J Nanostruct Chem (2014) 4:83
DOI 10.1007/s40097-014-0083-4

ORIGINAL

Antibacterial activity of ruthenium nanoparticles synthesized using *Gloriosa superba* L. leaf extract

Kasi Gopinath · Viswanathan Karthika · Shanmugam Gowri · Venugopal Senthilkumar · Subramanian Kumaresan · Ayyakannu Arumugam

Received: 8 December 2013 / Accepted: 25 January 2014 / Published online: 15 March 2014

Abstract This work reports an ecofriendly approach for the synthesis of Ruthenium nanoparticles (Ru NPs) using aqueous leaf extract of *Gloriosa superba*. *G. superba* contains cholidonic, superbine, colchicine, gloriosol, phytosterils and stigmasterin, which are found to be responsible for the bio-reduction of Ru NPs. The synthesized Ru NPs were characterized using UV–Vis spectroscopy, Fluorescence spectra, FTIR, XRD, SEM and EDX analyses. UV–Vis spectra of the aqueous medium containing Ru NPs showed a gradual decrease of the absorbance peak observed at 494 nm. Fluorescence spectra of Ru NPs emission (λ_{em}) exhibited at 464 nm are attributed to the Ru=N π bonds transition. The biomolecules responsible for the reduction of Ru NPs were analyzed by FTIR. XRD results confirmed the presence of Ru NPs with hexagonal crystal structure. The calculated crystallite sizes using Scherrer formula are in the range from 25 to 90 nm. Scanning electron microscopy ascertained spherical nature of the Ru NPs. The EDX analysis showed the complete elemental composition of the synthesized Ru NPs. The synthesized Ru NPs exhibited good antibacterial performance against gram-positive and gram-negative bacterial strains, which was studied using standard disc diffusion method. The synthesis of Ru NPs by this method is rapid, facile and can be used for various applications.

K. Gopinath · V. Karthika · S. Gowri · A. Arumugam (✉)
Department of Nanoscience and Technology, Alagappa University, Karaikudi 630 004, Tamil Nadu, India
e-mail: sixmuga@yahoo.com

K. Gopinath
e-mail: gopiscientist_1986@rediffmail.com

V. Senthilkumar · S. Kumaresan
Department of Plant Biology and Plant Bio-Technology, R.K.M.V. College, Chennai 600 004, Tamil Nadu, India

Keywords Green synthesis · *Gloriosa superba* · Leaf extract · Ruthenium nanoparticles · Antibacterial activity

Background

The field of nanotechnology is one of the most innovative research areas in modern era. Size and shape have most important role in physical, chemical, electrical and optical properties of metal nanoparticles namely Ag, Au, Pt, Pd and Ru NPs. Ruthenium (Ru) is a 4d transition metal, which belongs to the platinum group [1, 2]. It is a low-cost material than that of Pd and Pt. Ruthenium nanoparticles were used in many applications such as catalytic dehydrogenation [3], methanol fuel cells [4], synthesis of diesel fuels [5], azo dye degradation [6], removal of organic pollutants from water [7] and so on. Synthesis of Ru NPs is usually carried out by various physical and chemical methods such as microwave irradiation [6, 8], sonochemical method [9], hydrothermal method [10] and electrochemical method [11]. However, most of these techniques are complex, power and time consuming, expensive, hazardous and employed by toxic chemicals. Therefore, simple and cost effective methods are needed to synthesize Ru NPs. The development of 'green' chemistry approach is an environmentally benign process for the synthesis of nanoparticles evolving as an important area of nanotechnology. Only a very few reports are available on the microbial synthesis of Ru NPs using *Pseudomonas aeruginosa* SM1 [12]. Hence, an attempt was carried out in the present study to synthesize Ru NPs using leaf extract. This method offers enormous benefits as cost effectiveness, biomedical, pharmaceutical applications and in large-scale commercial production.

Gloriosa superba L., belongs to Colchicaceae family. It is a perennial, greenish, climbing herb and nativity of

South Africa. Every part of this plant is being used in Siddha, Ayurveda and Unani system of medicine. It is a tuberous plant with L–V shaped cylindrical tubers. The tuber powder was effectively used against paralysis, rheumatism, snake bite, insect bites, against lice, intermittent fevers, wounds, anti-fertility, gonorrhea, leprosy, piles, debility, dyspepsia, flatulence, hemorrhoids, helminthiasis and inflammations [13]. It contains two major alkaloids namely colchicines ($C_{22}H_{25}NO_6$) and colchicosides ($C_{27}H_{33}O_{11}N$). The seeds consist of colchicines, which are 2–5 times higher than in the tubers [14]. Leaves contain cholidonic, superbine, colchicine, gloriosol, phytosterils and stigmasterin [15].

In the present study, we report the green synthesis and characterization of Ru NPs using *G. superba* leaf extract and their potential application of antimicrobial activity. To the best of our knowledge, this is the first report on the synthesis of Ru NPs using *G. superba* leaf extract.

Results and discussion

A reduction of Ru NPs was clearly observed when *G. superba* leaf extract was added with $RuCl_3$ solution heated at 100 °C for 20 min. The solution was changed from brown to light blackish yellow color, which indicates the Ru NPs formation in the range from 25 to 90 nm.

UV–Vis spectroscopy and fluorescence analysis

The $RuCl_3$ solution was subjected to UV–Vis spectroscopy analysis that showed a peak at 494 nm. In addition, plant extract was heated to reflux and absorbance was monitored by UV–Vis spectra, which indicates the gradual decrease of the absorbance in the interval of 2 and 5 min. This implies that the Ru^{3+} has completely reduced to Ru^0 (Fig. 1). Similarly, the $RuCl_3$ absorbance peak disappeared in the same region [6, 9, 16]. The fluorescence emission spectra of the synthesized Ru NPs were recorded in water and the fluorescence emission peak was observed at 464 nm which is attributed to the Ru=N π bonds transition (Fig. 2) and this is consistent with the previous report [17].

Fourier transform infrared spectroscopy and X-ray diffraction analysis

FTIR analysis was performed to identify the possible biomolecules responsible for the reduction of the Ru^+ ions and capping of the reduced Ru NPs synthesized using *G. superba* leaf extract (Fig. 3). The strong IR band at 3,418 cm^{-1} corresponds to N–H stretching vibration of primary amines, whereas the band at 2,922 cm^{-1} corresponds to aliphatic C–H stretching. The bands at 1,642 and 1,384 cm^{-1} are due to the C=C stretching and NO_2 stretching, respectively. The IR bands observed at 1,249 and 1,076 cm^{-1} correspond to the C–O stretching

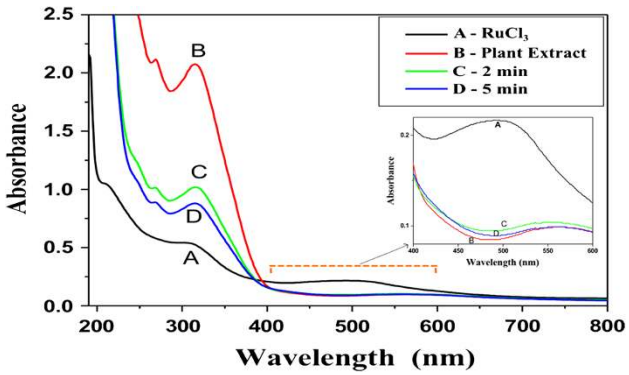

Fig. 1 UV–Vis spectrum of Ru NPs synthesized using *G. superba* leaf extract

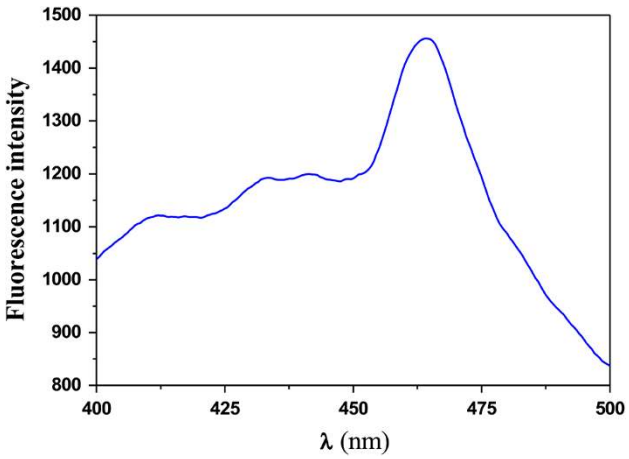

Fig. 2 Fluorescence spectra of Ru NPs emission (λ_{em}) wavelength at 464 nm

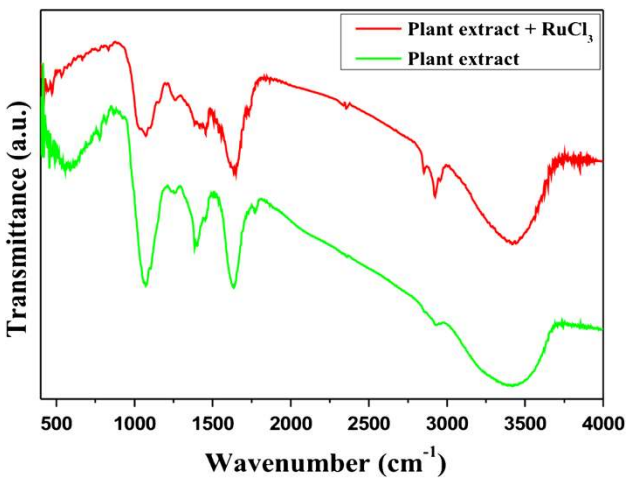

Fig. 3 FT-IR spectra of Ru NPs synthesized using *G. superba* leaf extract

and –C–O–C stretching, respectively. The band at 587 cm^{-1} corresponds to C–Cl stretching. Hence, the main components such as, cholidonic, superbine, colchicine, gloriosol, phytosterils and stigmasterin, were present in the leaf extract of *G. superba* and responsible for reduction and capping during the synthesis of Ru NPs. The two new strong bands recorded at 832 and 470 cm^{-1} in the spectra of synthesized material were assigned to C–H bending and metal (Ru), respectively. The C–H bending peak may be raised due to the reduction of $RuCl_3$ to Ru NPs.

X-ray diffraction pattern was recorded for the synthesized Ru NPs (Fig. 4). Five distinct diffraction peaks at 38.42°, 42.12°, 43.98°, 58.32° and 69.42° were observed and indexed with the planes (1 0 0), (0 0 2), (1 0 1), (1 0 2) and (1 1 0) for the hexagonal structure of Ru (JCPDS card no. 89-3942). The well-resolved and intense XRD pattern clearly showed that the Ru NPs formed by the reduction of Ru^{+} ions using *G. superba* leaf extract are crystalline in nature. In addition, the unassigned peaks suggested the crystallization of bioorganic phase occurs on the surface of the nanoparticles. Similarly, unassigned peaks were observed at other metal nanoparticles (Ag and Au) synthesized by geranium leaf extract [18] and *Murraya koenigii* leaf extract [19].

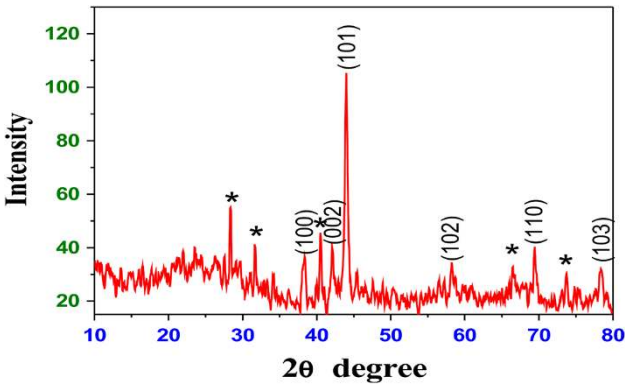

Fig. 4 XRD pattern of synthesized Ru NPs (*asterisk* shows unassigned peaks)

Scanning electron microscopy and energy dispersive X-ray spectroscopy analysis

The SEM image (Fig. 5) further ascertained that the Ru NPs are predominantly spherical in morphology with the sizes ranging from 25 to 90 nm and has an average size of about 36 nm. Energy dispersive X-ray spectroscopy (EDX) (Fig. 6) illustrated the chemical nature of synthesized Ru NPs using *G. superba* leaf extract. The peak obtained at the energy of 2.6 keV for Ru and also some weak peaks for C, O, Na, Al, P and K have also been found. The emission energy at 2.6 keV indicates the reduction of Ru ions to element of ruthenium. Similarly, sonochemical synthesis of Au-Ru bimetallic nanoparticles showed an EDX spectrum, emission energy at 2.6 keV which confirmed the presence of ruthenium metal [9].

Antibacterial assay

Green-synthesized Ru NPs were tested against three gram-positive and four gram-negative bacteria to determine its ability as an antibacterial agent and were compared with antibiotic vancomycin to ascertain its true potential. *Klebsiella pneumoniae, P. aeruginosa* and *Shigella dysenteriae* have not exhibited zone of inhibition for vancomycin. Similarly, Ru NPs were also inactive against *K. pneumoniae* and *S. dysenteriae*, whereas they have significant effect on *P. aeruginosa* with zone size (2.67 ± 0.33 mm). *E. coli* and *Staphylococcus aureus* exhibited zone of 3.33 ± 0.33 mm compared to the standard at 5.67 ± 0.33 mm as well as *Bacillus subtilis* and *Streptococcus pneumoniae* showed

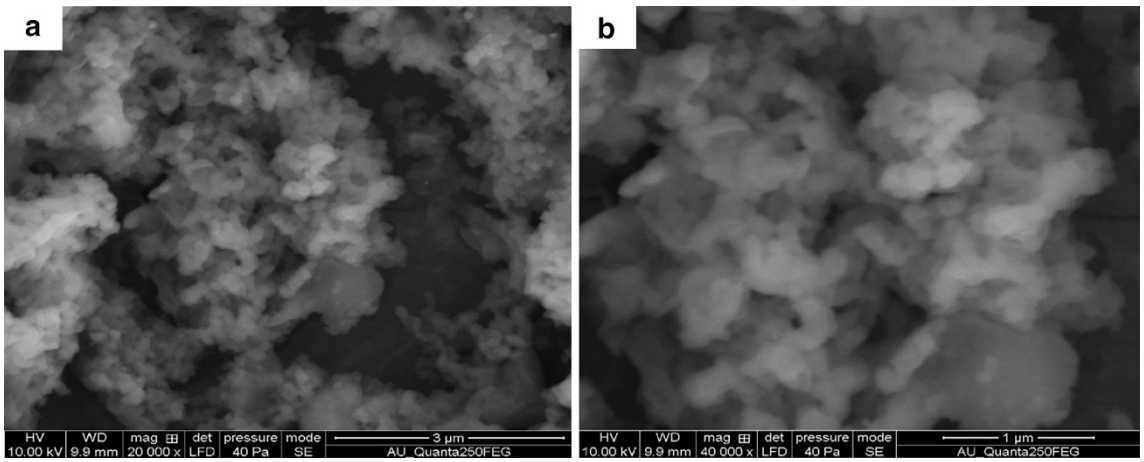

Fig. 5 **a**, **b**—SEM image of Ru NPs synthesized using the *G. superba* leaf extract

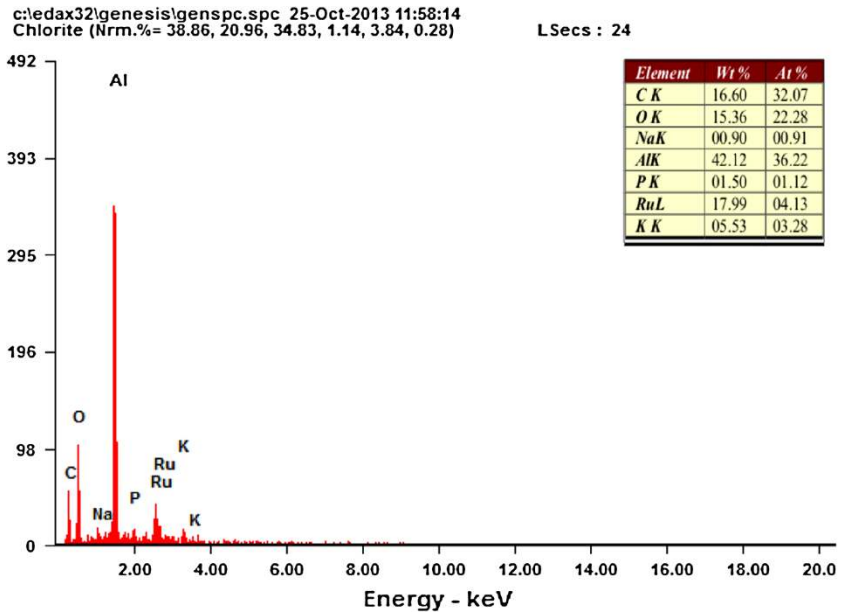

Fig. 6 EDX analysis of Ru NPs

modulated effect of 2.33 ± 0.33 mm compared to standard at 6.67 ± 0.33 mm (Fig. 7). The exact mechanism of metal nanoparticles on antibacterial activity has not yet been understood clearly. Ability of Ru NPs to attach onto the bacterial membrane by electrostatic interaction between the negatively charged bacterial cell and the positively charged nanoparticles is crucial for the activity of the nanoparticles as bactericidal material and this disrupts the integrity of the bacterial membrane and subsequently cell death takes place due to this structural change. Ru NPs interference with the bacterial cell membrane and their binding with mesosome will there by reduce the mesosomal function and increase the reactive oxygen species generation, which leads to cell death. In general, gram-negative bacteria are comparatively susceptible to cell wall damage than gram-positive bacteria and this is attributed to the nature of cell wall present in the bacteria; however, in this study gram-positive bacteria were prone to cell wall damage than the gram-negative bacteria (Fig. 8). The actual mechanism behind this action is not clear but still the earlier researchers recorded the same behavior. Green-synthesized gold nanoparticles using *Terminalia chebula* seed extract showed a better antibacterial activity on gram-positive bacteria compared to gram-negative bacteria [20]. Hence, Ru NPs could be used in pharmaceutical industry to develop drugs for gram-positive bacterial diseases.

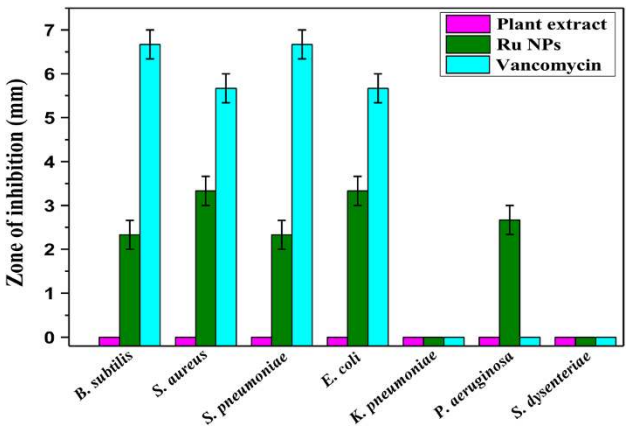

Fig. 7 Antibacterial activity of Ru NPs compared to the vancomycin antibiotic against gram-positive and gram-negative bacteria

Conclusion

The present study reports the green synthesis of Ru NPs using *G. superba* leaf extract. The SEM image substantiated that the particles are spherical shaped with the average size of 36 nm. The antibacterial activity of Ru NPs has significant effects against the gram-positive bacteria compared to gram-negative bacteria. This green synthesis is rapid, facile, convenient, less time consuming and environmentally safe. We propose this green synthesis method to be used for metal and other metal oxide nanoparticles.

Methods

Collection of plants

The *G. superba* explants were collected from Science Campus, Alagappa University, Karaikudi, Tamil Nadu, India. The taxonomic identification was made by Dr. S. John Britto, The Rapinat Herbarium and Centre for Molecular

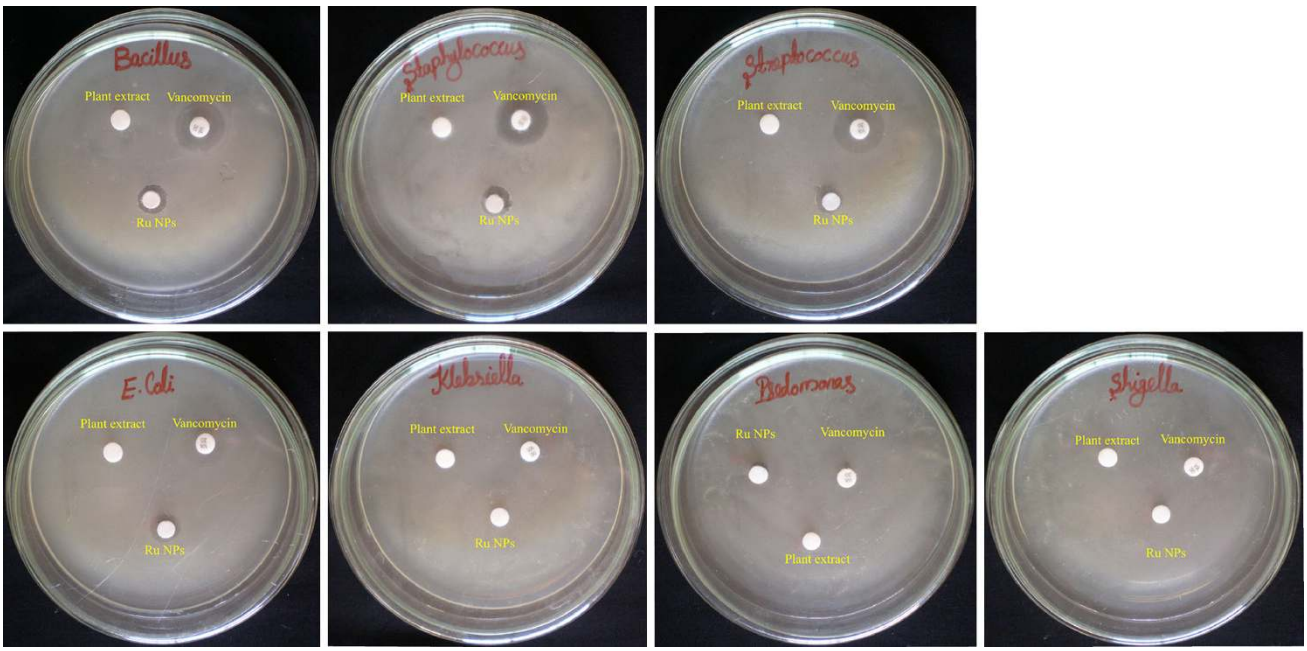

Fig. 8 Antibacterial activity of Ru NPs against gram-positive and gram-negative bacteria

Systematics, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The voucher specimen was numbered (KG-001) and is kept in the Department of Nanoscience and Technology, Alagappa University, Karaikudi.

Synthesis of Ru NPs using *Gloriosa superba* leaf extract

Fresh *G. superba* leaves were cleaned in running tap water, and then by double distilled water. 10 g of leaves was added with 100 ml of double distilled water and boiled at 50–60 °C for 5 min. The obtained extract was filtered using Whatman No. 1 filter paper and the filtrate was collected in 250-ml Erlenmeyer flask and stored at room temperature for further usage. Thereafter, 1 ml of *G. superba* leaf extract was added to 100 ml of 2 mM $RuCl_3$ solution and stirred at 100 °C for 20 min. The reduction of Ru NPs was clearly observed within 20 min. The brown solution was changed to light blackish yellow color, which indicates the formation of Ru NPs.

Characterization

The synthesized Ru NPs were subjected to UV–Visible spectroscopy in the wavelength range of 200–800 nm using Shimadzu spectrophotometer (Model UV-1800) operated at a resolution of 1 nm. The fluorescence study was carried out using an Elico SL 174 spectrofluorometer in the range of 400–500 nm. Moreover, Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out in the range of 400–4,000 cm^{-1}. XRD pattern was recorded using Cu Kα radiation ($\lambda = 1.54060$ Å) with nickel monochromator in the range of 2θ from 10° to 80°. The average crystallite size of the synthesized Ru NPs was calculated using Scherrer's formula ($D = 0.9\lambda/\beta\cos\theta$). Scanning electron microscopy and energy dispersive X-ray spectroscopy analysis were performed for a thin film sample prepared using the Ru NPs by spin coating (1,500 rpm) method on a aluminum foil (1 cm × 1 cm) by dropping 100 µl of the sample and allowed to dry for 30 min at room temperature and was further subjected to SEM analysis (Instrument model: FEI Quanta 250, Czech Republic) operated at an accelerating voltage of 10 kV.

Antibacterial activity of Ru NPs

The biocidal property of the green-synthesized Ru NPs was examined against three gram-positive (*B. subtilis, S. aureus, S. pneumoniae*) and four gram-negative bacteria (*Escherichia coli, K. pneumoniae, P. aeruginosa, S. dysenteriae*) by disc diffusion method. These seven bacterial strains were grown in nutrient broth at 37 °C until the bacterial suspension has reached 1.5×10^8 CFU/ml. Approximately 20 ml of molten nutrient agar was poured into the Petri dishes and cooled. All the bacterial suspension was swapped over the medium, the disc loaded with 100 µl of Ru NPs and vancomycin disc 30 mcg were placed over the medium using sterile forceps. Plant extract (100 µl) was used as a control. The plates were then incubated for 24 h at 37 °C. The inhibition zone formed around each discs was measured. Each experiment was performed for three times. The data shown represent the

mean ± SE. The data were analyzed statistically using SPSS software.

Acknowledgments Authors gratefully thank School of Physics, Alagappa University for extending the XRD facility and also the Department of Industrial Chemistry, Alagappa University for providing the fluorescence analysis and EDX with SEM facilities. We thank secretary, RKM Vivekananda College, Chennai, for providing infrastructure and moral support.

Conflict of interest The authors declare that they have no competing interests.

Author contribution KG, VK, SG and VS carried out the ruthenium nanoparticles synthesis, characterization and antimicrobial activity. SK and AA carried out the manuscript preparation. All authors read and approved the final manuscript.

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