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Green Synthesis and Charcterization of Silver Nanoparticles From Leafs Extracts of *Rosa indica* and its Antibacterial Activity Against Human Pathogen Bacteria

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ABSTRACTS

This work was carried out for green synthesis of silver nanoparticles and investigation of its antibacterial activity. *Rosa indica* is of considerable interest and is well known compound because of its antioxidant, antidibetic, anti-inflammantry, antimicrobial activities. In the present study rapid and facile synthesis of silver nanoparticles at room temperature has been shown. On addition of plant extracts to Silver nitrate solution the change in color of the reaction mixture was obsereved which proved the formation of nanoparticles. Further, the green synthesised silver nanoparticles were characterized UV-Vis spectrophotometer, XRD analysis, FTIR, DLS, SEM with EDX. The average particles size of silver nanoparticles show between 1-100 nm through DLS anaylsis. Antibacterial activity of the Silver nanoparticles was evaluated by testing against *Gram negative (Pseudomonas aeruginosa*) and *Gram positive(Bacillus subtilis*) bacteria.

Keywords: Green synthesis, *Rosa indica*, Silver nanoparticles, Antibacterial activity.

INTRODUCTION

Nowdays, we are witnessing the development and advancement of a new interdisciplinary scientific field nanoscience.[1] As was shown by numerous example in physics, chemistry and biology, a transition from macrosizes to those of 1-100 nm gives size to qualitative change

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in physicochemical properties of individual compounds and system. The dependence of physicochemical properties on the partical size was magnetic properties, thermodynamics, electrochemistry, conductivity and electron transport. Nanochemistry is related with the production and reactions of nanoparticles and their compounds.[2-4] It is concerned with the unique properties associated with assemblies of atom or molecules on a scale between that of the individual building blocks and the bulk material (from 1 to 1000),[5] At this level, quantum effect can be significant, and also new ways of carrying out chemical reation become possible.[6] The science we methodologies from the synthetic chemistry and the material chemistry to obtain nanometerial with specific size, shapes, surface properties. Cross disciplinary nanoscience research involving physicts, chemists and engineers is concerned about the need of developing ecofriendly and sustainable methods for the synethesis of nanomaterials.[7-9]

There are many method for the synthesis of nanopartilces but one of the most conventional method is green synthesis because it is eco-frendily, non-toxic, very less expensive and very pure method. Nanoparticles having unique properties arising from their nanoscale dimensions.[10-12] Nanoparticles has many important properties and various application in many areas such as drug, food, nutrition, electronics etc.[13] The biological synthesis of nanoparticles which is very monodispersied particles with very specific sizes and shapes. The huge application in application in phamacutical company for successful remedical treatment as for disease. Green synthesis nanoparticles have great properties, which is synthesised by every part of plant such as, root, leaf, stem, flower, bark etc.[14] In Synthesis of nanoparticles dried from are used. In Every plant have terpenoids ,alkanoids, Flavanoid, total phenolic content, which is help to synthesied the nanoparticles.[15]

The most researchers studied about nanoparticles. Today those nanoparticles are synthesised which are noble metals like silver ,zincoxide, gold , lead etc. But among the nanoparticles, silver and zincoxide nanopartilces play a excellence role in the field of biological and drug industry. There are two type of approach for the synthesis of nanparticles –[16]

- Top – down approaches
- Bottom – up approaches

The very good effect show bottom – up approach as compared to top - down approaches. The bottom – up approaches show best results to produced the nanoparticles without any defects.[17]

Their many potential for human health.Biosynthesis of silver and zincoxie nanoparticles by plant, bacteria, fungi, and yeast. There are many routes for the synthesis of nanoparicles such as biological, chemical, physical, hydrothermal, electrochemical, irradiative, photochemical method.but green synthesis method has one of the best method for the synthesis of nanoparticles.[18-19]

Rosa indica is a traditional medicinal plants and its belonging family Rosaceae.[20] This plant have showing greatest advantages for stomach problem, and are being investigation for controlling cancer growth, constripation, inflammation, leucorrhea, heart and eye disease. Buds and Petal of *Rosa indica* used for removal of gal bladder and kidney and flower are used against asthma. It is commonly cultivated for fiber and most edible purposes and many used in medicinal purposes. The plant have more medicine value show like antioxidant, blood pressure, anti-dipression, anticancer, diuretic, antifungal, anti-immammatory anti-carcinogenic activity.

MATERIALS

Plant collection

The fresh leaves of *Rosa indica* were collected from Department of Horticulture , SHUATS Allahabad (State University), India.

Chemicals

Silver nitrate was analytical grade. Ultra-purified water used throughout the research work to prepare solution from a Merkmillipore-Milli-Q water purification System. All glassware have been washed with sterile double distilled water and dried in an oven before use.

METHODS

Preparation of plant extract[21]

*Rosa indica*leaves were washed thoroughly under running deionised water and then rinsed thoroughly with ultra-purified water and dried at room temperature. The leaves are grinded to make a fine powder and used for experimental studies. The solution of leaf broth was prepared by taking 15 g leaves powder in a 150 ml ulta-purified water (deionized water) and then boiled 30 min. at the temperature 60 ^{0}C. Then the leafs broth was cooled at room temperature then the leaf broth were filtrered using whatman filter paper before centrifuged at 3000 rpm for 10 min. for remove heavy metal and then leaf broth kept in refrigerators at 4 ^{0}C for further experimental use.

Phytochemical analysis[22-23]

Phytochemical analysis of leafs extracts of *Rosa indica*ananlysis by the spectra of UV-Vis spectrophotometer and FTIR .

Green synthesis of silver nanoparticles[21]

5 ml of leaf broth was added drop by drop to 45 ml of 1mM aqueous silver nitrate solution using magnetic stirrer at room temperature, the solution become brown in color. Then the solution was centrifuged 5000 rpm for 15 min. Then it show formation of silver nanoparticles.

Characterization of silver nanoparticles UV-Vis Spectrophtometer

UV-Vis spectroscopic studies were carried out on a Shimadzu UV-3600 Plus spectrophotometer at MNNIT Allahabad.

Dynamic light scatterring

The average Particle size of the synthesized silver nanoparticles was evaluated with the help of a Nano Microtrac total solution in particles at MNNIT Allahabad.

X-Ray Diffraction

Silver nanoparticles was examined by X-Ray diffraction analysis using Rigaku Smart Lab. X-ray diffractometer with Cu Kα radiation monochromatic filter in the range of 10-90^0 at MNNIT Allahabad Fourier transform- infra red FTIR spectra of silver nanoparticles was recorded by Perkin Elmer Spectrum Version 10.4.00.at MNIT Jaipur.

Scanning electron microscopy with EDX

SEM study was carried out to investigation the shape, size, and the surface area of the silver nanoparticles by ZEISS at IIT KANPUR.

Anti-bacterial assay[24]

Anti-bacterial activities were studied against *Bacillus subtilis* (MTCC 121) and *Pseudomonas aeruginosa (*MTCC 1688) strain. The antibacterial activity of Green synthesized of silver nanoparticles was testedagainst variou spositiove and negative bacteria by the agar well diffusion method. To examine the antibacterial activity of green synthesied AgNPs Muller- Hinton agar plates were sterilized and allowed to solidify. After solidification, 10µl of each bacterial suspension was inoculated on the petriplates by a sterile glass rod. Then, using sterile borer pounched four well into agar plates. Then we take three concentration, 25 µl, 50 µl, 75 µl of silver nanoparticles was poured into well by the using of micropittete. Then we take a control (Amoxicillin) and the concentration of control is 25 µl, which is poured in one well of the agar plates. After that incubated for 24 h and at 37 ^{0}C,The were observed around the discs. Antibacterial activity was investigation by measuring the diameter of the zones of inhibition .

Statistical analysis

Two way of analysis variance (ANOVA) using in the result of antibacterial activity.

RESULT AND DISCUSSION

The first confirmation of green synthesis of silver nanoparticles by observation, During the visual observation, when addition of leaves extract of *Rosa indica to* 1mM solution of silver nitrate then the color of the solution changed from pale yellow to brown. This color change indicate the formation of silver nanoparticles.[27] In this reation show the reduction of silver ions to silver nanoparticles. The solution of color arises due to the excitation of surface plasma vibration in silver nanoparticles (Figure.1).

Phytochemical ananlysis of leafs extracts-

The phytochemical ananlysis of leaves extract was examined spectroscopically. Phytochemical analysis of leafs broths was confirm by the instruments of UV-Vis Spectroscopy and

FTIR. It is given confirmation that total phenolic group, antioxidant content, flavonoids, amines etc. formed in the plant extracts, these compounds confirm by IR band Which is used to as capping agent for the reduction of silver ion to silver nanoparticels.showen in Fig. 2 (A) and 3(B) .The UV-Vis spectroscopy confirm the presence of polyphenol in theleafs extracts. The absorbance of leafs extracts presented peak at 270 nm as characteristic of polyphenol molecules. This result also in according of the reported literature.[22] In FTIR Showing different functional groups in leaves extracts, that confirm the polyphenol groups present in plant extrats. Also this result are according of reported literatue.[23]

Fig. 1. Color Change indicating show formation of silver nanoparticles (A)*Rosa indica* leaves extracts (B) 1mMSolution of silver nitrate (C) and (D) show change the color from pale yellow to brown

UV-Vis Spectrophotometer

After observation, then the Green synthesisedof silver nanoparticles was first characterized from UV-Vis spectrophotometer. At the different time interval, the complete the reaction of green synthesied of silver nanoparticles the maximum absorbance peak are observe at 425 nm. In the green synthesied silver nanoparticles showing

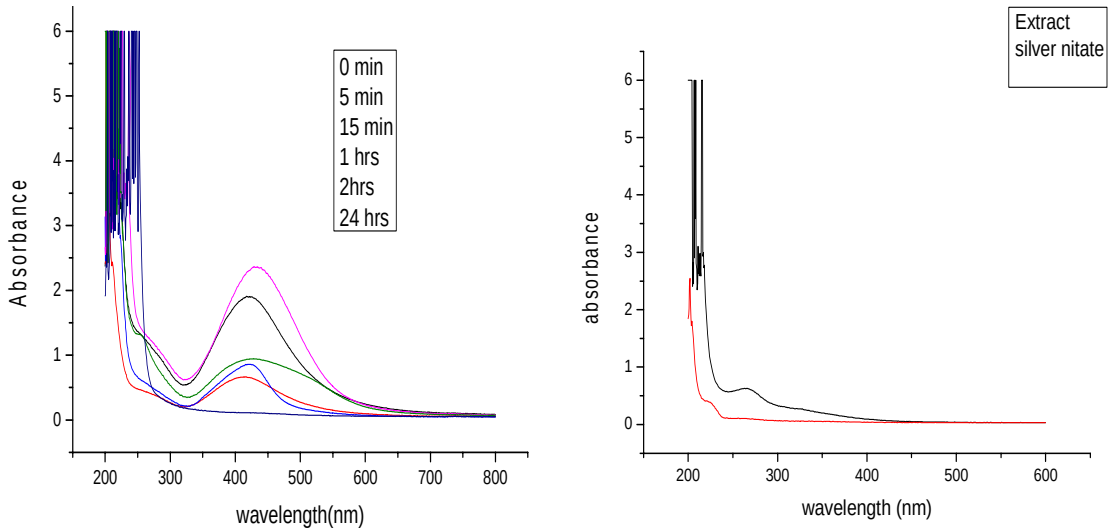
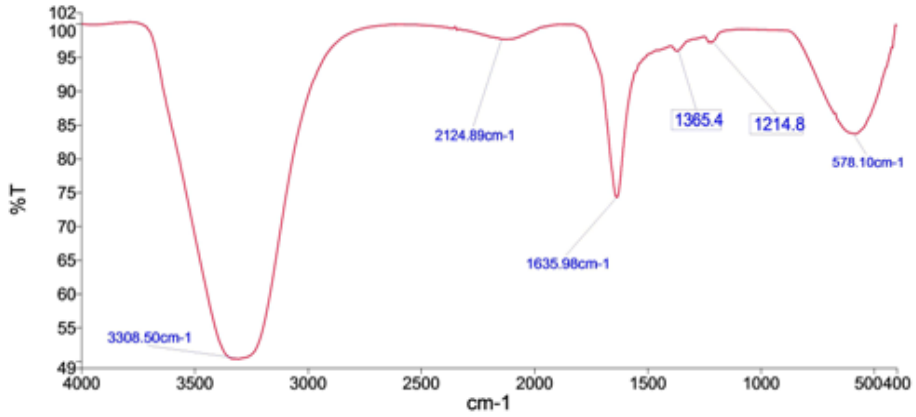


Fig. 2. A) UV-vis spectrophotometer showing peaks of leaves extracs and silver nitrate, B) Confirmation of silver nanoparticles at different time interval.

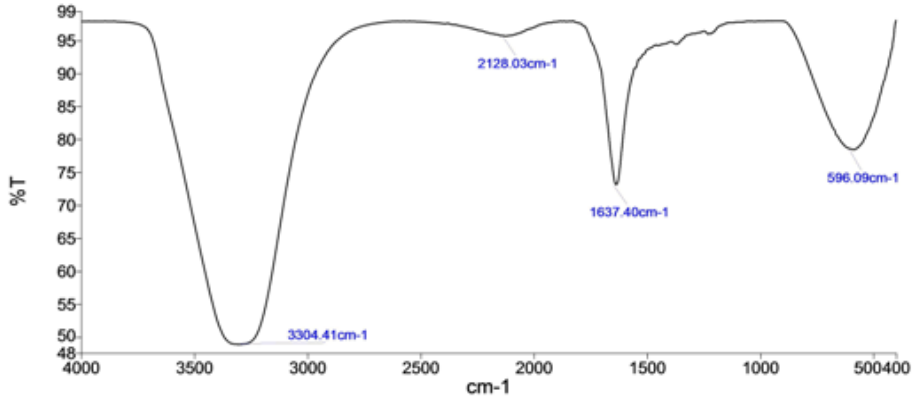
strong surface plasma resonace band at 420 nm using *Rosa indica* leaves extrats.[25] UV-Vis absorbance spectrophotometer is an most important chacterization for the showing the stability and formation of silver nanoparticles. In Fig B showing complete conversion of silver ion to silver nanoparticles.

Fourier transform- infra red –

FTIR is the one the most important characterization technique for the detecting the functional group in plant extract and silver nanoparticles,[26-30] which is indicating to confirmation of silver nanoparticles. For, the reduction of silver



(a)



(b)

Fig. 3. FTIR Spectra A) Silver nanoparticles B) leaves extracts

nanoparticles the functional group of leaves extract is responsible for the reduction of silver ions.

The peak intensity of the spectrum showing different region of functional group in leaves extract and silver nanoparticles are analysed and showing in Figure. 3.

In FTIR spectra of silver nanoparticles and leaves extracts having more similarties with some difference between of position of peak.[31-32] The most intense and broad peak was come in spectra at 3304 cm^{-1} corresponds to OH stretching vibrations of phenol and – COOH gorup present in leaves extracts. The peak located at 1635 cm^{-1} it was showing and indicating C=O sterching and amide binding. At 2124 cm^{-1} peak obsevedby the alkanes present due to stretching . The nitro N-O bending is assigned the peak at 1365 cm^{-1} and at 1214 cm^{-1}

peak showing to C-O-C streching aromatic ring. At 578 cm^{-1} peak inicating to allkyl halides band for C-Cl. In FTIR spectra showing various gourp like carboxyl, carbonyl, amide, alkyl halide, phenol group of *Rosa indica* leaves extracts are used for the Green synthesis of silver nanoparticles. All goup are use as capping and stabilization agent in reduction of silver ion to silver nanoparticles .

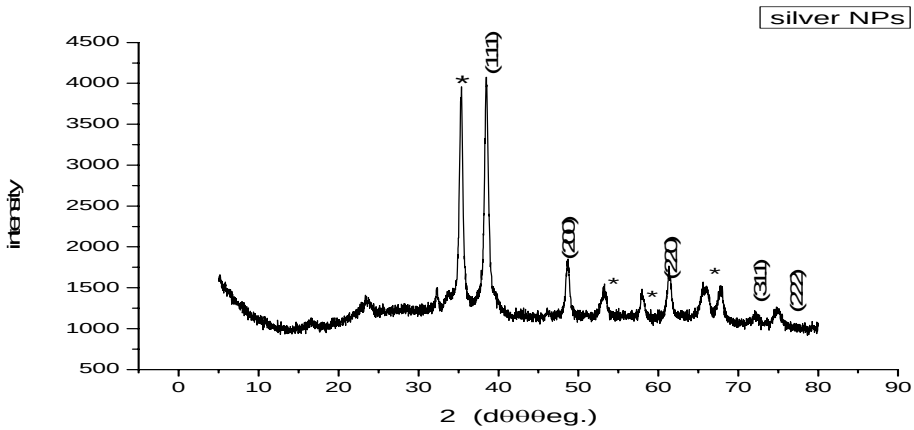


Fig. 4. X- Ray diffraction analysis of green synthesied nanoparticles

X- Ray diffraction (XRD)

The X-Ray diffraction characterization of sample was carried out to confirmation of green synthesied silver nanoparticles.

The five stong Bragg reflections formed at 2 theta value of 38.21°, 44.11°, 64.19°, 77.56°, 81.67° corresponding to (111), (200), (220), (311), (222). Silver nanoparticles indicating strong, narrow and crystalline nature.[33-34] The size of silver nanoparticles was formed by Debye - Scherrer formula given by the equation-

$D=K\lambda/(\beta \cos \theta)$

Where-

D- the crystal size

λ- the wavelength of the X-ray radition (λ= 0.15406 nm) for CuKa,

K- usually taken as 0.89

B- the line width at half- maximum height

In XRD analyisis showing structural peaks and approximately size of crystalline of nanoparticles around 45-85 nm. the green

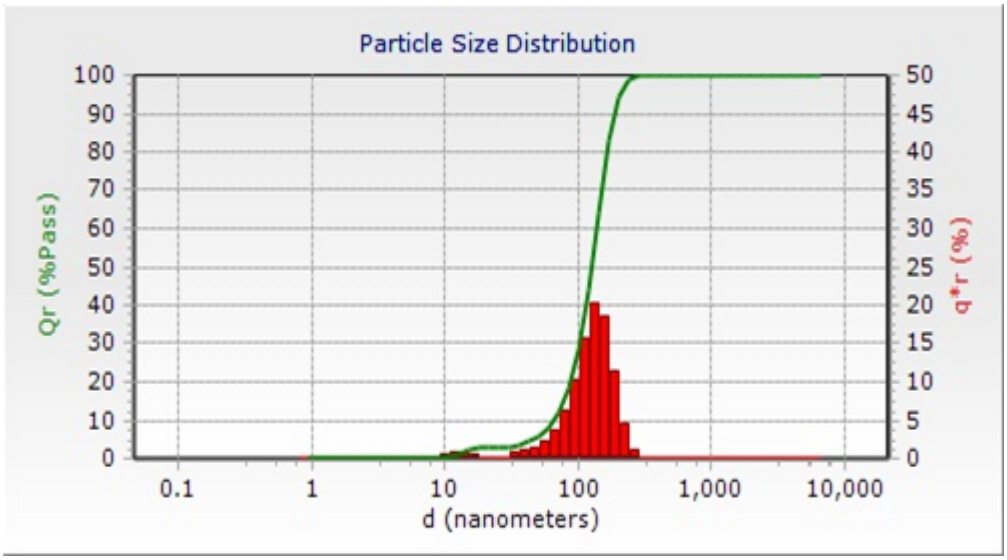


Fig. 5. Showing particles size distrubutionofgreensynthesied nanoparticles

synthesied of silver nanoparticles show nano crystalline in nature.[35] In XRD spectra show some extra peak, which is showed by stars, this peaks observed that the crystallization of bioorganic phase formed on the surface of Ag NPs. similar result was reported in silver nanoparticles using edible mushroom extract.[38]

Dynamic Light Scattering (DLS)

In dynamic light scattering used suspension of silver nanoparticles green synthesis from leaf exract from *Rosa indica*. The particle size distrubution though the DLS indicate the size of green synthesied nanoparticles between around 10 to 127 nm. In this Fig V the very small amount of green syntheised silver nanoparticles are agglomerated showing in Figure.5.

Scanning electron microsopy with EDX

The solution of green synthesied of silver nanoparticles was formed by centrifuged at 15000 rpm at 20 min. then the suspension and pellet are seperated the pellets again redispersed in ultrafine deionised water. The highly pure pellet was freeze then dried, then used for analysis. The surface morphology of silver nanoparticles was ananlysis using scanning electron microscopy with EDX. This figure show the confirmation of silver nanoparticles, which is synthesied by leafs extracts. The green synthesied silver nanoparticles were small amount

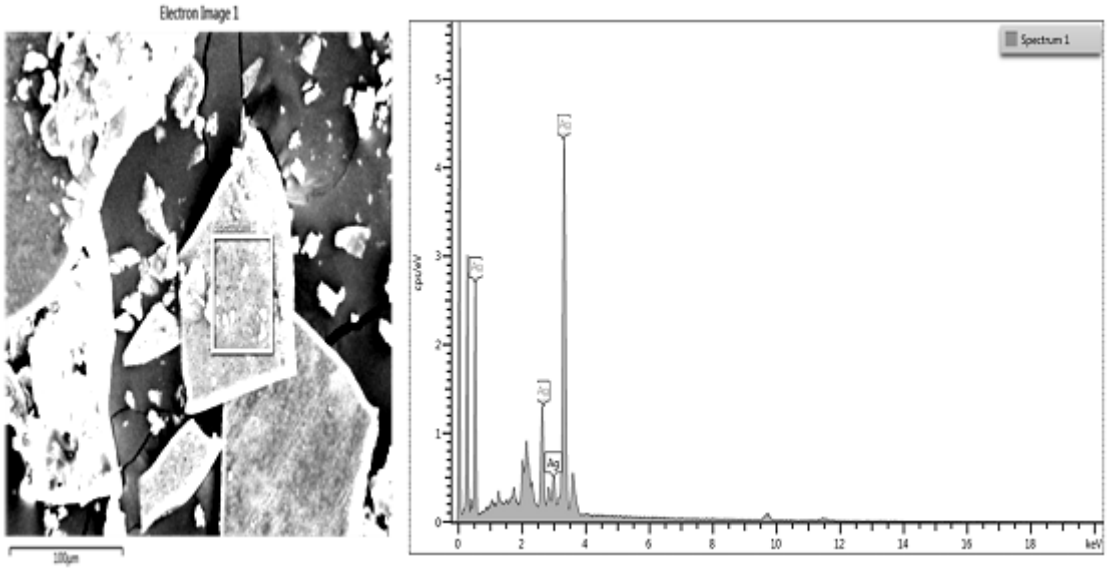


Fig. 6. Scanning electron microscopy with EDX

agglomerated. The green synthesied of silver nanoparticles are spherical in shape.[36] silver nanoparticles was futher confirmation by EDX. In EDX Fig showing strong peak which is indicating confirmation of AgNPs.

Antimicrobial activity

Antimicrobial acitivity was done by well diffusion method. The antimicrobial activity of green synthesied AgNPs was accomplished against *Gram positive* and *Gram negaitive* bacteria. The green synthesied Silver nanoparticles having excellent antimicrobial activity against *Gram positive* and *Gram negaitive* bacteria. In this paper, the *Gram positive* bacteria indicating the higestzone of inhibition and most effective as compared to *Gram negaitive* bacteria. The higest zone of inhibition was observed for 15mm in *B.subtilis* and 14.5 in *P. aeruginosa* at the higest concentration 7.5 µg/µl. According to Fig 25 µl concentration of amoxicillin showed mamxium radius of zone of inhibition of 21 against *B.subtilis* and 22.5 against *P.aeruginosa*. Silver nanoparticles have show most efficiency to inhibit the bacteria growth by the formation of

P.aeruginosa ***B. subtilis***

Fig. 7. Antibacterial activity of Silver nanoparticles synthesied against *P. aeruginosa* and *B. subtilis*

Table. 1: Zone of inhibition (mm) obtained from well diffusion methods

Concentrations µg/µl	Zone of inhibition (mm)	
	P.aeruginosa	*B.subtilis*
25	7.9	8
5	13.9	14.5
75	14.9	15

Disc's diameter- 5 mm

ANOVA :

Source	d. f.	S.S.	M.S.S.	F. Cal.	F. Tab. 5%	Result	S. Ed. (±)	C.D. at 5%
Due to zone inhibition	1	0.107	0.107	2.56	18.51	NS	0.167	0.344
Due to Concentration	2	59.083	29.542	709	19.00	S	0.204	0.421
Error	2	0.083	0.042	-	-	-	-	-
TOTAL	5		-	-	-	-	-	-

reactive oxygen species and accumulation of nanoparticles. Silver nanoparticles act as bactericidal and bacteriostatic agents.[37] In this result, using the statistical analysis by the annova software.

CONCLUSION

In this paper we study the green synthesied of silver nanoparticles from leaves of *Rosa indica.* In this rection leafs extracts used as capping agent for the stability of nanoparticles. Silver nanoparicles having sheperical shape and size of 1-100 nm. Its show excellent antibacterial activity against *Gram positive* and *Gram neagaitive* bacteria. We also agree to the methods of green synthesis of silver nanoparticles by UV-Vis, XRD, DLS, FTIR, SEM, EDX. This is eco-friendly, non-toxic, very conventional method.

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