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Synthesis and Characterization of Silver Nanoparticles using *Ricinuscommunis* Plant and Study of their Biological Activity

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***Abstract*:-- The main aim of this study is to evaluate the Green synthesis of silver nanoparticles synthesized from aqueous plant extract of Ricinuscommunis and study its Antimicrobial activity. The synthesized silver nanoparticles were characterized by UV-VIS spectroscopy, Fourier Transform Infra-Red spectroscopy, Scanning Electron Microscopy and X-ray diffraction. The antimicrobial activity of synthesized silver nanoparticles was compared with their respective plant extract by agar well diffusion method was calculated. The zone of inhibition varied in range of 12 to17 mm with 100 µg/ml silver nanoparticles concentration. The antimicrobial activity of synthesized silver nanoparticles was higher than that of the standard drug i.e. streptomycin (for bacteria). The synthesized nanoparticles of Ricinuscommunis have shown good antimicrobial efficacy as compared to plant extract and may prove to be better antimicrobial agent against wide range of microbes.**

***Index Terms*:— Ricinuscommunis; Silver nanoparticles; Plant extract; Antimicrobial**

I. INTRODUCTION

Nanotechnology is one of the most active research in material science and its capability of modulating metals into their nano size, which drastically changes the optical , physical and chemical properties of metals. Nanoparticles are the small particles its size range of 1-100 nm. Nanoparticles are fundamental buildings blocks of nanotechnology. The most important and distinct property of nanoparticles have found uses in many applications in different field , such as catalysis , photonics and electronics. Silver nanoparticles as an arch product from the field of nanotechnology, because of its distinctive properties, those are good conductivity , catalytic, antifungal, chemical stability, antiviral, antibacterial activity, and anti-inflammatory,…etc[1]. Nobel metal nanoparticles such as silver(Ag), platinum(Pt), gold(Au) and palladium(Pd) is an emerging field of research due to their important applications in the fields of metal-based consumer products , textile engineering, water treatment, biotechnology, bioengineering and other areas, optoelectronics, magnetic, electronic, information storage and Medical [2]. In medical industry such as topical ointments to prevent infection against burn and open wounds [3]. The antibacterial activities of silver nanoaparticles are related to their size , with the smaller particles having higher activities on the basis of equivalent silver mass content.

The content of this work is collection and identification of plant species, isolation and identification of silver nanoparticles and their characterization by UV-Visible spectroscopy, Fourier Transform Infra-Red spectroscopy, X-ray diffraction, Scanning Electron microscopy and to study its Biological activities. Castor oil has many uses in medicine and other applications. An alcoholic extract of the leaf was shown, in lab rats, to protect the liver from damage from certain poisons.[4][5][6]Methanolic extracts of the leaves of Ricinuscommunis were used in antimicrobial testing against eight pathogenic bacteria in rats and showed antimicrobial properties. The extract was not toxic.[7] The pericarp of Ricinus showed central nervous system effects in mice at low doses. At high doses mice quickly died.[8] A water extract of the root bark showed analgesic activity in rats.[8] Antihistamine and anti-inflammatory properties were found in ethanolic extract of Ricinuscommunis root bark.[9]

II. MATERIALS AND METHODS

Plant Materials:

Ricinuscommunis, the castor bean[10] or castor-oil-plant,[11]is a species of flowering plant in the spurge family, Euphorbiaceae. It is the sole species in the monotypicgenus, Ricinus, and subtribe, Ricininae. The evolution of castor and its relation to other species are currently being studied using modern genetic tools.[12]It

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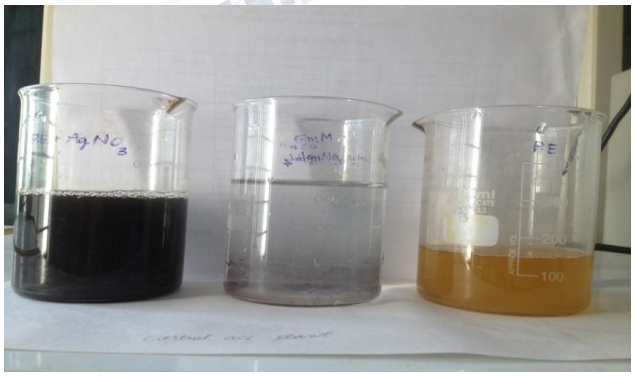
reproduces with a mixed pollination system which favor selfing by geitonogamy but at the same time can be an out-crosser by anemophily or entomophily.Its seed is the castor bean, which, despite its name, is not a true bean. Castor is indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions (and widely grown elsewhere as an ornamental plant).[13]

- Scientific classification
- Kingdom: Plantae
- Order: Malpighiales
- Family: Euphorbiaceae
- Sub-family: Acalyphoideae
- Tribe: Acalypheae
- Sub-tribe: Ricininae
- Genus: Ricinus
- Species: R.communis
- Binomial name: Ricinus communis
- Collection and preparation of plantmaterials:

Fresh leaves of Ricinuscommunisplant free from diseases were collected from S.I.E.T college campus, tumkur, Karnataka and then washed thoroughly 2-3 times with tap water and once with sterile water. 20 gm of fresh leaves was finally chopped and added to 100 ml of distilled water and stirred at 600 C for 1 h. After boiling, the mixture was cooled and filtered with Whatman filter paper number 1. Filtrate was collected [14]

Synthesis of Silver Nanoparticles [15]. 5mM aqueous solution of silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 5ml of leaf extract of Ricinuscommuniswas added to 45 ml of 5mM AgNO3 solution for bioreduction process at room temperature.



III. CHARACTERIZATION

UV-Vis spectroscopy: Leaf extract were challenged to 100ppm AgNO3 solution, The mixture were observed visually for an colour change and one mL of reaction mixture were withdrawn periodically for analysis of surface Plasmon resonance of silver nanoparticles using a UV-Vis spectrophotometer (Shimadzu 1601 model, Japan) at the resolution of 1 nm in range of 340 to 900 nm. X-Ray diffraction analysis: The scintillation detector present in the instrument moved with required angle at specific counts and sample was scanned with a straight angle at 100C and stopping angle at 700C. The output was obtained in the form of a graph with 2θ on x-axis and intensity on y-axis. The obtained graph from analysis having peaks corresponding to different planes of the crystal was compared with the standard data in JCPDS card. From the subsequent data obtained, the average size of particle was calculated by Scherer's formula [14].
$D=0.9\lambda/\beta\cos\theta$, where, λis wavelength ,θ is Braggs diffraction angle, β is full width at half maximum of peak and D is average particle size.

FT-IR analysis:FT-IR analyses were performed using Shimadzu FT- IR model number 8400. Approximately three mg of lyophilized flower extract under study was mixed with 300 mg of dried KBr, crushed well in mortar and pestle to prepare thin pallet for analysis, Same procedure was performed for synthesized Ag NPs using flowers extract, 16 scans per sample were taken in range of 400-4000 cm-1[16]. Scanning electron microscopy (SEM) : A drop of aqueous solution containing purified silver nanomaterials obtained after repetitive centrifugation was placed on the carbon coated copper grids and dried under infrared lamp for characterization of their morphology using FEI Quanta 200 Scanning electron microscope at accelerating voltage of 20 KeV.

Assay for antimicrobial activity of silver nanoparticles :The silver nanoparticles is deionized water were tested for their antibacterial activity by the agar diffusion method. Pseudomonas aeroginosa, Shigellaflexineria,KlebsiellapneumoniaeSalmonella typhi, Enterobactorand Vibrio choleraewere used for this analysis . These bacteria were grown on nutrient broth (NB) media for 24 hours prior to the experiment, seeded

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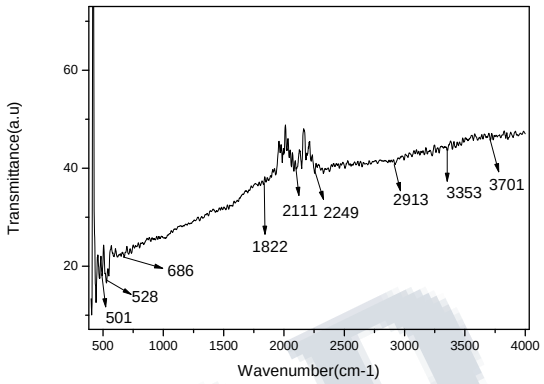
in agar plates by the pour plate technique. Phytochemical analysis: Test was performed for the detection of alkaloids, flavonoids, cardenolides, saponins, phenols,tannins, anthraquinones, cardiac glycosides, phlobatannins, terpenoids.[17,18]

IV. RESULTS AND DISCUSSION

A reduction of Ag NPs was clearly observed when Ricinuscommunis leaf extract was added with AgNO3 solution within 20 min. The colorless solution was changed to brown color which indicates the formation of silver nanoparticles. UV-Vis-spectroscopy and Fourier transform-infrared spectroscopy analysis

The mixture of leaf extract and AgNO3 solution was subjected to ultraviolet-visible (UV-vis) spectroscopy analysis in the recorded spectra, and showed a observable peak at 340 nm which corresponds to the wavelength of the surface Plasmon of Ag NPs (Figure 1). Various reports have established that the resonance peak of silver nanoparticles appears around this region [15]. Fourier transform-Infrared (FT-IR) analysis was performed to identify the possible biomolecules responsible for the reduction of the Ag+ ions and capping of the reduced Ag NPs synthesized using Ricinuscommunis leaf extract, The strong IR bonds were observed at 3,702, 3354, 2,913, 2249, 2111, 1823, 688, 528, and 500 cm-1. The bands which appeared at 3,702 cm-1 corresponding to N-H, 3354and 2913 cm-1 –OH stretching and alkyl –C-H stretching, respectively [19]. The bands at 2249 and 2111 cm-1 are due to the alkyl C=C and alkyne C≡C stretching, respectively. The IR band observed at 1823 cm-1 may be ascribed to carbonyl –C═O stretching mode, respectively. The low band at 688 cm-1 corresponds to alkyl halide C-Cl stretching. The two new strong bands recorded at 528 and 500 cm-1 in the spectra of the synthesized material were assigned to Alkyl halides of C-Br and C-I bending peaks may be raised due to the reduction of AgNO3 to Ag nanoparticles (Figure 2).

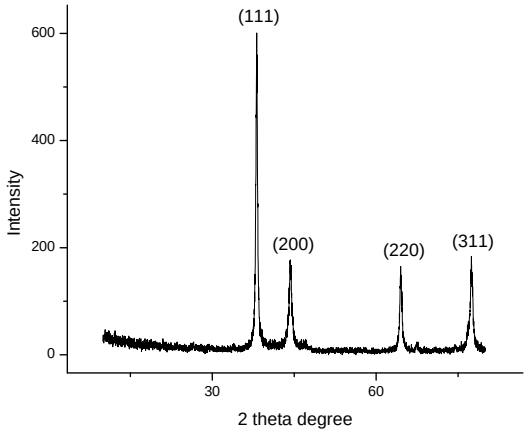
(Figure 1)UV-vis spectrum of Ag NPs synthesized by Ricinuscommunis leaf extract.



(Figure 2)IR spectra of Ag NPs synthesized using Ricinuscommunis leaf extract.

X-ray diffraction

X-ray diffraction pattern (XRD) was recorded for the synthesized Ag NPs (Figure 3). Three distinct diffraction peaks at 38^0, 44^0, and 64^0 were indexed with the planes (111), (200), and (220) for the face-centered cubic silver as per the JCPDS card no. 89-3722.The well resolved and intense XRD pattern clearly showed that the Ag NPs formed by the reduction of Ag^+ ions using *Ricinuscommunis* leaf extract are crystalline in nature. Similar results were reported for Ag NPs in the literature [20]. The low intense peak at 77^0belongs to (311) plane. Average particle size (D) of synthesized NPs is found to be 27-29 nm using Scherer's formula.



(Figure 3) XRD pattern of synthesized silver nanoparticles

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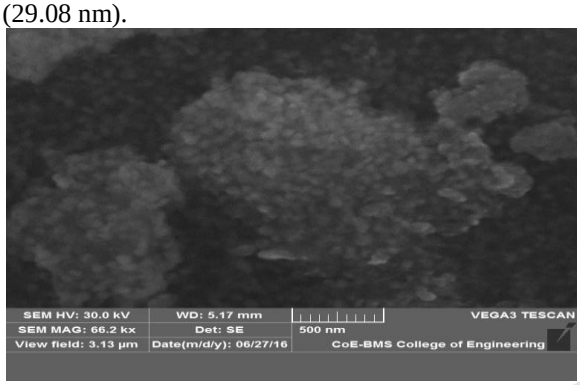
Scanning electon microscopy (SEM) analysis:

The scanning electron microscopy (SEM) image (Figure 4) further ascertains that the silver nanoparticles are pre-dominantly spherical in morphology.The average size of synthesized AgNPs is obtained in SEM analysis (29.08 nm).



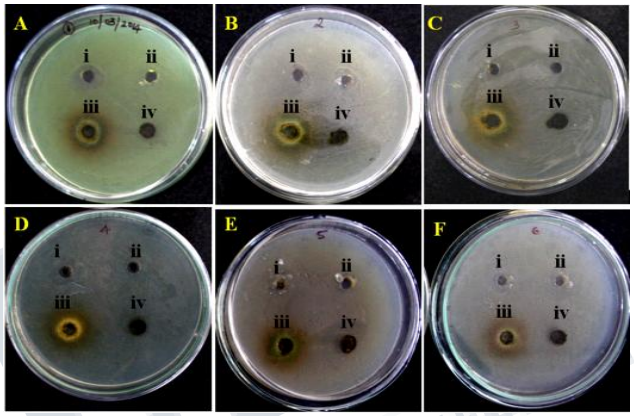
(Figure 4) SEM image of Ag NPs using leaf extract of Ricinuscommunis.

Antibacterial Assay:

antibacterial assay was performed against bacterial pathogens like nosocomial pathogens such as *Pseudomonas aeroginosa*, *Shigellaflexineria*,*KlebsiellapneumoniaeSalmonella typhi*, *Enterobactor*and *Vibrio cholerae*by standard disc diffusion method, briefly agar medium was used to cultivate bacteria .Fresh overnight culture of inoculum (0.1 ml) of each culture was spread on to Muellar Hinton Agar (MHA) plates. The plates were incubated at 37^0C overnight. Next day the inhibition zones around the discs were measured. . For *Ricinuscommunis* the Zone of inhibition was found to be mm for *Pseudomonas aeroginosa*,*Shigellaflexineria*,*KlebsiellapneumoniaeSalmonella typhi*, *Enterobactor*and *Vibrio cholerae*whereas the study done by the Zone of inhibition found was 12-17mm for these specie[21].

Table 1: Result for antibacterial activity by disc diffusion method

Plate	Bacterial Species	Aqueous extract (mm)	AgNPs (mm)
A	*Pseudomonas aeroginosa*	NS*	16
B	*Shigellaflexineria*	NS*	13
C	*Klebsiellapneumoniae*	NS*	17
D	*Salmonella typhi*	NS*	16
E	*Enterobactor*	NS*	14
F	*Vibrio cholera e*	NS*	16



(Figure 5)Zone of inhibition of A) Pseudomonas aeroginosa, B) Shigellaflexineria, C)Klebsiellapneumoniae D) Salmonella typhi E) EnterobactorF) Vibrio cholera for i)Standard ii)Control iii)AgNPs iv)Aqueous extract.

V. PHYTOCHEMICAL ANALYSIS:

Qualitative phytochemical analysis:

The ethyl alcohol as well as petroleum ether extracts of *Ricinuscommunis* were evaluated for qualitative and quantitative determination of major phyto constituents i.e. tannins, cardenolides, terpinoids, flavonoids, cardiac glycosides, saponins and phenols.The results of phytochemical analysis of *Ricinuscommunis* plant are presented in table 2, and some photographs of phytochemical analysis are shown in fig 1,2,3,4,5,6,7,8 and 9 . Both extracts yielded the presence of flavonoids, saponins, cardiac glycosides tarpinoids .including these phytochemicals ethyl alcohol extract shown the presence of phenols, antraquinones alkaloids and cardinoloids.

Table 2. Results of phytochemical analysis of Ricinuscommunis(leaf)

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Sl. No	Phytochemicals	Solvents used for extraction	
		Petroleum ether	Ethanol
1	Flavonoids	+++	+++
2	Saponins	+	+
3	Phenols	-	+
4	Tannins	+++	-
5	Anthraquinones	-	++
6	Cardiac glycosides	+++	+++
7	Terpenoids	++	++
8	Cardenolides	-	++
9	Alkaloids	-	++

Repeated the experiment two times for each replicates. -: absent, +:indicates, ++: presence, +++:confirms

Fig 1: Test For Tannins

Fig 2: Test For Flavonoids

Fig 3: Test For Alkaloids

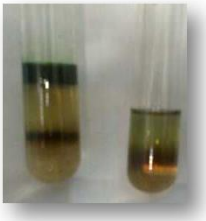

Fig 4: Test For Cardiac Glycosides

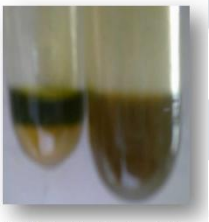

Fig 5: Test For Cardenoloids

Quantitative phytochemical analysis:

- Flavonoids: 1.794g in 10g of dry sample.
- Alkaloids: 0.308g in 5g of dry sample.
- Phenol: 162.5mg/L.

VI. CONCLUSION

The present work indicates the green-synthesized AgNPs using Ricinuscommunis leaf extract was done and confirmed by UV-Visible spectrometer, FT-IR ,SEM and XRD techniques. The average size of synthesized silver nanoparticles is found to be 27-29nm using XRD data by Scherrer's formula, which is approximately similar as the size obtained in SEM analysis (29.08nm). The antimicrobial activity depends upon the concentration of Ag NPs to produce the most significant effects against the gram-positive and gram-negative bacteria. The phytochemical analysis shows that the more number of phytochemicals are present in the Ricinuscommunis leaf extract.This green-synthesized method is rapid, facile, convenient, less time consuming, environmentally safe, and can be applied in variety of existing applications. This plant leaf extract compounds can be extended to the synthesis of the other metal and non- metal oxide nanoparticles.

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