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# GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM TRACHYSPERMUM ROXBURGHIANUM

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## ABSTRACT

Nanoparticles have become a matter of great interest in the recent times due to their unique properties and varied applications in different fields. Although there exist an array of physical and chemical methods for nanoparticle synthesis, the industry is on a lookout for a cleaner and greener alternative route for the production of nanoparticles. Synthesis of nanoparticles using microbial or phytochemical route have gained importance in this regard. This study focuses on biogenic production of silver nanoparticles. The aqueous seed extract of *Trachyspermum roxburghianum* was used for one step synthesis or biosynthesis of silver nanoparticles. The phytochemicals or the secondary metabolites present in the seed extract act as reducing agent and capping agent for producing zero-valent metal (ZVM) silver nanoparticles. The synthesized Silver nanoparticles were characterized by UV-VIS Spectrophotometer, X-Ray Diffraction (XRD), Fourier Transform Infra Red spectroscopy (FTIR) and Field Emission Gun-Scanning Electron Microscope (FEG-SEM). Silver nanoparticles have garnered a lot of attention as antimicrobial agent and thus bear significance in the field of Nutraceutical and food storage & packaging. However, the use of nanosilver in food coating is in its quiescent stage in nutraceutical delivery and nano carriers.

**KEYWORDS:** Silver Nanoparticles, Biosynthesis, Trachyspermum Roxburghianum, Field Emission Gun-Scanning Electron Microscope (FEG-SEM).

## INTRODUCTION

Nanoparticles have a wide range of applications in diverse fields (Brown, 2013; Cardinal et al, 2008; Chaudhary, 2011; Jain, 2003; Jeffrey et al, 2006; Liu et al, 2011; Loo et al, 2005;

Misra et al, 2010; Syed, 2014; Wang et al, 2010; Zhou et al, 2010). As a result, their synthesis on large scale through eco-friendly routes have gained momentum in recent times (Logeswari et al, 2013). Among other methods, biosynthesis of metal nanoparticles is an environment friendly method without use of harsh, toxic and expensive chemicals unlike the conventional reduction methods. The conventional methods use chemicals like sodium borohydride, DMF, ethylene glycol etc., which may raise the toxicity of the nanoparticles due to absorption of these chemicals on the surface. The use of plant materials (inactivated plant tissue, plant extracts and living plant) as construct for nanomaterial has received more attention as compared to physical and chemical methods. This is mainly because they have proved to be a cheap and economical tool for production of nanomaterials on a large-scale. The extracts from plants can act as both reducing and capping agents in nanoparticle synthesis. Moreover, biological reduction by combination of biomolecules found in plant extracts (e.g: enzymes, proteins, amino acids, phytochemicals and phytopigments) is environmentally benign. Besides, they could be involved in the stabilization of the nanoparticles, so formed (Vinod, 2017).

The emergence of biogenic production of nanoparticles has created an environmentally benign and green route approach in the field of nanotechnology, that has found its way in allied sciences and technologies. The phytoconstituents and active components of the plants such as polyphenols, terpenes and alkaloids etc. (from its parts, tissues and debris) possess good reducing capabilities as antioxidants. This property of the plant phytochemicals has been exploited in eco-friendly biosynthesis of nanoparticles and has laid the foundation of green chemistry in biogenic production of nanoparticles. The purpose of this study was to exploit the reducing capabilities of the aqueous seed extract of *Trachyspermum roxburghianum* in the biosynthesis of nanoparticles at lab-scale, which could be used in the industrial applications of Nutraceutical, Pharmaceutical and Food Technology.

The production of silver nanoparticles from natural sources can be done using either microbial sources or plant sources. However, plant sources have been found to be suitable candidates for biosynthesis of nanoparticles. Nanoparticles produced by plants are more stable and the rate of synthesis is faster than in the case of microorganisms. In comparison to microorganisms, the phytosynthesis method is devoid of complex and multistep processes like microbial isolation, culturing, maintenance etc. Phytosyntheis is a very rapid and cost-effective approach that can be easily scaled up for bulk production of nanoparticles (Shankar

et al, 2004). Moreover, the nanoparticles vary in shape and size in comparison with those produced by other organisms. The advantages of using plant and plant-derived materials for biosynthesis of metal nanoparticles have interested researchers to investigate mechanisms of metal ion uptakes and bioreduction by plants, and to understand the possible mechanism of metal nanoparticle formation by plants (Iravani, 2011). These newer techniques for greener silver nanoparticle synthesis using biorenewable materials appear promising as they do not have any toxic materials deployed during the production process.

The production of nanoparticles majorly involves physical and chemical processes. Silver nanomaterials can be obtained by both the so-called 'top-down' and 'bottom-up' methods. The top-down method involves the mechanical grinding of bulk metals and subsequent stabilization of the resulting nanosized metal particles by the addition of colloidal protecting agents. The bottom-up methods, on the other hand, include reduction of metals by electrochemical methods and sonodecomposition.

Biosynthesis of silver nanoparticles is a bottom-up approach that mostly involves reduction/oxidation reactions. It is majorly the microbial enzymes or the plant phytochemicals with antioxidant or reducing properties that act on the respective compounds and give the desired nanoparticles. The major components involved in the preparation of nanoparticles using biological methods are the solvent medium for synthesis, the environmentally friendly reducing agent, and a nontoxic stabilizing agent (Prabhu and Poulose, 2012).

The nanoparticles are of great interest due to their extremely small size and large surface to volume ratio. They exhibit utterly novel characteristics as compared to the large particles of bulk materials (Perez et al, 2005). Nanoparticles of nobel metals eg: gold, silver, platinum and palladium have been synthesized using plants. As stated earlier, plants are known to possess a broad range of metabolites that can be exploited successfully for rapid and extracellular biosynthesis of nobel metal nanoparticles with promising implications in the near future.

Highly stable and crystalline silver nanoparticles were reported using geranium leaf extract of size 16-40 nm (Shankar et al, 2003). In a comparative study of biosynthesis of nanoparticles from leaf extracts of different plants viz. *Helianthus annus, Basella alba, Oryza sativa, Saccharum officinarum, Sorghum bicolar* and *Zea mays,* it was concluded that *H. annus*

exhibited the strongest and rapid potential of bioreduction of silver ions (Leela and Vivekanandan, 2008; Njagi et al, 2010). Similarly, many methods have been reported for leaf-mediated biosynthesis of silver nanoparticles viz. Black tea leaf extracts (Begum et al, 2009), extracellular synthesis using leaf extracts of Pine, Persimmon, Gingko, Magnolia and Platanus plants (Song and Kim, 2009) and evaluation of their antibacterial and antimicrobial activities viz. *Eucalyptus hybrida* leaves (Dubey et al, 2009), *Allium cepa* (Saxena et al, 2010), *Euphorbiahirta* (Elumalai et al, 2010), *Acalypha indica* (Krishnaraj et al, 2010), *Cassia auriculata* (Udayasoorian et al, 2011). Synthesis of silver nanoparticles using the fruit extract of *Carica papaya* were found to be highly toxic against different multidrug resistant human pathogens (Jain et al, 2009).

A comparative test of *Curcuma longa* was carried out to check the compatibility of the bark and powder extracts of *Curcuma longa* toward the formation of silver nanoparticles and it was reported that bark extract could produce a higher amount of silver nanoparticles compared to the powder extract. The resulting nanoparticles varied in shape and size but had strong antibacterial activity against *Escherichia coli* (Sathishkumar et al, 2010). Similarly, other workers also reported the formation of silver nanoparticles using leaf extract of *Argemone maxicana* (Singh et al, 2010), bran powder of *Sorghum spp*. (Niagi et al, 2010).

Furthermore innumerable works on plant-mediated biosynthesis of silver nanoparticles have been reported that have several applications in different sectors. Silver has long been recognized for its inhibitory effect towards various microorganisms commonly present in medical and industrial processes. Silver nanoparticles have been employed in sensor technology, biological labeling and many other biomedical applications (Akhtar et al, 2013).

The unique optoelectronic and physicochemical properties of silver nanoparticles have been successfully exploited for the purpose of drug delivery, tissue/tumor imaging, biosensing, catalysis, and surface-enhanced Raman scattering-based sensors (Khan et al, 2013).

In the present paper, silver nanoparticles were synthesized from the aqueous extract of *Trachyspermum roxburghianum* seeds. The objective of using this plant source for biosynthesis of nanoparticles was to distinguish and draw out a comparative relation between silver nanoparticles synthesized from the plants of the same genera (*Trachyspermum sps*) i.e. *Trachyspermum ammi* (Vijayaraghavan et al, 2012) and *Trachyspermum copticum* (Deshpande et al, 2011).

*Trachyspermum ammi* mediated synthesis resulted in formation of nanoparticles of size range between 87nm-998nm with the majority of 87 nm. In this method, specimen was ground in a burr mill and incubated with 1 mM silver nitrate solution with constant shaking at 120 rpm for 24 hours. As against this, minimal conditions were employed for *Trachyspermum roxburghianum* in the present study.

A much more comprehensive and complex step was employed in case of *Trachyspermum copticum*, using Microwave radiation. Though, a biogenic route, it involved a mixture of water and ethanol, an organic solvent for extraction. Moreover, the aqueous phase was extracted for 4 hours on a rotary shaker and subsequently the extract was incubated with 1mM $AgNO_3$ solution followed by exposure to Microwave unifrequency radiation. The majority yield of nanoparticles using this ethanolic extract ranged in size between 6-60 nm. Although a one step and rapid synthesis, this method follows the principles of Green chemistry route partially and incoherently.

In the present study, a less complicated and alternative green route has been employed for the synthesis of silver nanoparticles from *Trachyspermum roxburghianum* with mediocre conditions and using simpler equipments for the course of investigation, thereby yielding similar, analogous and comparable results.

## MATERIALS AND METHODS

### Plant material

Seeds of *Trachyspermum roxburghianum* were purchased from a local market in Mumbai city. The seeds were sieved to remove impurities and adulterants, washed under running water and sundried until they were completely dried. Further, they were oven dried at 37°C for 3 hours to remove any residual moisture. Seeds were finely powdered in a mixer grinder and the powdered sample was stored in an air tight container until further use.

### Preparation of the plant extract

5 g of the powdered seed was mixed in 25 ml of distilled water and extracted on a boiling water bath for 5-10 minutes with constant stirring. The extract was cooled and centrifuged at 4100 rpm for 15 minutes. The supernatant was filtered through Whatmann Filter paper no. 41. This aqueous extract was preserved at 4°C for all further studies and analysis.

**Qualitative test for phenols**

A 5% aqueous solution of ferric chloride is used for the detection of phenols. 1ml each of filtrate is diluted with distilled water and two drops of aqueous 5% $FeCl_3$ is added.

**Preparation of silver nitrate solution**

8mM silver nitrate solution was freshly prepared using distilled water and stored in an amber colored flask in dark.

**Biosynthesis of silver nanoparticles**

The aqueous plant extract was mixed with 8mM silver nitrate solution in 1:9 ratio [2.5 ml of aqueous plant extract was taken in a 25 ml amber colored volumetric flask and the volume was made up using 8mM silver nitrate solution]. This reaction mixture was incubated at 37°C in hot air oven for 3 hours. Subsequently, the reaction mixture was incubated in dark for 40-48 hours for the synthesis of silver nanoparticles. The supernatant is subjected to UV-Vis spectrometry. The precipitated silver nanoparticles were characterized by X-Ray Diffraction (XRD), Fourier Transform Infra Red (FTIR) spectrometry and Scanning Electron Microscopy (SEM).

**CHARACTERIZATION OF NANOPARTICLES**

**UV-Vis Spectrum Analysis:** The reduction of ionic silver was measured after 3 hours of incubation. The incubated mixture was centrifuged and a small aliquot of the supernatant was diluted with distilled water in 1:9 ratio. The sample was analysed using UV-VIS spectrophotometer (UV 3000+ LABINDIA) in spectrum mode within a range of 200-800 nm at 1nm resolution. Further UV-Visible characterization was also carried out after 48 hours by scanning from 300-500 nm for comparing the bio-reduction of the ionic silver.

**XRD (X-Ray Diffractometer) Analysis:** XRD analysis was carried out for studying the crystalline structure of the silver nanoparticles. The precipitated silver nanoparticles, after centrifugation were transferred onto a whatman filter paper no. 42 for absorption of any excess unreacted free moiety and dried to obtain a powder form. The filter paper with the powder coated on it was cut to the required size (Rectangle 1" X 1") and used for XRD analysis from 0° to 110° at 2 theta angles. The X-Ray Diffractometer (Model: XPERT-PRO) was operated at a voltage of 40 kV and a current of 30 mA with Cu K-alpha radiation.

**FTIR (Fourier Transform InfraRed) Analysis**

The sediment layers were characterized by FTIR (Model: 3000 Hyperion Microscope with Vertex 80 FTIR System, Bruker, Germany) in order to determine the functional groups in the *Trachyspermum roxburghianum* extracts and its possible involvement in bio-reduction and synthesis of silver nanoparticles. The sample after precipitation was dried, mixed with potassium bromide crystals and pressed using a hydraulic press to form a pellet. FTIR spectrum of the pellet was recorded in the mid IR region of 500-4000 $cm^{-1}$.

**FEG-SEM (Field Emmision Gun-Scanning Electron Microscope) Analysis**

To determine the morphology of the synthesized silver nanoparticles, the sample was analysed by Field Emission Gun-Scanning Electron Microscope [FEG-SEM (Model:JSM-7600F by JEOL). Thin film of sample was evenly spread on a carbon coated copper grid. The film on the SEM grid was allowed to dry for 5 minutes by putting it under a mercury lamp and the morphological characters were deciphered by FEG-SEM.

**RESULTS AND DISCUSSION**

**UV-Vis Spectroscopy**

Visual observation of the reaction medium after 3 hours of incubation at 37°C showed a change in color from pale yellow to brown indicating the formation of silver nanoparticles. Biological methods of synthesis of silver nanoparticles exhibit strong absorption of the electromagnetic waves in the visible range due to (SPR) Surface Plasmon Resonance (Kreibig and Vollmer, 1995; Link and El-Sayed, 2003). The change in color is due to surface plasmon vibrations / surface plasmon resonance in silver nanoparticles.

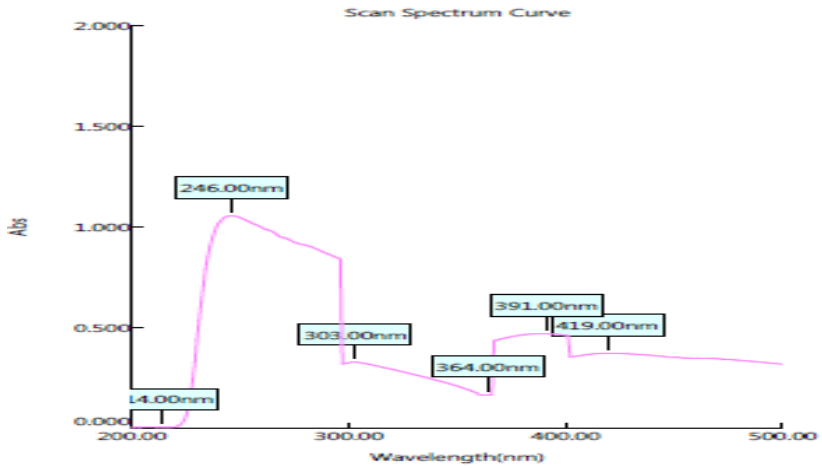


**Figure. 1: UV absorption spectra after 3 hours of bio-reduction by *T. roxburghianum* extract.**

UV-Vis analysis was done to detect the formation of silver nanoparticles. **Figure 1** shows the UV-Vis spectrum of the reaction medium after 3 hours of incubation. The 200-800 nm spectrum scan shows the absorption band at 419 nm confirming the formation of silver nanoparticles. The broad peak indicates that the silver nanoparticles are in polydispersed phase. An absorption band is clearly visible at 246 nm. This absorption band is due to the electronic excitation of organic moieties present in the extract. This is also indicative of the activity of organic moieties in bio-reduction of silver ions to silver nanoparticles. The peaks/ absorption bands obtained at 303 nm, 364 nm and 391 nm are indicative of uncapped silver colloids which are not stabilized (He et al, 2001).

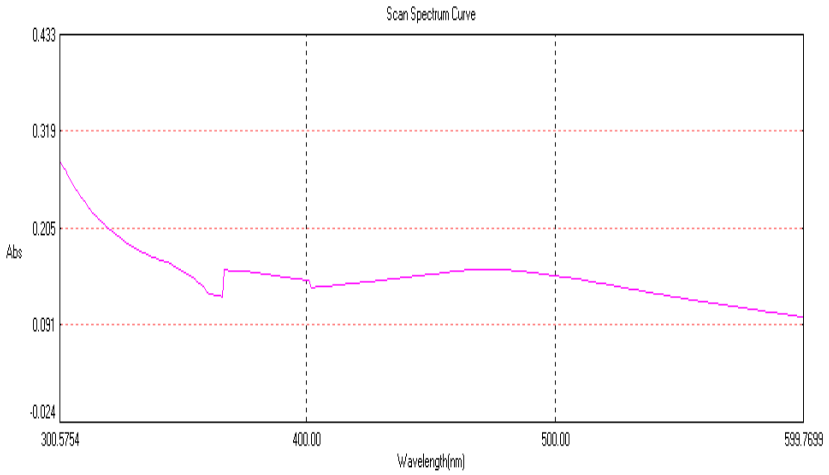


**Figure. 2: UV absorption spectra after 40-48 hours of reduction of $Ag^+$ ions into Silver nanoparticles.**

UV-Vis spectrum scan after 48 hours is illustrated in **Figure 2**. In the UV-Vis spectroscopy, the peak was obtained at 474 nm confirming the presence of silver nanoparticles. The broadened and wide peak is indicative of their polydispersed nature. In comparison to the peak generated after 3 hours (**Figure 1**), the absorption peak recorded after 48 hours shows a shift to longer wavelength with peak broadening. This shift in wavelength can be explained by the fact that spectral response of silver nanoparticles is a function of diameter (Oldenburg, 2018). As the particle size increases, the peak plasmon resonance shifts to longer wavelengths/ red shift and broadens. This implies that the diameter or particle size increases with time of incubation.

**X-Ray Diffraction Analysis**

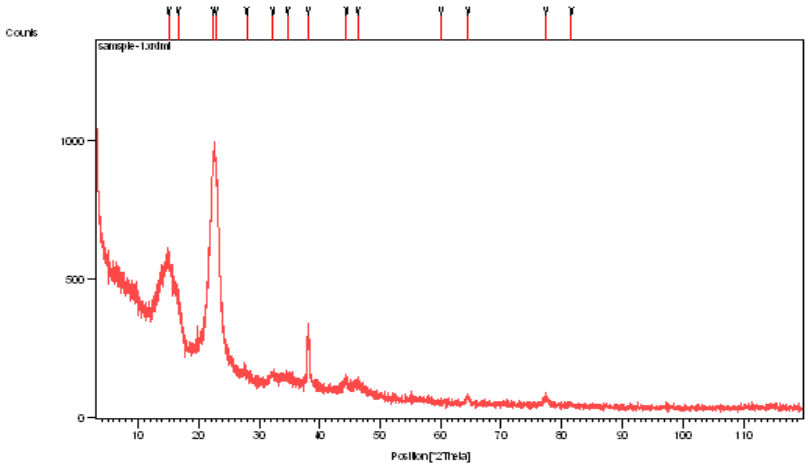


**Figure. 3: XRD pattern for silver nanoparticles.**

| Pos.[°2q] | Height[cts] | FWHM.[°2q] | d-spacing[Å] | Rel.Int.[%] |
|---|---|---|---|---|
| 15.0619 | 440.66 | 0.6691 | 5.88225 | 52.58 |
| 16.5865 | 299.53 | 0.8029 | 5.34483 | 35.74 |
| 22.4592 | 838.10 | 0.6022 | 3.95878 | 100.00 |
| 22.8133 | 804.49 | 0.3346 | 3.89813 | 95.99 |
| 27.8823 | 34.29 | 0.8029 | 3.19991 | 4.09 |
| 32.2186 | 38.75 | 0.4015 | 2.77845 | 4.62 |
| 34.6843 | 29.41 | 1.0706 | 2.58637 | 3.51 |
| 38.1094 | 226.25 | 0.2676 | 2.36143 | 27.00 |
| 44.3847 | 53.89 | 0.3346 | 2.04104 | 6.43 |
| 46.2873 | 39.38 | 1.0706 | 1.96148 | 4.70 |
| 60.0328 | 7.39 | 0.2007 | 1.54111 | 0.88 |
| 64.4767 | 30.99 | 0.2007 | 1.44521 | 3.70 |
| 77.4211 | 28.02 | 0.4015 | 1.23274 | 3.34 |

The phase identification and crystalline structures of the nanoparticles were characterized by X-ray Diffractometer. The X-Ray Diffraction pattern obtained for silver nanoparticles is shown in **Figure 3**. It is found that there exist diffraction peaks with 2q values of 38°, 44.3°, 64.4° and 77.4° that correspond to the crystal planes of 111, 200, 220 and 311 planes of a Face-Centred Cubic (FCC) lattice of silver nanoparticles respectively. The observed 2q values are in agreement with the JCPDS silver file no. 04-0783, thus confirming the formation of silver nanoparticles.

**Fourier Transform Infra Red (FTIR) Analysis**

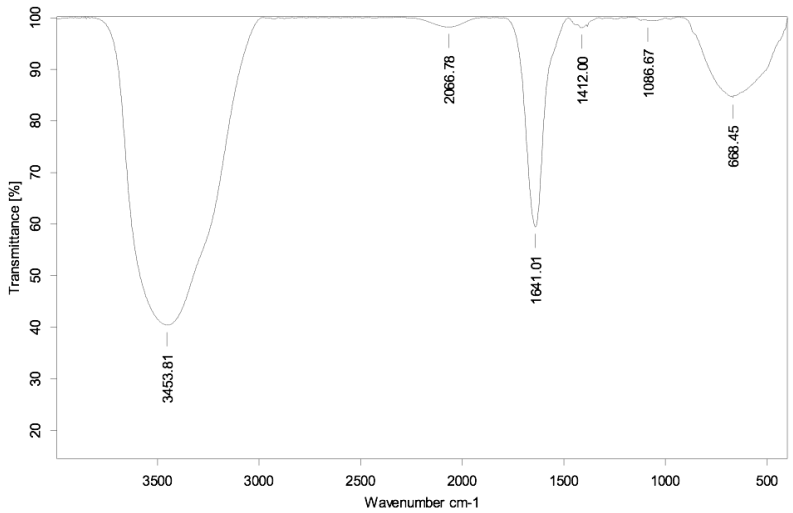


**Figure. 4: FTIR of silver nanoparticles synthesized using the seed extract of *Trachyspermum roxburghianum*.**

In order to identify the possible biomolecules responsible for the reduction of the $Ag^{+}$ ions and capping the bio-reduced silver nanoparticles synthesized using *Trachyspermum roxburghianum* seed extract, FTIR spectrum was recorded. The representative spectrum of the nanoparticles obtained in the present study is shown in **Figure 4**. FTIR shows peaks at 3543.81 $cm^{-1}$, 2066.78 $cm^{-1}$, 1641.01 $cm^{-1}$, 1412.00 $cm^{-1}$, 1066.67 $cm^{-1}$ and 688.45 $cm^{-1}$. The narrow peak obtained at 1641$cm^{-1}$ suggest the presence of C=C bonds of aromatic ring. The broad peak at 3453.81 $cm^{-1}$ corresponds to O-H bond indicating the presence of a phenol. Weak bands at 1412 $cm^{-1}$ (C-C stretch in aromatic ring) (Marimuthu et al, 2011) and 1066.67 $cm^{-1}$ (C-N stretch) are known to arise due to carbonyl stretch and amine stretch in proteins (Arulkumar and Sabesan, 2010). It is well known that proteins can bind to silver nanoparticles either through free amino groups or cystene residues in the proteins, thereby stabilizing them. Besides, one or more of these proteins may be enzymes that reduce silver nitrate ions and form the silver nanoparticle by reduction technique. Hence, it can be inferred that the biomolecules are responsible for reducing and capping the silver nanoparticles.

**Field Emission Gun-Scanning Electron Microscope (FEG-SEM) Analysis**

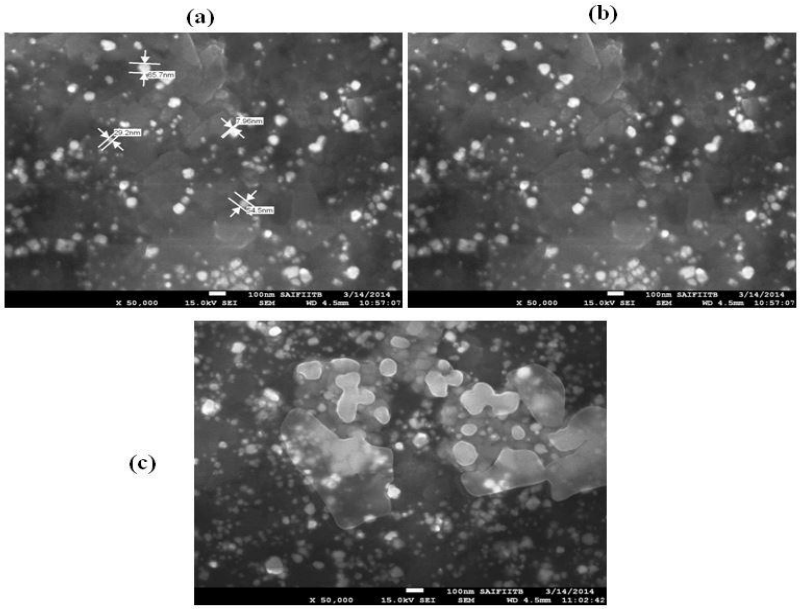


**Figure 5: FEG-SEM of silver nanoparticles synthesized using the seed extract of *Trachyspermum roxburghianum.***

The SEM analysis finally confirmed the morphology of the synthesized silver nanoparticles as seen in **Figure 5(a)** and **5(b).** The silver nanoparticles are polydispersed, roughly spherical in shape, grey white in color with varied sizes, a very few of them of minute sizes as marked with arrows, 7.96 nm, 29.2 nm and most of them in the range 54.5 nm-67.5 nm. The average range of the particles is roughly visualized to be between 50-70 nm in addition to a few measuring about 100 nm. In **Figure 5(c)**, thin and sensitive layer adsorbed onto the surface of the nanoparticles are very well seen (marked by an arrow). These are the organic moieties adsorbed on the surface, which are responsible for binding and stabilizing the silver nanoparticles.

## CONCLUSION

Based on the investigations carried out in this study it could be inferred that bio-reduction of silver metal ions from seed extract of *Trachyspermum roxburghianum* could yield Silver nanoparticles of fairly well-defined dimension. Though there are reports describing the synthesis of silver nanoparticles using the same genera of apiaceae i.e. *Trachyspermum ammi* and *Trachyspermum copticum*, present study demonstrates a simpler method of obtaining silver nanoparticals using *Trachyspermum roxburghianum*. Hence, it could be ascertained that the seed extract of *Trachyspermum roxburghianum* could be a conducive source of producing nanoparticles through cleaner, safer and greener approach.

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