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ORIGINAL ARTICLE **OPEN ACCESS**

Green synthesis of silver nanoparticles using leaf extract of *Cordia gharaf* along with guar gum

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ABSTRACT
Green synthesis has gained attention as a well-grounded approach to the synthesis of metallic nanoparticles. The present study used the aqueous extract of leaves of Cordia gharaf as a reducing agent to synthesize silver nanoparticles. The prepared nanoparticles were further stabilized with a hydrocolloid guaran, commonly called guar gum,which has a unique structural arrangement of adjacent hydroxyl groups. The synthesized silver nanoparticles were characterized by Ultraviolet-visible (UV-vis) spectrophotometer, Dynamic Light Scattering (DLS), Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The UV-visible spectra revealed the formation of silver nanoparticles with an absorption maximaat 410 nm. The DLS study showed nanoparticles with an average particle size of 50 nm, whereas SEM micrographs displayed spherical and cuboid-shaped silver nanoparticles. The antibacterial study showed that the silver nanoparticles stabilized with guaran showed an excellent inhibition zone against E. coli.
Keywords: Silver nanoparticles, green synthesis, Cordia gharaf, guar gum, antibacterial.

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INTRODUCTION

Nanotechnology is the most dynamic branch of material science study [1]. The field of nanotechnology has introduced metal nanoparticles that have unique physicochemical properties such as low melting point, strong surface reactivity, magnetization, and a substantially larger surface area [2]. In recent years, silver nanoparticles (AgNPs) have drawnsignificant interest as a potential source of antimicrobials. Besides their use in the healthsector, AgNPs have been widely employed in the areas of food storage, textile coatings and many environmental applications[3] as antibacterial agents [4]. They exhibit excellent antibacterial activity against Gram-positive and Gram-negative bacteria as well as multidrug-resistant bacteria. AgNPs can increase bacterial membrane permeability, infiltrate into the bacterial cytoplasm, denature bacterial proteins, and interfere with DNA replication, ultimately killing the bacteria [5].

The current work aims to produce AgNPs with excellent antimicrobial properties.This study showed the antibacterial action of AgNPs against the gram-negative bacterium *Escherichia coli*. It is a facultative anaerobe found in the human and animal gastrointestinal tracts[6]. Infection with this bacteria is one of the major issues threatening the profitability of poultry businesses [7].

Green synthesis, also known as biogenic synthesis, is a promising method for producing AgNPs.It has spawned a new field called "Phyto nanotechnology," which deals with the green synthesis of metal nanoparticles using plant resources, including its optimization and applications [8]. Green synthesis has many advantages over traditional methods, including low cost, environmental friendliness, and the absence of high pressure, energy, as well as the employment of harmful chemical reagents [9,10].

The currentwork used the aqueous extract of leaves of *Cordia gharaf*, commonly known as Gonda, as a reducing agent to synthesize AgNPs. The plant *Cordia gharaf* has forage values for livestock [11]. The leaves and fruits of the plant have traditionally been used for medicinal purposes. The phytochemicals present in the leaves act as reducing agents. The other species of this plant, like *Cordia dichotoma*[12–14], *Cordia sebastena*[15], *Cordia obliqua willd*[16], *Cordia macleodii*(Griff.) Hook. F & Thomas and *Cordia myxa*[17,18], etc., are also known for their Ayurvedic properties. They have been employed for the synthesis of metal nanoparticles.Bharathi et al. used fruit extract of Cordia dichotoma for the synthesis of silver nanoparticles. They investigated phyto-compounds responsible for capping and reduction of silver

ions. They further found the average size of AgNPs was 2-60 nm with a spherical shape, which showed significant antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*[13]. Ramalingam and co-workers investigated the synthesis of AgNPs using floral extract of Cordia sebastena. This study revealed that the flower of Cordia sebastena contains alkaloids, flavonoids, phenols, tannins, cardiac glycosides, terpenoids, and phlorotannin, which may serve as reducing agents. The floral extract applied at 1: 10 concentrations to 2 mM silver nitrate at pH 9 resulted in AgNPs with an average particle size of 85.2 nm[15]. An attempt was madeby Dhal et al.for the synthesis of AgNPs using stem extracts of Cordia macleodii (Griff.) Hook. F & Thomas revealed that the synthesized AgNPs were spherical with a size range of 5–50 nm. The AgNPs synthesized using this technique showed strong antibacterial effects against nine different pathogenic bacterial strains such as Pseudomonas aeruginosa, *Staphylococcus aureus*, Citrobacter sp., *E. coli*, CONS (Coagulase-negative Staphylococci), Acinetobacter sp., Enterobacter sp., Proteus vulgaris, and Klebsiella sp.[19].

Another important part of this study is the use of a natural hydrocolloid, guar gum, to improve the stability of AgNPs. Guar gum has several properties that make it a good choice for stabilizing nanoparticles.Although there are certain reports of its use as an NP stabilizer, we have attempted to find the way guar gum stabilizes nano particles. Our study further indicates its role in determining the morphology of synthesized nano particles.Guar gum is an environmentally benign material because of its easy biodegradability. It is a high molecular-weight natural polysaccharide composed of two monosaccharides, galactose and mannose. The structure involves a linear chain of β1→4 linked mannose residues with galactose residues linked α1→6 at every second mannose unit, forming a short side branch [20]. Its aqueous dispersion has a high solution viscosity, distinguishing it from other commonly known polysaccharides like cellulose and starch. Both the galactose and mannose units of guaran possess a pair of cis-hydroxyl groups on adjacent carbon atoms. This structural feature is responsible for highly effective hydrogen bonding. Hence, guaran interacts more effectively with the surfaces of other foreign materials and can stabilize distinct particles against merging. Guar gum, therefore, effectively coats the nanoparticles and provides steric repulsion to prevent particle growth. It has been discovered to improve the stability and mobility of nanoparticles [21,22].

MATERIAL AND METHODS

All of the chemicals and reagents used in this investigation were AR grade, purchased from a local supplier. The fresh leaf sample of *Cordia gharaf* was collected from CAZRI, Jodhpur. The taxonomic identity of the plant material was validated by the taxonomists. Guar gum powder was procured from the local guar processing industry.

A stock solution of 1mM of silver nitrate was prepared in nano-pure water. A 1% aqueous dispersion of guar gum was prepared in nano-pure water with the help of a mechanical stirrer. The leaf sample was thoroughly washed with tap water and then rinsed with nano-pure water. After washing and drying it at room temperature, 1 gm of the leaf sample was crushed into small pieces using a pestle and mortar. The paste was then dispensed into a R B flask containing 100 mL of nano-pure water.The above solution was heated for 15 minutes on a heating mantel with a distillation unit. The prepared extract was then cooled and filtered through Whatman No. 42 filter paper. The solution so prepared was made up to 250 mL in a volumetric flask.

Two samples of AgNPs were prepared at room temperature. One sample was simply prepared by mixing silver salt solution into leaf extract, while another was prepared by using an aqueous 1% dispersion of guar gum.

10 mL of $AgNO_3$ solution was mixed with 5 mL of leaf extract, labeled as sample A, and kept at room temperature. Another sample, labeled sample B, was made by mixing 10 mL of AgNO3 solution with 5 mL of leaf extract containing 10 mL of 1% aqueous dispersion of guar gum and leaving it at room temperature. The progressive shift in color from light yellow to reddish-brown within 5 minutes indicates the emergence of the AgNPs. Sample A and B were kept at room temperature in the dark for future analysis.

RESULTS AND DISCUSSIONS:

The UV-visible spectra of samples were obtained using an ELICO® SL 210 Double Beam UV-Visible Spectrophotometer in the range of 300 to 600 nm, as shown in Fig-1. The different absorption maxima of both samples were observed in the UV-visible spectra. Sample A showed an optical absorption peak at 410 nm, while sample B displayed absorption at 423 nm, which is typical for AgNPs. The corresponding absorbance values are 0.6012 and 0.3395, respectively.

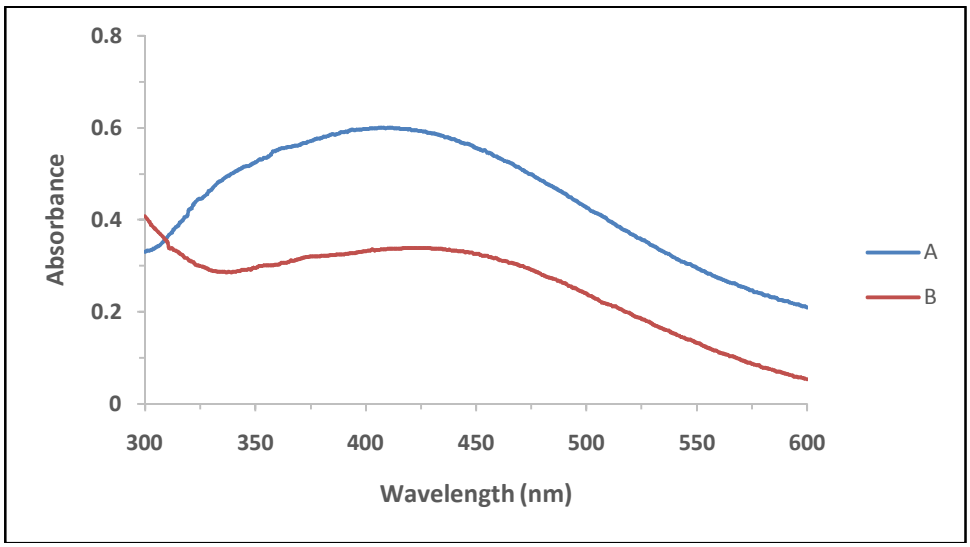

Fig-1: UV-visible absorption spectrum of AgNPs (A) in absence of guar gum, (B) in presence of guar gum.

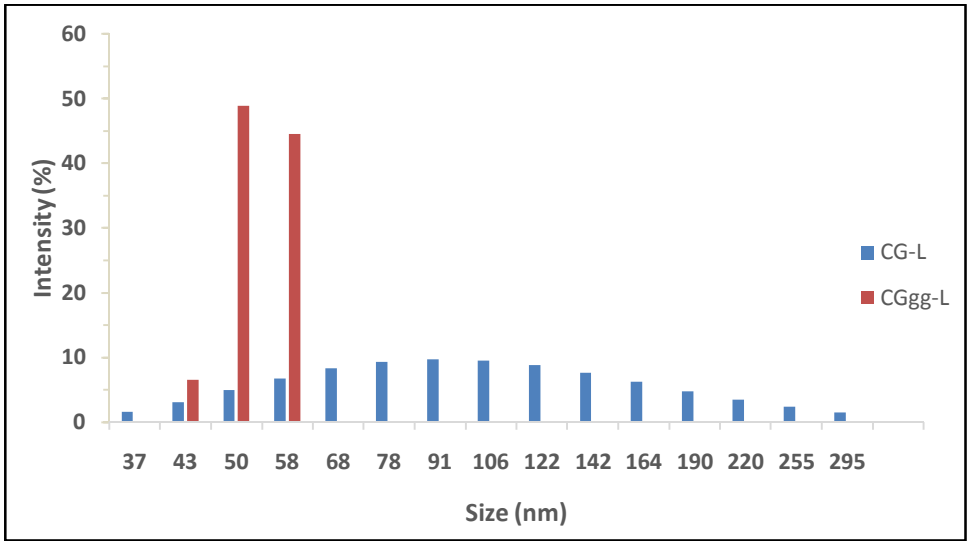

Fig-2: Particle size distribution histogram of silver nanoparticles (A) in absence of guar gum, (B) in presence of guar gum, determined from DLS.

As shown in fig-2, the particle size distribution histograms of both samples were recorded using the Malvern Instrument Zetasizer Nano ZS90. The DLS data revealed the particle size distribution of AgNPs within the size range of 37 nm to 295 nm. The figure demonstrates that sample B has a narrow particle size distribution in the range of 43 to 58 nm, which confirms the excellent cappingproperty of guar gum. Sample A has particles of an average size of 91 nm, whereas Sample B exhibits nanoparticles of an average diameter of 50 nm.

The FTIR instrument (Agilent technology) was used to record FTIR spectra. The FTIR spectra(fig-3) reflects different structural units present in the biomolecules of the aqueous leaf extract of *Cordia gharaf*. The band at 3365 cm^{-1} in the FTIR spectrum can be attributed to the O–H stretching of alcohols and phenols. The band at 1065 cm^{-1} is associated with C–O stretching of alcohol. The C–H stretching of sp^3 carbon was also shown in the region of 2925 cm^{-1}. The band at 1446 cm^{-1} is associated with C=C bending of aromatic ring. The aromatic C–H out of plane bending (in the ring) is indicated by the overtone at 1861cm^{-1}. The C=O stretching of carbonyl is shown by the band at 1695 cm^{-1}. The band below 900 i.e., 812 cm^{-1} indicates the presence of aromatic compounds in the extract. The bioreduction of silver ions into silver nanoparticles is clearly attributed to these functional groups, according to this study. In addition to the other bands, a band in the region below 500 cm^{-1} indicates presence and hence confirmation that Ag nanoparticles are present in the reaction mixture.

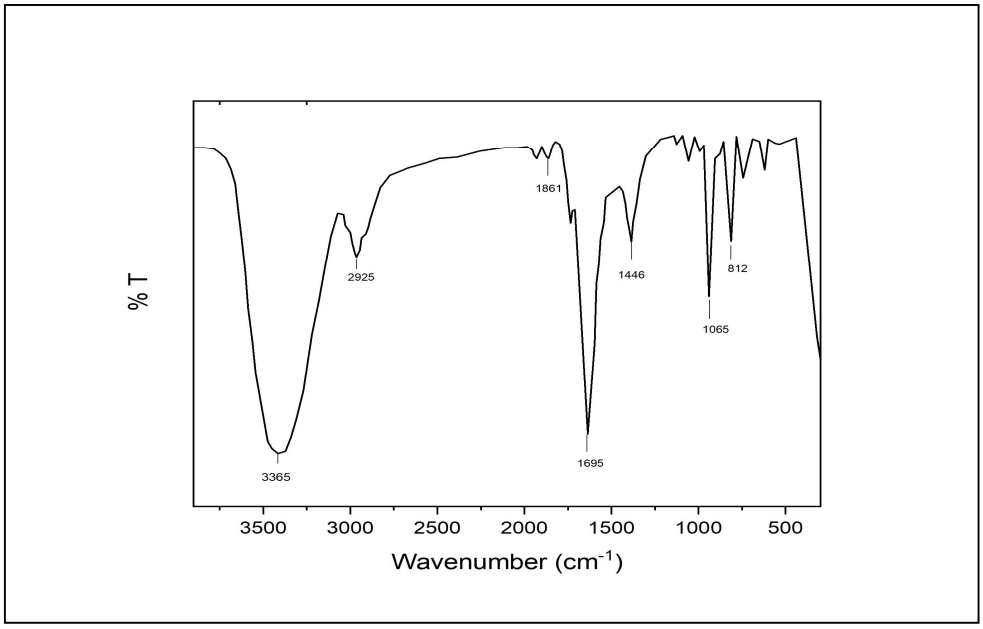

Fig-3: FTIR spectra of AgNPs formed using leaf extract of *Cordia gharaf* in presence of guar gum.

The SEM analysis was conducted using Bruker XFlash® 6-30. Fig-4 shows micrographs of AgNPs formed using leaves along with guargum at different resolutions. The SEM study revealed the presence of uniformly-dispersed AgNPs. A clear separation of these particles can also be observed.AgNPs formed are nearly uniform in shape, varying from cuboids to hexagonal.Another important observation is the appearance of flowery structures that must be ultrathin.The formation of such flowery structures indicates that these are highly crystalline structures which are in conformity with the fact that these particles are formed relatively at a slower rate as compared to other conditions.Since guargum molecules are polymeric hydrocolloids, therefore, their presence on nanoparticle surfaces increases interparticle separation. It may be concluded that it is because of the presence of hydrated guargum adsorbed to their surfaces. These observations and conclusions are in good agreement with DLS and spectrophotometric data.

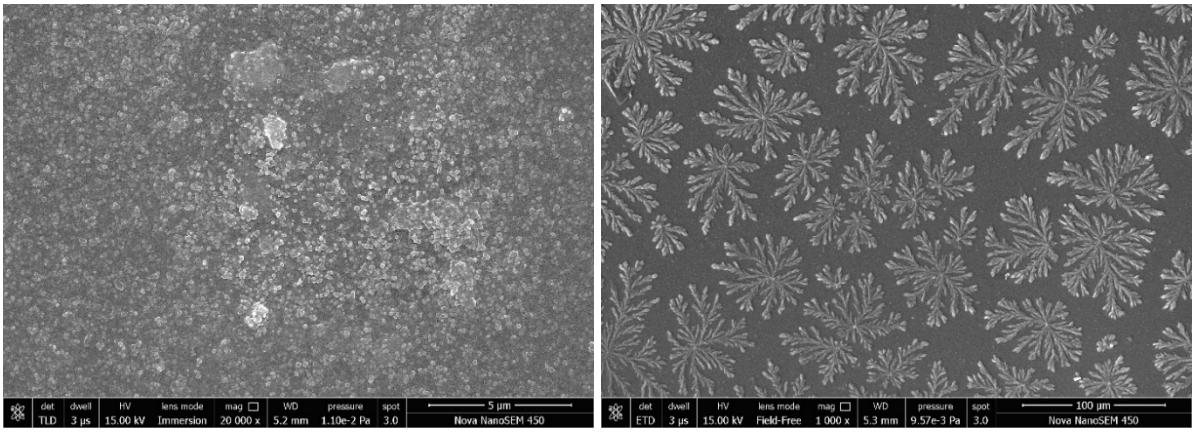

Fig-4: SEM micrographs of AgNPs formed using leaf extract of *Cordia gharaf*in presence of guar gum at different resolutions; (A) at 5 µm and (B) at 100 µm.

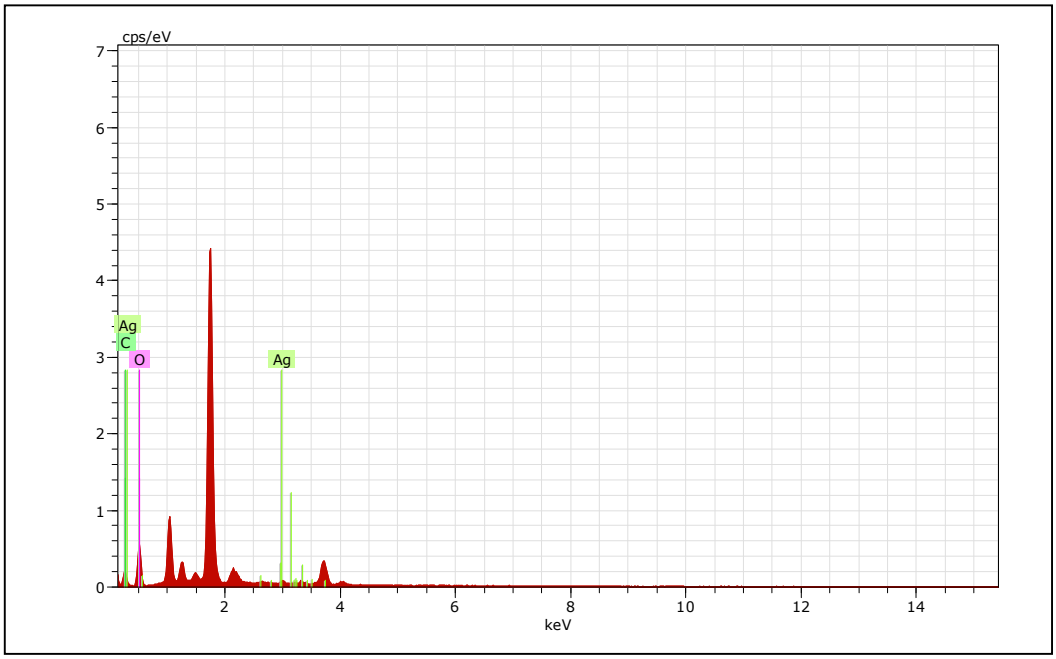

Fig-5: EDX spectra of AgNPs formed using leaf extract of *Cordia gharaf* in presence of guar gum.

The elemental analysis of AgNPs was performed using EDX.Fig-5 displays the EDX graph of AgNPs formed using leaf extract of *Cordia gharaf* in the presence of guar gum. The strong peak around 3 keV corresponds to the binding energies of silver metal nanoparticles. Table-1 reveals the % weight composition of AgNP samples formed using leaf extract of *Cordia gharaf*along with guar gum.

Table-1:EDX data of AgNP sample formed using leaf extract of *Cordia gharaf*and guar gum.

Element	Atomic number	Series	Unnormalized concentration [wt%] of element	Normalized concentration [wt%] of element	Atomic wt%	Concentration error [wt%] (1 sigma)
O	8	K-series	12.18	53.19	75.59	2.04
C	6	K-series	1.98	8.64	16.36	0.50
Ag	47	L-series	8.74	38.17	8.05	0.34
		Total	22.90	100.00	100.00	

Table-2: Inhibition zones of leaf extract, sample A and sample B against *Escherichia coli*.

Samples	Inhibition zones (mm)
Standard (ciprofloxacin)	39
Leaf extract	13
Sample A	14
Sample B	16

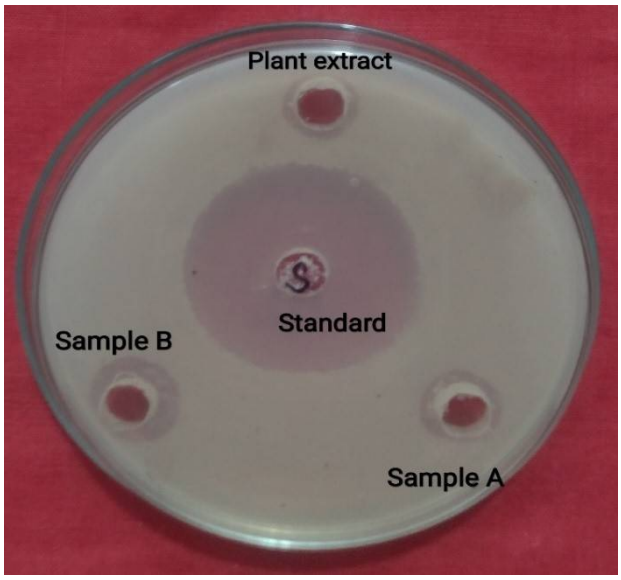

Fig-6: Inhibition zones of leaf extract, sample A and sample B determined using the agar well diffusion method.

For the antimicrobial study, a pure culture of ***E. coli*** was provided by S.M.S. Medical College, Jaipur. The agar well diffusion method was used to test the antibacterial activity of the AgNPs solution in vitro against a gram-negative bacteria strain **of** *Escherichia coli*. The antibacterial spectrum of silver nanoparticle solution was measured in terms of zone diameters surrounding the well for the bacterial species. The inhibition zone diameters produced by plant extract, sample A, and sample B were compared to those produced by ciprofloxacin, a commercially available antibiotic.

Table-2 shows the inhibitory zones of plant extract, sample A, and sample B against ***E. coli*** obtained using the agar well diffusion method at a concentration of 90 µL.Fig-6 reveals that the smaller AgNPs synthesized using guar gum as a stabilizing agent showed considerable antimicrobial activity. AgNPs prepared with the help of guar gum (sample B) showed the highest activity against gram-negative bacteria, ***E. coli***. Plant extract alone had a smaller inhibitory zone when compared to silver nanoparticle samples.

Another important fact to keep in mind while interpreting these results is that guargum itself is susceptible to bacterial attack, i. e., it supports bacterial growth. Hence, inspite of this adverse situation, any improvement in anti-microbial properties is well appreciated. AgNPs produced with leaf extract of *Cordia gharaf* as a reducing agent and guar gum as a stabilizing agent were found to exhibit promising antimicrobial activity against *E. coli*.

CONCLUSION

The therapeutic properties of *Cordia gharaf* assist in the reduction of metal ions to nano-size, which introduces a more efficient biosynthetic approach to the synthesis of AgNPs. The efficacy of AgNPs was further improved with the help of a hydrocolloid guaran, which worked as an excellent stabilizing agent. The silver nanoparticles produced with this technique were stable for more than two months, even at room temperature. The use of the arid region's natural polysaccharide guar gum enhances its bioactivity in yielding stable, uniform-sized, and well-structured AgNPs. All the associated essential characteristics of a nanoparticle are significantly improved by the presence of guar gum. Some scope remains for further study on controlling the particle size and surface structure with the help of chemical constituents present in guar gum.

The antimicrobial study showed that the AgNPs prepared using aqueous leaf extract of *Cordia gharaf*exhibit excellent antibiotic activity against ***E. coli***. All the characterization techniques revealed the formation of small AgNPs with a larger surface area where the improved stability was aided by guar gum.

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