



Research Article

BIOSYNTHESIS OF GOLD NANOPARTICLES USING *MUKIA SCABRELLA* AND ITS ANTIMICROBIAL APPLICATIONS**Samyvel Periyasamy and Kandasamy Prabakar***Postgraduate and Research Department of Zoology, Jamal Mohamed College (Autonomous),
Tiruchirappalli-620020, Tamil Nadu, India**Article History:** Received 4th March 2018; Accepted 23rd March 2018; Published 24th March 2018**ABSTRACT**

The present study was performed for the one pot green synthesis of gold nanoparticles (AuNPs) at room-temperature using the leaf extract *Mukia scabrella*. AuNPs were characterized with different spectroscopy techniques such as UV-visible spectroscopy (UV-vis), Fourier Transform Infra-red spectroscopy (FTIR), and Energy dispersive X-ray (EDAX) spectroscopy SEM analysis presented that synthesized particles were predominantly round in shape with a size range of 30- 50 nm. Zeta potential analysis showed - 32.2 mV characteristic for stable AuNPs. Antimicrobial activity results presented that synthesized AuNPs have potent antimicrobial activity against gram negative bacteria. The results suggested that *M.scabrella* could be used for the synthesis of gold based nano drug against bacterial pathogens.

Keywords: *M. scabrella*, Biosynthesis, AuNPs, Antibacterial activity.**INTRODUCTION**

Green synthesis of metallic nanoparticles has been considered as a safe and an ecofriendly approach than chemical method. Several studies have shown that gold nanoparticles have many applications including biomedical, pharmaceutical, catalytic and optoelectronic applications (Rai *et al.*, 2010). Furthermore, gold nanoparticles are non-toxic and photo stable (Jayaseelan *et al.*, 2013). Due to this, recent methods aimed at get desired shape of nanoparticles in a cost effective and sustainable method. Consequently, in the past year, there has been an exponentially growing interest in the biosynthesis of gold nanoparticles using medicinal plants (Chandran *et al.*, 2006; Narayanan and Sakthivel, 2008) AuNPs have also considered for important applicatios such as drug delivery, tissue/tumor imaging, photothermal therapy and immunochromatographic identification of pathogens in clinical specimens (Huang, 2006).

Globally, the emerging antimicrobial resistance bacterial pathogens urgently need an effective treatment without any harmful effects. The present day drugs are outdated and nanotechnology provides the basis for the

drug formulation to overcome the various drug resistant mechanisms that showed by pathogenic organisms. In this view, medicinal plants have attracted more attention.

Mukia scabrella has several health benefits such curative effect on respiratory infections, cough, cold and digestive disorders in traditional Ayurveda medicine. However, there are no studies on biosynthesis of gold nanoparticles.

Considering the above facts, in the present study we report the biosynthesis of AuNPs by *M. scabrella* leaf extract. AuNPs were characterized using UV-visible spectroscopy, FTIR, EDAX, SEM, and zeta potential. The antimicrobial activity was evaluated against *Escherichia coli* causing intestinal illness in humans.

MATERIALS AND METHODS**Test bacterial strains**

Clinical isolates of *Escherichia coli*, was obtained from Doctors Diagnostic Center, Trichy. All test strains were cultured at 37°C in Luria broth or on Luria agar.

*Corresponding Author: Dr. K. Prabakar, Assistant Professor of Zoology, Jamal Mohamed College, Tiruchirappalli -620020, Tamil Nadu, India, Email: drprklab@gmail.com, Mobile: +91 9659796999

Collection of plant material

Healthy plant (leaf) of *M. scabrella* was collected from foothills of Pacahimalai Mountains, Tamil Nadu, India. Dried leaves of *M. scabrella* were finely ground. The powder was used for the preparation of leaf extract. Briefly, 5% of *M. scabrella* leaf extract was prepared with sterile deionized water and heated at 60°C for 20 min. The resulting mixture was cooled and filtered with Whatman paper No. 1 and used for biosynthesis of AuNPs.

Biosynthesis of AuNPs

For the fabrication of AuNPs, 5 ml of leaf extract was added to 95 ml of 1 mM aqueous final concentration of HAuCl₄ (aq). The reaction mixture was incubated at room temperature. The bioreduction process, color change to purple confirmed the formation of AuNPs. With this preliminary observation, UV-visible spectrometric measurements were performed.

Characterization of AuNPs

Preliminary characterization of AuNPs was performed with visual observation for change of color in the reaction mixture. Reduction of Au³⁺ to Au⁰ was carried out on UV-vis spectroscopy at wavelength range between 300 and 700 nm. The spectra were recorded at 24 h. FTIR spectra were recorded on a spectrum RX 1-one instrument in the diffuse reflectance mode at a resolution of 4 cm⁻¹ in KBr pellets. The surface morphology and elemental composition of AuNPs was analysed in scanning electron microscope (FESEM-ZEISS) equipped with an EDAX. Energy dispersive spectrometer spectrum was measured at 20 kV accelerating voltage. The potential of AuNPs carried out using Zetasizer Nano-ZS (Malvern, UK).

Antibacterial activity of AuNPs

Antimicrobial activity was tested for biosynthesized AuNPs clinical isolates *E. coli*, on Muller-Hinton agar plate using a disc diffusion method. Zone of inhibition (ZoI) was determined to study the growth inhibition of test strains. Sterile standard antibiotic discs with diameter of 6 mm were purchased from HIMEDIA Laboratories, India. 60 µl of test samples were loaded on the sterile disc and air dried completely. MHA agar was spread plated about 10⁶ CFU/ml of test pathogens, impregnated with the sample loaded disks incubated at 37°C for 24 h. Following incubation, ZoI in millimeters was measured to interpret the results Prabakar *et al.* (2013 and 2015).

RESULTS AND DISCUSSION

Biosynthesis of AuNPs

In view of developing safe nano formulation, in the present study we performed plant-mediated synthesis of gold nanoparticles. Several studies reported the use of herbal plant materials for the synthesis of AuNPs. However, reaching the desired shape and charge of the particles is the primary importance whilst formulation of Nano based materials. A reaction mixture of 5% leaf extract and HAuCl₄ changed gradually from light yellow to purple color indicated the formation of AuNPs (Figure 1). The color change in the reaction mixture was due to the excitation of surface plasmon vibrations and characteristic of the synthesized nanoparticles (Song *et al.*, 2009). In contrast no color change observed in control experiments that contain aqueous HAuCl₄ only (Figure 2 [A]). There may be involvement of enzymes or secondary metabolites in the formation of gold nanoparticles by *M. scabrella* leaf material.



Figure 1. Color change profile of biosynthesized gold nanoparticles.

A: Control (No leaf extract); B: 1mM Auric chloride and 5% leaf extract (Contain reductant)

Characterization of biosynthesized AuNPs

The UV-vis spectra recorded for the reaction mixture to study the kinetics of bioreduction process at 36 h. The spectra revealed a strong absorption peak at 535 nm (Figure 2) corresponding to the surface plasmon resonance in AuNPs after 24 hrs of incubation at room temperature (Song *et al.*, 2009). The increase in the absorption peak with time around 535 nm without any shift after incubation in the reaction mixture indicate the particles were in similar size and dispersed (Binupriya *et al.*, 2010). FTIR

measurements were carried out to identify the possible interactions between gold nanoparticles and possible capping agents in the leaf extract of *M. scabrella*. FTIR spectrum of AuNPs synthesized by leaf extract of *M. scabrella* shows intense bands at 3967 cm^{-1} , 3431 cm^{-1} , 2070 cm^{-1} , 1638 cm^{-1} and 680 cm^{-1} . The bands at 3431 cm^{-1} and 1638 cm^{-1} could be attributed to free N-H and -C=C- vibrations respectively, which corresponds to heterocyclic compounds like proteins (Figure 3). This serves as support for proteins present in the leaf extract as capping agents for the biosynthesized AuNPs.

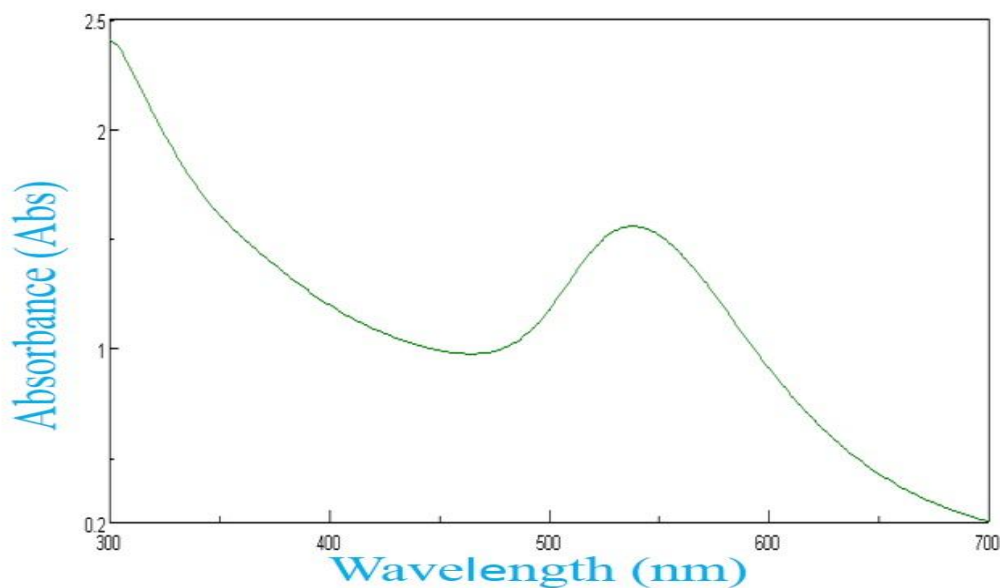


Figure 2. UV-Vis spectra of biosynthesized AuNPs.

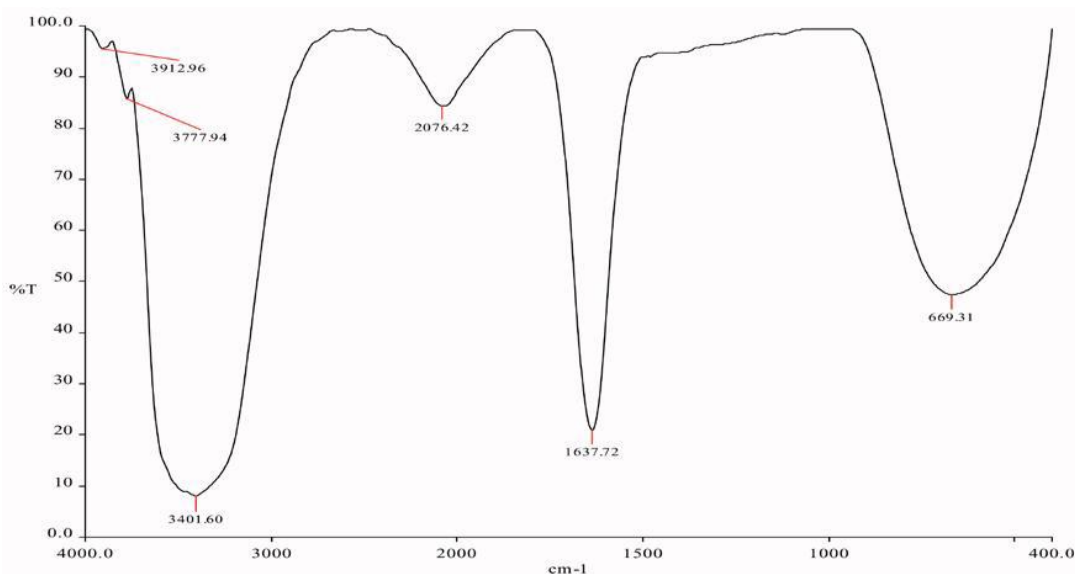


Figure 3. FT-IR spectrum of AgNPs synthesized by using Rhizome extract of *M. scabrella*.

The FESEM micrograph observations showed the size and shape of the nanoparticles (Figure 4). The shape of the synthesized nanoparticles was observed predominantly to be spherical in shape. The size of the nanoparticles was in the range of 20 - 35 nm.

EDAX analysis showed strong peak at 2.1 and 9.5 keV confirmed the presence of metallic gold crystals as shown in (Figure 5). The zeta potential of synthesized AuNPs was found to be -32.2 mV (Figure 6) indicating that the surface

of the nanoparticles was negatively charged that dispersed in the colloidal solution. The high negative potential of nanoparticles causes electrostatic repulsive forces between them and prevent from aggregation which might be attributed for the stable nature of AuNPs (Sathishkumar *et al.*, 2009).

Thus, the analysis proved the presence of nanocrystalline gold particles in favor of the UV-vis spectra, EDAX and FESEM analysis.

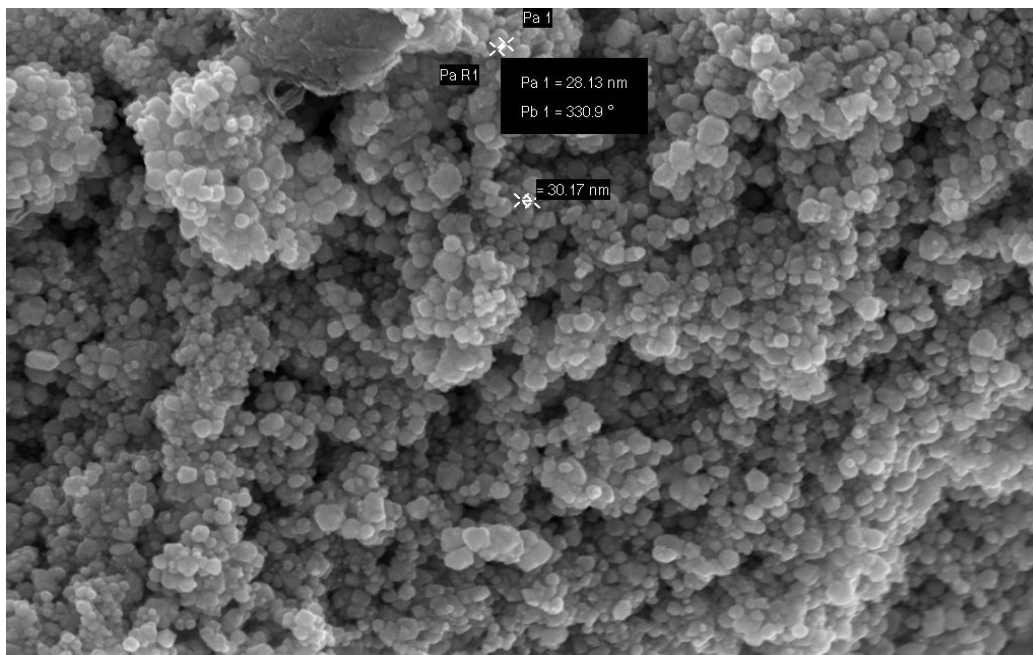


Figure 4. FESEM observation of biosynthesized AuNPs using leaf extract (5%) of *M. scabrella* at a scale bar of 200 nm.

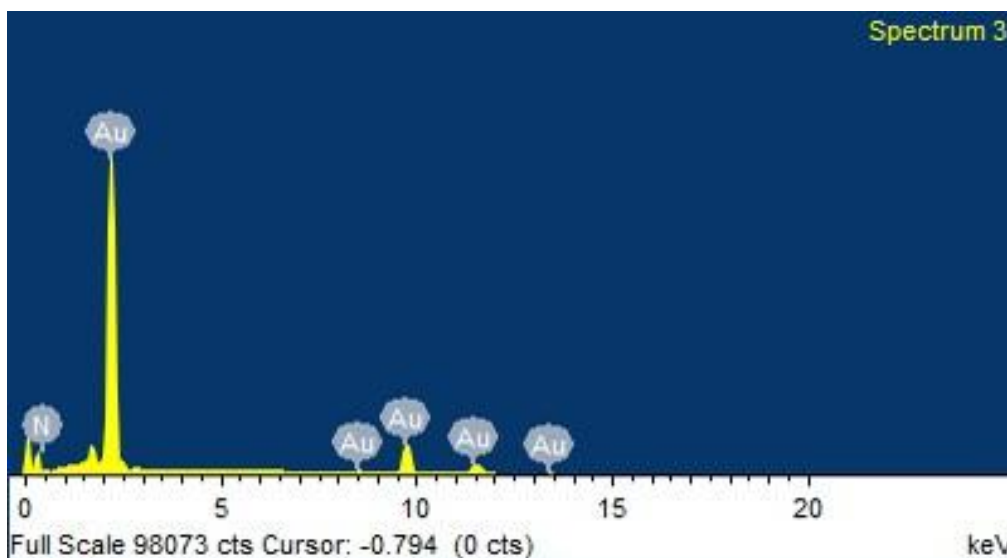


Figure 5. EDAX analysis of biosynthesized AuNPs using leaf extract (5%) of *M. scabrella*.

Antibacterial activity of AuNPs

In the present investigation, antibacterial activity of synthesized AuNPs was evaluated against three different gram negative enteric pathogens of *Escherichia coli* (MTCC 1687) using disc diffusion method.

The bactericidal effect of AuNPs was evaluated by measuring the clear zone of inhibition after 24 h incubation at 37 °C. It was observed that AuNPs synthesized from leaf extract (5%) exhibited enhanced antibacterial activity than cell free supernatant alone Prabakar *et al.*, (2013 and 2015). In contrast leaf extract (5%) did not indicate any clear visible zones of inhibition at the same concentration used for synthesis (Figure 6 and 7). The results indicated that larger surface area of the nanoparticles acts better on the bacterial cells and had bactericidal activity (Drogat

et al., 2011). Though the exact mechanism for the bactericidal activity of AuNPs has not been elucidated yet, several mechanisms have been proposed. Rai *et al.* (2010) reported that AuNPs may bind to the DNA and inhibit the uncoiling of and transcription of DNA resulting in the death of bacteria. Another proposed mechanism involves the interaction of gold nanoparticles with bacterial outer membrane lipopolysaccharide and protein resulting in disruption of membrane integrity (Rai *et al.*, 2010). Previous studies reported that antibiotics coated AuNPs lead to enhanced antimicrobial activity (Gu *et al.*, 2003; Grace and Pandiyan, 2007). Similarly, we believe that antibacterial secondary metabolite of the leaf extract capped on the surface of the nanoparticle might be the possible reason for elevated antimicrobial activity.

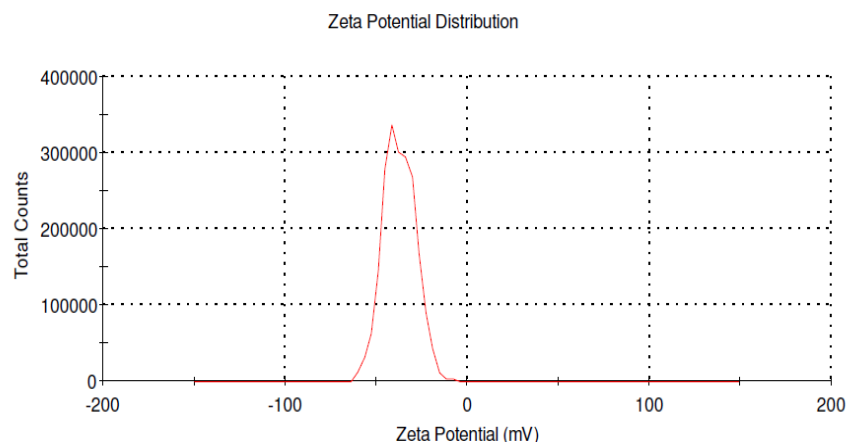


Figure 6. Zeta potential of biosynthesized AuNPs using leaf extract (5%) of *M. scabrella*.

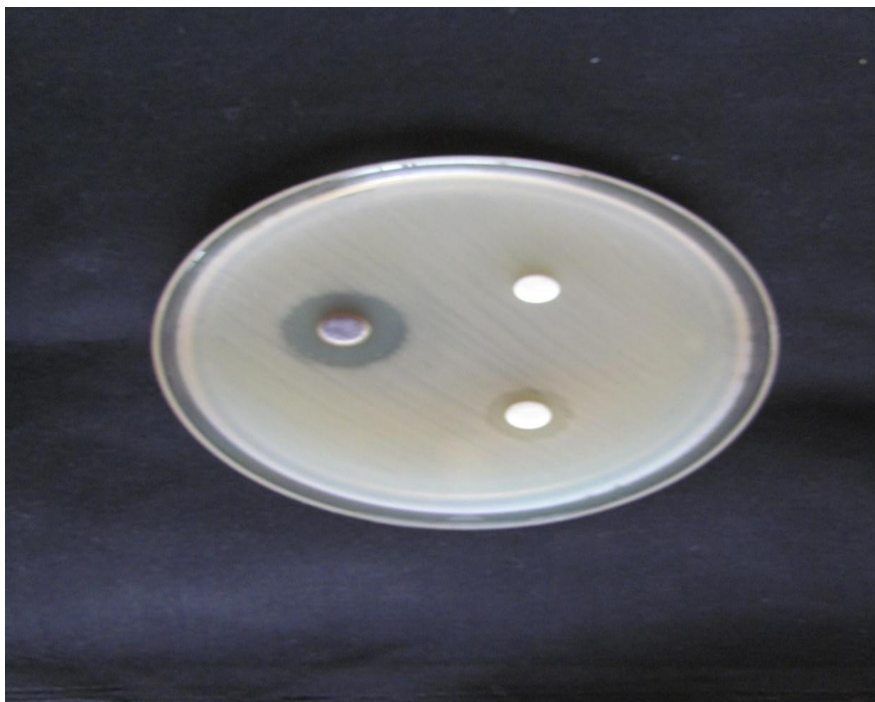


Figure 7. Antimicrobial activity of biosynthesized AuNPs using leaf extract (5%) of *M. scabrella*.

CONCLUSION

In conclusion, we presented a potentially safe, ecofriendly and cost effective methodology to synthesize gold nanoparticles using the leaf extract of *M. scabrella*. The synthesized AuNPs were characterized using Uv-vis spectroscopy, FTIR, EDAX, Zeta potential, and SEM. Antibacterial activity of fabricated AuNPs was evaluated against gram negative bacteria. The results showed significant antibacterial activity against the test bacterial pathogens. Thus the present study proves that *M. Scabrella* could be used for the sustainable production of gold nanoparticles for antimicrobial applications.

ACKNOWLEDGEMENT

Authors are much thankful to the UNIVERSITY GRANTS COMMISSION (UGC) New Delhi for the financial assistance under the Major Research Project Scheme [UGC F.No. 42-608/2013 (SR)]. Also my sincere thanks Dr. A.K. Khaja Nazeemudeen Secretary and Correspondent and Dr. S. Ismail Mohideen, Principal, Jamal Mohamed College and Dr. Mohamed Shamsudin, Associate Professor and Head, The Department of Zoology and former Principal's Dr. Khadermohideen, Dr. A.M. Mohamed Sindhasha and Dr. S. Mohamed Salique for providing all facilities to carry out my project successfully.

REFERENCES

- Binupriya, A.R., Sathishkumar, M. and Yun, S.I., 2010. Biocrystallization of silver and gold ions by inactive cell filtrate of *Rhizopus stolonifer*. *Colloids Surf B Biointerfaces*, 79, 531- 534.
- Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A. and Sastry, M., 2006. Synthesis of gold nanotriangles and silver nanoparticles using aloe vera plant extract. *Biotechnol. Progr.*, 22, 577-583.
- Drogat, N., Granet, R., Sol, V., Memmi, A., Saad, N., Koerkamp, C.K., Bressollier, P. and Krausz, P., 2011. Antimicrobial silver nanoparticles generated on cellulose nanocrystals. *J. Nanopart. Res.*, 13, 1557-1562.
- Grace, A.N. and Pandian, K., 2007. Antibacterial efficacy of aminoglycosidic antibiotics protected gold nanoparticles-a brief study. *Colloids Surf. A*, 297, 63-70.
- Gu, H., Ho, P.L., Tong, E., Wang, L. and Xu, B., 2003. Presenting vancomycin on nanoparticles to enhance antimicrobial activities. *Nano Lett.*, 3, 1261-1263.
- Huang, S.H., 2006. Gold nanoparticle-based immunochromatographic test for identification of *Staphylococcus aureus* from clinical specimens. *Clinica Chimica Acta*, 373, 139-143.
- Jayaseelan, C., Ramkumar, R., Rahuman, A.A. and Perumal, P., 2013. Green synthesis of gold nanoparticles using seed aqueous extract of *Abelmoschus esculentus* and its antifungal activity. *Industrial Crops and Products*. 45, 423-429.
- Narayanan, K.B. and Sakthivel, N., 2008. Coriander leaf mediated biosynthesis of gold nanoparticles. *Mater. Lett.*, 62, 4588-4590.
- Prabakar, K., Sivalingam, P., Mohamed, R., Muthuselvam, M., Devarajan, N., Arjunan, A., Karthick, R., Suresh, M.M., Pote, J. 2013. Evaluation of antibacterial efficacy of phyto fabricated silver nanoparticles using *Mukiascabrella* (*Musumusukkai*) against drug resistance nosocomial gram negative bacterial pathogens. *Colloids and Surfaces B: Biointerfaces*. 104:282-288.
- Prabakar, K., Samyvel Periyasamy and Charli Deepak 2015. Phytochemicals screening, antioxidant and antibacterial potential of *Mukiascabrella* (*Musumusukkai*) against nosocomial bacterial pathogens. *Int. J. of Pure and Appl. Zoology*. 3(1): 1-8.
- Rai, A., Prabhune, A. and Perry, C.C., 2010. Antibiotic mediated synthesis of gold nanoparticles with potent antimicrobial activity and their application in antimicrobial coatings. *J. Mater. Chem.*, 20, 6789-6798.
- Sathishkumar, M., Sneha, K., Won, S.W., Cho, C.W., Kim, S. and Yun, Y.S., 2009. Cinnamon zeylanicum bark extract and powder mediated green synthesis of nanocrystalline silver particles and its bactericidal activity. *Colloids Surf. B*, 73, 332-8.
- Song, J.Y., Jang, H.K. and Kim, B.S., 2009. Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extracts. *Process Biochem.*, 44, 1133-1138.