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Synthesis of Fenugreek leaves & seeds mediated Silver Nanoparticles and Their Antibacterial Coating on Cotton Cloth

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ABSTRACT

In the present work, Fenugreek (Trigonellafoenum-graecum) leaves and seeds extract mediated silver nanoparticles have been synthesized. These nanoparticles are characterized by ultraviolet-visible spectroscopy and dynamic light scattering (DLS). The size of the synthesized silver nanoparticles found between 10-50 nm. These nanoparticles show a very good bactericidal influence on E. coli and B. subtilis. In this work the cotton cloth was coated withSilver nanoparticle to enhance antibacterial properties of cotton cloth which can further be used for medical purposes.
Keywords: *Silver nanoparticles, Trigonellafoenum-graecum, UV-Vis. spectroscopy, Antibacterial,Coating of cotton cloth.*

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INTRODUCTION

Fenugreek *(Trigonellafoenum-graecum*) is a green vegetable, highly nutritious for human health and is generally known as methi (in Hindi). This plant belongs to the family Fabacecae. It has been used from ancient time as a flavoring agent in cooking spice, and as a medicinal plant. Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses (anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, antimicrobial,etc.)This is used as impenetrable in food toheighten the flavor and colour to make food tasty. The largest producer of this spice in the world is India. "KasuriMethi" is another variety of fenugreek generally grown in Pakistan and is very well known for its enticing fragrance. It contains many beneficial nutrients like potassium, lysine,L-tryptophan, and saponins. Fenugreek seeds have been known for their medicinal values from ancient time. The steroid diosgenin of Fenugreek seeds is of great pharmaceutical importance.The polyphenolic compounds and amino acids present in seeds are responsible for these applications[1].These seeds are used in Iranian traditional medicine for lowering of blood sugar[2]. The main amino acids present in fenugreek seeds are arginine, lysine,isoleucine, and histidine.
As the biologically synthesised nanoparticles are eco-friendly and have remarkable properties over bulk materials, this field is attracting more attention of researchers [3,4]. Biological methods are more beneficial than chemical and other methods[5]. In these biological methods plant extracts work as reducing as well as capping agents for the synthesis of nanoparticles [6,7]. Because of the antimicrobial properties of silver, silver nanoparticles(AgNPs)are more widely being used in medical applications than other metals nanoparticles[8]. As the silver is nontoxic to animals cells but highly toxic to bacterial cells this makes the silver as safe bactericidal metal [9–11]. Silver is being used as disinfectant from ancient time [12]. Fenugreek is a pragmatic, cost effective plant and their phytochemicals are used as reducing agents to synthesise metal nanoparticles.
In many medical and healthcare products, cotton is used as raw material[13]. The high moisture absorption capability of cotton fibres makes them prone to the vast development of microbial growth, so there is a need of research to produce textile materials coated with antibacterial agents[14–16].Therefore cotton fibres are treated with number of chemicals to get antimicrobial cotton fibres[17–19].Silver nanoparticles show good effectiveness for strong antimicrobial effects[20]**.** Disc diffusion method was followed antimicrobial treatment against gram positive bacteria *B.subtilis* and zone of inhibition was measured to find antimicrobial activity. Silver nanoparticles get attach to the negatively charged cell

surface of bacteria and disturb the electron transport and respiration of cell [21]. Also these nanoparticles interact with the bacterial cell DNA [22] and produce silver ions which has biocidal effect on microorganisms.

MATERIAL AND METHODS

Collection of the material

The leaves and seeds of Fenugreek were collected from local market of Mathura city, India. All the chemicals used were of AR grade, purchased from CDH and used without further purification.

Preparation of Plant extracts

The leaves and seeds were thoroughly washedwith triple distilled water.20 gram of seeds were crushed and 20 gram of leaves were cut into small pieces with the scissor. These were boiled in 80ml of distilled water for 10 minutes. These both solutions were filtered with the Whatmann filter paper and stored at 4°C until further use for present investigation.

Synthesis and Characterization of Silver Nanoparticles

The 8 ml of fenugreek leaf extract (FLE)and fenugreek seed extract (FSE) was then added to the 100 ml of 1mM silver nitrate solution drop wise. Then this mixture was refluxed at 80-90°Cfor 2 hours to obtain colloidal silver nanoparticles. The synthesis of AgNPs was confirmed by the change in color of the reaction (light yellow to light brown) as shown in Figure 1. The surface plasma resonance (SPR) vibration is responsible for this color change.

(a) **(b)**

Figure1: Silver nanoparticles (a) before addition of $AgNO_3$ (b) AgNPs

Preparation of Media

The media was prepared as per the guidelines given in Bacteriology Manual[23]. All the dry ingredients given in the manual were taken in beaker and dissolved in distilled water. The so prepared medium was sterilized by keeping this in autoclave at 121°C for 30 minutes. 15 ml of this medium was poured in petri plate and this plate was incubated for 24 - 48 hours at 37°C.

Composition

peptone - 0.5%
NaCl - 0.5%
agar -1.5%
beef extract - 0.3%
distilled water
pH - (6.8) at room temparature.

Collection of Bacteria

Both the bacteriawere procured from CSIR-IMTECH , Chandigarh

Characterization

Initially, the synthesis of AgNPs was confirmed by color change of solution and by absorption spectrum produced by UV-Vis spectrophotometer at 200-700 nm wavelength. The reduction of silver ions to the nanoparticle was confirmed by the UV-Visible spectra of the solutions[24]. Dynamic light scattering (DLS) was employed to determine the size using Zetasizer Nano ZS (Malvern Instruments, UK). All the analysis was carried out in an automatic mode.

Antibacterial assay

Antibacterial activity of FLE and FSEmediated nanoparticles was tested by the disc diffusion method against gram-negative *E. coli* and gram positive *B.subtilis* bacterial culture, prepared by the standard process. Before the use , petri plates and media were autoclaved. 10 μL of pure bacterial culture was uniformly spread on nutrient agar media in petri plates using L-rod. 10 μL of each sample of AgNPs was poured on a sterile disc. Four sterile discs were placed on the bacterial culture in both petri plates.These plates were incubated for 48 hours at 37°C. After 48 hours results were observed. The zone of inhibition was measured in mm and streptomycin antibiotic is used as control.

Coating of Silver nanoparticle colloid over the cotton fiber

20ml of fenugreek leaves extract(FLE)and 20ml of fenugreek leaves extract mediated nanoparticles colloidal solution were taken in two saparate beakers. 3cm long cotton cloth is taken and washed first with tapwater and then with distilled water. Cotton cloth is dipped into the respective solution and heat for 10-15minutes. After 10-15 min, the cotton cloth is taken out with squeezing out the extra solution and washed again with distilled water. Agar Petri plate was prepared by culturing 10µl of *B.subtilis* bacteria and allow it to dry for 10 minutes. Cotton cloth dipped in FLE and FLE mediated nanoparticles were placed on this cultured plate[25].This plate is incubated at 37°C for 48 hours.

RESULTS AND DISCUSSION

Physicochemical characterization

The free electrons are responsible to produce an SPR absorption band[26–29]. These free electrons in metal nanoparticles jump freely between the conduction and valence band which are close to each other. Bioreduction of silver ions present in their solution into respective nanoparticles by the phytocompounds present in the fenugreek leaves and fenugreek seeds extractwas studied using UV-Vis spectrophotometer[30,31]. The highest absorbance peak was observed at 420 nm and 440 nm for FLE and FSE mediated silver nanoparticles respectively (Figure 2).The size of the obtained silver nanoparticles was in the range of 10-50 nm (Figure 3).

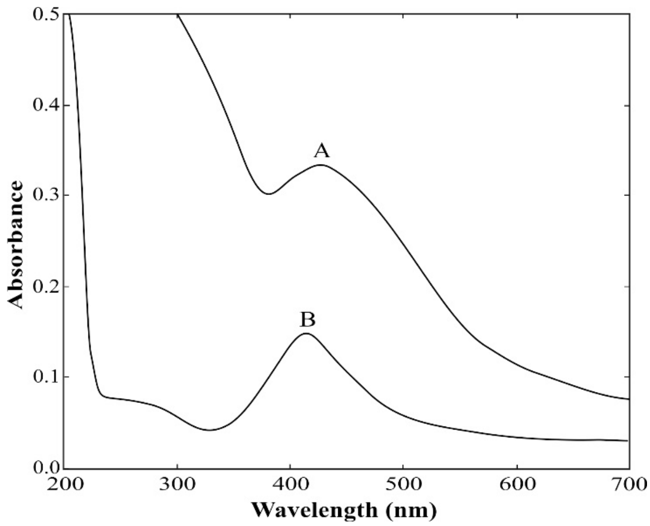

Figure2: UV-Vis spectra of silver nanoparticles
(A) Fenugreek seed extract mediated
(B) Fenugreek leaf extract mediated

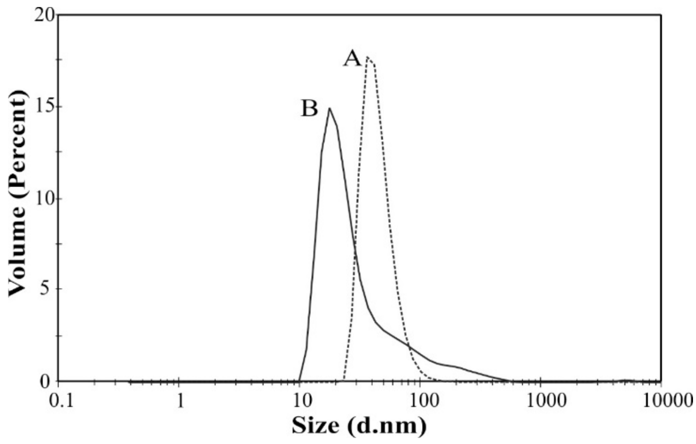

Figure 3: Size analysis of silver nanoparticles
(A) Fenugreek seed extract mediated
(B) Fenugreek leaf extract mediated

Antibacterial Activities

Because of the enhanced surface area of nanoparticles than atomic size, nanoparticles can easily interact with bacterial cells. The interaction of AgNPs with bacterial cells, kill the bacteria by attacking the respiratory chain and cell division as AgNPs get attached with sulfur and phosphorous constituents of the bacterial cells[32]. This result is clear from Fig. that AgNPs synthesized by the FLE extract are better bactericidal agents than FSE extract nanoparticles. This bactericidal effect is better in the case of *E.coli* than *B. subtilis*(Figure 4). The zone of inhibition was measured in mm(Table 1). AgNPs have better antibacterial activity than the free silver ions[33].

(a) **(b)**

Figure 4: Zone of inhibition against for FLE and FSE mediated AgNPs against **(a)***E. coli***(b)***B. subtilis*

Table 1: Antibacterial Assay- Zone of inhibition (in mm)

Plant Extract	*E.coli*				*B.subtilis*		
	$AgNO_3$	Streptomycin	AgNPs		$AgNO_3$	Streptomycin	AgNPs
FLE	25mm	30 mm	23mm		15mm	30 mm	16 mm
FSE	25mm	30 mm	19 mm		15mm	30 mm	12 mm

Coating of Silver nanoparticle colloid over the cotton fiber

Silver nanoparticles are stable for a long time. When cotton cloths dipped in the solution of silver nanoparticles colloidal solution,they easily coated on cloth and stable even after washing. It is clear from the Fig. that cotton cloth coated with silver nanoparticles show very good zone of inhibition while this zone is very less in case of FLE (Figure-5). So these coated cotton cloth can be easily used for various medical purposes.

Figure 5: Plate showing the in vitro antimicrobial activity of the cotton cloth on *B.subtilis* culture (1) Fenugreek leaves extract(FLE) coated cotton cloth (2) FLE mediated AgNPs coated cotton cloth

CONCLUSION

The green synthesis of nanoparticles has been successfully carried out using the leaves and seed extract of *Fenugreek (Trigonellafoenum-graecum)*. The size of the obtained silver nanoparticles (AgNPs)was in the range of 10-50 nm. The phytocompounds present in *Fenugreek (Trigonellafoenum-graecum)*extract are responsible for the bioreduction of metal ions into metal nanoparticles. AgNPs synthesized by *Fenugreek (Trigonellafoenum-graecum) leaves*extract showed better bactericidal effect than AgNPs synthesized by *Fenugreek (Trigonellafoenum-graecum)* seedsextract against gram–ve bacteria *E.coli* as well as gram +ve bacteria *B. subtilis*. Thus green approach of synthesizing nanoparticles can be utilized in antibacterial action. The cotton cloth coated with these silver nanoparticle can be used for various medical purposes.

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