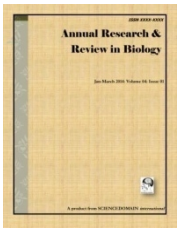

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Novel Scientific Appraisal of *Elaeocarpus ganitrus* the Rudraksha: Nano Silver Synthesis with Aspects of Variation in Concentration Antimicrobial Activity and *In vitro* Biocompatibility

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Authors' contributions

Author PD understood the concept, conducted the experiments, collected all the data, and drafted the manuscript, under the guidance of the authors SSN and RPT. The authors have read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The remarkable non-degradable distinctive feature of the Rudraksha bead renders reutilization of its resources and inexhaustibility of its potentiality to mediate innumerable silver nanoparticle syntheses. The present study is a step forward towards high prospects of manoeuvred nano silver manufactured for many essential applications.
Study Design: In this nanoregime we bring forth a glorious green route for flabbergasting silver nanoparticle fabrication, with the intervention of the five faced seed of the plant *Elaeocarpus ganitrus* Roxb, the Rudraksha bead. Aspects, of the variation in concentration of the precursor as well as the mediator on the physico-chemical nature of the silver nanostructure, its antimicrobial activity, together with the issues of its biocompatibility, have been dealt here.
Place and Duration of Study: Chemistry Department and Biotechnology Department, Motilal Nehru National Institute of Technology, India, between April 2011 and August 2012.
Methodology: The Rudraksha extracts were prepared and biosyntheses of silver

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nanoparticles were conducted and different parameters were studied together with various characterization.
Results: The silver nanoparticles produced are chiefly spherical with variable size. There exists also variation in size and silver content depending upon the concentration of precursor and mediator. The antimicrobial activity of the nano silver produced is high with good biocompatibility.
Conclusion: The inexhaustible characteristic of the non-degradable Rudraksha facilitates incessant biocompatible nano silver production.

Keywords: Nanotechnology; nanoparticle; biosynthesis; antimicrobial; cytotoxicity.

1. INTRODUCTION

As the field of nanotechnology has boomed [1], with blooming strategies to knock out lingering therapeutic challenges [2-4]; the green biosynthesis of nanoparticles has blossomed [5,6], with promising essence especially in nanomedicine [7]. Silver since ages, have myriads of applications, also popularly known and excessively used as antimicrobial agents [8,9]; so specifically silver nanoparticles hold a special space in science, technology and especially medicine [10-13]. We have therefore endeavoured to bring forth a majestic green route to enormous, economic and eco-friendly silver nanoparticle generation, eliminating obnoxious reagents; enabled by the five faced seed of the plant *Elaeocarpus ganitrus* Roxb. (syn: *Elaeocarpus sphaericus*), commonly called the Rudraksha [14-18], for the first time ever. The Rudraksha as a mediator is superior and privileged over other plant materials as it stands non-degradable, the same bead is capable of giving extracts innumerable times, can withstand repeated boiling; while most of the plant materials degrade after extract being once taken out. The mechanism of silver nanoparticle biosynthesis is 'bottom-up' approach, through phyto-chemicals mediated bio-reduction of $Ag^{+} \rightarrow Ag^{o}$ and there after their self assembling with colloidal aggregation. The results of varying concentration of the precursor as well as the mediator, on the physico-chemical properties of the silver nanoparticles are discussed here. Extent of the biocompatibility of the manoeuvred silver nanoparticles manufactured employing phyto-fabrication method exploring the Rudraksha bead is determined by the cytotoxicity testing [19-23].

2. MATERIALS AND METHODS

2.1 MATERIALS

Silver nitrate ($AgNO_3$) of analytical grade, nutrient agar and nutrient broth, were purchased from Thomas Baker (Chemical) Pvt. Ltd. India. Five faced seeds of the plant *Elaeocarpus ganitrus* Roxb.i.e. the Rudraksha beads, were purchased from Rudraksha World, Allahabad, India. All solutions were made in deionized water.

2.2 Preparation of the Rudraksha Extracts

The Rudraksha beads were washed, air dried and weighed to be 3.9 g. The beads were boiled in 100 ml of deionized water in a 500 ml Erlenmeyer flask for 30 min at 100ºC. The crude colourless, clear and transparent extract was filtered using Whatman No. 41 filter paper and stored in closed bottle at 4ºC for further use. This was marked as extract RE(A). The beads were air dried, and weighed again to be of 4.3 g, possibly due to slight moisture

retention. Then the above mentioned procedure was followed again taking 50 ml of sterile deionised water for boiling and preparing the water coloured Rudraksha extract with the previously used and air dried beads. This was marked as extract RE(B).

2.3 Biosyntheses of Silver Nanoparticles

50 ml of the aqueous Rudraksha extract RE(A) was added to 100 ml of 1 mM $AgNO_3$ solution and this was marked as RM(A). Then 10 ml of Rudraksha extract RE(B) was added to another 100 ml of 1 mM $AgNO_3$ solution and marked as RM(B). Again 10 ml of extract RE(A) was added to 100 ml of 2 mM $AgNO_3$ solution and marked as RM(C). The reaction mixtures were colourless and transparent immediately after mixing which were further allowed to incubate at room temperature. Within a short duration of time ~ 1 h colour transformation of all the reaction mixtures took place indicating formation of silver nanoparticles. Allowing stabilization for 7 days, after complete colour development the reaction mixtures were progressively studied.

2.4 Characterization of Silver Nanoparticles

The reduction of Ag^+ to Ag^o was monitored by measuring the UV-visible spectrum of each reaction mixture using UV-visible spectrophotometer (Shimadzu UV – 2450), from 200 to 800 nm. Scanning electron microscopic (SEM) study was done using (JEOL JXA 8100) instrument. The chemical composition identification of the nanoparticles was enabled by energy dispersive X-ray analysis (EDX); performed with (HR TEM TECNAI 20 G^2) instrument operated at an accelerating voltage of 200 kV.

2.5 Antimicrobial Assay

The silver nanoparticle pellet obtained from the suspension RM(A) was assayed for antimicrobial activity against *Staphylococcus aureus* (gram positive), the most virulent bacterial strain causing majority of the infections. Disc diffusion method [24] was used to find out the standard zone of inhibition (ZOI). Well was bored having 7 mm diameter on cultured agar plate and the test sample of nano silver pellet was placed. Nutrient agar was used as culture media and inoculation was done with 500 µl of bacterial organism containing suspension. The inoculated plate containing the test sample was incubated at 37ºC for 48 h. The plate was then examined for evidence of zone of inhibition (ZOI), which appear as a clear area around the well. The diameter of such (ZOI) was measured using a meter ruler.

2.6 *In vitro* Cytotoxicity/biocompatibility Testing

In vitro cytotoxicity test for biocompatibility determination was done through MTT assay against the J774A.1 murine macrophage cell line. Silver nanoparticle suspension from RM(A) was placed as the test sample. For positive control only macrophage cells while for negative control RPMI media were considered.

3. RESULTS AND DISCUSSION

3.1 Observation of Colour Development

The process of colour development was within ~ 1 h in all the three reaction mixtures put under constant observation. Colour transformation was gradual in RM(A) and RM(B), while it

was comparatively rapid in RM(C). After 24 h the colour changes were noted. The colour transformed from colourless to light greenish brown with very little turbidity in RM(A), brown in RM(B), also with very little turbidity and brown in RM(C) but with high transparency and clarity. (Fig. 1) clearly displays the finally developed colours.

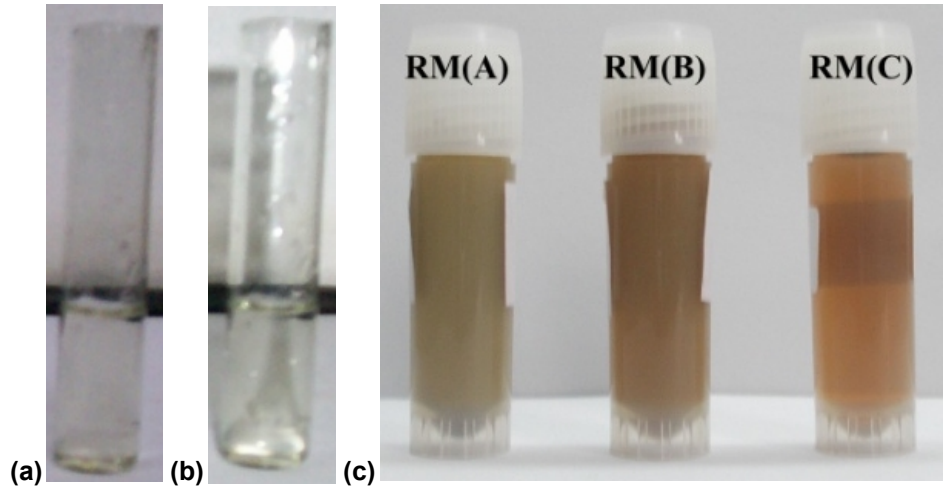


Fig. 1. Photographs; (a) $AgNO_3$ solution, (b) Rudraksha extract, (c) the developed colour in reaction mixtures

3.2 UV-visible absorbance spectroscopic studies

UV-visible absorption spectra recorded from the nanoparticle suspensions of the reaction mixtures are given in (Fig. 2). A surface plasmon resonance (SPR) band absorption peak appears between 420-480 nm, characteristic of silver nanoparticles [25]; for RM(A) at 475 nm, RM(B) at 471 nm, and RM(C) at 435 nm. According to the generalized theory the absorption peak of silver nanoparticles due to SPR shifts towards longer wavelength with increasing particle size [26]. Therefore nanoparticles obtained from RM(C) are having the minimum size and are comparatively smaller while that from RM(A) are slightly larger.

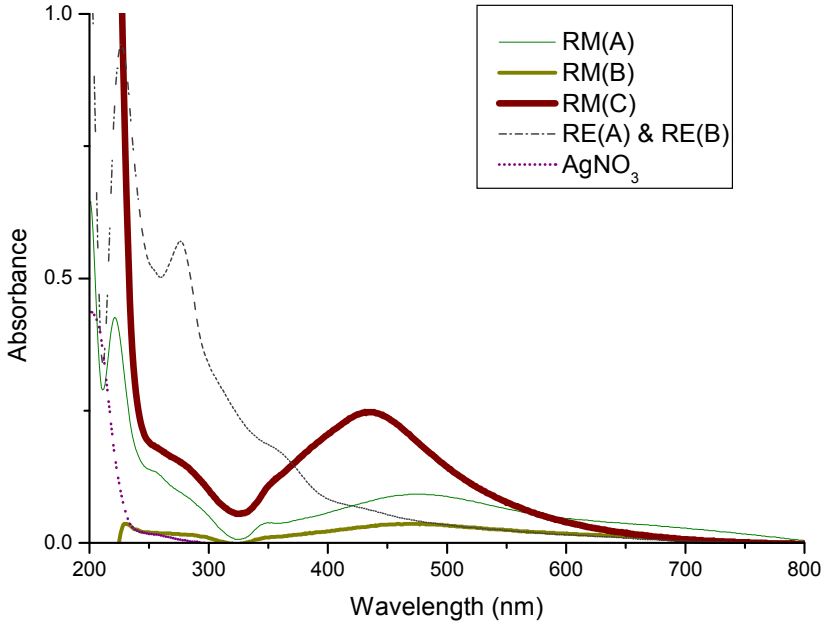


Fig. 2. UV-Visible spectra of silver nanoparticles in suspensions RM(A), RM(B), RM(C); Rudraksha extracts RE(A), RE(B) and $AgNO_3$ solution

3.3 SEM observations with analysis of elemental composition of the nanoparticles

The physical properties and morphological features of silver nanoparticles were studied through SEM images shown in (Fig. 3), which reveals that they are chiefly spherical but also irregular in shape as well as non-uniform in size, and nanoparticles obtained from RM(C) are comparatively smaller sized. Identification of the chemical composition by EDX, gives evidence of the presence of silver in the nanoparticles. Details given in (Table 1) and (Fig. 4), indicates highest silver content for nanoparticles from suspension RM(C). The presence of silver in the nanoparticles from all the suspensions are in variable quantity is because of the variation in concentration of the mediator and the precursor while the presence of elements other than silver in the spectra is due to possible interference of ions, during intervention of the bio-fabrication reaction.

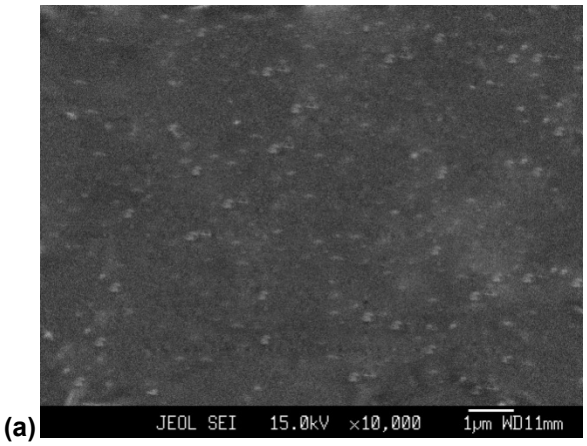
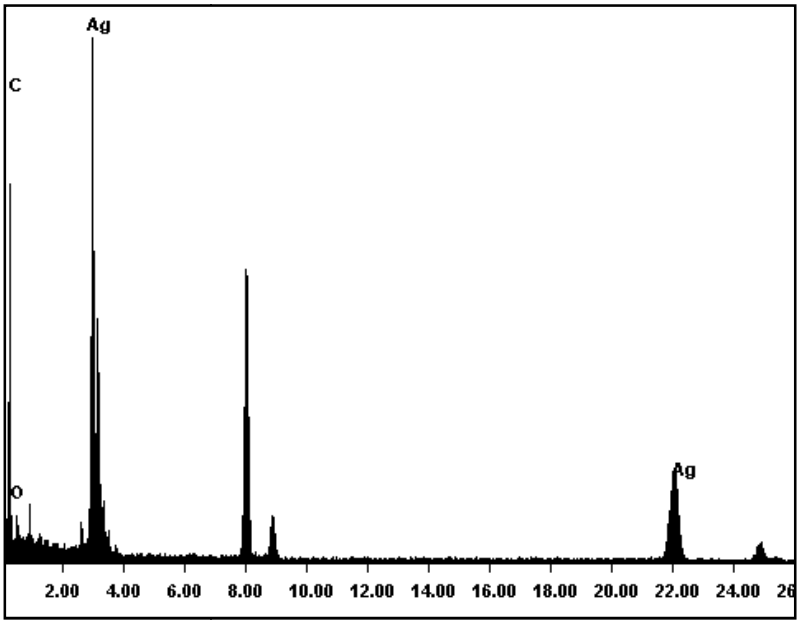
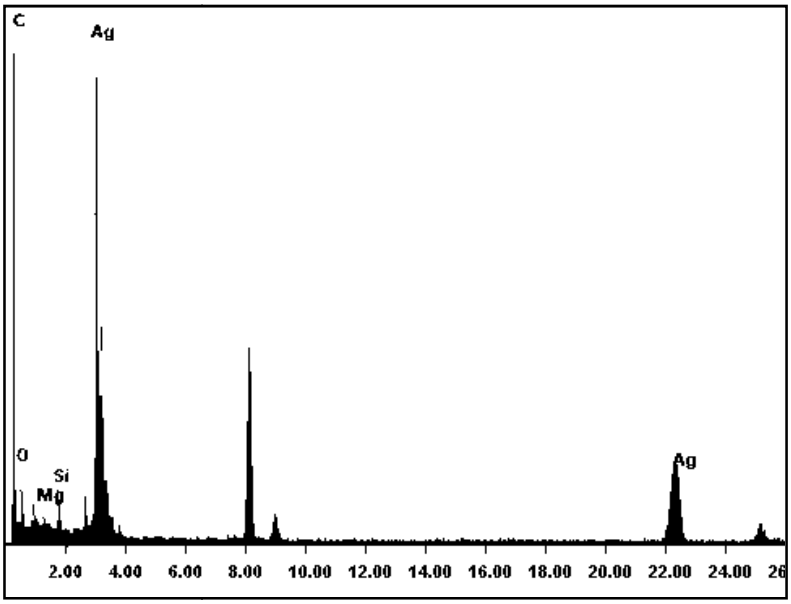


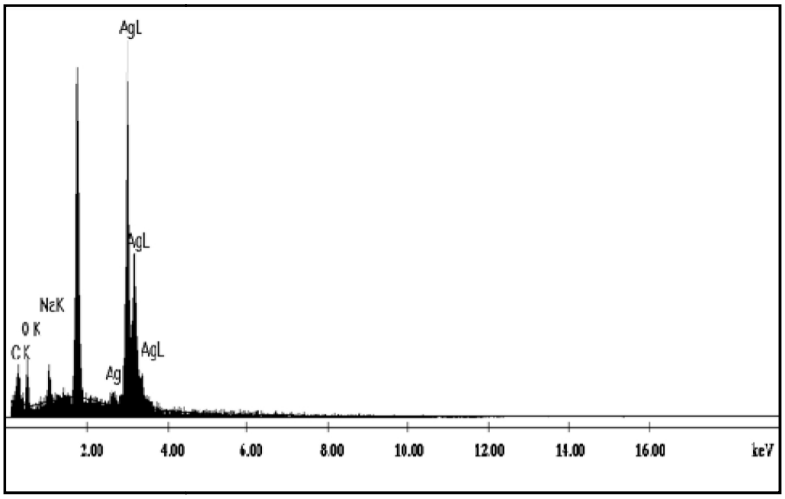
Fig. 3. SEM micrographs of silver nanoparticles in suspensions (a) RM(A), (b) RM(B) and (c) RM(C)



(a) Energy (keV)



(b) Energy (keV)



(c) Energy (keV)

Fig. 4. EDX spectra and compositions of nanoparticles in suspensions (a) RM(A), (b) RM(B) and (c) RM(C); the horizontal axis represents energy (keV).

Table 1. Composition of nanoparticles in suspensions (a) RM(A), (b) RM(B) and (c) RM(C) obtained through EDX

	(a)			(b)			(c)	
Element	**Weight %**	**Atomic %**	**Element**	**Weight %**	**Atomic %**	**Element**	**Weight %**	**Atomic %**
C K	44.00	83.70	C K	42.47	63.84	C K	07.19	22.20
O K	03.70	05.20	O K	04.50	06.00	O K	20.27	46.95
Ag K	52.30	11.10	Ag K	49.34	12.14	Ag K	67.87	23.32

3.4 Antimicrobial Activity

It has been observed that the effect of nano silver was noticeably pronounced against the stern strain, *Staphylococcus aureus* gram positive bacteria, which lack the outer membrane but has a prominent peptidoglycan layer of about 30 nm thickness. ZOI obtained was 21 mm; (Fig. 5) shows the photograph giving a vivid view of the remarkable result.

The antimicrobial activity of silver has been known since ages, even Hippocrates recognized the role of silver in the prevention of diseases and clinicians have accepted it for over the years [27]. Metallic silver when exposed to aqueous environment releases silver ions (Ag^+) that binds with the thiol groups of certain amino acids, inhibits the enzymes of respiratory cycle and also interferes with the DNA replication of the microbes. Smaller sized particles having larger surface to volume ratio and are more effective in the mode of antimicrobial action. The main mechanism through which silver nanoparticles manifest antibacterial properties is by anchoring to and penetrating into the bacterial cell, thereafter modulating cellular signaling by dephosphorylating vital key peptide substrates on tyrosine residues and finally causing cell lysis by rupturing the cytoplasmic membrane [28].

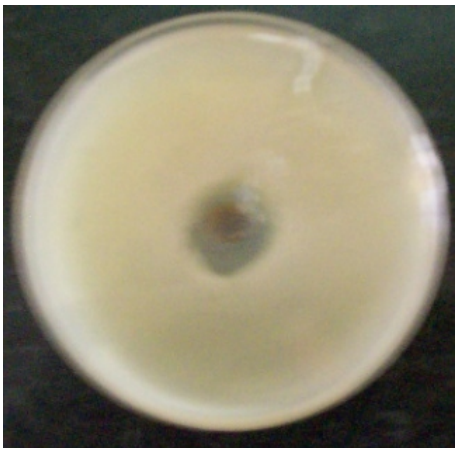


Fig. 5. Photograph showing the antibacterial activity of nano silver through zone of inhibition (ZOI) against *Staphylococcus aureus*

3.5 Biocompatibility/cytotoxicity

The silver nanoparticles synthesized can be considered reasonably biocompatible and safe to be used in regulated quantity on the basis of the cytotoxicity test done. The CC_{50} value of the silver nanoparticle suspension obtained was 0.25 ± 0.01 µM. Results of the analysis are given (Table 2) and displayed in (Fig. 6).

Table 2. Linear regression analysis of silver nanoparticles

S No.	CC_{50}
1	0.2511
2	0.2511
3	0.2578
4	0.2673
Mean	0.25
Standard deviation	0.01

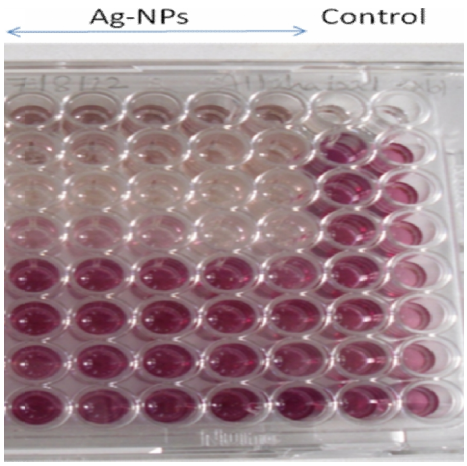


Fig. 6. MTT reading plate after incubation

4. CONCLUSION

Far reaching goals of various arrays can be efficiently achieved through this facile and flabbergasting technique of silver nanoparticle phyto-fabrication presented here. The inherent inexhaustible characteristic of the non-degradable Rudraksha, endowed with repeatedly exploitable extensive resources within, provides it the prominence for incessant, instant and incisive capability of potential nano silver production inundating enormous yield. This is undoubtedly a novel scientific appraisal of the divine bead. Further insight involving other faceted seeds of *Elaeocarpus ganitrus* Roxb, the Rudraksha beads, may impart light, bestowing impressive results, following this route and include different innovative applications. Rudraksha, hitherto holding a high place in mythology [29,30] should since now have a special space in science and technology.

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COMPETING INTERESTS

There exist no financial or non-financial competing interests.

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