantity of ZnNPs was resuspended in distilled water and dried on a copper grid carbon with the help of hot air (50–60°C; 5 minutes). This leads to the formation of a thin film coating. Using gold coating through a sputter coater (SPI, USA) for 120s at 30 mA, the images were obtained by Scanning Electron Microscope (SEM JSM–5910 Japan).

Energy dispersive x-ray: Energy Dispersive X-Ray paired with SEM JSM–5910 JEOL, Japan, Model No. INCA200 was used to study the elemental composition of ZnNPs. On to dual sided gummed tape, the synthesized particles were spread and placed over a microscopic end made of aluminum (Al).

X-ray diffraction: In this study, an X-Ray Diffractometer (JDX–3532, JEOL Japan) was used for analyzing the shape and size of ZnNPs. A layer of ZnNPs was prepared by dipping a glass plate into the respective solution. The diffracted intensities were Figureured at 2θ angles at 0.02°/min with a time constant of 2s from 10˚ to 80˚. Measurement condition were; 30mA current, wavelength of 1.5418 A° in θ- 2 θ configureuration and voltage (V) ranging 20–40kV in the presence of Cu Kα radiation.

Thermogravimetric/differential thermal analysis: For determining the physical and chemical properties of the synthesized ZnNPs, Simultaneous Thermo-gravimetric and Differential Thermal Analysis (Shimadzu DTG–60/DTG–60A) was used. By increasing the temperature, mass gain and loss of ZnNPs was analyzed.

Antibacterial activity: The bacterial strains used in current research were; *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Morgella morganii, Acinetobacter baumannii, Proteusvulgaris* and Methicillin Resistant *Staphylococcus aureus* (MRSA). The nutrient broth and nutrient agar media were prepared and selected bacterial strains were grown in sterile nutrient broth at 37ºC for 24 hours. To make bacterial lawn, bacterial cultures from the nutrient broth was transferredto sterile nutrient agar plates. The wells were then made using sterile borer followed by introduction of 100µl test samples (methanolic, ethanolic and EtOAc) from the stock solution (3mg/ml of Dimethyl Methyl Sulfoxide (DMSO). The plates were incubated at 37ºC for 24 hours. Amoxicillin and DMSO were as positive and negative control respectively. After incubation, percent zone of inhibition was measured in comparison to positive control (Salmon *et al.*, 1998). To determine the antibacterial activity of ZnNPs, the method of Bashir *et al*., 2016 was followed.

Antifungal activity: The test samples were screened for their antifungal activity against *Aspergillus parasiticus, Penicillium notatum, Hemimycena pseudocrispula, Aspergillus niger, Verticillium longisporum* and *Penicillium chrysogenum* using a published protocol (Bashir *et al*., 2011).

The stock solutions of the test samples were prepared at 24mg/ml in sterile DMSO. Four mL of Sabouraud Dextrose Agar (SDA) in test tubes was autoclaved and when the temperature was about50ºC, 67µl from each stock solution were added to each test tube to make slants. To sterile test tubes, seven days old fungal cultures were inoculated followed by incubation at 28±1ºC for seven days, Miconazole and DMSO being positive and negative controls respectively. In comparison with negative control, results were measured in percentage as linear growth in the slanted test tubes.To determine the antifungal activity of ZnNPs, the method of Bashir *et al*., 2016 was followed.

Phytotoxic activity: *Lemna minor* assay was used to check the phytotoxic activity of test samples (Bashir *et al*., 2011). Stock solutions of the test samples were prepared at 20mg/ml of methanol and from stock solutions 1000, 100

and 10µl were introduced into flasks. After evaporation of methanol, 20mL of E-media and 16 healthy *L. minor* were introduced to each flask. Results were noted after incubation at 28±1ºC for seven days by counting the number of damaged plants. To determine the phytotoxic activity of ZnNPs, the method of Bashir *et al*., 2016.

Heamaglutination activity: The heamaglutination activity of test samples was carried out against ABO blood groups. Different dilutions; 1:2, 1:4, 1:8 and 1:16 in phosphate buffer were made from stock solutions (1mg/ 1ml of DMSO). In a test tube, two percent RBC's suspension and 1ml of each sample was incubated at 37ºC for 30 minutes. The test tubes were centrifuged after incubation and examined for rough and smooth button formation indicating positive and negative results, respectively (Ahmad *et al*., 2009). To determine the heamaglutination activity of ZnNPs, the method of Bashir *et al*., 2016 was followed.

Results

Characterization of Synthesized ZnNPs

Ultra violet-visible spectroscopy: The aqueous extracts were characterized by scanning the wavelength emission over the range of 300–600nm, to observe the absorbance peaks for ZnNPs. A broad peak was found at 455nm, clearly indicating the formation of ZnNPs (Fig. 1).

Fourier transform infrared spectroscopy: Strong absorption bands observed at 3298.28 and 3338cm^{-1}in the IR-spectrum are due to the stretching vibrations of hydroxyl (OH) or carboxyl (COOH) groups. The absorption band appeared at 1593$cm^{-1}$is believed to be associated with stretching vibration of C=C bonds. Absorption peaks at 1116.78 and 1386$cm^{-1}$are attributed ton-plane C—H bending vibration and tertiary alcohol. Due C—O stretching vibration, peak appeared at 1066 cm^{-1}, which indicates the presence of epoxy or alkoxy group. The peak due to C-N stretch (aliphatic amines) appeared at 1083 cm^{-1}while, at 653cm^{-1}mightbe attributed to Zn (Figs. 2-4).

Scanning electron microscopy: The surface morphology and particle size of the synthesized ZnNPs was determine, using three different magnification powers (X=15000, 30000 and 50000) where, enhancing the magnification lead to more prominent images of the ZnNPs. The size of ZnNPs ranged between 30–60nm (Figs. 5-7).

Energy dispersive x-ray: In the present work elemental Zn was confirmed from the EDX profile (Fig. 8) bya signal at 8.2 keV. Furthermore, the EDX profile of ZnNPs showed absorption bands at 0.6 keV for Magnesium, 1.2 for Silicon, 1.9 for Sulfur, 2.9 for Potassium, 2.2 for Chlorine and at 3.3 for Calcium (Figure. 8). The ZnNPs were found to comprise percent composition of Zn (42.20), C (41.10), Cu (47.06), Mg (0.79), S (3.66) and O (4.28), respectively by weight, while were found to contain 51.40, 44.18, 0.49, 1.72 and 1.01 percentage of above elements respectively. The EXD provided an intense peak for Zn, followed by C (Table 1).

X-ray diffraction: The indexing of the diffraction pattern is done and *Miller indices* (h, k l) were assigned to each peak, while degree of crystallinity of ZnNPs was determined from the peak intensity. Debye-Scherrer formula was used to estimate size of the ZnNPs and was found to be 33nm.

E. japonica was found effective to stabilizes and reduce the Zn ions to their corresponding ZnNPs, as showed by the XRD pattern (Fig. 9). Strongest peaks of ZnNPs were observed at around 2θ values of 38°, 44° and 69° corresponding to the crystallographic planes (002), (101) and (103,110), respectively. The intense peaks observed in XRD proved the presence of ZnNPs. Impurity peaks were observed in the XRD pattern. Peaks corresponding to ZnONPs were observed at 2θ=31° and 57° while average particle size was 33nm, based onthe angular position of Bragg peaks. Hence, this study confirms that extracts of *E. japonica* can reduce Zn ions into crystalline ZnNPs.

Thermogravimetric/differential thermal analysis: The results demonstrated that with an increase in temperature in the range of 40–1000ºC, there is a reduction in the mass and activity, thereby concluding that ZnNPs are thermally sensitive. The TG/DTA analysis was done in temperature ranges from 48.3-1020°C, using the TG/DTA thermal analyser instrument (Fig. 10). The TGA curve shows that at 250.100, 100.034 and 45.134°C, the weight reduction of ZnNPs was 5.710, 5.890 and 6.100mg, respectively. The significant weight reduction started from 400 to 560.324 °C and weight reduced from 4.001 to 2.103 mg, but no further weight loss beyond 600°C. This weight reduction was correlated to the organic matrix ignition, which behaved as a capping and stabilizing agent.

Antibacterial activity: The results of antibacterial activity of crude methanolic, ethanolic and EtOAc extract of *E. japonica*are summarized in Fig. 11. The percent antibacterial activity of crude methanolic, ethanolic and EtOAc extract against *P. aeruginosa* was 44.4, 40.7 and 37, *E. coli* 62, 59.2, 44.4, *S. aureus* 69.2, 46.1, 50, *M. morganii* 59, 40.9, 68, *A. baumannii* 52.1, 43.4, 0, MRSA 73.6, 52.6 and 0 and *P. vulgaris* 60, 52, 64. The results of antibacterial activity of ZnNPs are presented in Fig. 12. The order of antibacterial activity in terms of percentage was; MRSA (94.7), *P. vulgaris* (92), *S. aureus* (73), *M. morganii* (63.6), *E. coli* (48.1), *A. baumannii* (43.4) and *P. aeruginosa* (37).

Antifungal activity: The test samples were screened for their antifungal potentials against the selected fungal strains and the results are given in Figure. 13. The crude extract of methanol have shown low potential against *H. pseudocrispula* (10%) while it was inactive against the rest of fungal test pathogens. The ethanolic extract exhibited low activity against *A. niger* (23%) and *P. notatum* (13%) while no activity against other fungal strains. The EtOAc extract showed low activity against *A. niger* (12%) while it was inactive against the rest of fungal test pathogens. The antifungal activity of ZnNPs against the selected fungal strains was; *H. pseudocrispula* (60%), *P. chrysogenum* (55%), *A. niger* and *P. notatum* (40%), *A. parasiticus* (30%) and no activity against *V. longisporum* shown in Fig. 14.

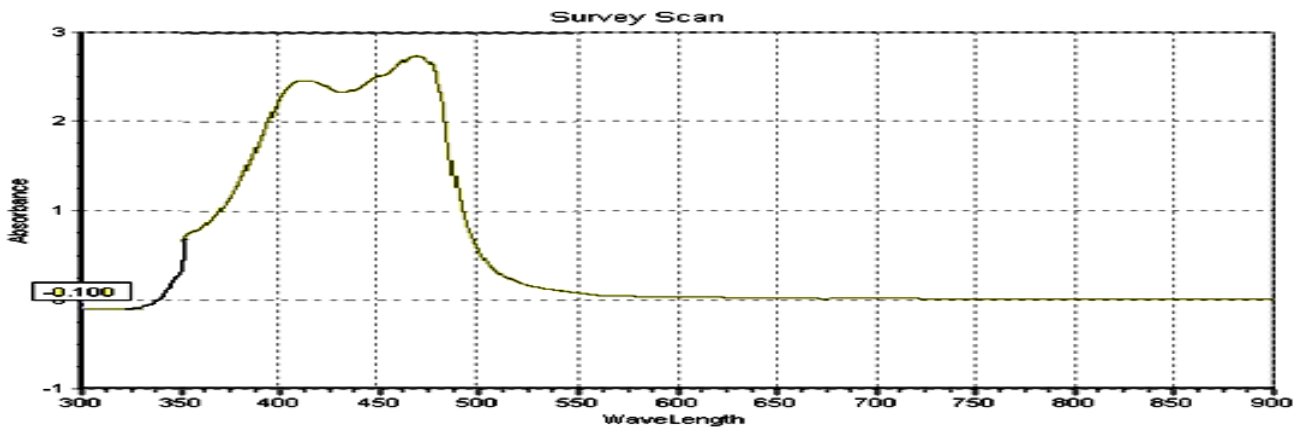

Fig. 1. UV-Vis spectra of synthesizedZnNPs from aqueous extract of *E. japonica*.

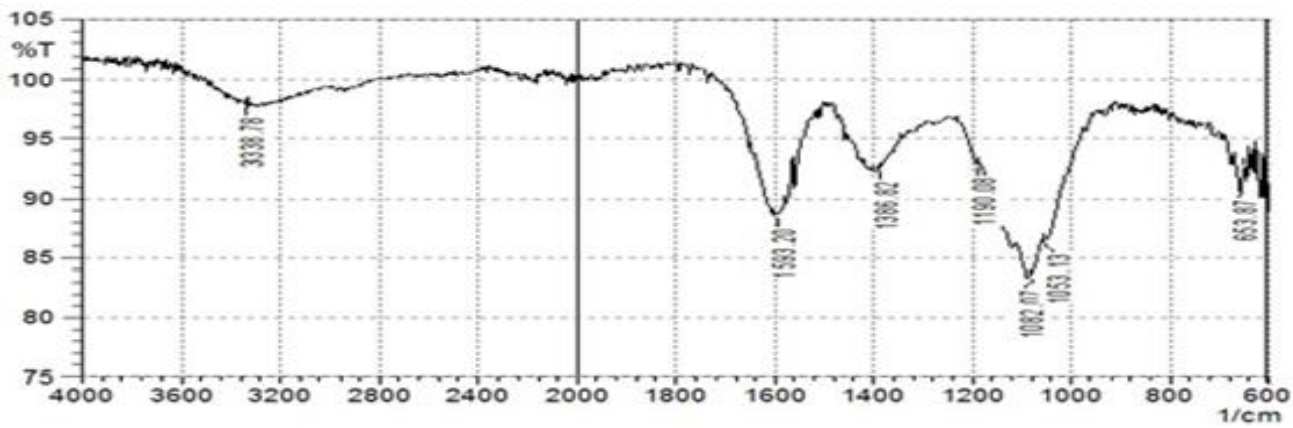

Fig. 2. FTIR spectra of synthesized ZnNPs from aqueous extract of *E. japonica*.

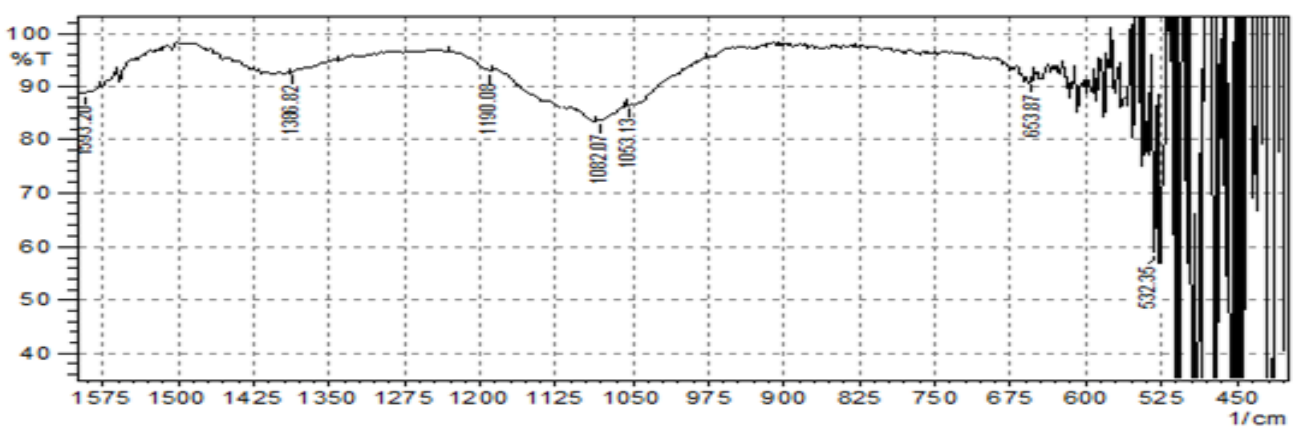

Fig. 3. FTIR spectra of synthesized ZnNPs from aqueous extract of *E. japonica*.

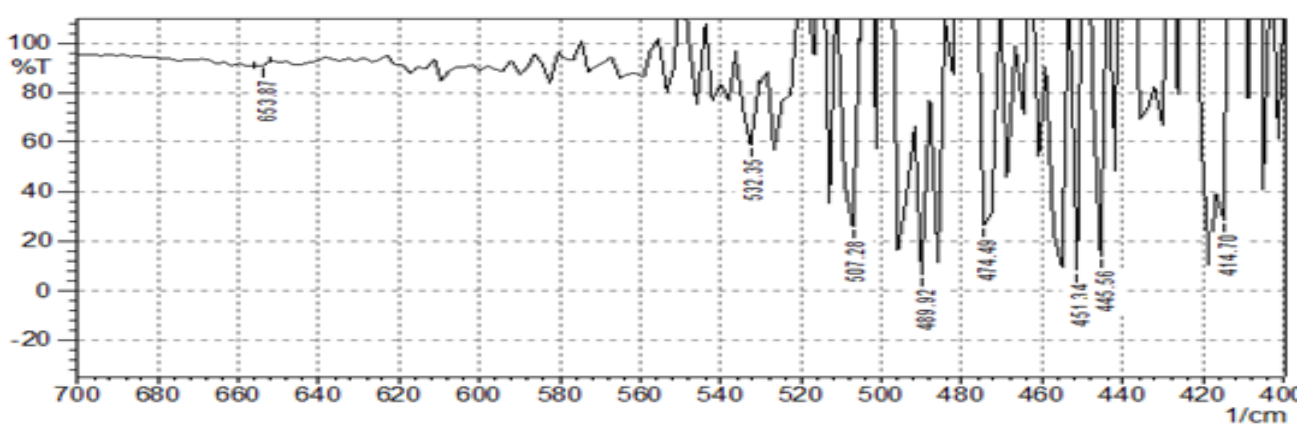

Fig. 4. FTIR spectra of synthesized ZnNPs from aqueous extract of *E. japonica*.

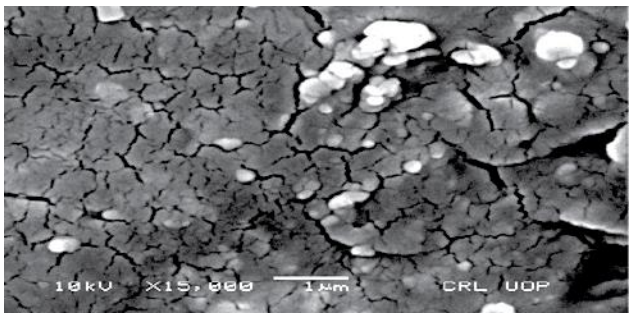

Fig. 5. SEM image of synthesized ZnNPs from aqueous extract of *E. japonica*at 15000x.

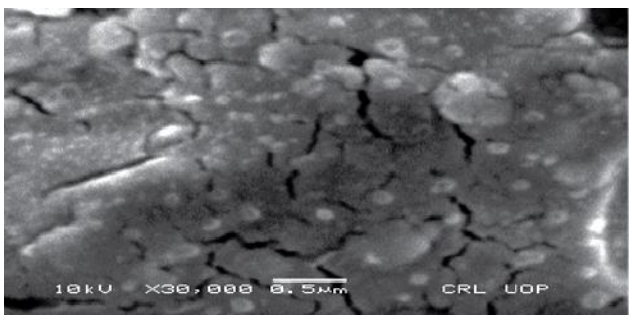

Fig. 6. SEM image of synthesized ZnNPs from aqueous extract of *E. japonica* at 30000x.

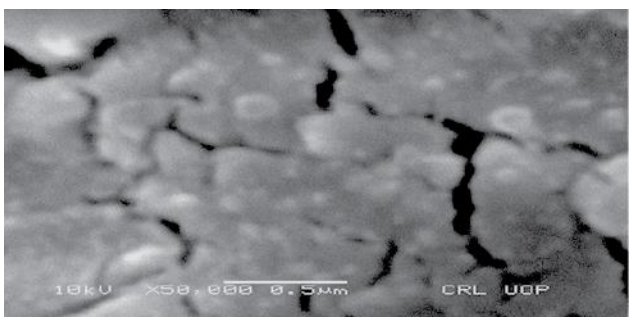

Fig. 7. SEM image of synthesized ZnNPs from aqueous extract of *E. japonica* at 50000x.

Table 1. Mass and atomic weight percentage of synthesized ZnNPs.

Name of element	Mass (%)	Atom (%)
C K	45.23	42.36
Zn K	42.20	45.23
Mg K	1.06	0.65
S K	2.56	1.20
K K	2.59	0.98

Phytotoxic activity: The phytotoxic activity of the crude methanolic, ethanolic and EtOAc extracts are summarized in Fig. 15. The methanolic, ethanolic and EtOAc extract showed (50, 40 and 20), (50, 40 and 30) and (50, 30, and 10) percent growth regulation at1000, 100 and 10 µg/ml, respectively against *L. minor*. The ZnNPs of the selected plant showed 60, 50 and 30% growth regulation at 1000, 100 and 10 µg/ml respectively (Fig. 16).

Heamaglutination activity: All the test samples and ZnNPs of *E. japonica* showed no heamaglutination activity against ABO blood, indicating lack of phytolectins in the selected plant (Table 2).

Discussion

In an earlier study, the UV-Vis spectra of ZnNP s prepared with 0.5% soluble starch was analyzed. The absorption peak was detected at a wavelength of 360nm. A significant widening in peaks was observed between 350–480nm, supporting our study [Ashe, B. 2011]. Our results can be compared to that of a previous research, where the FTIR spectrum of ZnNPs was studied revealing an absorption band at 3458.04cm^{-1}, because of the presence of O-H group. The absorption peak at 875.23nm confirms the tetrahedral dexterity of Zn (Sagar *et al*., 2015). In previous studies, some researchers have reported ZnNPs in the size range of 53-65 nm and 44–62 nm with our size being 30-60nm (Sadauskas *et al*., 2009). The present study can be compared to a previous study, where EDX plot analysis demonstrated that Zn and O were present in the sample. The EDX detected the presence of C, Si, and Ca in the EDX image which is in line with our study[Ashe, B. 2011].The XRD pattern of ZnNPs was found in close proximity with the published XRD pattern for the ZnNPs (Przybyszewska & Zaborski, 2009). A previously reported study showed that TG-DTA analysis of ZnNPs have an initial weight reduction in the range of 30-100°C, the highest being 17.87% at 150–280°C, showing the presence of ZnNPs which is comparable with our results (Romero *et al*., 2002). The results of antibacterial activity of the current study can be compared to the study of Mahesh *et al*. (2008) where the antibacterial activity of *n*-hexane and dichloromethane extracts of *Zingiber officinale*, *Psidium guajava*, *Caryophyllus aromaticus*, *Allim sativum*, *Caryophyllus aromaticus*, *Cymbopogon citrates* and *Mikania glomerata* were screened against *S. aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The results indicated that the selected plant extracts exhibited significant activity against the selected pathogens while in our study good activity was observed against certain bacterial pathogens making them comparable. The antibacterial activity of ZnNPs of our study exhibited significant and good activity against the selected pathogens most of the time which was in line with antibacterial activity of ZnNPs of another published study (Jiang *et al*., 2009). Silver nanoparticles extracted from *Dioscorea bulbifera* had shown good activity against *Bacillus cereus*, *Bacillus subtilis*, *S. aureus*, *P. aeruginosa* while no activity was observed against *Salmonella typhimurium* and *P. vulgaris*. The AgNPs exhibited antibacterial activities by interacting with bacterial DNA, cell wall or plasma membrane and proteins making it in line with our study (Chaloupka & Malam, 2010). The test samples of our study (organic extracts and ZnNPs) showed low and no activitiy against the test fungal pathogens; *H. pseudocrispula*, *A. niger* and *P. notatum*. Different extracts of *Cynara scolymus* were screened for their antifungal activity against *Alternaria* sp. The results indicated that crude extracts exhibited good activity (75%) against *Alternaria* sp., which was in contrast to our study where we found low activity in most of the cases (Diaz *et al*., 2011).The antifungal activity of ZnNPs against the *H. pseudocrispula* was good while no activity was observed against *V. longisporum*. Comparing it with another study where antifungal activity of Copper nanoparticles was investigated against *Botrytis cinerea* and

Penicillium expansum. These nanaoparticles showed good activity against the selected fungal pathogens and may be recommended as fungicide in agriculture and food industry (He *et al*., 2010). The current study also included phytotoxic activity of the test samples and ZnNPs against *L. minor*. The results indicated that test samples showed moderate activity at higher concentration while low activity at lower concentration. The ZnNPs of the selected plant showed 60, 50 and 30% growth regulation at 1000, 100 and 10 µg/ml respectively. The results of the current study are in contrast with another reported study where different fraction of *Ricinus communis*, *Chrozophora tinctoria*, *Peganum harmala*, *Tribulus terrestris* and *Fagonia cretica* was effective against *L. minor* (Samanta *et al*., 2011). The results revealed that the test samples and ZnNPs of the selected plant showed no heameagglutination activity. In can be concluded that *E. japonica* can be a potential source of active antibacterial and phytotoxic compounds. The ZnNPs of the current study also hold promise to be an effective agent against certain bacterial strains.

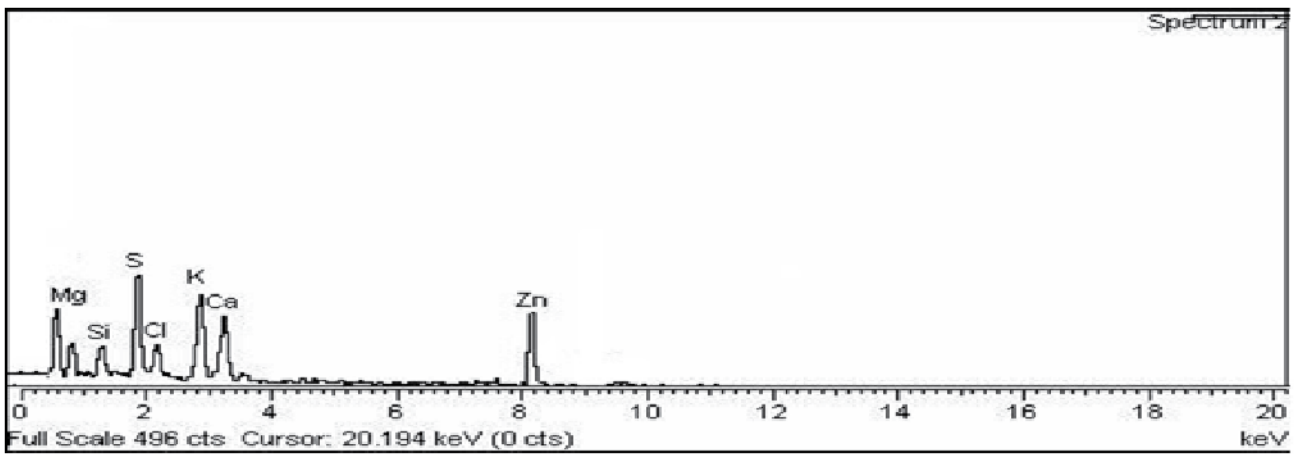

Fig. 8. EDX spectra of synthesized ZnNPs from aqueous extract of *E. japonica*.

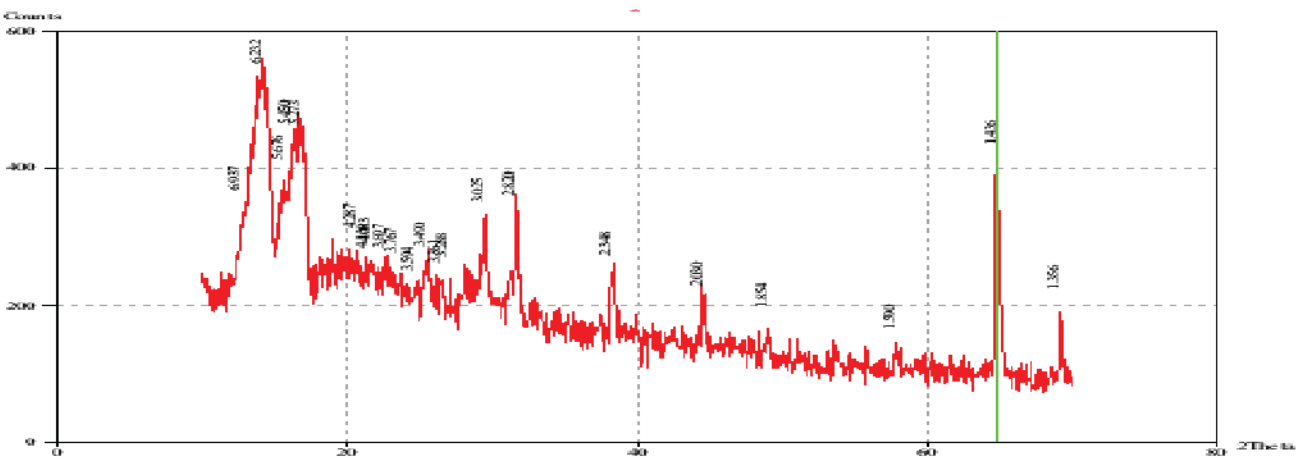

Fig. 9. XRD of synthesized ZnNPs from aqueous extract of *E. japonica*.

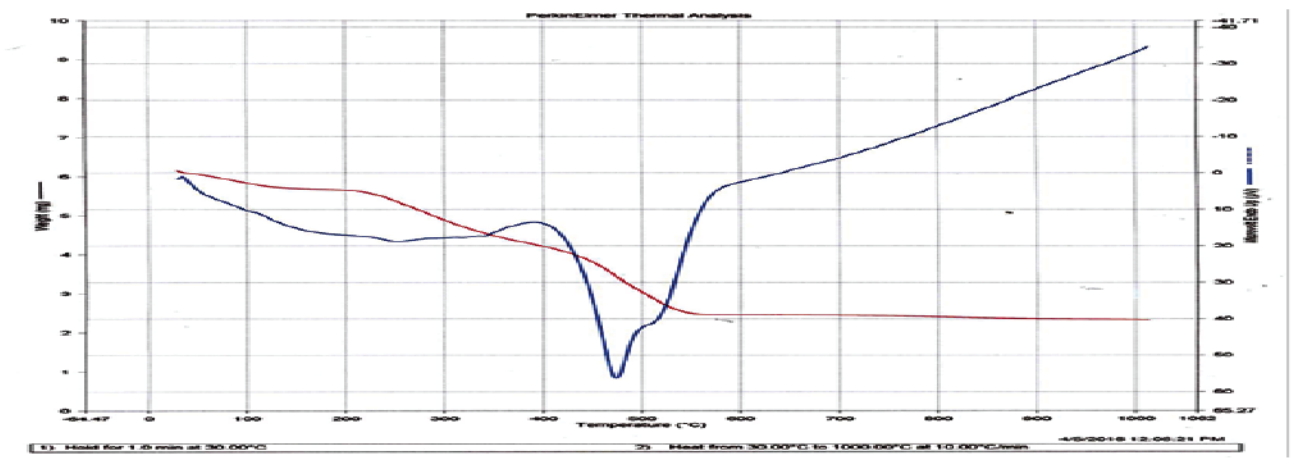

Fig. 10. TGA/DTA of synthesized ZnNPs from aqueous extract of *E. japonica*.

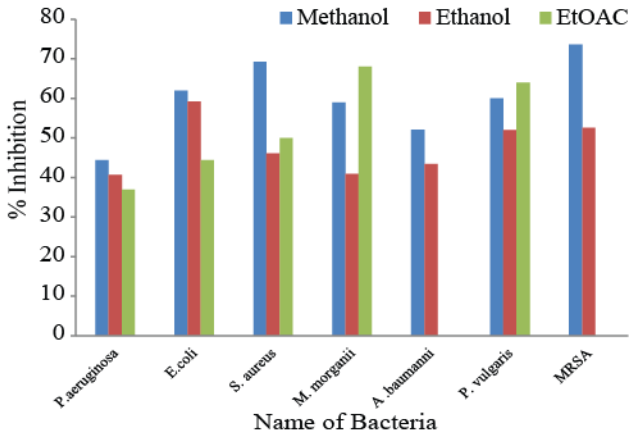

Fig. 11. Antibacterial activity of crude methanolic, ethanolic and EtOAc fractions of *E. japonica*.

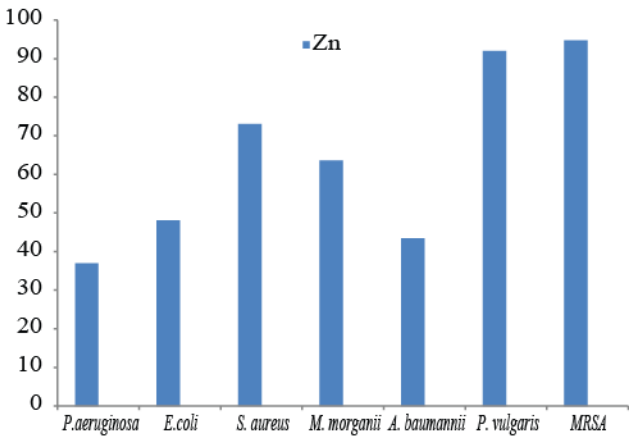

Fig. 12. Antibacterial activity of ZnNPs of *E. japonica* against selected bacterial strains.

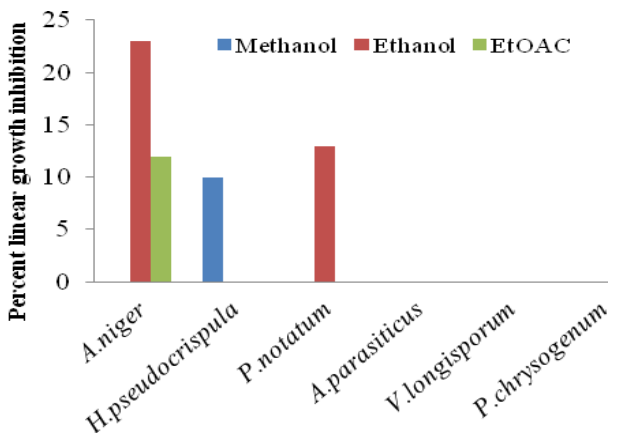

Fig. 13. Antifungal activity of crude methanolic, ethanolic and EtOAc fractions of *E. japonica*.

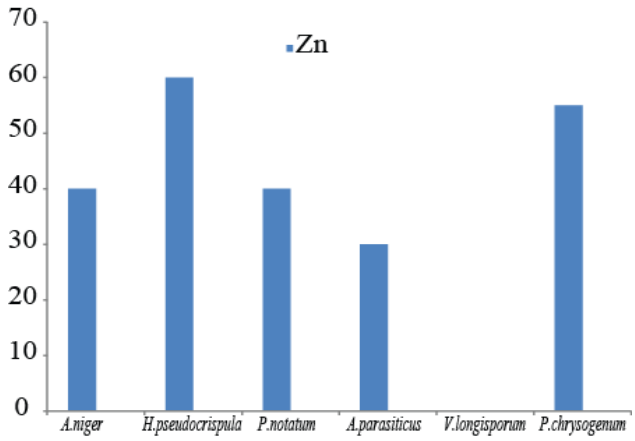

Fig. 14. Antifungal activity of ZnNPs against selected fungal strains.

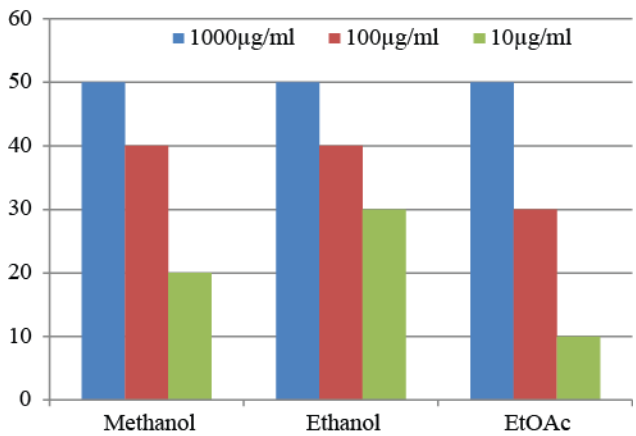

Fig. 15. Phytotoxic activity of *E. japonica* against *Lemna minor*.

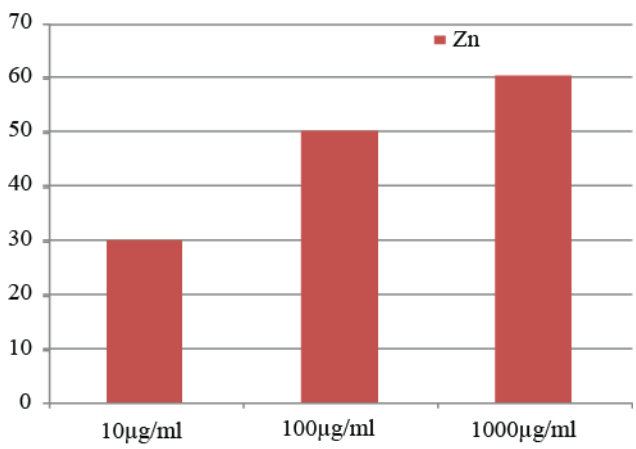

Fig. 16. Phytotoxic activity of ZnNPs against *Lemna minor*.

Table 2. Heamaglutination of methanolic, ethanolic, EtOAc and ZnNPs of *E. japonica*.

Blood groups	**AB +ve, AB –ve, B +ve, B-ve , O +ve, O –ve, A +ve, A–ve**			
Dilutions	1:2	1:4	1:8	1:16
Methanol	-	-	-	-
Ethanol	-	-	-	-
EtOAc	-	-	-	-
ZnNPs	-	-	-	-

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References

Agar, G., S. Ercisli, N. Yildirim, A. Esitken and E. Orhan. 2008. Identification of apricot (*Prunus armeniaca* L.) cultivars in Turkey using RAPD markers. *Turk J. Biol.*, 32: 1-7.

Ahmad, B., N. Ali, M.I. Choudhary, S. Azam and I. Khan. 2009. Parasiticidal, antifungal and antibacterial activities of Onosma griffithii Vatke. *Afr. J. Biotechnol*., 8: 5084-5087.

Ashe, B. 2011. A detail investigation to observe the effect of zinc oxide and Silver nanoparticles in biological system. *National Institute of Technology. M. Sc. (Roll NO-607bm004)*.

Bashir, A., F. Shireen, S. Bashir and I. Khan and A. Sadiq. 2016. Green synthesis, characterisation and biological evaluation of AgNPs using Agave americana, Mentha spicata and Mangifera indica aqueous leaves extract. *IET Nanobiotechnol*., 1-7.

Bashir, A., I. Khan, A. Sadiq, S. Bashir and F. Hussain. 2011. Screening of *Acacia modesta* for antifungal, anti-termite, anti-oxidant and brine shrimp cytotoxic activities. *J. Med. Plant Res.*, 5(15): 3380-3386.

Chaloupka, K.Y. and A.M. Malam. 2010. Nanosilver as a new generation of nanoproduct in biomedical applications. *Trends Biotech*., 28(11): 580-588.

Chan-Yeung, M.N. 2000. Air pollution and health. *J.Hong Kong Med.*, 6: 390-398.

Derfus, A.M., W.C.W. Chan and S.N. Bhatia. 2004. Probing the cytotoxicity of semiconductor quantum dots. *NanoLetters*, 4: 11-18.

Diaz, D.P., A. Cabrera1, D. Alem, P. Larrañag, F. Ferreira and M.D. Rizza. 2011. Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. *Chil. J. Agric. Res.*, 231-239.

El-Abhar, H.S. and M.F. Schaalan. 2014. Phytotherapy in diabetes: Review on potential mechanistic perspectives. *World J. Diabetes*, 5: 176-197.

Elumalai, K., S. Velmurugan, S. Ravi, V. Kathiravan and S. Ashokkumar. 2015. Green synthesis of Zinc oxide nanoparticles using *Moringa oleifera* leaf extract and evaluation of its antimicrobial activity. *Spectrochimica Acta Part A: Spectrochim. Acta Mol. Biomol. Spectrosc.*, 64(1): 143-158.

Ghosh, S., S. Patil, Ahire, M. Kitture and R. Jabgunde. 2011. Synthesis of goldnano-anisotrops using *Dioscorea bulbifera* tuber extract. *J. Nanomater*, 1-8.

Ghosh, S., S. Patil, M. Ahire, R. Kitture, S. Kale and K. Pardesi. 2012. Synthesis of silver nanoparticles using *Dioscorea bulbifera* tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. *Int. J. Nanomed*., 7: 483-96.

He, L., L. Yang Azlin and M.L. Mengshi. 2010. Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. *Microbiol. Res*., 3: 207-215.

Hoet, P.H., M.A. Nemmar and B. Nemery. 2004. Health impact of nanomaterials., *Nature Biotechnol*., 22(1): 19.

Jiang, W., H. Mashayekhi and B. Xing. 2009. Bacterial toxicity comparison between nano- and micro-scaled oxide particles. *Environ. Pollut.*, 157: 1619-1625.

Khalil, A., T.Z.K. Shinwari,Q. Muhammad and B.M. Khan. 2014. Phyto-Therapeutic claims about euphorbeaceous plants belonging to Pakistan. *An Ethnomedicinal. J. Bot*., 46(3): 1137-1144.

Kleiner, K. and J. Hogan. 2003. How safe is nanotech? *New Scientist*, 14-15.

Lead, J.R. and E. Smith. 2009. Environmental and human health impacts of nanotechnology. Wiley, UK.

Lee, M.H., K. Cho, A.P. Shah and P. Biswas. 2005. Nanostructured sorbents for capture of cadmium species in combustion environments. *Environ. Sci. Technol.*, 39: 8481-8489.

Lin, S.Q. 2007. World loquat production and research with special reference to China. *Acta Hort*., 750: 37-44.

Mahesh, B., & Satish, S. 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J. Agri. Sci.*, 4(5): 839-843.

Przybyszewska, M. and M. Zaborski. 2009. The effect of zinc oxide nanoparticle morphology on activity in crosslinking of carboxylated nitrile elastomer. *Express Polym. Lett.*, 3: 542-552.

Romero-Romero, T., A.L. Anaya and R. Cruz-Ortega. 2002. Screening for effects of phytochemical variability on cytoplasmic protein synthesis pattern of crop plants. *J. Chem. Ecol*., 28(3): 617-629.

Sadauskas, E., N.R. Jacobsen, G. Danscher, M. Stoltenberg, U. Vogel, A. Larsen and H. Wallin. 2009. Biodistribution of gold nanoparticles in mouse lung following intratracheal instillation.*Chem. Cent. J*., 20(3): 16.

Sagar, R., P.V. Thorat and T. Rohini. 2015. Green synthesis of zinc oxide (ZnO) nanoparticles using ocimum tenuiflorum leaves. *IJSR*., 4(5): 1225-1228.

Salmon, S.A., J.L. Watts, J.W.Pankey and R.J. Yancey. 1998. Minimum inhibitory concentrations for selected antimicrobial agents against organisms isolated from themammary glands of Dairy Heifers in New Zealand and Denmark. *J. Dairy Sci*., 81: 570-578.

Samanta, A., G. Das and S.K. Das. 2011. Roles of flavonoids in plants. *J. Pharm. Sci*., 1: 12-35.

Shinwari, Z.K. and A. Nasim. 2014. Ethnobotany in Pakistan In: *Encyclopaedia of the History of Science, Technology, and Medicine in Non-Western Cultures*. pp. 880-886.

Shinwari, Z.K. and S. Gilani. 2003. Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (Northern Pakistan). *J. Ethnophormacology*, 84(2003): 289-298.

Shinwari, Z.K., M. Rehman, T. Watanabe and Y. Yoshikawa. 2006. Medicinal and aromatic plants of Pakistan (A Pictorial Guide). pp. 492 Kohat University of Science and Technology, Kohat, Pakistan.

Xiao, J. 2015. Phytochemicals in medicine and food. *Phytochem Rev*., 14: 317-320.

Zeng, F., C. Hou, S.Z. Wu, X.X. Liu, Z. Tong and S.N. Yu. 2007. Silver nanoparticles directly formed on natural macroporous matrix and their anti-microbial activities. *Nanotechnology*., 18 (8): 55- 65.

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