Green Synthesis, Structural Characteristics of *Rhodomyrtus tomentosa* Plant Leaf Extract and Antimicrobial Application on *Labeo rohita*

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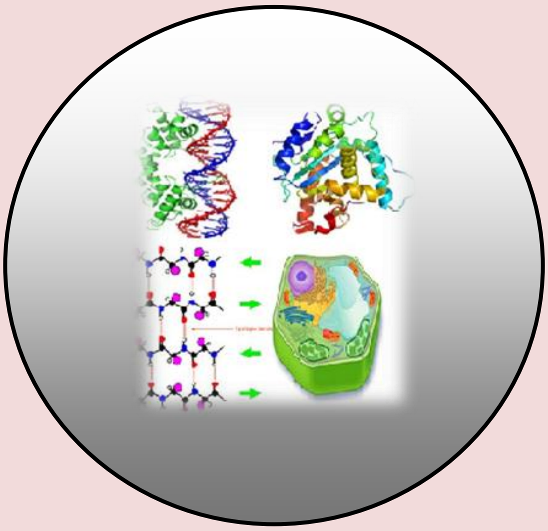

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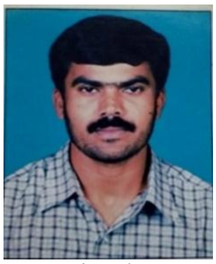

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RESEARCH PAPER

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ABSTRACT

Green syntheses of silver nanoparticles have fascinated currently and simple method instead of physical and chemical approaches. In the present study, bioengineered nanoparticles were synthesized using fresh leaves of Rhodomyrtus tomentosa. Synthesized nanoparticles were characterized and confirmed by UV Spectrophotometer, followed by EDX, SEM, XRD and FTIR. Biomolecule-mediated nanoparticle synthesis has recently gained the attention of researchers due to its eco-friendly and non-toxic nature. UV-Visible spectroscopy and Scanning electron microscopy (SEM) & energy dispersive X-ray (EDX) to fulfill the growing demand for non-hazardous nanoparticle synthesis routes. Metal and plant extract nanoparticles have an extensive range of applications. Since metabolites from Rhodomyrtus tomentosa plant extracts represent a better alternative to chemical methods the present study was explored on biofabrication of selenium nanoparticles (Se-NP) using leaf extracts of Rhodomyrtus tomentosa. The leaves have the potentiality to reduce sodium selenite and synthesize stable SeNP within 5 days, which was indicated by the colour change of reaction medium from colorless to brick red colour. The mechanism of reduction was analysed through X-ray diffraction depicting peaks of selenium. And we have done antimicrobial property of the synthesized SeNPs on Labeo rohita against bacterial pathogens. Further the synthesized nanoparticles showed high efficiency against drug resistant microbes in the laboratory.

Key word: Rhodomyrtus tomentosa, XRD, FTIR, UV, SEM and Nanoparticles.

INTRODUCTION

In the present era, Nanotechnology is playing a vital role in the research of material Sciences due to its specific characteristics such as morphology, size, shape and distribution. Metallic nanoparticles having innovative applications (Murphy *et al.*, 2008; Smith *et al.*, 2004). The manifestation of nanotechnology has provided a wide research in recent years by intersecting with diverse other branches of science and creating impact on all forms of life (Baker and Satish, 2012).

In nanotechnology nanoparticles research is an important aspect due to its innumerable applications. Nanoparticles have expressed significant advances owing to wide range of applications in the field of bio-medical, sensors, antimicrobials, catalysts, electronics, optical fibers, agricultural, bio-labeling and in other areas (Salam *et al*., 2012). Asia has abundant species of medicinal and aromatic plants and traditional medicines have practiced in Asia since ancient times. India has made use of medicinal plants to cure ailments of thousands of years. According to World Health Organization the goal of health for all can't be achieved without herbal medicines, while the demand for herbal medicine is growing in developing countries, there are indications that consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives in traditional medicine (Tamizhazhagan and Pugazhendy, 2017; Tamizhazhagan *et al*., 2017a, 2017b). Nanoparticles have wide applications in the field of biomedicine such as to deliver pharmaceuticals, for diagnostic purposes as well as for the therapeutic approaches because of its small size (Khan *et al*., 2017). Silver nanoparticles were synthesized for the first time in the living system like Alfalfa sprouts by Gardea Torresdey *et al*., (2003). Green syntheses of silver nanoparticles using plants are faster than the microbes quite rapidly. Shankar *et al*., (2003) results showed that synthesis reaction has taken a longer time when compared to Geranium leaves. Various methods for synthesizing nanoparticles have been developed to formulate such nanoparticles, including chemical, physical and biological methods. The use of plant extract for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining cell cultures (Rajakumar *et al*., 2017). Selenium nanoparticles (SeNPs) are gaining importance in the field of medicine owing to their antibacterial and anticancer properties. The SeNPs are biocompatible and non-toxic compared to the counterparts, selenite and selenate. Most of the medicinal plant parts are used as raw drugs and they possess varied medicinal properties (Mahesh and Satish, 2008). Medicinal plants are easily available, less expensive and also have no side effects (Passini *et al*., 2011). Calixto, (2005) collected the information from WHO and reported that 65% - 80% of the world's population in developing countries depend on the medicinal plants for their primary health care due to the poverty and lack of access to modern medicine. Medicinal plants represent a rich source of the antimicrobial agent (Mahesh and Satish, 2008). They can be synthesized by physical, chemical, and biological methods and have distinct bright orange-red color SeNPs are stable and do not aggregate owing to natural coating of the biomolecules. Selenium (Se) is an essential element in human and animal body in low concentration. It is a necessary dietary constituent of at least 25 human selenoproteins and enzymes containing selenocysteine (Zhang *et al*., 2011). The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction (Prabhu *et al*., 2018). Biological synthesis is clean, non-toxic, and eco-friendly; additionally it utilizes less energy than chemical and physical synthesis with an efficient use of material without generation of harmful by-products. Despite the fact the therapeutic efficiency of available drugs and treatment has been well proven, incompetent and deprived delivery of antimicrobial agents could consequence in insufficient therapeutic index and causing adverse effects like nausea, vomiting, irritation, scaling and gut microbial flora reduction. Along with developing multidrug resistant microbial strains, however, variations in the society, environment, technology and developing microorganisms are contributing to the emergence of new diseases and development of antimicrobial resistance (Lam *et al*., 2017). In addition, there are two other major advantages of biologically synthesized NPs over those chemically synthesized: increased biocompatibility and much greater stability. SeNPs exhibit low cytotoxicity compared to selenium (Se) compounds and possess excellent anticancer and therapeutic activities making them apt for medicinal applications (Surwase *et al*., 2013; Wang *et al*., 2011). The hydroxyl and carboxyl groups present in the plants were able to bind with the metals and the flavonoids and phenols have exclusive power to warp nanoparticles to avoid the agglomeration (Huarte *et al*., 2010).

MATERIAL AND METHODS

Synthesis of Silver Nanoparticles

The leaves of *Rhodomyrtus tomentosa* (Figure 1) are the explants used in the present study to synthesize Selenium nano particles. Fresh and healthy leaves were collected from Krishnagiri, Tamilnadu. The leaves are washed, and weighed.

They are crushed in aqueous medium, and were centrifuged at 10,000 rpm for 20 minutes. The leaf extract is used in this green approach (Sheela and Deepa, 2013). The obtained leaf extract is colorless 20 ml of freshly prepared leaf extract was added to 100 ml of 5mM aqueous solution of sodium selenite. This reaction medium was kept in an incubator cum shaker with 250 rpm at 36 degree C for five days. Spectral analysis of the reaction medium was done with a small amount of diluted reaction medium using UV-Visible spectrophotometer (Bharani *et al*., 2012; Heath, 1989). Fresh leaves of *R. tomentosa collected* from the Krishnagiri, Tamil Nadu, India. The fresh leaves were washed rapidly with milli-Q water. Five to ten grams of leaf was boiled with 100 mL milli-Q water for 5 to 10 minutes. The obtained extract was filtered through Whatman No 1 filter paper. The filtrate was collected in 250 mL Erlenmeyer flask and stored at 4 ºC for further use. 1mM $AgNO_3$ solution was added in 9:1 ration to *R. tomentosa* plant extract and kept in dark condition to avoid photo-oxidation at room temperature. The color change of the plant extract from pale green to dark brown was observed periodically and the aqueous portion was collected for characterization. The formation of brown color indicates the synthesis of silver nanoparticles. The synthesized sample was centrifuged at 5000 rpm for 15 minutes and the pellet was stored for further characterization (Jain *et al*., 2009).

Collection of fish

The fresh water fish *Labeo rohita* were collected from the fish farm located in Krishnagiri, India. The fish were brought to the laboratory and transferred to the rectangular cement tanks (100 × 175) of 500 liters capacity containing chlorine free aerated well water- fishes of the same size and weight were used irrespective of their sex for the experiments.

Figure 1. Fresh leaves of *Rhodomyrtus tomentosa.*

Figure 2. *Labeo rohita.*

Antibacterial activity

The standard well diffusion method was followed for the antibacterial activity of the synthesized silver nanoparticles against multi drug resistant clinical isolates such as *Staphylococcus aureus*, *Streptococcus sp*., *E. coli* and *Pseudomonas sp*. obtained from Microbiology laboratory, Annamalai University, Approximately 20 to 25 ml of Nutrient agar was poured in sterilized Petri dishes and kept in room temperature for overnight to avoid contamination.

For maintaining the virulence, the pathogens were grown in Mueller–Hinton broth at 15 days interval. Further, 24hrs old bacterial pathogens were swabbed in Muller Hinton agar seeded plates and allowed to dry for 10 minutes. A standard cork borer of 6 mm was used to cut six uniform wells on the surface of the agar.

RESULTS AND DISCUSSION

Fresh leaves of *Rhodomyrtus tomentosa,* collected from Krishnagiri are used in present research. The leaves were washed, wiped and weighed, before preparing plant extract. Reduction of metal salts into metal nanoparticles by the biomolecules is accompanied by the colour change of the reaction medium. In the present research, the colourless solution of sodium selenite acquired brick red to ruby red colour due to the addition of leaf extract of *R. tomentosa*, plant. As the reduction proceeds, the colour of the reaction medium gradually changed from colourless to brick red colour after five days.

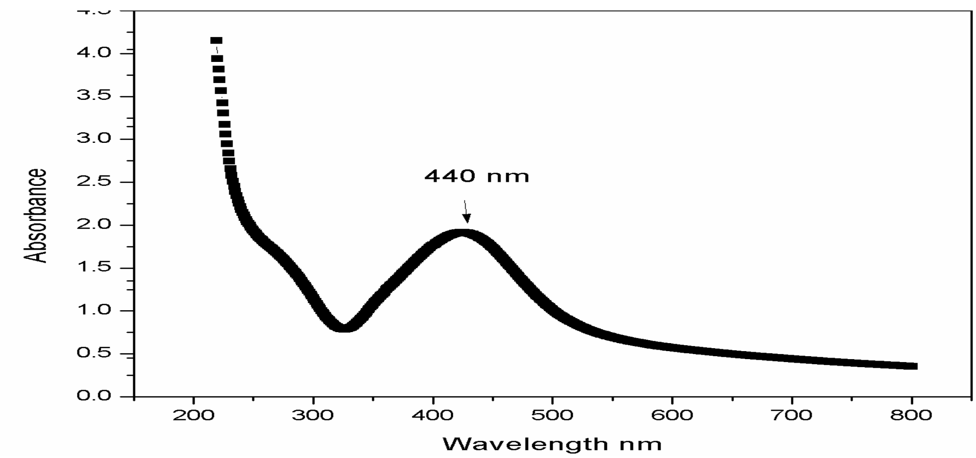

Figure 3. UV-visible absorption spectra for selenium nanoparticles synthesized with leaf extract of *Rhodomyrtus tomentosa* plant.

As the reduction proceeds, there will be increase in Surface Plasmon Resonance (SPR) bands with increasing reaction time. The appearance of strong bands in the spectral patterns is due to the excitation of the localized surface plasmons which causes strong light scattering by an electric field at a wavelength where resonance occurs (Bharani *et al.*, 2012). The technique outlined above proved to be very useful for the analysis of nanoparticles. As illustrated in upper spectrum, strong absorption bands with a maximum (218, and 248 nm) located between 200nm and 250nm was observed due to formation of selenium nanoparticles produced during reduction of selenium ions (Se+4). This clearly indicates that the absorption is maximum with decrease in the size of the particles (Heath, 1989). Powder X-ray diffraction is one of an important analytical technique which aids to identify and characterize unknown material using monochromatic X-rays.

The XRD pattern of selenium nanoparticles synthesized using plant extract of agle mermelos (Figure 4) showed number of Bragg's reflections. This confirmed the crystalline nature of Selenium nanoparticles. The average crystalline size of SeNPs was calculated by using Scherer's formula for peak (101) (Samanta and Sarkar, 2011). The average calculated crystalline size of biosynthesized SeNPs was 37.4 nm. These set of planes had been indexed on the basis of the face-centered cubic structures (fcc) of standard selenium PDF card -00-001-0848 of International centre for diffraction data. The functional groups involved in the synthesis of selenium nanoparticles were detected with the help of FT-IR analysis. A comparative study on FT-IR spectrum of *R. tomentosa,* leaf extract and synthesized selenium nanoparticles predicted in the Figure 5.

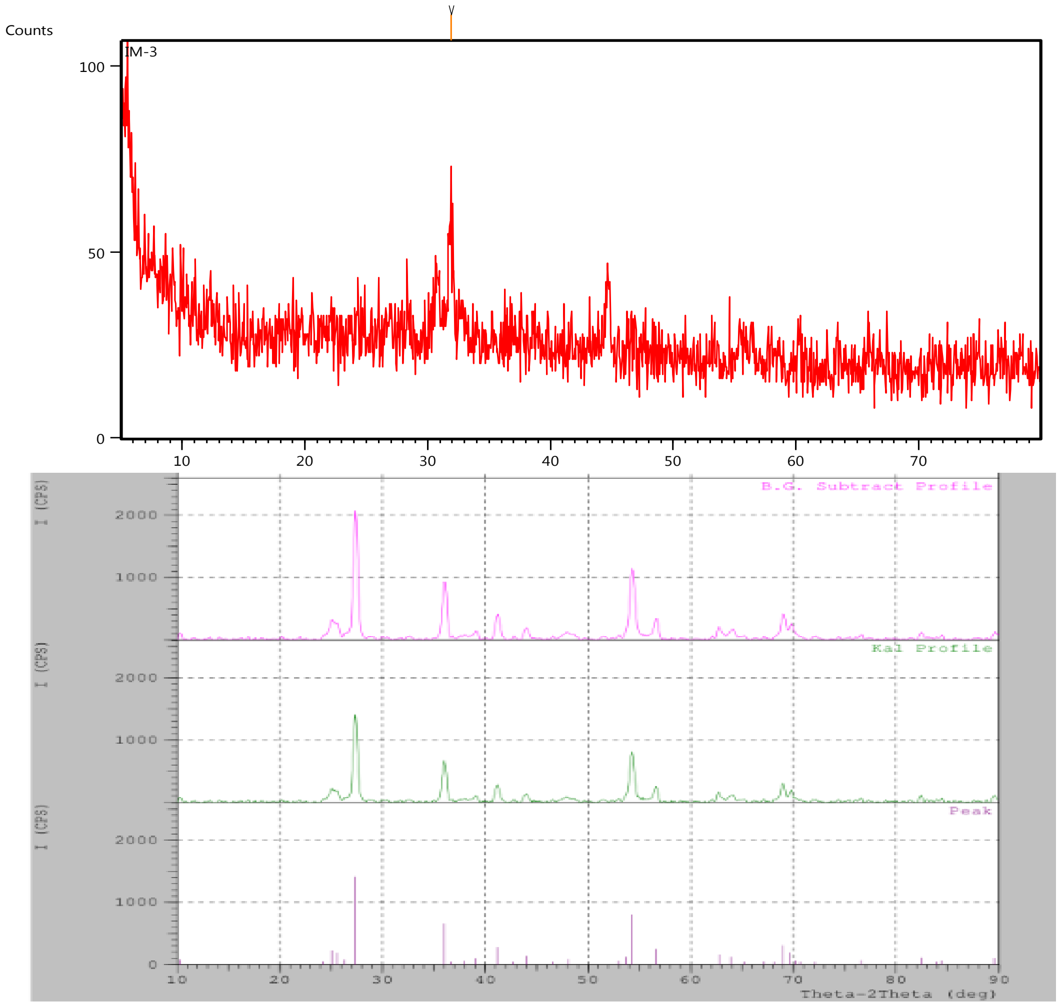

Figure 4. XRD Pattern of synthesized selenium nanoparticles.

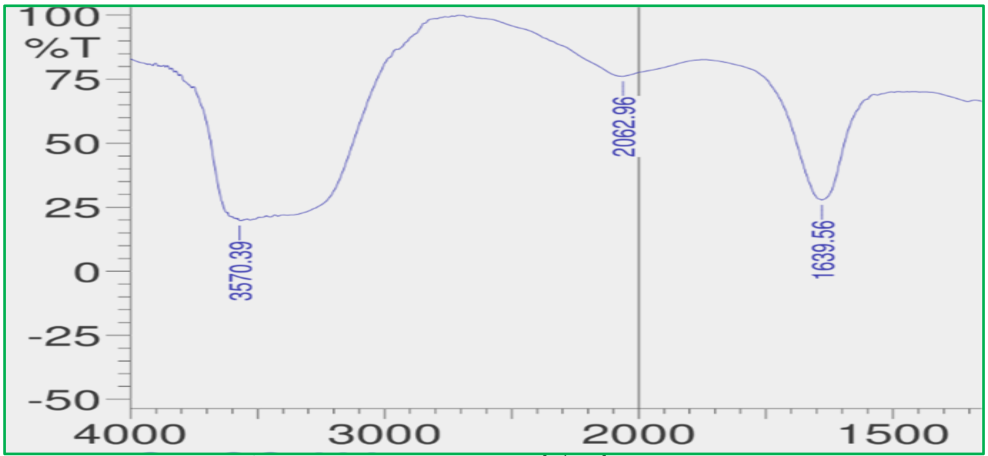

Figure 5. FT-IR Spectra of leaf extract.

FT-IR spectrum confirms the presence of various functional groups in the plant extract, which may possibly influence the reduction process and stabilization of nanoparticles. The result of FT-IR spectrum of the leaf extract is presented in Figure 5 and FT-IR analysis of synthesized SeNPs is presented in Figure 5. Absorption peak at 3404 was shifted to 3412; additionally, two new peaks were identified at 2,959 and 2,934 in Se containing leaf extract.

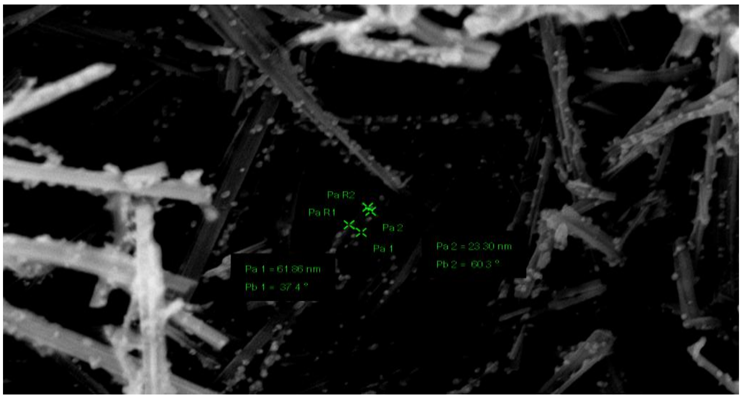

Figure 6. EDAX analysis in EDAX analysis, the vertical axis shows the amount of X-ray counts and horizontal axis shows energy emitted in keV.

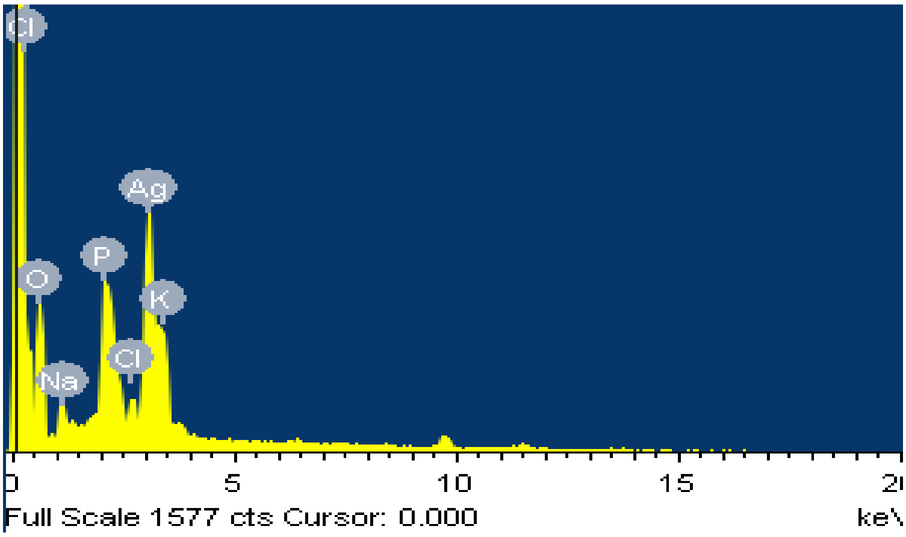

Figure 7. EDAX analysis.

Table 1. Bacterial Zone of Inhibition in mm.

Sample in µg	Bacterial Zone of Inhibition in mm			
	Staphylococcus aureus	*Bacillus cereus*	*Pseudomonas*	*E-coli*
100 µg	-	-	-	-
200 µg	19	23	39	26
300 µg	23	30	41	24
400 µg	13	31	47	29

Peak positioned at 1,648, 1,404 and 1,258 was shifted to 1,646, 1,415, and 1,256 cm−1, respectively. There was complete absence of peak at 1,164 positions while peak at 1,077 was shifted to 1,089 followed by the development of a new peak at 1,048 (46 cm−1). Figure 5 further indicated the presence of a completely new absorption peak in fingerprint region at different wave number, e.g., 814, 779, 710, 679, and 613 cm−1, respectively. The shift in peak located around 3,400 can be assigned as an involvement of OH (of alcohol) and N–H group information of Se nanosphere. Similarly, shift and appearance of peak at 2,959 and 2,934 cm−1 suggested the involvement of C-H and O–H group of leaf extract in particle formation. The peak at 1,647 cm−1 was shifted to 1,646 cm−1with reduction in height.

The change at this wavenumber indicated that group, C=O of ketones, of leaf extract could interact with sodium selenite. Peaks around 1,200 and 1,000 cm−1 may be due to stretching and vibration between N–H. Peaks at 1,315.41, 1,419.54, 1,280.45 and 1,048.46 cm−1 emerged due to vibrations in bond between C–O and N–H elements. Absorption peaks at 887.13, 819.80, 785.98, 719.64, 697.90, and 617.05 cm−1 in leaf extract containing Se may be due to the partial deutriation of amine or carboxyl group. A strong physical adsorption of O–H groups on Se surfaces, leading to a highly stable structure of SeNPs in water, has been reported. These biomolecules may be the functional groups that involved in the reduction and stabilization of nanoparticles. The biomolecules may be derived from the phytochemicals such as alkaloids and flavonoids present in the aqueous leaf extract of *Rhodomyrtus tomentosa.* Quantitative estimation of phytochemicals (mg/g) is been done in *R. tomentosa*, which indicates the presence of Alkaloids-14.79 ± 0.20 Flavonoids -23.5 ± 0.225 Phenols 13.22 ± 0.082- Saponins- 6.89 ± 0.55 Tannins- 3.05 ± 0.2. There is a relationship between the size of the particles and biological activity. The culture supernatant of *Aspergillus terrus* produced spherical selenium nanoparticles of 47 nm (Zare *et al*., 2013), *Klebsiella pneumonia* produced selenium nanoparticles of 245nm (Fesharaki *et al*., 2010).

Elemental microanalysis depicted in Table 1 derived from the synthesized Selenium Nanoparticlesi ndicate 66.46 % of Selenium Nanoparticles (Salam *et al*., 2012) Table-1. 10 mg of the sample dissolved in 1mL of DMSO (Dimethyl sulfoxide) in sterilized appendroff. From this stock, sample was made 100µg, 200µg, 300µg, 400µg of concentration pipetted out in sterile Eppendorf for bacterial plates respectively and the final volume was made up to 50µL by adding DMSO. Mahesh and Satish, (2008) characterized the *Mimusops elengi* synthesized silver nanoparticles through FTIR spectral analysis and they obtained the characteristic peak at 3415, 2928, 118, 1445 and 1041cm^{-1}. Similar results were observed in the present study silver nanoparticles synthesized by *R. tomentosa*, and the FTIR spectral analysis revealed the presence of =C-H stretch and bend strong sharp peak, C-H bend sharp beak, C-H stretch weak very strong nitro and carboxyl beak and hydroxyl beak (Sheela and Deepa, 2013; Zare *et al*., 2013). The band at 3415cm^{-1} was responsible for OH stretching (Gardea-Torresdey *et al*., 2003; Heath, 1989). The crystalline nature of silver nanoparticles was confirmed through XRD analysis and the diffraction of 100% intensity peak was observed in the range of 31.8836. Antibacterial activity of *R. tomentosa*, synthesized silver nanoparticles showed maximum zone of inhibition in *Pseudomonas sp.* (39 ± 0.0 mm) followed by *S. aureus* (19.3 ± 0.6 mm), *Streptococcus* sp. (19 ± 0.0 mm) and they exhibited moderate activity against *E. coli* (26.8 ± 0.00 mm) respectively. These results corroborate with (Huarte *et al*., 2010; Khan *et al*., 2017).

CONCLUSION

Rhodomyrtus tomentosa Biogenic SeNPs have wide applicability in the field of nanomedicine is gaining considerable importance in recent years. FT-IR spectroscopic study indicated the involvement of functional groups like carboxyl (−C=O), hydroxyl (−OH), and amine (−NH) of leaf extract in synthesis of nanoselenium. The biomolecules may be derived from the phytochemicals such as alkaloids and flavonoids present in the aqueous leaf extract of *Rhodomyrtus tomentosa*. Herein we have reported a facile ecofriendly biogenic synthesis of Selenium Nanoparticles using leaf extract of *Rhodomyrtus tomentosa* plant. The synthesized Se NP showed absorption maximum at 250 nm revealed by UV –Vis spectroscopy. XRD results showed the formation of polydispersed, crystalline SeNPs. SEM analysis elucidates that the synthesized selenium nanoparticles ranged in the size of 37 nm to 61 nm and are spherical and hollow in nature. Selenium possesses several applications in Medicine, chemistry and electronics. Hence, appropriate methods should be developed and tested, especially for the recovery of these nanoparticles from natural resources such as plants. To the best of our knowledge, this report of biogenesis of SeNPs using leaf extract of *Rhodomyrtus tomentosa*, characterization and their antimicrobial application on *Labeo rohita*.

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