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Synthesis of AgNPs of Cuminum cyminum seed extract, Characterization and Antimicrobial Activity

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Abstract:
The Green synthesis was carried out using the aqueous solution of Cuminum cyminum (Cumin seed) spice extract and $AgNO_3$. A fixed ratio of spice extract to metal ion was prepared and the formation of silver nanoparticle was confirmed by observing the colour changes from colorless to dark brown colour, which proved the formation of nanoparticles. An intense peak was obtained in the UV-Vis spectrophotometer at 404 nm. The possible functional groups like alcohols and alkenes present in the cumin extract are responsible for capping and reducing of silver nanoparticles, were identified by FTIR analysis. From the XRD data, the crystalline nature of the biosynthesized silver nanoparticles is confirmed and the average particle size is found to be 13 nm. Finally, the antimicrobial activity of biosynthesized AgNPs shows a promising zone of inhibition, over the E.coli bacteria.

I. INTRODUCTION

The field of nanotechnology is the most active areas of research in modern materials science and technology. It provides the ability to create materials, devices and systems with fundamentally new functions and properties. Metals in nanometer size will exhibit special properties that differ from bulk metals. Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes [1]. An array of physical, chemical and biological methods has been used to synthesize nanomaterial. Specific methodologies have been used to synthesize noble metal nanoparticles of particular size and shape which are previously done in plant material such as **Cardamom [2], Azadirachta indica [3]**, **Nigella sativa [4]**, **Myristica fragrans [5], Fenugreek seed [6]**, **Syzygium aromaticum [7]**, **Lemon [8], Piper nigrum [9]** etc. Spices provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. The project work has been reported the synthesis of silver nanoparticles using the ***Cuminum cyminum*** (cumin seed) spice extract. Due to the presence of aromatic flavor compounds in the cumin seed were likely to be responsible for the reduction of silver ions to silver Nanoparticle [10]. The present work reports the synthesis of silver nanoparticle by using the spice extract of Cuminum cyminum (cumin seed). Further, silver Nanoparticle were characterized by UV-vis spectroscopy, XRD, FTIR. Finally, the antimicrobial activity of phytosynthesized AgNPs against bacteria was observed.

II. MATERIALS AND EXPERIMENTAL METHODS

Preparation of Seed Broth

About 5 g of fresh cumin seed was added in 50 ml of distilled water and it was boiled at 60°C for 15 minutes. The extract was filtered through the filter paper and it was stored at 4°C. The cumin extract was used for the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0).

Preparation of Precursor from $AgNO_3$

For the preparation of precursor, 3.34mg of $AgNO_3$ salt was suspended in 30ml of distilled water, to obtain 1mM of $AgNO_3$. The solution was allowed to stir in a magnetic stirrer for 15 min. Thus, an aqueous solution of 1mM of silver nitrate was prepared; this solution was used for the synthesis of silver nanoparticles.

Synthesis of Silver Nanoparticles

For the synthesis of silver nanoparticles, Cuminum cyminum seed extract was added to $AgNO_3$ aqueous solution in the ratio of 1:1. The mixture was stirred in a magnetic stirrer and boiled at 80°C for an hour. After an hour of reaction, the colour of the reaction mixture changes to dark brown colour indicating the reduction of Ag^+ ions to AgNPs (**Fig** 1). Finally, the solution mixture was centrifuged at 5,000 rpm for 20 min and the resulting substrate was collected and the pellets are dried in a hot air oven at 65ºC for 20 min.

Figure.1. Reduction of Silver ions to AgNPs

CHARACTERISATION OF SILVER NANOPARTICLES

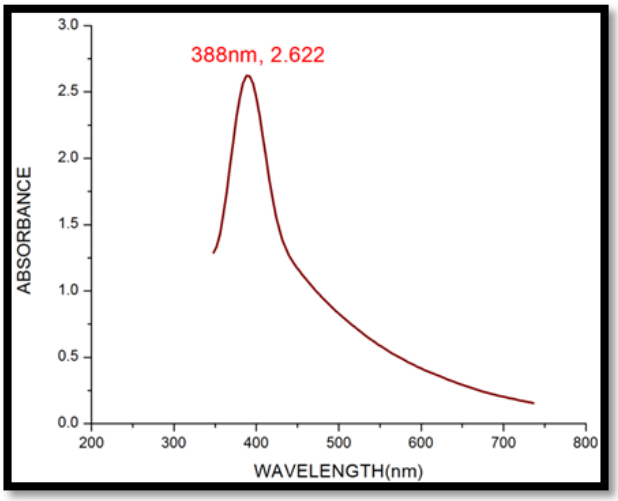
The formation and the stability of metal nanoparticle in aqueous solution are monitored by UV-Vis Spectroscopy [11]. The spectrum was taken for the reaction mixture immediately when it is prepared. The stability, formation and the shape of the synthesized silver nanoparticles was compared. The chemical

composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer. FTIR is taken for the cumin seed extract and also for the silver nanoparticles prepared from cumin extract, to identify the functional groups present in the cumin extract, that are responsible for reduction of silver nanoparticles [12]. The dried silver nanoparticles synthesized from Cuminum cyminum extract are characterized by X-Ray diffractometer; the XRD pattern proposes the crystalline nature of the metal nanoparticle and also the crystallite nature of the biosynthesized nanoparticles can be determined from Debye Scherrer's equation [13].

III. RESULTS AND DISCUSSION

UV-Vis Spectrum Analysis

In the present investigation silver nanoparticles are synthesized with the trial of two different concentrations of cumin extract. Concentration A: 5g of cumin seed in 50ml of distilled water. Concentration B: 1g of cumin seed in 50ml of distilled water Silver nanoparticles are prepared from 1mM aqueous $AgNO_3$ and cumin extract of 1:1 ratio. The absorption peak of AgNPs from A was obtained at 388 nm and for B at 380 nm (**Fig** 2, 3) which shows the red shifting of peaks, at high concentration. Since the SPR band for silver is 396 to 420 nm, concentration A is taken to be a best for further studies. The absorption peak is found to be narrow and single peaked, shows that the shape of the silver nanoparticles reduced by cumin extract is found to be spherical and the particles are monodispersed. UV spectrum of as prepared silver nanoparticles (from A) exhibits an absorption peak at 404 nm. After two days, UV-Vis spectrum depicts the peak at 424 nm, shows that the reduction of Ag ions by cumin extract was not yet completed. After 6 days, peak at 416 nm reveals the reducing activity of cumin extract. After 10 days, the active reduction of Ag ions was found by depicting a peak at 412 nm. Thus, cumin extract reduces the silver ions to silver nanoparticles within a short period of time and also improves the stability of the silver nanoparticles (**Fig** 4).



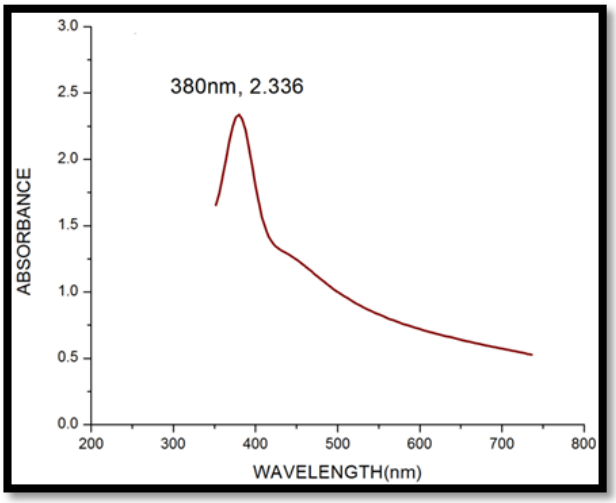


Figure.2. UV-Vis Spectrum for AgNPs in Concentration A Figure.3. UV-Vis Spectrum for AgNPs in Concentration B

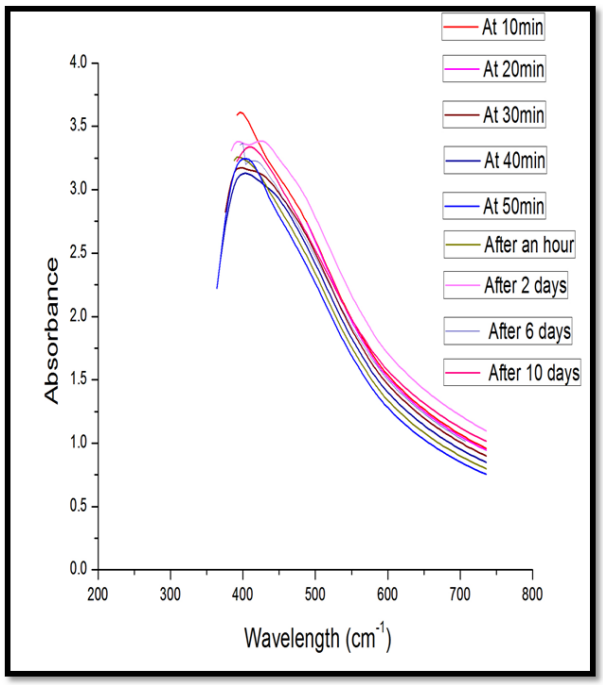


Figure.4. UV-Visible Spectra of the synthesized silver nanoparticles

FT-IR Spectrum Analysis

Results of FT-IR study showed transmittance peaks located at about 3318 cm^{-1}, 2361 cm^{-1} and 1635 cm^{-1} (**Fig** 5). From the spectrum the major peak was assigned at 3318 cm^{-1}, which indicates OH stretching in alcohols and phenols. The alcohols present in the Cuminum cyminum extract is cuminyl alcohol. The FTIR spectrum assigned C = O stretch of Amide I band at 1635cm^{-1}, due to the stretching of C = O in Cuminaldehyde ($C_{10}H_{12}O$) which is a carbonyl group present in the protein of cumin seed. The absorption peak at 1635 cm^{-1} is close native to proteins present in cumin seed gets interacted with the biosynthesized silver nanoparticles.

The carbonyl group of amino acid residue has the capacity to prevent agglomeration, thereby increasing the stability of the reaction mixture [14]. A small peak at 2361 cm^{-1} is assigned for –NH stretch mode was found to be enhanced when compared with the FTIR spectrum of cumin extract. Thus, the overall observation confirms the presence of proteins in cumin extract and phenols act as a reducing and stabilizing agent for silver nanoparticles.

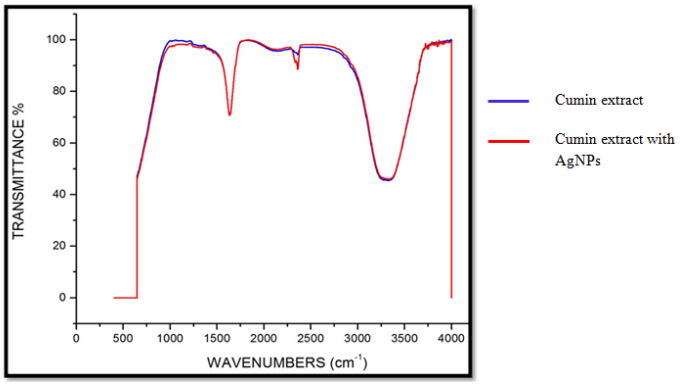


Figure.5. FT-IR spectrum for Cumin seed mediated silver nanoparticles

XRD Analysis

The dry powders of silver nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 10º to 90º at 2θ angles. The XRD peaks for the green synthesized silver nanoparticles were observed at 38º and 46º, which correspond to 111 and 200 Bragg's plane (**Fig** 6). From these Bragg's plane it was concluded that the silver crystallites are **FCC** structure.

The average crystallite size of the prepared samples was determined

using Debye Scherrer's equation $\mathbf{D} \approx \frac{0.9\lambda}{\beta\cos\theta}$ where **D** is the

crystallite size, $\boldsymbol{\lambda}$ is the wavelength of X-ray, $\boldsymbol{\theta}$ is the Braggs angle in radians and $\boldsymbol{\beta}$ is the full width at half maximum of the peak in radians. The average crystallite size estimated was 13 nm. The broadening of the peak resembles the formation of silver nanoparticle.

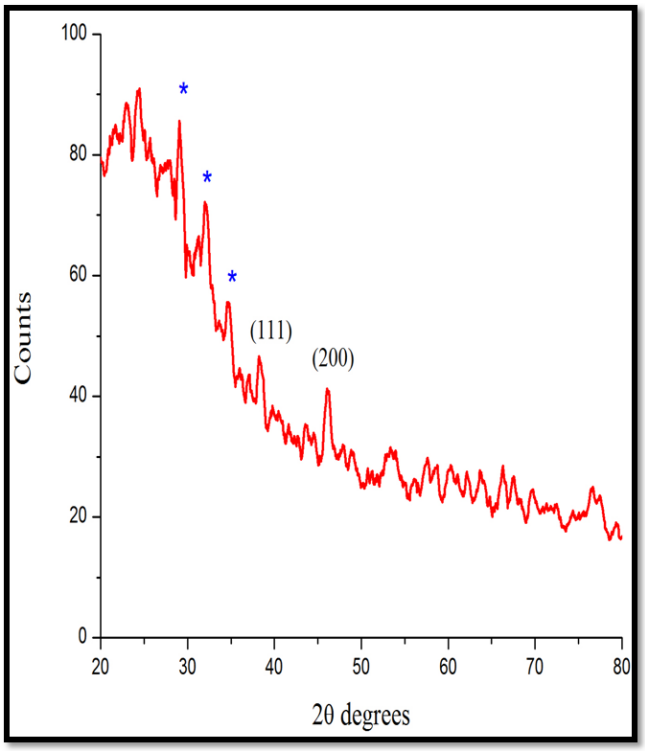


Figure.6. XRD patterns of Ag NPs synthesized using the seed extract Cuminum cyminum

Note: The extra peaks found in the XRD data are due to the presence of various crystalline biological biomolecules in the cumin extract [15]. Strong X-Ray scattering at 32º arises from metalloproteinase in the broth [16].

Antimicrobial Studies The effect of AgNPs of Cuminum cyminum over E.coli bacterium was compared with the standard antibiotic amikacin. The silver nanoparticles reduced by concentration A of C.cyminum giving a zone of inhibition of 8 mm and for concentration B zone of inhibition were not observed (**Fig** 7). The zone of inhibition for the standard antibiotic was 16 mm. Thus, the silver nanoparticles of C.cyminum seeds can significantly inhibit the pathogens when the concentration of C.cyminum extract is high. Antimicrobial activities of the synthesized silver nanoparticles over E.coli bacterium are as follows (**Table** 1)

Table.1. Results of the antibacterial activity of the synthesized silver nanoparticles

S.No	Antibacterial Agents	Inhibition zone diameter (mm)
1	AgNPs of C.cyminium	8
2	Amikacin (antibiotic)	16

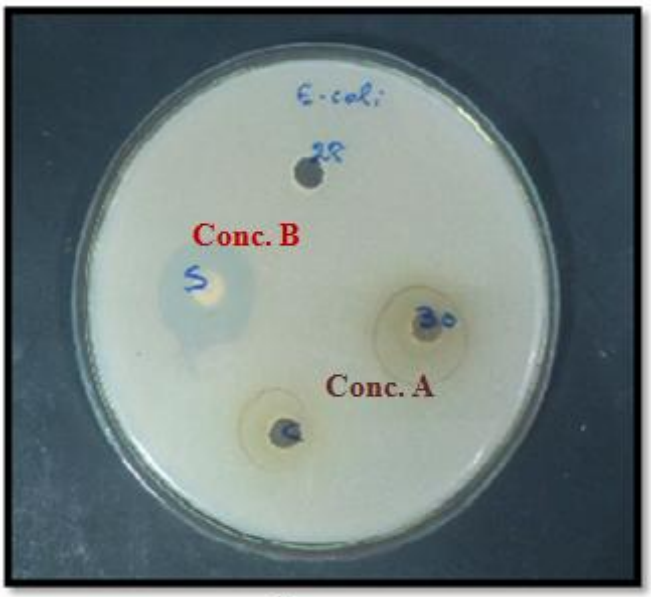


Figure.7. Antibacterial activity of silver nanoparticles of C.cyminum over E.coli

IV. CONCLUSION

Green synthesis of silver nanoparticles using Cuminum cyminum extract was found to be environmentally friendly approach for reducing silver nanoparticles without the involvement of toxic chemicals. The metal ions reduction occurs very rapidly, and the reduction of Ag ions will be completed within an hour. From UV-Vis spectrum, the synthesized silver nanoparticles were found to be monodispersed, with an absorption peak at 404 nm. From FT-IR spectrum, they were found to be stable due to the presence of proteins which act as capping agent and prevent the particle from aggregation. From XRD data, the crystalline nature of the synthesized nanoparticle is confirmed and they show an average crystallite size of about 13 nm. Assessment on the antibacterial effect of nanosized silver colloidal solution against *Escherichia coli* reveals the antimicrobial activity of synthesized silver nanoparticles. Thus, the silver nanoparticles synthesized from the present method have various applications in biomedical field.

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