International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 819-826
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

ASSESSMENT OF GREEN SYNTHETIC ROUTE OF SILVER NANOPARTICLES SYNTHESIS BY MANGROVE SPECIES *RHIZOPHORA APICULATA* OF ANDAMAN COAST

***Tijo Cherian, Suneelkumar Yalla and R.Mohanraju**

Department of Ocean Studies and Marine Biology, Pondicherry University, Brookshabad, Port Blair-744112, Andamans, India

***Corresponding Author Email: tvarghese891@gmail.com**

ABSTRACT

Silver nanoparticles (AgNPs) were fabricated by using Rhizophora apiculata aqueous leaf extract (RALE) and their antimicrobial potential was assessed. Their studies of characterization was done by UV-Visible spectrophotometry, X-ray diffraction, HR-TEM, SAED pattern and FTIR analysis. The characteristic peak of RALE-AgNPs was found at a intensity of 421 nm which when further validated by HR-TEM depicted their size ranging from 14.46-29.58 nm. The antagonistic and inhibitory activity of RALE-AgNPs was analysed by concentration dependent zone inhibition patterns against pathogenic strains, thereby, formulating a green eco-friendly, economical perspective for synthesizing bio-functionalized RALE-AgNPs as potential applicants for clinical settings.

KEY WORDS

Nanotechnology, Green synthesis, Silver nanoparticles, Rhizophora apiculata, Antibacterial activity

INTRODUCTION

Nanobiotechnology is a multidisciplinary sphere, offering a dynamic working platform, intersecting, transforming and investigating material sciences with the aspects of biology which uniquely put forwards wide and diverse inspirational biologically active models and various bio-assembled systems. This unique novel remodel of Nanobiotechnology, renders the principles of nanosciences to various biological systems and processes further devising out new devices and integrating systems at nanoscale (MubarakAli *et al*., 2011).

This fascinating field of science has paved a novel path for the introduction, discovery and invention of various nanostructural materials of nanoscale dimensions. Conventional chemical and physical approaches usually foster large amounts of nanomaterials in short time span but the reacting molecules are usually toxic and generates non-eco-friendly byproducts. Due to these ill-effects, a safer, cheaper, environmentally benign, green synthetic technology has to be devised out for the generation of diverse nanostructures (Ahmed S *et al*., 2015). Thus, an advancement in green synthetic process has marched forward as an important and indispensable realm of nanobiotechnology using various biological entities like microorganisms, plant extract or plant biomass as "Nanomanufacturing units" leading to a promising green and clean alternative of nanogeneration (Reddy *et al*., 2012)

Till date, a vast sum of literature reviews has been outlined on bioinspired nanoparticles synthesized via microbial cellular biosystems or plant mediated synthetic procedures which primarily undertakes their reducing and antioxidative properties in bioreduction of metallic solutions to their specific nanoforms. Among these procedures, microbial cell mediated synthetic approach is more appealing but has a limitation of low industrial feasibility on broad scale because of the

constant maintenance of microbial strains under high aseptic sterile conditions. Therefore, the plant mediated process offers an advantage over microbial cells because of process of ease of improvement, nullifying elaborate mechanisms of cell culture maintenance and no biohazard (Ahmed S *et al*., 2015).

The present investigation describes the potentiality of an mangrove *Rhizophora apiculata* from coastal realms of Andaman for biosynthesis of phyto-extract functionalized silver nanoparticles; their characterization and antibacterial activity.

MATERIALS AND METHODS

Growth conditions and bacterial strains

Four bacterial pathogenic strains (Enterotoxicogenic *Escherichia coli* O114, *Vibrio cholerae* MTCC 3906, *Shigella sonnei*, *Shigella flexneri*) maintained in our laboratory, were tested. The pure cultures were cultured and streaked on nutrient agar and maintained as slants, stabs and glycerol cultures at -20°C.

Preparation of the Rhizophora apiculata leaf extract (RALE)

Fresh leaves of *R.apiculata* were collected from mangrove region of Carbyn's Cove, South Andaman (GIS coordinates: 11°38'35.0''N, 92°44'49.1''E) thoroughly washed with deionized water, air dried and chopped into small pieces. Chopped leaf pieces (50g) were thoroughly grounded in a grinder and the resulting thick paste was dispersed in 100 ml deionized sterile water. Thus, the obtained extract, was filtered via Whatman filter paper No.1 and stored for further analysis.

Synthesis of RALE capped AgNPs

For the bioreductive synthesis of RALE-AgNPs, 4 ml of *R.apiculata* leaf extract (RALE) was added to 16 ml 1mM $AgNO_3$ solution, pH 8.0 and incubated for 24 h at room temperature under dark conditions (to avoid photochemical reactions). A control setup (flask with same quantity of RALE) was run under identical conditions. The obtained coloured solution was dried further in an oven (Yorco, India) at 40°C for 20-24 h and the resulting dried content was pulverized and stored for further analysis.

Characterization of RALE-AgNPs

UV-visible spectroscopy

UV-Visible spectroscopy was employed for analyzing the unique and characteristic surface plasmon resonance peak of biosynthesized RALE-AgNPs by UV-Vis spectrophotometer (double beam, UV5704S Electronics, India ltd) at 350–700 nm wavelength at 0.5 nm resolution.

X-ray diffraction (XRD) peak measurements

The X-ray diffraction peak patterns of RALE-AgNPs was recorded by MiniFlex II XRD benchtop system unit (Rigaku Corporation, Tokyo, Japan) operational at 40 kV, 30 mA current with a Cu Ka radiation (k = 1.54 Å) and diffracted intensities of 20° to 80° 2Θ angles. The size of crystal lattice of the NPs was calculated by Debye–Scherrer's formula: $D= 0.9\lambda/\beta\cos\theta$: where λ is the X-ray wavelength (1.541Å), β is full-width-at-half maximum of diffraction peak and D is crystal lattice size of NPs.

High resolution transmission electron microscopy (HR-TEM)

HR-TEM was performed on JEOL 100/120 kV (JEOL 3010, Tokyo, Japan) with a voltage of 200 kV. For microscopic visualization, samples were readily made by dropping 10µl of RALE-AgNPs on a copper grid and were dried for 6 h at 80°C in an oven and analysed.

Fourier transform infrared (FTIR) spectroscopy

Assessment of chemically defined functional groups on RALE and RALE functionalized AgNPs was undertaken by FTIR spectroscopy. The air-dried powdered samples of RALE and RALE-AgNPs were diluted with KBr (spectroscopic grade with mass ratio of about 1:100) and the spectral lines were recorded. The subsequent peak measurements were analyzed by Perkin Elmer FT-IR spectrometer Spectrum Two (Perkin Elmer Life and Analytical Sciences, USA) at a diffused reflectance mode at 4 cm^{-1} resolution in KBr pellets.

Antibacterial activity

The dose dependent RALE-AgNPs antibacterial activity against pathogenic isolates was ascertained by well diffusion method as mentioned by Ali *et al.* (2015). Bacterial culture (0.1 ml, cell density 2×10^8 CFU/ml) was plated uniformly on nutrient agar plates and wells were created by cutting agar with gel puncture. Subsequently, variable concentrations of RALE-AgNPs (0, 25, 50, 75, 100 µl) solution were added to the pre-cut wells in the plates and incubated for 24 h at 37°C. The size of inhibition zones were measured by determining the zone radii.

Statistical analyses

The experiments were performed in triplicate and the data was expressed as mean ± S.D. One-way analysis of variance (ANOVA) Holm-Sidak method was used to perform statistical analysis, multiple comparative data

values versus control group value (Sigma Plot 11.0, USA).

RESULTS

Biosynthesis of RALE-AgNPs

The reduction and biotransformation of aqueous form silver ions was studied by monitoring the variations in color of the solution with UV-Visible absorption spectroscopy. The change in color of the solution was fast and instant, an preliminary and primary indication of RALE-AgNPs formation, which signifies the interaction of electromagnetic waves with metallic nanoparticles leading to collective oscillations and excitations of conduction band electrons. The collective resonance of charged outer shell electrons in a solid lattice excited by incident light is described as Surface plasmon resonance phenomena (SPR), which strictly depends upon shape, size, dielectric constant and charge distribution of metal and its surrounding medium (Eustis *et al*., 2006). A sharp, stable and distinct absorptive peak at 421 nm (Fig.1) was observed which indicated the generation of stable RALE-AgNPs in the solution, thereby, indicative of the role of phyto-compounds present in *R.apiculata* leaf extract which resulted in the ion reduction of Ag^{+} to stable AgNPs and their effective wrapping around the AgNPs to provide excellent stabilizer to avoid agglomeration.

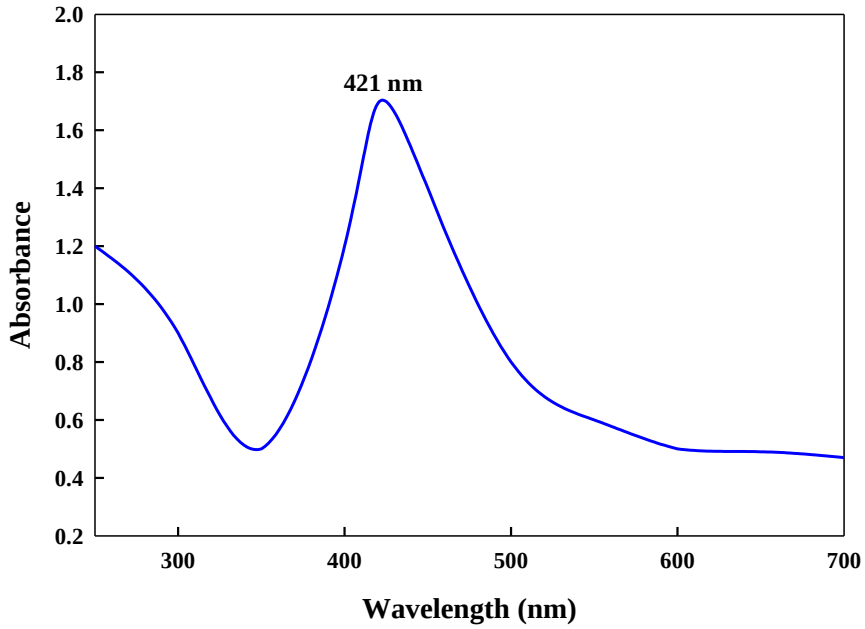


Fig. 1 UV-Visible spectrum of *Rhizophora apiculata* synthesized silver nanoparticles (RALE-AgNPs) showing characteristic peak at 421 nm.

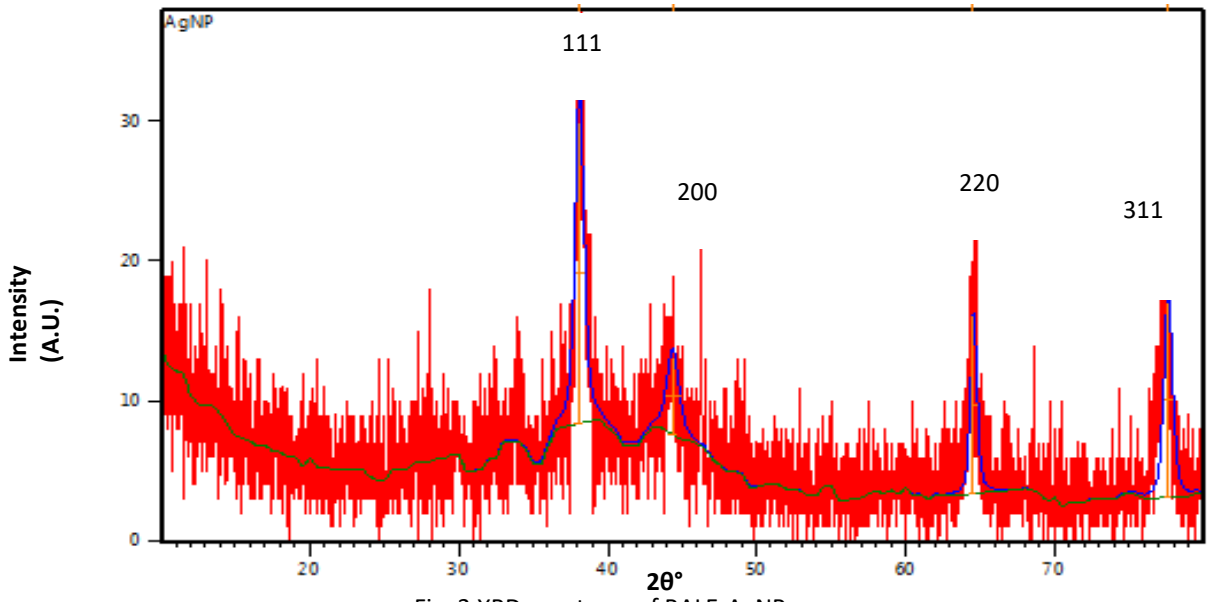


Fig. 2 XRD spectrum of RALE-AgNPs.

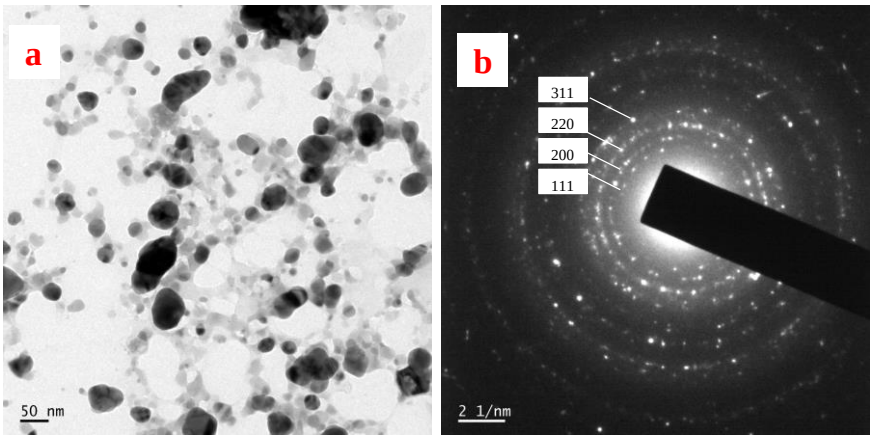
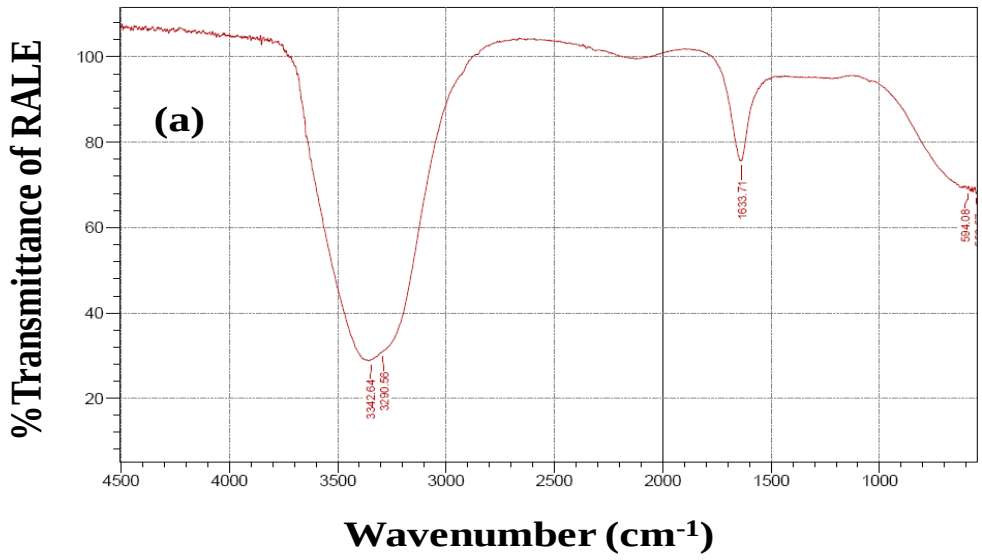


Fig. 3 HR-TEM images of biofunctionalized RALE-AgNPs (a) at 50 nm (b) SAED pattern



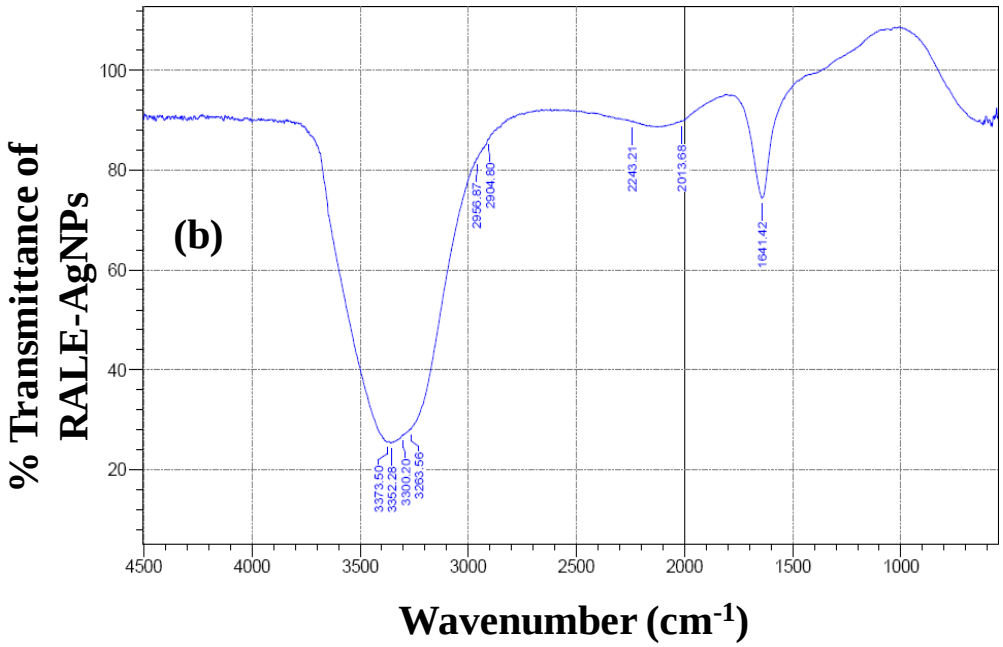


Fig. 4 FT-IR spectra of (a) leaf extract (RALE) and (b) biosynthesized RALE-AgNPs.

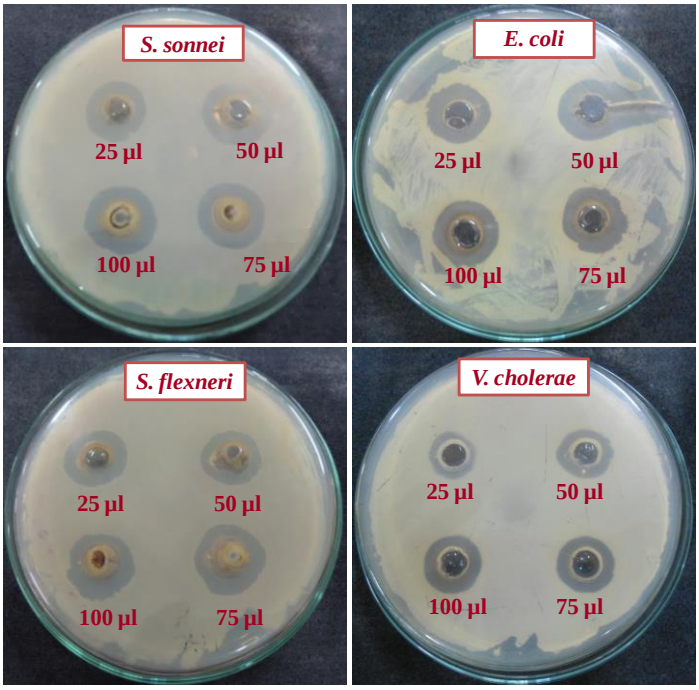


Fig. 5 Assessment of antibacterial activity of RALE and RALE-AgNPs by well diffusion assay.

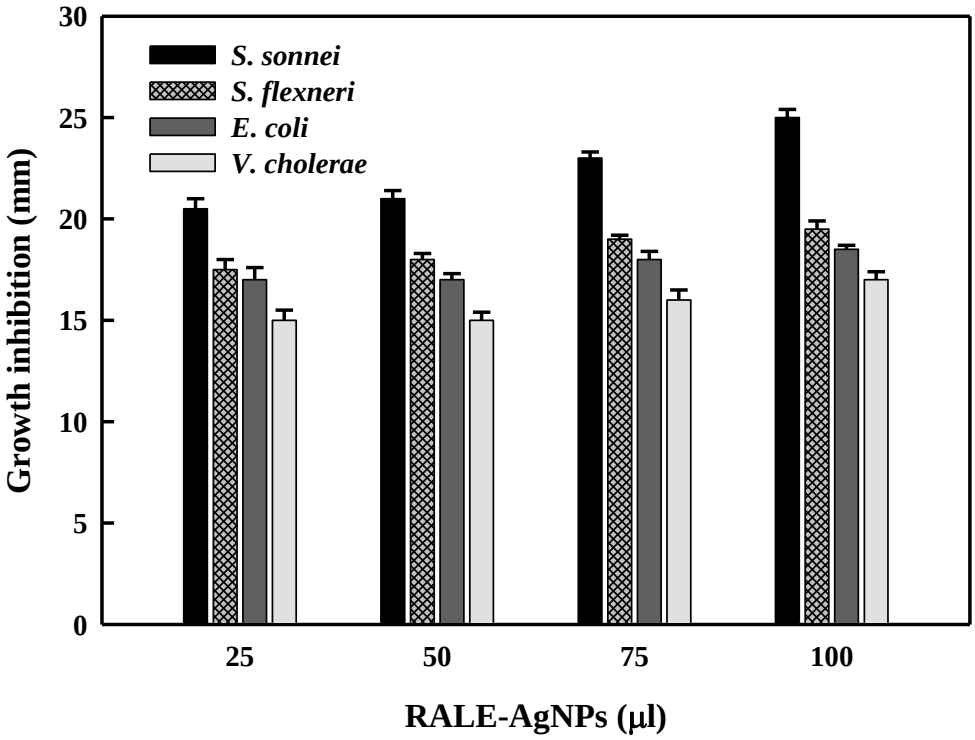


Fig. 6 Comparative antibacterial activity with increasing amounts (25-100 µl) of RALE-AgNPs against the tested clinical isolates.

Characterization of RALE-AgNPs

The XRD peak pattern (Fig.2) of biosynthesized RALE-AgNPs exhibited four sharp graphical peaks in spectral graph at 2θ values of 20 to 80. The diffraction peaks at 38.09°, 44.35°, 64.53° and77.57° indexed to [111], [200], [220] and [311] face centred cubic lattice (fcc) plane of silver, respectively exhibiting the crystalline lattice nature of RALE-AgNPs (JCPDS File No. 03–0921). The average particle size of RALE-AgNPs was determined to be 19.23 nm, calculated on full-width-at-half-maximum (FWHM) value at (111) reflection plane. Gnanadesigan *et al.* (2011) have reported *Rhizophora mucronata* aqueous leaf extract mediated biosynthesis of colloidal AgNPs (60-95 nm) which are much larger in size compared to RALE-AgNPs produced in our experiments. The shape and size of synthesized RALE-AgNPs were well documented by High Resolution TEM micrographs exhibiting variable and spherical RALE-AgNPs in the size range of 14.46-29.58 nm (Fig 3a). The selected area electron diffraction (SAED) pattern of biosynthesized RALE-AgNPs indicated face centered cubic lattice structure of silver (Fig 3b).

FTIR analysis (Fig.4) was performed for the evaluation of the different types of various phytocompounds and metabolites of *R. apiculata* fundamentally responsible for the synthesis of stable AgNPs. The multiple broad peaks at 3373.5-3263.56 cm^{-1} may be attributed to the stretching vibrations of hydroxyl (-OH) group. The weak absorptions at 2956.87-2904.8 cm^{-1} could be assigned as stretching vibrations of aliphatic C-H while the multiple peaks at 2243.21-2013.68 cm^{-1} corresponds to alkane group. The peak of absorption at 1641.42 cm^{-1} may be attributed to stretching vibrations of C=C in aromatic ring. All these bands of vibrations are the result of various biocompounds such as alkaloids, flavonoids and many bioactive phytochemicals present in the leaf extract.

Antibacterial activity of RALE-AgNPs

The antimicrobial activity of RALE-AgNPs were assessed against Enterotoxicogenic *Escherichia coli* O114, *Vibrio cholerae* MTCC 3906, *Shigella sonnei*, *Shigella flexneri*. Fig 5 shows the results of well diffusion assay of RALE and RALE-AgNPs at a constant dose of 100 µl each against the test strains. A marked increase in zone inhibition size (15–25 mm) with RALE-AgNPs, were

observed with pathogenic test strains. The results show that growth inhibition induced by RALE-AgNPs follows the order *S. sonnei* >*S. flexneri* >*E. coli* ≈ *V. cholerae*. The antimicrobial effect differs depending upon the type of species and its cellular constitution. The pronounced effect was observed at 100 µl RALE-AgNPs with inhibition zone recorded as 25 and 20 mm for *S. sonnei* and *S. flexneri*. Though, both Gram negative strains of *E.coli* and *V.cholerae* were inhibited but effect was less pronounced (Zone of inhibition as 19 and 17 mm, respectively) as compared to the Gram positive cells. These findings are well in validation with the research results of Umashankeri *et al*., 2012 and Ali *et al.*, 2015. Fig 6 denotes the concentration dependent effect of RALE-AgNPs (25–100 µl) on the pathogenic test strains with maximum cytotoxic effect at 100 µl RALE-AgNPs.

DISCUSSION

Varied forms and types of NPs are being immensely used as potential antimicrobial agents. Unlike other metals, nano or ionic form of silver is found to be most toxic for microorganisms (Sondi *et al*., 2004). Moreover, there is an view concerning the presumptive role of ionic Ag^+ release from nanosilver and its toxic effects against different microbial species (Tolaymat *et al*., 2010). Several researchers have postulated that dissolved Ag^+ ions govern toxic nature of nanosilver (Navarro *et al*., 2008; Miao *et al*., 2009). Many studies have reported, in contrast, that the nanosilver toxicity not only depends on the effects of Ag^+ ions released alone but the size, shape, peripheral surface coating and its surface charge distribution on biosynthesized nanosilver particles also plays an important role in affecting the toxicity through indirect mechanisms that controls the location, rate, extent and release timing of Ag^+ ions (Fabrega *et al*.,2009; Xiu *et al*., 2012). Various hypotheses are proposed which exemplifies the steps involved of antimicrobial toxicity of AgNPs. It is widely assumed that AgNPs are incorporated in the cellular membrane, causing intracellular ionic disturbance leading to the leakage of intracellular contents and ultimately cell lysis (Sondi *et al.*, 2004; Jain *et al*., 2005; Cho *et al*., 2005). Moreover, Paredes *et al.* (2014) suggested that amino acids may serve as reducing agents in the nanosilver synthesis. The carbonyl groups from the residual proteins and amino acids are found to have stronger affinity towards metal binding thereby, denoting that the proteins could be one of the constituents involved in the capping and stabilizing AgNPs to prevent agglomeration (Musarrat *et al.*, 2010). A report by Gericke and Pinches (2006) suggested the surface absorption of terpenoids or flavanones, without strong agents of ligation, on the metal NPs via interaction of carbonyl groups (>C = O) or π-electrons.

CONCLUSION

Rhizophora apiculata leaf extract has the potential of synthesizing stable extracellular AgNPs. The organo-chemical biological compounds present in RALE are the active molecular stabilizers, acting on surface and rendering stability to AgNPs. The green approach demonstrates a rapid and efficient method involving environmental benign and cost-effective natural resource reductant, thus providing a safe, viable and inert option to chemical procedures, for production of nanoparticles. The green biofunctionalized RALE-AgNPs were found as potent antibacterial agents against four different pathogens viz, *S. sonnei, S. flexneri*, *E.coli* and *V.cholerae*. Future studies are required for the optimization and standardization of the conditions for large-scale industrial production and formalization for ascertaining the efficacy, effectivity and concentration response of RALE-AgNPs for clinical settings, as broad spectrum nanoantibiotics against the conventional classic medication therapy.

ACKNOWLEDGEMENTS

Both the authors thank the authorities of Pondicherry University for providing required facilities; School of Pure and Applied Physics, School of Life Sciences and IIUCNN, Mahatma Gandhi University, Kottayam for providing the facilities of characterization.

REFERENCES

Ahmed S *et al*. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise, J Adv Res (2015) http://dx.doi.org/10.1016/j.jare.2015.02.007.

Ali K., Ahmed B., Dwivedi S., Saquib Q., Al-Khedhairy A.A., Musarrat J., Microwave accelerated green synthesis of stable silver nanoparticles with *Eucalyptus globula* leaf extract and their antibacterial and antibiofilm activity on clinical isolates. PLoS ONE, 10(7) (2015) DOI: 10.1371/journal.pone.0131178

Cho K., Park J., Osaka T., Park S. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim Acta, 51(9): 56–60, (2005)

Eustis S., El-Sayed M.A., Why gold nanoparticles are more precious than pretty gold: noble metal surface Plasmon resonance and its enhancement of the radiative and non-radiative properties of nanocrystals of different shapes. Chem Soc Rev, 35: 209-217, (2006)

Fabrega J., Fawcett S.R., Renshaw J.C., Lead J.R., Silver nanoparticles impact on bacterial growth: effect of pH, concentration, and organic matter. Environ Sci Technol, 43:7285–90, (2009).

Gericke M., Pinches A., Biological synthesis of metal nanoparticles. Hydrometal, 83: 132–140, (2006)

Gnanadesigan M., Anand M., Ravikumar S., Maruthupandy M., Vijayakumar V., Selvam S., Dhineshkumar M., Kumaraguru A.K., Biosynthesis of silver nanoparticles by using mangrove plant extract and their potential mosquito larvicidal property. Asian Pac. J. Trop. Med., 4: 799–803, (2011)

Jain P. and Pradeep T., Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnol Bioeng, 90: 59–63, (2005)

Miao A.J., Schwehr K.A., Xu C., Zhang S.J., Luo Z., Quigg A., Santschi P.H., The algal toxicity of silver engineered nanoparticles and detoxification by exopolymeric substances. Environ Pollut, 157: 3034–3041, (2009)

MubarakAli D., Thajuddin N., Jeganathan K., Gunasekaran M., Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids and Surfaces B: Biointerfaces, 85: 360–365, (2011)

Musarrat J., Dwivedi S., Singh B.R., Al-Khedhairy A.A., Azam A., Naqvi A., Production of antimicrobial silver nanoparticles in water extracts of the fungus *Amylomyces rouxii* strain KSU-09. Bioresource technology, 101(22): 8772–8776, (2010)

Received:04.05.18, Accepted: 07.06.18, Published:01.07.2018

Navarro E., Piccapietra F., Wagner B., Marconi F., Kaegi R., Odzak N,. Toxicity of Silver Nanoparticles to *Chlamydomonas sreinhardtii*. Environ Sci Technol, 42: 8959–8964, (2008)

Paredes D., Ortiz C., Torres R., Synthesis, characterization and evaluation of antibacterial effect of Ag nanoparticles against *Escherichia coli* O157:H7 and methicillin-resistant *Staphylococcus aureus* (MRSA). Int J Nanomed, 9:1717–1729, (2014)

Reddy M.C., Rama Murthy K.S., Srilakshmi A., Sambasiva Rao K.R.S., Pullaiah T., Phytosynthesis of eco-friendly silver nanoparticles and biological applications–A novel concept in Nanobiotechnology. J African Journal of Biotechnology, 14 (3): 222–247, (2015)

Sondi I., Salopek-Sondi B., Silver nanoparticles as antimicrobial agent, a case study on *E. coli* as a model for Gram-negative bacteria. J Colloid Interface Sci, 275: 177–182, (2004)

Tolaymat T.M., El Badawy A.M., Genaidy A., Scheckel K.G., Luxton T.P., Suidan M., An evidence based environmental perspective of manufactured silver nanoparticle in syntheses and applications: asystematic review and critical appraisal of peer-reviewed scientific papers. Sci Total Environ 408 (5): 999–1006, (2010)

Umashankeri J., Inbakandan D., Ajithkumar T.T., Balasubramanian T., Mangrove plant, *Rhizophora mucronata* (Lamk, 1804) mediated one pot green synthesis of silver nanoparticles and its antibacterial activity against aquatic pathogens. Saline Systems, 8:11, (2012)

Xiu Z.M., Zhang Q.B., Puppala H.L., Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett, 12: 4271–4275, (2012)

***Corresponding Author:**
Tijo Cherian*
Email: **tvarghese891@gmail.com**