Open Access Contents
Int. J. Drug Dev. & Res.
| April - June 2014 | Vol. 6 | Issue 2 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

# Screening of zinc oxide nanoparticles for cell proliferation synthesized through *Adhatoda vasica* nees

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**Abstract:**

Nano science is the basic Study of systems and materials at the nanoscale. The objective of the present study focuses on the Phytoconstituent mediated biological synthesis of ZnO-NPs by Zinc acetate and sodium hydroxide by utilizing the biocomponents of leaves of *Adhatoda vasica* .The samples are characterized by UV-Vis Spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDAX), X-ray diffraction (XRD) and FT-Raman Spectroscopy. The Synthesized ZnO-NPs were found to be discoid in Shape with an average size of 19 - 60 nm .The diffraction pattern also confirmed that the higher percentage of ZnO with fine particle size. Phytochemicals present in the plant were responsible for the quick reduction of $Zn^{+}$ ion to metallic Zinc Oxide nanoparticles. The synthesized ZnO-NPs had the potential to mitigate the bacterial cell proliferation particularly *Escherichia coli, Bacillus thuringiensis, Pseudomonas aeurogonisa* and *Staphylococcus aureus. Adhatoda vasica* is a good source for rapid reduction of metallic Zinc oxide in to nanoparticles with antibacterial activity. These green synthesized ZnO-NPs are possess therapeutic values.

**Keywords**: *Adhatoda vasica,* Zinc Oxide naoparticles, XRD, Raman spectroscopy.



## Introduction

Nanotechnology is the creation and utilization of materials through the control of the properties and structure of matter at the nanometric scale. Nanoparticles fall in the transition zone between individual molecules and the corresponding bulk materials, which generate both positive and negative biochemical effects in living cell [1]. Any material which enters into a plant system should prove three fundamental aspects that they are biologically active degradable and safe. Hence, there is need to explore different chemical and biological methods for this purpose. Nanoparticles due to their smaller size and large surface to volume ratio exhibit remarkable novel properties and methodical applications in the field of biotechnology, sensors, medical, catalysis, optical devices, DNA labeling, drug delivery [2] and they are rewardingly treated as a bridge between bulk material and atomic and molecular structures.

Zinc oxide (ZnO) belongs to a group of metal oxides with photo-oxidizing and photo catalytic ability against chemical and biological species [3]. ZnO-NPs have found fabulous application in biomolecular detection, diagnostic and micro electronics [4]. ZnO-NPs have been used to remove arsenic and sulphur from water even when bulk Zinc oxide cannot absorb arsenic. It is because; nanoparticles have much larger surface areas than bulk particles [5]. Several studies have been investigated the use of natural materials for ZnO-NPs synthesis such as DNA[6], albumen [7], orange juice [8], pea starch [9] and peptide structures [10] etc.

ZnO-NPs are used in the preparation of substances processing medically as well as cosmetically. Due to its antibacterial properties, ZnO is applied on the skin irritation, diaper rash, dry

skin and blisters. ZnO is used along with iron oxide to prepare calamine lotion and with eugenol to prepare ZnO eugenol which is used for dental applications [11]. ZnO-NPs can also be used for selective destruction of tumor cells and has a great potential in drug delivery applications [12]. ZnO-NPs have also been shown to exhibit strong protein adsorption properties, which can be used to modulate cytotoxicity, metabolism or other cellular responses [13]. Apart from these many applications, ZnO, due to its low toxicity is listed as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration (21, CFR 182, 8991).Green Synthesis that includes a clean, safe, eco-friendly and environmentally nontoxic method of nanoparticles synthesis and this method there is no need to use high pressure, energy, temperature and toxic chemicals [14]. The qualitative and quantitative analysis is very essential for identifying and quantification of active principles present in the medicinal plants which is important for medicinal action and drug preparation. The phytochemicals responsible for the synthesis of nanoparticles are terpenoids, flavonoids, phenols, carbohydrates, saponins, alkaloids and proteins[15]. The term phenolic compound embraces a wide range of plant substances that bear in common an aromatic ring with one or more hydroxyl substituents. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. *Adhatoda vasica* is an important medicinal plant belongs to the family Acanthaceae. *Adhatoda vasica* has been used in Ayurvedic medication to take care many skin diseases, malarial fever, intrinsic hemorrhage, cough, leprosy and piles [16]. In modern medicine it is reported to be an expectorant [17]abortifacient [18], antimicrobial [19], antitussive [20] and anticancer [21]. Important chemical constituents in *A.vasica* are adhatonine, vasicinone, pyrrloquinozoline, alkaloids, vasicine, vasicol, vasicinolone. Biological synthesis of ZnO-NPs was carried out by using plants such as *Calotropis gigantean* [22] and *Aloe barbadensis* [23]. Lot of work had been carried out on *A. vasica* for isolation of secondary metabolites but synthesis of nanoparticles particularly ZnO-NPs are scanty. Hence, in the present study, we have explored the green synthesis of ZnO-NPs by using the leaves of *A. vasica* and characterized these NPs with SEM, EDAX, XRD and FT Raman spectroscopy. Furthermore, the synthesized ZnO-NPs were evaluated for the bacterial cell proliferation against *Bacillus thuringiensis, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeuroginosa*

## MATERIALS AND METHODS

Zinc acetate dehydrate (99% purity) and sodium hydroxide (Pellet 99%) were used as introductory materials supplied by Sigma-Aldrich chemicals (Bangalore, India). *A. vasica* leaves were collected from S.V.U. Botanical Garden, Tirupati, Andhra Pradesh, India.

### Plant extract and qualitative analysis

Plant leaf extract was prepared by mixing 10g of dried leaf powder with 100 mL deionized water in 500 mL of Erlenmeyer flask and boiled for 20 min at $100^0$C. Then the leaf extract was collected in separate conical flask by Standard filtration method. This extract was used for further studies. Identification of active phytoconstituents (Table-1) of *A.vasica* was done by following the methods of Gibbs [24] and Herbone [25].



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### Biological synthesis of ZnO-NPs

The aqueous leaf extract of *A. vasica* was added to 0.025 M aqueous zinc acetate and adjusted the pH 12. The resulted solution was pale white in color. After stirring, the precipitate was washed against with distilled water followed by ethanol to get free of impurities. The solution was vacuum dried and used for characterization of ZnO-NPs.

### UV-Vis Spectra analysis

The reduction of pure Zinc Oxideions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 h after diluting a small aliquot analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu).

### SEM analysis of ZnO-NPs

Scanning Electron Microscope (SEM) analysis was carried out by using Hitachi S-4500 SEM Machine. Thin film of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry.

### EDAX measurements

In order to carry out the EDAX analysis, the drop of leaf extract with reduced ZnO nanoparticles was dried on coated carbon film and performed on Hitachi S-3400 N SEM instrument equipped with thermo EDAX attachments.

### X-Ray diffraction (XRD) analysis

The particle size and nature of the ZnO-NPs were determined using XRD. This was carried out with Shimadzu XRD-6000/6100 model with CuK $\alpha$ radians at $2\theta$ angle. X-ray powder diffraction is rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The size of the ZnO-NPs was determined by Debye Sherrer's equation.

$$D = 0.94\ \lambda/\beta\ \cos\theta$$

### FT-Raman Spectroscopy analysis

FT-Raman spectra were obtained on a Bruker RFS-100 instrument. It is equipped with an ND: YAG laser (1064 nm line) and the laser power can be controlled using the OPUS software. The main instrument is connected to a microscope using which small areas of samples can be analyzed. In the analysis of ZnO-NPs, the powder is placed under the microscope, the energy of the laser photons being shifted up or down. The shift energy gives information about the vibration modes in the system.

### Antibacterial assay

The following bacterial strains were used in this study viz., *Bacillus thuringiensis* (ATCC 10792), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeuroginosa* (ATCC 15442). Disc diffusion method was carried out by using standard protocol [26]. Overnight bacterial cultures (100 µL) were spread over Muller Hinton Agar (Hi Media Laboratories Private Limited, Mumbai, India) plates with a sterile glass L-rod. 100 µL of the each extracts were applied to each filter paper disc Whatmann No. (5 mm dia) and allowed to dry before being placed on the agar. Each extract was tested to triplicate and the plates were inoculated at $37^0$C for 24 hours after incubation. The diameter of inhibition zones were measured and tabulated.

## Result and Discussion

The phytochemical analysis of *A. vasica* leaf extract Table-1 revealed that this plant contains biomolecules, including alkaloids, proteins, tannins, glycosides, steroids, phenols, reducing sugars, carbohydrates, proteins and lignins. These phytochemicals are responsible for the immediate

reduction of ions and formation of AgNPs [27], particularly phenols and alkaloids which could be used as reductant to react with silver ions and therefore used as Scaffolds to direct the formation of ZnO-NPs in the solution [28].The chemical reactions which proceed in the aqueous extract may be as follows.

Green synthesis of ZnO-NPs by using *A. vasica* revealed that the pale white precipitate was appeared (Fig-1). ZnO-NPs typically have neutral hydroxyl groups attached to their surface, which plays a key role in their surface change behavior [29]. In aqueous medium and at basic pH, the chemisorbed protons (H+) move out from the particle surface leaving a negatively charged surface with partially bonded oxygen atoms ($ZnO^-$). At acidic pH, protons from the environment are likely transferred to the particle surface, leading to a positive charge from surface $ZnOH^+_2$ groups. The iso-electronic point indicates that ZnO-NPs will have strong positive surface charge under physiological conditions. *A. vasica* has been used as the reducing material as well as surface stabilizing agent for the synthesis of ZnO-NPs.

$$Zn(CH_3COO_2)2H_2O + 2NaOH \longrightarrow Zn(OH_2) + 2CH_3COONa + 2H_2O$$

$$Zn(OH)_2 + \text{Biomolecules of metabolites} \longrightarrow ZnO + H_2O$$

UV-Visible absorption Spectroscopy is widely being used technique to examine the optical properties of nano sized particles. The band was observed around 340-400nm Shown in Fig-2 due to the Plasmon resonance phenomenon which is the collection of Oscillation of electrons. Here the direct electro chemical process is involved that means the reduction or Oxidation in ZnO-NPs, the electrodes provide or accept the electron directly. In case of reduction, the Protons are supplied by the aqueous environment (i.e. Phenols which are presented in aqueous medium.

In SEM analysis a film beam of electron (10-40 KeV) is caused to scan the Sample in a Series of Parallel tracks. These electrons interact with electron in the sample, producing various Signals that can be detected and that Contain information about the samples. SEM analysis was used to determine the structure of the reaction products that were formed. SEM image has showed individual ZnO-NPs formed with diameter range 23.6 to 57.6 nm showed in Fig-3.

The elemental composition of green synthesized ZnO-NPs was analyzed through EDAX from Fig-4. EDAX Spectrum confirms the formation of elemental Zinc peak at 2.0 – 4 KeV., which is in congruence with the major emission peaks specified for metallic Zinc along with small peaks of Oxygen arisen due to the Capping of ZnO-NPs by biomolecule of *A. precatorius* leaf extract.

XRD analysis is used to determine the Phase distribution, Crystallinity and Purity of the synthesized nanoparticles .XRD spectra showed strong diffraction peaks at $30^0$, $38.28^0$, $44^0$ and $64.34^0$ degrees of $2\theta$ which corresponds to 111, 200, 220 and 311 crystal planes, which were significant agreement with the JCPDS file 36145 (a = b = 3, 249A°, C = 5.206A°) and indexed as the hexagonal wurtezite structure of ZnO (Fig-5). The average particle size of ZnO-Nps synthesized by the present biological method can be calculated using the Debye-Scherrer euation $D = K\lambda/\beta \cos\theta$ . It was found that the average size from XRD data and using the Debye-Scherrer equation was approximately 32nm. High purity and crystallinity of the prepared ZnO-NPs confirms the study and clear peak. For other impurities no characteristic peak was accessible.



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In order to find out the possible functional groups of capping agents associated in the stabilization of ZnO-NPs, FT-Raman spectrum of the NPs was recorded. Fig-6 gives the selective enhancement of Raman bands of the organic capping agents bound to the NPs. The spectrum shows a strong and sharp band at 500 $cm^{-1}$. The following absorption bands at 3350 $cm^{-1}$ stretching of N-H (or) OH (COOH), 2970-2500 $cm^{-1}$ stretching of $CH_3$, 1720-1700 $cm^{-1}$ stretching vibrations of C-C, 1420-1330 $cm^{-1}$ is stretching vibration of $CH_3$ deformation and the band between 500-400 $cm^{-1}$ correlated to ZnO-NPs.

Green synthesized ZnO-NPs were analyzed against bacterial cell proliferation by using two gram negative (*Escherichia coli* – ATCC 25922, *Pseudomonas aeuroginosa* – ATCC 15442) bacteria and two gram positive bacteria (*Staphylococcus aureus* – ATCC 6538, *Bacillus thuringiensis* – ATCC 10792) by disk diffusion method. Anti bacterial activity of *A.Precatorius* Fig.7 revealed that the highest potential was observed against *Staphylococcus aureus* followed by *Pseudomonas aeuroginosa*. The results were compared with the Ciprofloxacin as a positive control, zinc acetate as a negative control and plant extract as control.

ZnO-NPs can reduce the bacterial cell proliferation than traditional antibiotic ciproflaxacin. It has shown that, nano ZnO which has the average size between 20 nm and 45 nm can enhance the antibacterial activity of ciprofloxacin against *S. aureus* and *E. coli in vitro.* The enhancing effect of these nanomaterials is concentration dependent against all test strains. This effect may be due to two reasons first, Zinc oxide NPs interfere with Nor A protein. Nor A is a protein which is developed for conferring resistance in bacteria and has pumping activity that mediate the effluxing of hydrophilic fluroquinolone from a cell. Second ZnO-NPs can interfere with Omf which is a membrane protein that is responsible for the permeation of quinolones into the cell [30].

## Conclusion

*A. vasica* is a good medicinal plant for synthesis of ZnO-NPs by rapid reduction of Zn ions. ZnO-NPs showed strong potential against bacterial cell proliferation and can be used in the preparation of apoptotic agent and also in the synthesis of novel antibiotics as the bacterial strains are resistant to conventional antibiotics. Green methods are a good component for the chemical procedure, which are environment friendly, fast, cost effective and convenient.

**Table 1:** Qualitative phytochemical analysis of *A. vasica*

| S. No. | Phytoconstituents | Aqueous extract of *A. vasica* |
|---|---|---|
| 1. | Flavonoids | - |
| 2. | Tannins | + |
| 3. | Glycosides | ++ |
| 4. | Steroids | + |
| 5. | Saponins | - |
| 6. | Phenols | ++ |
| 7. | Terpenoids | - |
| 8. | Reducing sugars | ++ |
| 9. | Anthocyanins | - |
| 10. | Carbohydrates | + |
| 11. | Proteins | + |
| 12. | Alkaloids | ++ |
| 13. | Fatty acids | - |
| 14. | Lignins | ++ |
| 15. | Anthroquinones | - |

**Note**: `+' indicates present, `-' indicates absence

(a) (b)

**Fig 1:** Synthesis of ZnO NPs (Colour change) by using leaf extract of *A. vasica* (a) Plant extract, (b) Treated with ZnO

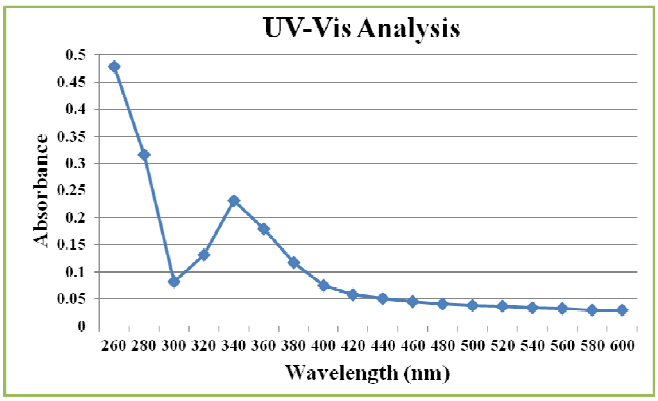


**Fig 2:** UV-Vis Spectroscopy analysis of synthesized ZnO-NPs using leaf extract of *A. vasica*

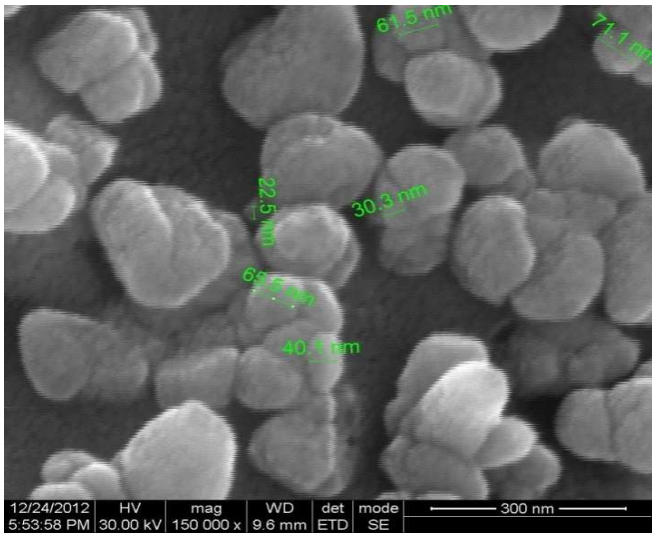


**Fig 3:** SEM images of ZnO nanoparticles synthesized by using leaf extract of *A. vasica*

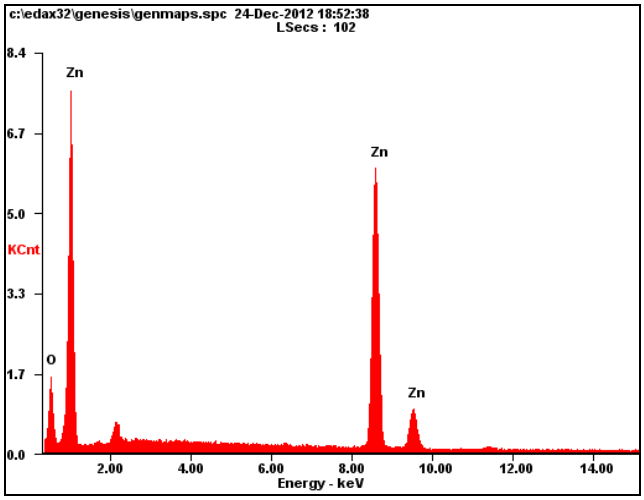


**Fig 4:** EDAX images of ZnO nanoparticles synthesized by using leaf extract of *A. vasica*

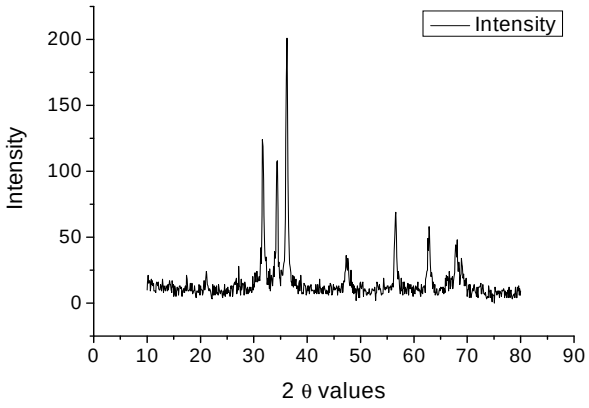


**Fig 5:** XRD pattern of ZnO nanoparticles of *A. vasica*

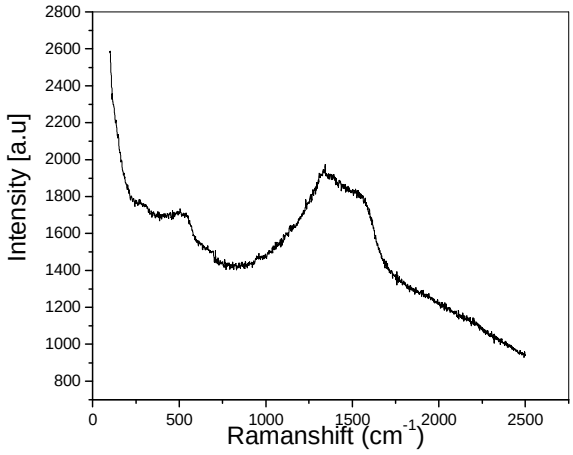


**Fig 6:** FT- Raman spectra of ZnO nanoparticles synthesized by using leaf extract of *A. vasica*

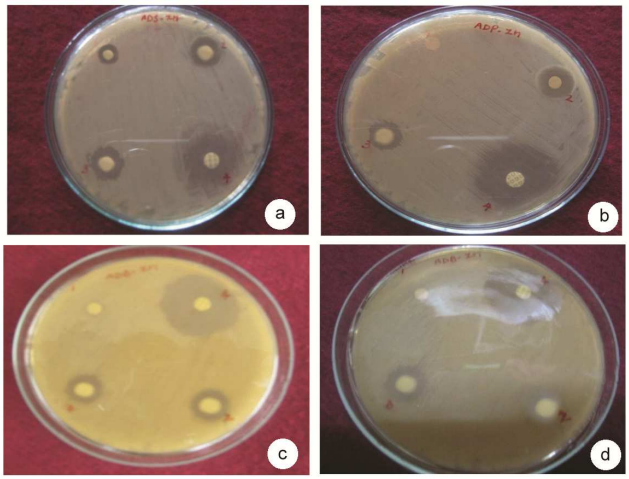


**Fig 7:** Antibacterial activity of leaf extract of *A. vasica*

a) *Staphylococcus*, b) *Pseudomonas*, c) *Bacillus*, d) *E. coli*
1) Plant extract, 2) Negative control, 3) Treated, 4) Ciprofloxacin

**Table 2:** Antibacterial activity of *A.vasica* leaf extract

| S. no | Bacterial Species | Negative Control (mm) | Treated (mm) | Ciprofloxacine (mm) |
|---|---|---|---|---|
| 1 | *Staphylococcus aureus* | 14 ± 0.01 | 15 ±0.07 | 23 ± 0.3 |
| 2 | *Pseudomonas aeuroginosa* | 12 ± 0.8 | 14 ± 0.1 | 24 ± 0.84 |
| 3 | *Bacillus thuringiensis* | 9 ± 0.2 | 12 ± 0.5 | 22 ± 4 |
| 4 | *Escherichia coli* | 7 ± 0.09 | 9 ± 0.29 | 20 ± 0.9 |


## ACKNOWLEDGEMENT

The first author is highly thankful to the DST for sanction of Inspire Fellowship.


## REFERENCES


1) Nel A, Xia T, Madle L, Li N. Toxic potential of materials at the nanolevel. *Science* 2006; **311**: 622-627.
2) Fan Z and.Lu J.G. *Journal of Nano Science and Nanotechnology* 2005; **5**(10), 1561-1573.
3) SzaboT, Nemeth J, Dekany I. Zinc Oxide nanoparticles incorporated in ultrathin layer silicate films and their photo catalytic properties. *In colloids and Surfaces.A* 2003; **203**: 23-25.
4) Singhal M, V. Chhabra, P. Kang, and D. O. Shah. Synthesis of ZnO nanoparticles for varistor application using Zn-substituted aerosol OT microemulsion. *Materials Research Bulletin,* 1997; **32**(2): 239-247.
5) Dhermendre K, Tiwari and Behari J, Prasenjit sen. Application of nanoparticles in waste water treatment. *World Applied Science Journal* 2008; **3**(3): 417-433.
6) Cai A-J, Wang Y-L, Xing S-T, Du L-Q, Maz-C. Tuned morphologies of DNA assisted ZnO struggling against pH. *Ceran Int* 2013; **39**(1): 605-609.
7) Nouroozi F, Farzaneh F. Synthesis and characterization of brush-like ZnO nanorods using albumin as biotemplate. *J Braz Chem Soc* 2011; **22**(3): 484-488.
8) Jha AK, Kumar V, Prasad K. Biosynthesis of metal and oxide nanoparticles using Orange juice. *Journal of Bio nanoscience*, 2011; **5**(2): 162-166.
9) Yu J, Yang J, Liu B, Max. Preparation and characterization of glycerol plasticized pea-Starch/ ZnO-Carboxy methyl cellulose Sodium nano composites. *Bio Resou Technol* 2009; **100**(11): 2832-41.
10) Tomczak MM, Gupta MK, Drummy LF, Rozenbak SM, Naik RR. Morphological c and assembly of Zinc oxide using biotemplate. *Acta Biometer* 2009; **5**(3): 876-82.
11) Ferracane, Jack L. Materials in Density: Principles and Applications, Lippincott Williams & Willkins 2nd Edition (2001).
12) Rasmussen JW, Martinez E, Louka P, Wingett DG. Zinc oxide nanoparticles for selective destruction of tumour cells and potential for Drug delivery applications. *Pubmed* 2010; **7**(9): 1063-1077.
13) Horie M, Nishiok, Fujit K et al. Protein adsorption of ultra fine metal oxide and its influence on cytotoxicity toward cultural cell. *Chem Res Toxicol*, 2009, 22: 543-53.
14) Savithramma N, Linga Rao M, Rukmini K and Suvarnalatha D. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. *International Journal of Chem Tech Research* 2011; **3**(3), 1394-1402 .
15) Sharma PV, Classical uses of medicinal plants, 1st ed. Varanasi, India. Chaukhambha Visvabharati; 1996; PP 304-3.
16) Ramy, M., Rameilah Sayed, Fayed, A., Ghada, I.M., Chemical compositions. Antiviral and antioxidant activities of Seven essential oils. J App Sci Re.6,50-62 (2010)
17) Atal CK, Chemistry and Pharmacology of Vasicine: A new oxytocic and abortifacient, R.R.L., Jammu Tawi, India: Published by the Director: 1980.
18) Wakhloo RL, Wakhloo D, Gupta OP, Atal CK. Vasicine hydrochloride: A new drug for interruption of pregnancy. *J obstet Gynaecol* 1979; 29: 939-40.







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19) Doshi JJ, Patel VK, Bhatt HV. Effect of *Adhatoda vasica* massage in Pyorrhea. *Int J Crude Drug Res* 1983; 21: 173-176.

20) Dhuley JN, Antitusive effect of *Adhatoda vasica* extract on mechanical or chemical stimulation induced coughing an animals. *J Ethnopharmacol* 1999; 67: 361-5.

21) Kulkarni AA, Ray of hope for cancer patents proceedings of International seminar on Holistic management of Cancer (Ayurveda Education Series No. 67) 1998. Pp. 5-11.

22) Vidya C, Shilpa Hiremath, MN Chandra Prabha, MA Lourdu Antony ray, Indu VenuGopal, Aayushi Jhain and Kokil Bansali of Zinc oxide nanoparticles by *Calotropis gigantea* Int *Journal of Current Engineering and Technology* (2013).

23) Gunalan Sangeetha, Sivaraj Rajeshwaria, Rajendran Venckatesh .Green synthesis of Zinc oxide nanoparticles by *Aloe barbadensis* Miller Leaf extract: Structure and Optical properties, *Materials Research Bulletin*, (2011); Vol **46**: No. 12, Pp. 2560-2566.

24) Gibbs RD. Chemotoxonomy of flowering plants, Mc Gill Queen's University Press, Montreal, London, 1974; , Vol.**1** ISBN 0773500987.

25) Herborne. Phytochemical methods. A guide to modern techniques of plant analysis. Chapmen and Hall, London, 1973.

26) Anonymous, Pharmacopiea of India (the Indian Pharmacopiea) 3rd ed. Delhi: Ministry of Health and Family Welfare, 1996.

27) Jha, A.K., Orasad, K., Prasad, K., Kulakarni, A.R., Plant system: natures nanofactory. Coll Surf B Biointer.73,219-223 (2009)

28) Chen, Z., Zhang, X., Cao, H., Huang, Y., Chitosan –capped Silver nanoparticles as a highly selective colorimeteric probe for visual detection of aromatic Ortho-trihydroxy phenols. Analyst.138,2343-2349 (2013)

29) Qu F, Morais PC. The pH dependence of the surface charge density in Oxide-based semiconductor nanoparticles immersed in aqueous solution. IEEE Transaction on Magnetics. *PMC* 2001; **37**: 2654-6.

30) Banoee M, Seif S, Nazari ZE, Jafari-Fesharaki P, Shahverdi HR, Moballegh A, Moghaddam KM, Shahverdi AR. ZnO nanoparticles enhanced antibacterial activity of Ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. *Journal mater Res B Appl Biometer* 2010; **93**(2): 557-61.

**Article History:** ----------

Date of Submission: 16-09-2013

Date of Acceptance: 29-09-2013

Conflict of Interest: NIL

Source of Support: NONE