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ANTICANCER EVALUATIONS OF SYNTHESIZED SILVER NANOPARTICLES USING STEM EXTRACT OF MORINDA CITRIFOLIA

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ABSTRACT: In the last decade, the advent of nanotechnology has spurred significant developments and innovations in this field of Research. Nanoparticles synthesis is an evergreen research field of 21st century. Therefore, it was thought interesting to synthesized Silver Nanoparticles. The present work focuses the use of the aqueous extract of stem of Morinda citrifolia. In producing silver nanoparticles (AgNPs) from silver nitrate aqueous. Phytochemical analysis of the extract reveals anticancer application. The AgNPs obtained was characterized by UV-visible spectroscopy, FT-IR spectroscopy. SEM images which revealed the presence of various shapes and sizes. In this study, we investigated anticancer activity of synthesized AgNPs.

KEYWORDS:
Morinda citrifolia; Stem extract; AgNPs; Anticancer; Nanotechnology; Phytochemistry.

INTRODUCTION: Metallic nanoparticles have attracted tremendous interest due to their unique physicochemical properties. Their applications include use in biosensing[i], media recording[ii], optics[iii], catalysts[iv], and environmental remediation[v-x]. The synthesis of nanoparticles has been proposed as a cost-effective environmentally friendly alternative to chemical and physical methods. Consequently, nanomaterials have been synthesized using microorganism[xi-xv] and plant extracts[xvi-xxii]. The use of plant extracts for synthesis of nanoparticles is potentially advantageous over microorganism due to the ease of scale up, the biohazards and elaborate process of maintaining cell cultures[xxiii,xxiv]. Nanotechnology deals with the synthesis of nanoparticles with controlled size, shape and dispersity of materials at the nanometer scale length[xxv]. Metal nanoparticle with at least one dimension approximately 1-100 nm have received considerable attention in both scientific and technological areas due to their unique and unusual physico-chemical properties with that of bulk materials. Due to specific size, shape and distribution nanoparticles are used in the production of novel systems.

Literature reveals a promising medical application of AgNPs synthesized using herbal extracts. Silver nanoparticles synthesized using herbal extracts have been reported to have good anti-bacterial, anti-fungal and antioxidant properties[xxvi,xxvii]. Nanoparticles synthesis by medicinal plants show more benefit; they may enhance the antibacterial activity of silver nanoparticles, because the medicinally valuable active biomolecule present in the plants may bind on the surface of the nanoparticles and reduce the silver ions to silver nanoparticles. The various types of nanomaterials of different metals such as copper, zinc, titanium, magnesium, gold and silver used for improvement of biosensors, gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) were proved to be most effective, as they have good antimicrobial efficacy against a wide variety of bacterial, viruses and other eukaryotic micro-organism[xxviii-xxxvi]. The use of plant extracts for synthesis of nanoparticles is potentially advantageous over microorganism due to the ease of scale up, the biohazards, and elaborates process of maintaining cell cultures[xxxvii-xxxix]. Plant extract was used for the synthesis of silver nanoparticles because it was proved to be less toxic and also need less purification as compared to chemical methods[xl, xli]. Silver nanoparticles were proven to be most efficient as they possess good antimicrobial and antioxidant activities[xlii-xlvi]. In this study, we successfully reported the synthesis of silver nanoparticles using stem extract of Morinda Citrifolia. Synthesized silver nanoparticles were applied to evaluate the anticancer activity.

MATERIALS AND METHODS

A. **PLANT MATERIALS:** The Stem of *Morinda Citrifolia* was collected from the bamboo Garden, Amravati, Dist. Amravati during December and January 2021.

B. **SYNTHESIS OF AGNPS:** The fresh stem extract used for the synthesis of AgNPs was prepared from 25gm weight of fresh stem was cut into fine pieces and thoroughly washed in a 500ml flask, boiled in 75ml of water for 20 min at 60^0C and the produced extract was subjected to freeze drying. Suspensions were filtered with Whatman No. 42 filter paper. 5ml of aqueous solution of stem extract mixed with aqueous NaOH. Silver nitrate ($AgNO_3$) was prepared in a flask and 2ml of stem extract was added at room temperature for 22h in the dark until the brownish color was developed which indicated the formation of AgNPs.

a) **UV-VIS ABSORBANCE SPECTROSCOPY ANALYSIS:** The reduction of silver nitrate ($AgNO_3$) to AgNPs was monitored periodically by UV-vis spectroscopy (Shimazu) after the dilution of the samples with deionized water. A UV-vis spectrograph of the silver and nanoparticles was recorded by using a quartz cuvette with water as reference. The UV-vis spectrometric readings were recorded at a scanning speed of 200-800 nm.

b) **FT-IR ANALYSIS:** PerkinElmer spectrometer FT-IR Spectrum one in the range of 4000-400cm^{-1} at a resolution of 4cm^{-1} was used. The sample was mixed with KBr procured from Merk chemicals. Thin sample pellet was prepared by pressing with the Hydraulic pellet press and subjected to FT-IR analysis.

c) **SEM ANALYSIS:** Morphological characterization of the samples was done using FEI Quanta 200scanning Electron microscope. A pinch of dried sample was coated on a carbon tape. It was again coated with platinum in an auto fine coater and then the material was subjected to analysis.

d) **ANTICANCER ACTIVITY:**

Materials: MDA MBA231 (Human Breast Cancer)
Dulbecco's Modified Eagle Media (DMEM) with glucose-Cat No-11965-092 (Gibco, Invitrogen)
Fetal bovine serum (FBS)- Cat No – 10270106 (Gibco, Invitrogen)

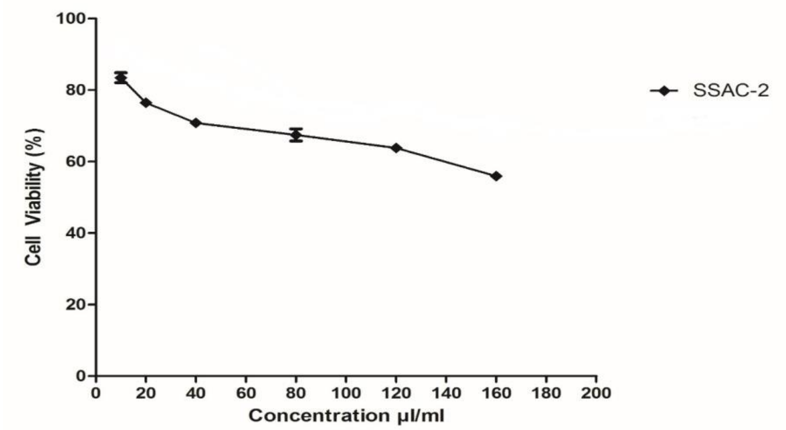
Antibiotic-Antimycotic 100X solution (Thermofisher Scientific)-Cat No-15240062 TACS Annexin V-FITC Apoptosis Detection Kit (R&D system) Cat No- 4830-01-K.

PROTOCOL: -

Cytotoxicity: The cells were seeded a 96-well flat-bottom micro plate and maintained at 37^0C in 95% humidity and 5% CO_2 for overnight. Different concentration (100, 50, 25, 12.5, 6.25, 3.125 µM/ml) of samples were treated. The cells were incubated for another 48 hours. The wells were washed twice with PBS and 20 µL of the MTT staining solution was added to each well and plate was incubated at 370C. After 4h, 100 µL of DMSO was added to each well to dissolve the formazan crystals, and absorbance were recorded with a 570 nm using micro plate reader.

Formula:

Surviving cells (%) = Mean OD of test compound / Mean OD of Negative control x 100 using graph pad Prism Version5.1, we calculate the IC 50 of compounds



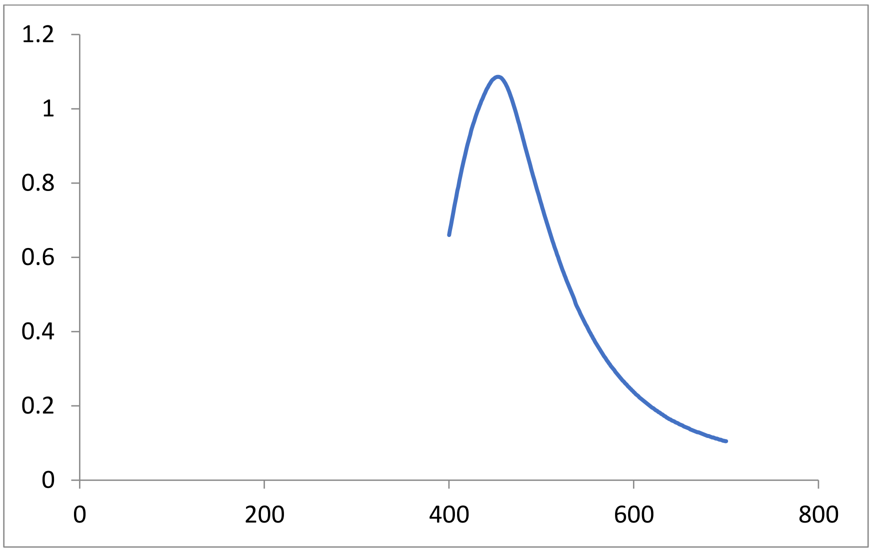
RESULTS

IC50 value of Compounds (µM/ml)

Sample Code	MDA MBA231	
	Mean	SD
SSAC-2	358.5	7.4

Cell Viability of MDA MBA231		
Concentration µM/ml	**SSAC-2**	
160	56.74	55.06
120	62.92	64.61
80	69.10	65.73
40	70.22	71.35

20	75.28	77.53
10	82.02	84.83
Negative Control	100	



Wavelength(nm)

Fig.1: UV-vis spectra of synthesized AgNPs

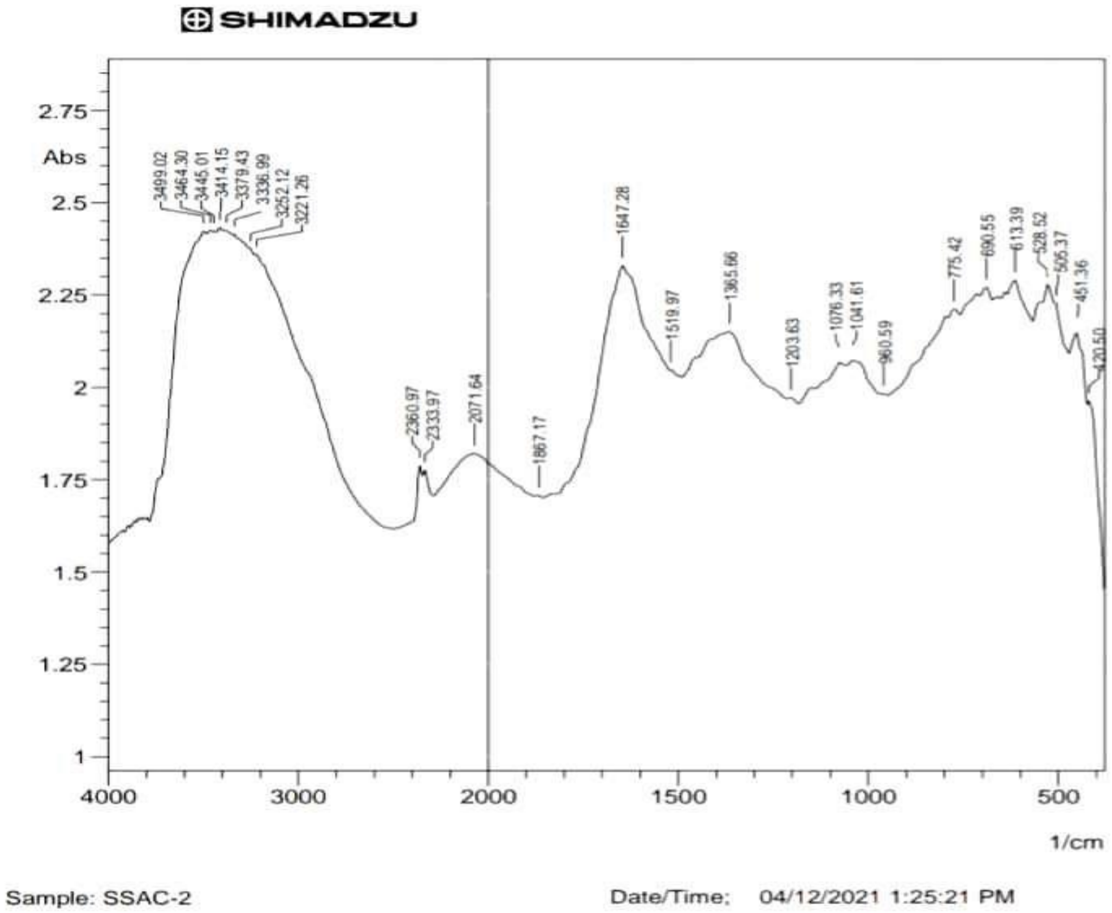


Fig.2: FT-IR absorption spectra of synthesized AgNPs.

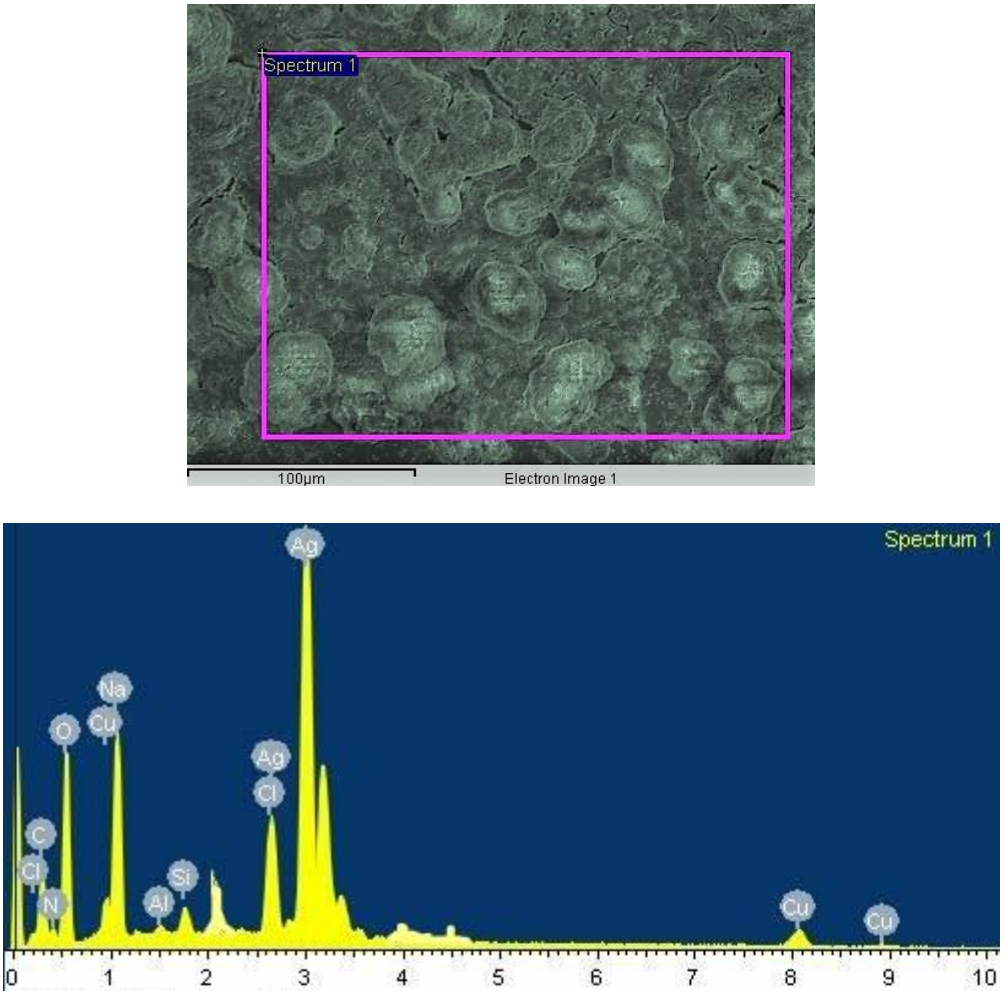


Fig.3.SEM images of silver nanoparticles synthesized from *Morinda citrifolia* stem extract show highly agglomerated shape.

RESULT AND DISCUSSION: When the stem extract of *Morinda Citrifolia* was mixed with AgNO3 solution, the yellow colour of aqueous extract changed to brownish colour immediately within 10 min, indicating the formation of silver nanoparticles.

The reduction of pure silver ions was confirmed by UV-vis spectra where the maximum absorbance was seen at 470nm (fig.1). The UV-visible characteristics can be associated with the size AgNPs. In addition, FTIR characteristics have confirmed the biomolecules bonding on AgNPs. FT-IR spectrum peak at 3445cm^{-1}, 3336cm^{-1}, 2360cm^{-1}, 2071cm^{-1}, 1647cm^{-1}, 1519cm^{-1}, 1365cm^{-1}, 1203cm^{-1}, 1076cm^{-1}, 1041cm^{-1}, 775cm^{-1}, 690cm^{-1} and 528cm^{-1} which shows many functional groups(fig.2). SEM images showed aggregation of nanoparticles (Fig.3).

CONCLUSION: The synthesis of silver nanoparticles using stem of *Morinda Citrifolia* extract has been demonstrated. The aim of this investigation is to evaluate the effectiveness of the procedure to synthesize AgNPs using *Morinda Citrifolia*. This method is simple, economic, non-toxic and efficient. Nanoparticles synthesis by medicinal plants shows more benefit, they may enhance the antimicrobial activity of silver nanoparticles, because the medicinally valuable active biomolecules present in the plants may bind on the surface of the nanoparticles and reduce the silver ions to silver nanoparticles. These silver nanoparticles were of high purity, making them potentially useful for biological applications. This biological method is potentially attractive for large scale synthesis of metallic and metal oxide nanomaterials. This work extends the knowledge for the exploration of natural resources particularly *Morinda Citrifolia* as reducing and stabilizing agents for a synthesis of AgNPs.

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REFERENCES:

i. Ren X.; Meng X.; Chen D.; Tang F.; J. Biosens Bioelectron.; 2005, 21,433-437.
ii. Sun S.; Murry C.B.; Weller D.; Folks L.; Moser A.; Science; 2000, 287,1989-1991.
iii. Kamat P.V.; Journal of Physical Chemistry; 2002, 106,7729-7744.
iv. Moreno-Manas M.; Pleixats R.; Account of Chemical Research; 2003, 36,638-643.
v. Kanel S.; Manning B.; Charlet L.; Choi H.; Environmental science and Technology; 2005, 39,1291-1298.
vi. Cao J.; Elliott D.; Zhang W.; Journal of nanoparticles Research; 2005, 7,499-506.
vii. Chen S.; Hsu H.; Li C.; Journal of nanoparticles Research; 2004, 6,639-647.
viii. Gillham R.W.; O'Hannesin S.F.; Ground Water; 1994, 36,958-967.
ix. Lin Y.; Wen C.; Chen F.; Separation and Purification Technology; 2008, 64,26-30.
x. Wang C.B.; Zhang W.X.; Environmental science and Technology; 1997, 31,2154-2156.
xi. Ahmad A.; Mukherjee P.; Mandal D.; Senapati S.; Khan M.I.; Kumar R.; Sastry M.; Journal of the American Chemical Society; 2002, 124,12108-12109.
xii. Ahmad A.; Senapati S.; Khan M.I.; Kumar R.; Sastry M.; Langmuir; 2003, 19,3550-3553.
xiii. Shahverdi A.R.; Minaeian S.; Shahverdi H.R.; Jamalifar H.; Nohi A.; Process Biochem (Amsterdam Neth.); 2007, 42,919-923.
xiv. Jha A.K.; Prasad K.; Biochemical Engineering Journal; 2009, 43,303-306.
xv. Bharde A.A.; Parikh R.Y.; Baidakova M.; Jouen S.; Hannoyer B.; Enoki T.; Prasad B.L.V.; Shouche Y.S.; Ogale S.; Sastry M.; Langmuir; 2008, 24,5787-5794.
xvi. Shankar S.; Ahmad A.; Sastry M.; Biotechnology Process; 2003, 19,1627-1631.
xvii. Shankar S.S.; Rai A.; Ahmad.; Sastry M.; Journal of Colloid and Interface Science; 2004, 275,496-502.
xviii. Ankamwar B.; Damle C.; Ahmad A.; Sastry M.; Journal of nanoscience and technology; 2005, 5,1665-1671.
xix. Chandran S.P.; Chaudhary M.; Pasricha R.; Ahmad A.; Sastry M.; Biotechnology Process; 2006, 22,577-583.
xx. Nadagouda M.N.; Verma R.S.; Green Chemistry; 2008, 10,859-862.
xxi. Nadagouda M.N.; Castle A.B.; Murdock R.C.; Hussain S.M.; Verma R.S.; Green Chemistry; 2010, 12,114-122.
xxii. Hoag G.E.; Collins J.B.; Holcomb J.L.; Hoag J.R.; Nadagouda M.N.; Verma R.S.; Journal of Material Chemistry; 2009, 19,8671-8677.
xxiii. Bar H.; Bhui D.K.; Sahoo G.P.; Sarkar P.; De S.P.; Misra A.; Colloids and Surface; 2009, 339,134-139.
xxiv. Sathishkumar M.; Sneha.; Kwak I.S.; Mao J.; Tripathy S.J.; Yun Y.S.; Journal of Hazardous Materials; 2009, 171,400-404.
xxv. X. Li.; H. Xu.; Chen Z.; Chen G.; Biosynthesis of nanoparticles by microorganism and their applications; Journal of Nanomaterials*;* 2011, Articles ID 270974, 16.
xxvi. Banerjee J.; Narendira kannan.; Dig J.; J. Nanomat. Biostruct; 2011, 6,961.
xxvii. Elumalai E.K.; Prasad K.V.; Kambala P.C.; Nagajyothi P.; David E.; Scholars Res. Library; 2010, 6,76.

xxviii. Wang G.; Wang W.; Wu J.; Liu H.; Shoufeng J.; Fang B.; Self-assembly of a silver nanaoparticles modified electrode and its electrocatalysis on neutral reduction; Microchim Acta; 2009, 164,149-155.

xxix. Gan X.; Liu T.; Zhong J.; Liu XJ.; Li GX.; Effect of silver nanoparticles on the electron transfer reactivity and the catalytic activity of myoglobin; ChemBioChem; 2004, 5,1686-1691.

xxx. Zeena S.P.; Kamat P.V.; What factor control the size and shape of the silver nanoparticles in the citrate ion reduction method; Journal of Physical Chemistry B; 2004, 108,945-951.

xxxi. Mallick K.; Witcomb J.M.; Scrurell M.S.; Self-assembly of silver nanoparticles formation of a thin silver film in a polymer matrix; Materials Science and Engineering; 2006, 26,87-91.

xxxii. Tripathi S.; Mehrotra G.K.; Dutta PK.; Silver-oxide nanocomposite film preparation and antimicrobial activity; Bulletin of Materials Science; 2011, 34,29-35.

xxxiii. Espinosa-Cristobal L.F.; Martinez-Castanon G.A.; Martinez-Martinez R.E.; Antimicrobial sensibility of spectrococcus mutans serotypes to silver nanoparticles; Materials Science and Engineering; 2012, 10,1016.

xxxiv. Liu Z.; Xing Z.; Zu Y.; Tan S.; Zhao Z.; Zhao L.; Sun T.; Synthesis and Characterization of L-histidine capped silver nanoparticles; Materials Science and Engineering; 2012, 10,1-31.

xxxv. Narang J.; Chauhan N.; Jain P.; Pundir C.S.; Silver nanoparticles/multiwalled carbon nanotubes/polyaniline film for amerometric glutathione biosensor; International Journal of Biological and Macromolecules; 2012, 50,672-678.

xxxvi. Litwack G.; Bothwell J.W.; Williams J.N.; Elvehjem C.A.; A colorimetric assay for xanthine oxidase in rat liver homogenates; Journal of Biological Chemistry; 1953, 200,303-310.

xxxvii. Ankamwar B.; Damle C.; Ahmad A.; Sastry M.; Journal of Nanoscience and Nanotechnology; 2005, 5,1665-1671.

xxxviii. Hoag G.E.; Collins J.B.; Holcomb J.L.; Hoag J.R.; Nadagouda M.N.; Verma R.S.; Journal of Materials Chemistry; 2009, 19,8671-8677.

xxxix. Bar H.; Bhui D.K.; Sahoo G.P.; Sarkar P.; De S.P.; Misra A.; Colloids and Surface; 2009, 71,113-118.

xl. Deshpande R.; Prashant A.B.; American Journal of Analytical Chemistry; 2011, 2,475.

xli. Bhattacharya D.; Gupta R.K.; Critical Review Biotechnology; 2005, 94,148.

xlii. Dwivedi A.D.; Gopal K.; Biosynthesis of silver and gold nanoparticles using Chenopodium album leaf extract; Colloides and Surfaces A; 2010, 369,27-33.

xliii. Bauer A.W.; Kirby W.M.; Sherris J.C.; Turck M.; American Journal of Society and Clinical Pathology; 1996, 45,493.

xliv. Darun N.; Priscyla Marcato D.; Journal of the Brazilian Chemical Society; 2010, 21,949.

xlv. Mahendra Rai.; Alka Yadav.; Aniket K Gade.; Silver Nanoparticles as a New Generation of Antimicrobials; Biotechnology Advances; 2008, 27(1),76-83.

xlvi. Virender K Sharma. et al.; Silver Nanoparticles: green synthesis and their antimicrobial activities; Advance Colloid Interface Science; 2009, 145,83.

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