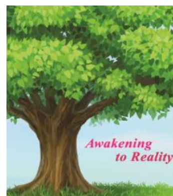

Available online at www.elixirpublishers.com (Elixir International Journal)

Electrical Engineering

Elixir Elec. Engg. 95 (2016) 41072-41076

ISSN: 2229-712X

Bio-fabrication of zinc oxide nanoparticles using leaf extract of Anisochilus carnosus, and to study their characterization and antibacterial activities

G.Parthasarathy[1,*], Dr. M. Saroja [1], Dr. M. Venkatachalam[1], V.K. Evanjelene[2] and Shruthi Mahalakshmi[2]

[1]Department of Electronics, Erode Arts and Science College (Autonomous),Erode-638 009.

[2]R and D Manager, Alpha Omega Hi Tech Bio Research Centre, Salem.

ARTICLE INFO

Article history:
Received: 15 May 2016;
Received in revised form: 13 June 2016;
Accepted: 18 June 2016;

Keywords
Anisochilus carnosus,
Zinc oxide nanoparticles,
XRD,
FTIR,
UV,
SEM,
Antibacterial.

ABSTRACT

In the present study, the objective was to study the synthesis and analyse the zinc oxide nanoparticles from *Anisochilus carnosus* leaf extract. The study revealed that the plant extract possessed significant phytochemicals. The nanoparticles were synthesized using the leaf extract and analysed using UV, FTIR, SEM and XRD. Different functional groups were found to be present indicating the presence of diverse compounds in the extract. The zinc oxide nanoparticles also possessed potent antibacterial activity against many pathogenic organisms.

© 2016 Elixir All rights reserved.

Introduction

Nanotechnology is evolving as a fast growing field as it finds application in Science and Technology to manufacture new materials at the nanoscale level (Albrecht *et al.,* 2006). Due to their distinctive features such as catalytic, optical, magnetic and electrical properties, metal nanoparticles have been of great interest (Singhal *et al.,* 2010). With a decrease in the size, distribution and morphology of the particles, nanoparticles have a higher surface area to volume ratio (Awwad *et al.,* 2012). In past few years, green synthesis of metal nanoparticles has become the focus of attention in the Nano science and Nano biotechnology field. There is an increasing attention on biosynthesis of the metal nanoparticles using organisms. The reduction of metal compounds into their respective nanoparticles are the result of the anti-oxidant or reducing properties of microbial enzymes or the plant phytochemicals.

From a green chemistry perspective, the three main steps that are to be evaluated in the preparation of nanoparticles are the choice of the solvent medium used for the synthesis, environmentally safe reducing agent and nontoxic material for the stabilization of the nanoparticles. Till date, most of the synthetic methods reported depend heavily on organic solvents. This is mainly because of the hydrophobicity of the capping agents used (Raveendran *et al.,* 2003). Synthesis of nanoparticles using bio-organisms is attuned with the green chemistry principles because the bio organism as well as the reducing agent and the capping agent employed in the reaction are eco-friendly (Li *et al*., 2009).The presence of some toxic chemical species adsorbed on the surface which may have adverse effects in medical applications often occur in chemical synthesis methods (Parashar and Srivastava, 2009).

Biosynthetic and environment friendly technology which is utilized for the synthesis of zinc oxide (ZnO) NPs are believed to be nontoxic, biosafe, and biocompatible and the nanoparticles have been used as drug carriers, cosmetics, and fillings in medical materials. (Rosiand Mirkin, 2005). Nevertheless most ZnO nano-particles which are used commercially have some advantages such as lower cost, white appearance over silver nano-particle (Vigneshwaran, 2006). The uses of plant extracts in the biosynthetic method have drawn attention as a simple and viable alternative to chemical and physical methods (Singh, 2011).

Nano-sized materials are used as novel antimicrobial agents. Due to increasing microbial resistances against various metal ions, various antibiotics, and the development of resistant strains in multiple ways the attention of the researchers are focused on high surface area to volume ratio (Chan and Tsai, 2008). Antibacterial activity is also observed against spores that are resistant to high temperature and high pressure. In the textile industry several classes of antimicrobial agents are used, many of which are biocides (Singh *et al.,* 2012).

The ZnO nanoparticles exhibit bactericidal properties due to electrostatic interaction between the nanoparticles and the cell surface and also cell damage is enhanced because of increased association of the nanoparticles. Upon prolonged contact between the bacterium cell membrane and the nanoparticles the toxic effects of ZnO nanoparticles towards the pathogenic species of bacteria are enhanced.

Tele:
E-mail address: sarathyae@gmail.com

© 2016 Elixir All rights reserved

Due to cytotoxic behavior of ZnO nanoparticles the bacterium and fungal lipid bilayer gets ruptured resulting in the drainage of the cytoplasmic contents (Feris *et al.,* 2010).

Anisochilus Carnosus (L) Wall is an annual herb commonly found in the Western Ghats distributed in the Southern moist mixed forests, grown among rocks, also known as Karpuravalli and Padukurkka. The stem is bluntly 4-angled, often tinged with red Leaves are simple, opposite, broadly ovate, obtuse, crenate, base subcordate or rounded, some- what fleshy, usually pubescent. Flowers are pale purple, in dense cylindric spikes. Seeds are small, suborbicular, compressed, and brown (Nambiar *et al.,* 1985; Jayaweera, 1981). Traditionally the plant has been used as hepatoprotective agent, stimulant, anti-ulcer, anti-inflammatory (Sirsi and Rao, 1956; Ravikumar and Santhosh, 2008; Grover *et al.,* 2001). The present study was aimed at synthesizing zinc oxide nanoparticles from *Anisochilus carnosus* leaf extract and to study their characteristics and antibacterial activity.

Materials and Methods

Extraction of the plant material

The fresh plant materials were washed with running tap water and shade dried. The leaves of *Anisochilus carnosus* were crushed to coarsely powdered by grinder. These coarse powders (25g) were then subjected to successive extraction in 250ml of each solvent (methanol) by using Soxhlet apparatus. The collected extracts were stored and then taken up for further investigations.

Phytochemical Screening

Preliminary phytochemical analysis was carried out for all the *Drynaria quercifolia* extracts as per standard methods described by Brain and Turner 1975 and Evans 1996.

Detection of alkaloids

*Anisochilus carnosus e*xtracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

a) **Mayer's test**: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

b) **Wagner's test**: Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

a) **Lead acetate test**: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

b) **H_2SO_4 test**: Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

Detection of Steroids

2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The colour changed from violet to blue or green in some samples indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test

0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

Detection of Anthraquinones

Borntrager's test

About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink colour indicates the presence anthraquinones.

Detection of Phenols

a) **Ferric chloride test**: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

b) **Lead acetate test**: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

Detection of Saponins

About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Detection of Tannins

A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

Detection of Carbohydrates

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Detection of Oils and Resins

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Quantitative phytochemical analysis

Estimation of Alkaloids

Alkaloid determination using Harborne (1973) method. 5g of the *Anisochilus carnosus* sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in methanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids

Ten grams of *Anisochilus carnosus* plant sample was repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250 ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference (Krishnaiah *et al*, 2007).

Estimation of Steroids

1 ml of *Anisochilus carnosus* extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at $70^0C \pm 20^0C$ for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Determination of Saponin

20g of *Anisochilus carnosus* plant sample was dispersed in 200 ml of 20% methanol. The suspension was heated over a hot water bath for 4h with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol.

The combined extracts were reduced to 40 ml over water bath at about 90^{0}C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of normal butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a constant weight. The saponin content was calculated in percentage (Nahapetian and Bassiri, 1975).

Antibacterial activity

Preparation of inoculums

Stock cultures were maintained at 4^{0}C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37^{0}C and 25^{0}C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/ml) for bacteria.

Antimicrobial susceptibility test

The disc diffusion method (Bauer *et al.,* 1966) was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{0}C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Synthesis of zinc oxide nanoparticles

Preparation of zinc oxide NPs For the synthesis of NPs, 50ml of plant leaves extract was taken and boiled at 60^{0}C - 80^{0}C by using a stirrer-heater. Then, 5 g of zinc nitrate was added to the solution as the temperatures reached at 60^{0}C. This mixture was then boiled until it converted to a deep yellow coloured suspension. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 400^{0}C for 2 h. A light white coloured powder was obtained and this powder was carefully collected and sent for different characterizations. The material was powered using a mortar and pestle so, that got a fine powder, which is easy for further characterizations.

Ultra- Violet Spectroscopy

The UV spectrum provides a useful means of detecting conjugated unsaturated chromophores within a molecule such as polyenes, α, β-unsaturated ketones and aromatic compounds. This can be particularly helpful in the identification of chromophores and flavones. The UV spectrum may be caused by the summation of chromophores from different parts of a polyfunctional molecule, and this should be considered in the light of deduction drawn from other spectroscopic methods and chemical degradation.

FTIR Spectroscopy

Infrared light from suitable source passes through a scanning Michelson inferometer and Fourier Transformation gives a plot of intensity versus frequency. When a powdered plant sample is placed in the beam, it absorbs particular frequencies, so that their intensities are reduced in the inferogram and the ensuing Fourier transform is the infrared absorption spectrum of the sample.

Scanning Electron Microscope

Scanning electron microscopic (SEM) analysis was performed using the Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by simply dropping a very small amount of the sample on the grid, with excess solution being removed using blotting paper. The film on the SEM grid was then allowed to dry by putting the grids under a mercury lamp for 5 min.

X-Ray Diffraction

ZnO nanoparticles were examined by X-ray diffractometer. The powdered metal was sticked in the cubes of XRD and then the result was taken in the XRD equipment.

Results and Discussion

Table 1. Qualitative phytochemical analysis of Anisochiluscarnosus.

Phytochemicals	Observations	Sample A
Alkaloids Mayer's test Wagner's test	Cream colour Reddish brown solution/ precipitate	 + +
Flavonoids Lead acetate test H_2SO_4 test	Yellow orange Reddish brown / Orange colour precipitate	- -
Steroids Liebermann-Burchard test	Violet to blue or Green colour formation	-
Terpenoids Salkowski test	Reddish brown precipitate	-
Anthroquinone Borntrager's test	Pink colour	-
Phenols Ferric chloride test Lead acetate test	Deep blue to Black colour formation White precipitate	+ +
Saponin	Stable persistent	-
Tannin	Brownish green / Blue black	-
Carbohydrates	Yellow / brownish / blue / green colour	+
Oil and Resin	Filter paper test	-

The qualitative phytochemical analysis of the leaf methanol extract of *Anisochilus carnosus* was done to test for presence of various phytochemicals. The plant was found to alkaloids, phenols and carbohydrates. Flavonoids, steroids, Terpenoids, anthroquinone, saponins, tannins, oils and resins were absent in *Anisochilus carnosus* extract. Kiruthiga and Sekar, 2014 reported that in the Anisochilus carnosus ethanol leaf extract alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins, phenols, triterpenoids, and anthraquinones, oils, fats and amino acids were absent.

Antimicrobial activity of Anisochilus carnosus

The antimicrobial activity of *Anisochilus carnosus* methanol leaf extract was studied at concentrations of 20, 30, 40 and 50µl against the organisms *S.typhi, S.aureus, B.subtilis, E.coli* and *P.aeruginosa.* There was no activity against any organisms at concentration of 20µl. Only *S.aureus, E.coli* and *P.aeruginosa* were inhibited at concentration 30µl. At concentration 40 µl and 50 µl, highest inhibition was found against *P.aeruginosa* and *B.subtilis* followed by *S.typhi.*

Table 2. Analysis of Antimicrobial activity of Anisochilus carnosus

S.No.	Name of Organism	Control	Concentration of Sample A			
			20 µl	30 µl	40 µl	50 µl
1.	*S.typhi*	22	00	00	09	11
2.	*S.aureus*	23	00	07	08	10
3.	*B.subtilis*	21	00	00	10	13
4.	*E.coli*	22	00	07	08	10
5.	*P.aeruginosa*	24	00	07	11	13

Fig.1 (a)

Fig.1 (b)

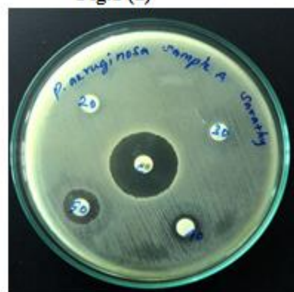

Fig.1 (c)

Fig.1 (d)

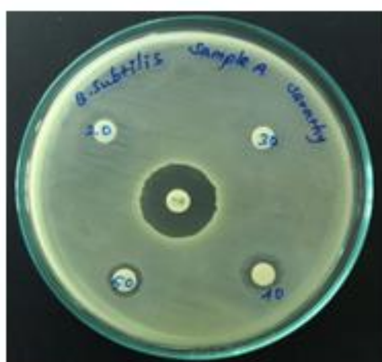

Fig.1 (e)

Fig 1. Antibacterial activity of ZnO Nanoparticles synthesised using 50ml of Anisochilus carnosus leaf extract.

Anbuvannan *et al.,* 2015 reported in their study that the antibacterial activity of the *Anisochilus carnosus* synthesized ZnO nanoparticles against *S. paratyphi, V.cholerae, S. aureus,* and *E. coli* showed inhibition zones of 6mm, 10mm, 7mm and 9mm respectively. When compared to control, *Anisochilus carnosus* synthesized ZnO NPs showed a smaller zone of inhibition.

UV analysis of *Anisochilus carnosus* extract

The UV analysis of the methanol extract synthesized zinc oxide nanoparticles of *Anisochilus carnosus* leaf. The maximum absorption peak was obtained at 219.24 nm wavelength.

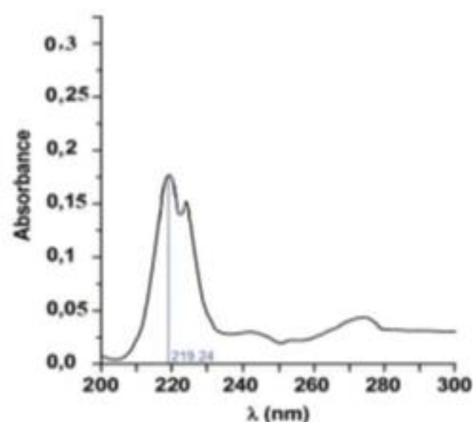

Fig 2. UV spectrum of ZnO Nanoparticles synthesised using 50ml of Anisochilus carnosus leaf extract.

FTIR analysis of Anisochilus carnosus extract

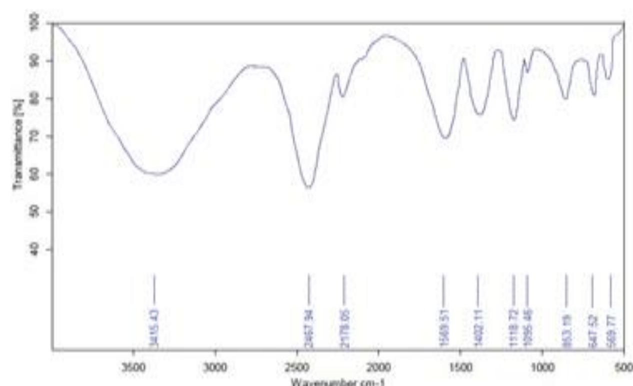

Fig 3. FTIR- spectra of ZnO Nanoparticles synthesised using 50ml of Anisochilus carnosus leaf extract.

The appearance of peaks at 569.77 cm^{-1}ascribed to Presence of Halogen atom combined with alkyl group. The C-H bending in alkyne group (strong stretching) appeared at 647.52 cm^{-1}. The intense band at 853.19cm^{-1} and 1095.46 cm^{-1}relates to the =CH-H Stretching and Aliphatic amine (C-N) respectively. The Hydroxyl group stretching (-OH) gives the band at 1118.72cm^{-1}. the strong absorptive peaks at 1402.11cm^{-1}and 1569.51 cm^{-1} are attributable to C-C Stretching (in ring) aromatic(-NO) nitro group asymmetric stretching (medium) respectively. The peaks at 2178.05 cm^{-1}indicates presence of triple bond stretching in alkyne compounds. The peaks at 2467.94 cm^{-1}and 3405.43 cm^{-1}are attributed to C-H medium stretching in aldehyde (HC=O) and N-H stretching (medium).

SEM analysis of *Anisochilus carnosus* extract

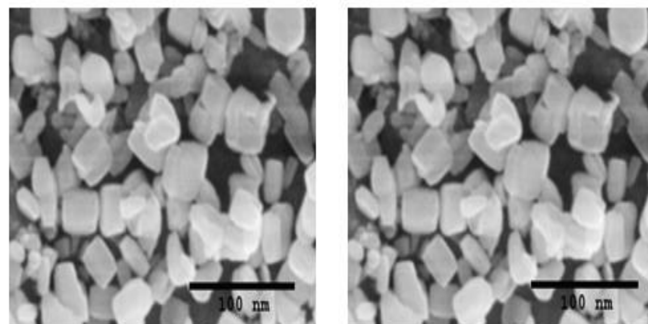

Fig.4 (a) Fig.4 (b)

Fig 4. SEM images of ZnO Nanoparticles synthesised using 50ml of Anisochilus carnosus leaf extract.

XRD analysis of *Anisochilus carnosus* extract

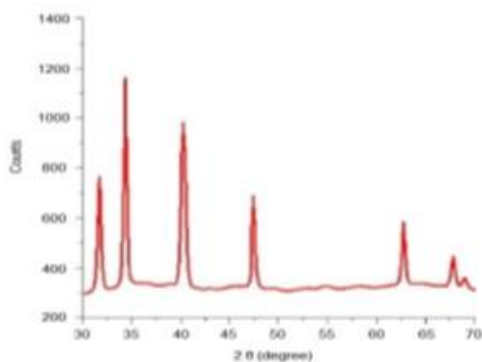

Fig 5. XRD spectrum of ZnO Nanoparticles synthesised using 50ml of Anisochilus carnosus leaf extract.

XRD patterns of ZnO synthesized for *Anisochilus carnosus* leaf extract are shown in the Fig. The diffraction peaks at 2θ=31.58, 34.49, 40.51, 47.56, 63.60, and 68.91corresponding to (100), (002), (111), (102), (103), and (201) planes respectively were observed and compared with the standard powder diffraction card of JCPDS No. 77-0191. According to the XRD data, the mean crystalline sizes (D) of the Zinc oxide nanoparticles calculated using Debye Scherrer's formula.

$$D = \frac{K\lambda}{\beta \cos\theta} \text{Å}$$

Where, λ=1.5406 Å is the wavelength of the X-ray radiation used. The term θ is the Bragg diffraction angle and β is the full width at half its maximum intensity of diffraction pattern (FWHM) in radian. The obtained sizes are 13.86, 8.156 and 11.85 nm

Conclusion

In the present study, the ZnO nanoparticles were synthesized using *Anisochilus carnosus* methanol leaf extract. The methanol leaf extract was found to possess alkaloids, phenols and carbohydrates. The nanoparticles were subjected to antibacterial study and were found to be effective. The nanoparticles were analysed using UV, FTIR, SEM and XRD. In UV analysis, the maximum absorption was at 219.24 nm. The FTIR analysis showed the presence of alkyl, alkyne, Aliphatic amine, Hydroxyl, aromatic, nitro and aldehyde functional groups. SEM analysis revealed that the nanoparticles were of cuboidal shape. The sizes of the nanoparticles are 13.86, 8.156 and 11.85 nm. From this study, it was evident that the plant *Anisochilus carnosus* can be used to synthesize nanoparticles using green chemistry methods for various applications.

References

1. Albrecht, M.A., Evans, C.W., Raston, C.L., (2006). Green chemistry and the health implications of nanoparticles. Green Chem. 8, 417–432.

2. Anbuvannan.M, M.Ramesh, G.Viruthagiri, N.Shanmugam, N. Kannadasan. *Anisochilus carnosus* leaf extract mediated synthesis of zinc oxide nanoparticles for antibacterial and photocatalytic activities. Materials Science in Semiconductor Processing. 39(2015)621–628.

3. Awwad, A.k.M., Nida, M., Salem, Amany, O., and Abdeen, 2012. Biosynthesis of Silver Nanoparticles using Oleaeuropaea Leaves Extract and its Antibacterial Activity. Nano science and Nanotechnology. 2 (6), 164-170.

4. Chan. H., Tsai. M. H., 2008. Synthesis and characterization of ZnO nanoparticles having prism shape by a novel gas condensation process. Reviews on Advanced Material Science 18. 734- 743.

5. Feris K., C. Otto, J. Tinker, D. Wingett, A. Punnoose, A. Thurber, M. Kongara, M. Sabetian, B. Quinn, C. Hanna, D. Pink, Langmuir 26 (2010) 4429.

6. Grover J.K., G. Adiga, V. Vats and S.S. Rathi, "Extracts of Anisochiluscarnosus (L) Wall prevent Development of experiment inflammation", J. Of Ethanopharm, 78, pp 159-164, 2001.

7. Jayaweera D. M. A., "Published by the National Science Council of Sri Lanka", COLOMBO pp 80 and 81, 1981

8. Kiruthiga N. and D. SathishSekar, Studies on Phytochemicals and Steroid Isolation from N-Hexane Extract of *Anisochiluscarnosus*. International Journal of Advanced Biotechnology and Research(IJBR).Vol5, Issue3, 2014, pp337-345.

9. Krishnan Nambiar V.P., N. Sasidharan, C. Renuka and M. Balagopalan, "Studies On The Medicinal Plants Of Kerala Forests", Report 42, Pages: 200, Dec 1985, Kerala Forest Research Institute Peechi, Thrissur.

10. Li, S.; Shen, Y.; Xie, A.; Yu, X.; Qui, L.; Zhang, L. & Zhang, Q. (2007). Green synthesis of silver nanoparticles using Capsicum annum L. extract. Green Chemistry. 9. 852-858

11. Parashar, U.K.S., S.P. & Srivastava, A., Bioinspired synthesis of silver nanoparticles. Digest journal of nanomaterials and biostructures, 2009. 4(1): p. 159- 166.

12. Raveendran, P.; Fu, J. & Wallen., S.L. (2003). Completely "Green" Synthesis and Stabilization of metal nanoparticles. Journal of American Chemical Society, 125(46).; 13940-13941.

13. Ravikumar J. and H. Y. Santosh, Pharma info.net, Volume 45, pp 979-981, 2008.

14. Rosi. N.L., Mirkin. C.A., 2005. Nanostructures in biodiagnostics. Chem Rev, 105:1547–1562.

15. Singh. G., Joyce. E. M., Beddow. J., and Mason. T. J., 2012. Evaluation of antibacterial activity of ZnO nanoparticles coated sonochemically onto textile fabrics, Journal of Microbiology, Biotechnology and Food Sciences 2 (1):106-120.

16. Singh. R.P., 2011. Biological approach of zinc oxide nanoparticles formation and its characterization. Mat Lett. (2):313–317.

17. SinghalGarima, RijuBhavesh, KunalKasariya, Ashish Ranjan Sharma and Rajendra Pal Singh, 2010. Biosynthesis of silver nanoparticles using *Octimum sanctum* (Tulsi) leaf extract and screaming its antimicrobial activity. Journal of nanoparticle Research, 13, 2981-2988.

18. Sirsi M. and R.S. Rao, Indian J med Res, Apr: 44(2), pp 283-7, 1956.

19. Vigneshwaran. N., 2006. Functional finishing of cotton fabrics using Zinc oxide soluble starch nano composites. Nanotechnology, (17): 5087–5095.

20. Brain, K.R., and Tuner, T.D., 1975. The practical evaluation of phytopharmaceuticals. Wright Scientectica Publishers, Bristol. 2, 57-58.

21. Evans, W.C., Trease, and Evans, 1996. Pharmacognosy. 14th Ed. London: WB Saunders Ltd; 119–159.