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Production of Silver Nanoparticles from *Carum copticum* Plant Extract and Identification of its Antimicrobial Activity

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Abstract

The nanotechnology develops well in the green synthesis of silver nanoparticles from the plants and microorganisms. Recently, silver nanoparticles were used against of disease management and its control measures. Various plants and its extract were used to synthesis of silver nanoparticles and these particles were identified by some advance techniques such as ultraviolet analysis, high-performance liquid chromatography analysis, X-ray diffraction, Fourier transform infrared, transmission electron microscopy, and scanning electron microscopy. These techniques were used for shape, size, and character analysis of the silver nanoparticles. In this work, silver nanoparticles were used to identify its potential antimicrobial against of urinary tract infection (UTI) pathogens which are produced extended-spectrum beta-lactamases. Both Gram-positive and Gram-negative bacteria will produce the UTI and in this work *Klebsiella* sp. used.

Keywords: Fourier transform infrared, Green synthesis, *Klebsiella*, silver nanoparticles, Transmission electron microscopy, Urinary tract infections

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Introduction

Silver and some silver ionic components have an inhibitory ability against of few pathogens.[1] Some silver-based components were used to control the development of bacterial population in the any one of objects. Recently, many peoples tried to prove the antimicrobial activity of various metals. Silver nanoparticles can inhibit the microbial growth in wide range. Even low concentration of silver nanoparticles can control the wide varieties of microorganisms due to its unique characters. This unique character attracts the attention of industries.[2,3] The silver nanoparticles possess very low toxic to the human cells.[4] Recently, many researchers using the both physical and chemical methods for the synthesis of silver nanoparticles from the various components. Nanoparticles attract the concentration of researchers from drug delivery, medicine development, biomedicines, and biosensors.[5] Synthesis of nanoparticles from the plants is very simple method and its create nanoparticles with better stability and perfect dimensions. This green synthesis of nanoparticles preferred because can produce large volume of silver nanoparticles with low-cost effective without any environmental damages.[6]

Materials and Methods

Preparation of the plant extract

Carum copticum seeds were obtained from local shop and make it more dry. The dried materials were washed with sterile distilled water and dry. The dried components were converted as powder.

Ethanolic extract

Fifty grams of powder were loaded into Soxhlet apparatus with ethanol and start the extraction process. Clear and light yellow color of liquid collected in beaker. The collected extract was stored in refrigerator at 4°C for further uses.[7] In other side, 5.0 g of dried powder material mixed well with 50 mL of sterile distilled water and the flask placed in shaker for 6 h then Whatman No. 1 filter paper used for the extract filtration. The collected material used for producing silver nanoparticles.

Biosynthesis of AgNPs

Ninety-five milliliters (1 mM) of aqueous solution of $AgNO_3$ taken and added with 5 mL of plant extract and incubated in normal room temperature. The reduction process was confirmed by color change from light brown to deep red or brown in color then by spectrophotometric absorption at 200–800 nm. The reacted component centrifuged in 10,000 rpm and the silver nanoparticle was obtained by precipitation.

Characterization of nanoparticles

Ultraviolet (UV)–visible absorbance was used to mention the formation of silver nanoparticles from the testing sample. A 200–800 nm range using absorbance of synthesized silver

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nanoparticles. The high-performance liquid chromatography (HPLC) analysis was performed for the identification of component present in the extract. The size of silver nanoparticle was measured by transmission electron microscopy (TEM) instrument and this instrument was operated at an accelerating voltage at 100 keV. The carbon coated TEM power grids coated by testing sample and proceeded. Fourier transform infrared (FTIR) methods performed for the identification of functional group of synthesized silver nanoparticles. In other side, freeze-dried sample was coated in X-ray diffraction (XRD) grid for the spectra analysis and the results were recorded.

Table 1: Zone against of isolated organism (SNP – silver nanoparticle)

Plant extract	*Sample volume in the disc and zone of inhibition*				
	20 µL	*30 µL*	*40 µL*	*50 µL*	*Control*
Isolate-1	12 mm	14 mm	19 mm	20 mm	-
Isolate-2	12 mm	13 mm	19 mm	21 mm	-
SNP solution	*Sample volume in the disc and zone of inhibition*				
	20 µL	*30 µL*	*40 µL*	*50 µL*	*Control*
Isolate-1	14 mm	17 mm	19 mm	21 mm	-
Isolate-2	14 mm	17 mm	19 mm	21 mm	-

SNP: Single-nucleotide polymorphism

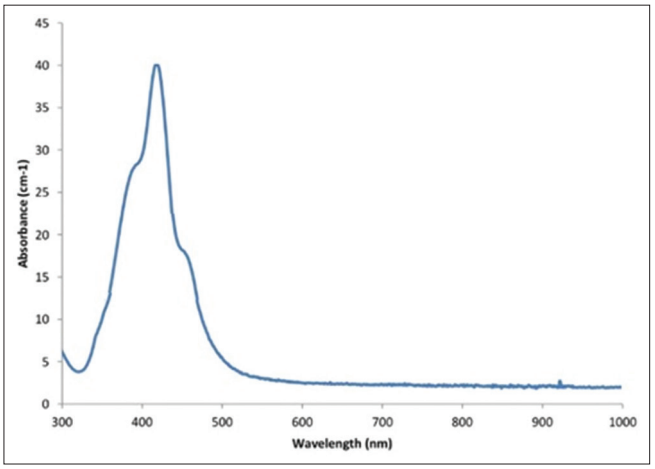

Figure 1: Ultraviolet analysis of silver nanoparticles

Antimicrobial assay

Bacterial strains: *Klebsiella* sp. were isolated from two urine sample. The collected plant extract and silver nanoparticles suspension separately used to identification of antibacterial effect against of pathogen in various concentrations. The results of plant extraction and silver nanoparticle suspension compare with control and recorded.

Disc diffusion assay

Mueller-Hinton agar plates were used for the determination of antimicrobial activity of synthesized silver nanoparticles and plant extract.[9] The discs were prepared with various concentrations such as 20 µl, 30 µl, 40 µl, and 50 µl. Standard of silver nitrate solution (50 µl) and empty disc was used as control. The test microorganisms were swabbed uniformly on MHA plates and the prepared discs were placed carefully. The inoculated plates were incubated at 37°C for 24 h and zone of inhibition was measured in the range of diameter.

Results and Discussion

Fresh and healthy seeds were collected and well dried in the shadow place. The dried materials were converted into powder form and loaded in Soxhlet apparatus for the collection of extract in the application of ethanol. The collected extract was used for the various analysis purposes.

Ninety-five milliliters (1 mM) of $AgNO_3$ solution and 5 mL of plant extract were added and incubated. Later that, mixed components color first changed into pale brown color and then brown in color. Samira Nasiri and Nasiri[10] also reported that the silver nanoparticle production was primarily confirmed by color change. The color changing indicated that the plant extract having ability to produce the silver nanoparticles.

The components were transferred to UV analysis and it produces peak level in the range of 430 nm and the absorbance range is 40 (Figure 1). The reliable results were interoperated by

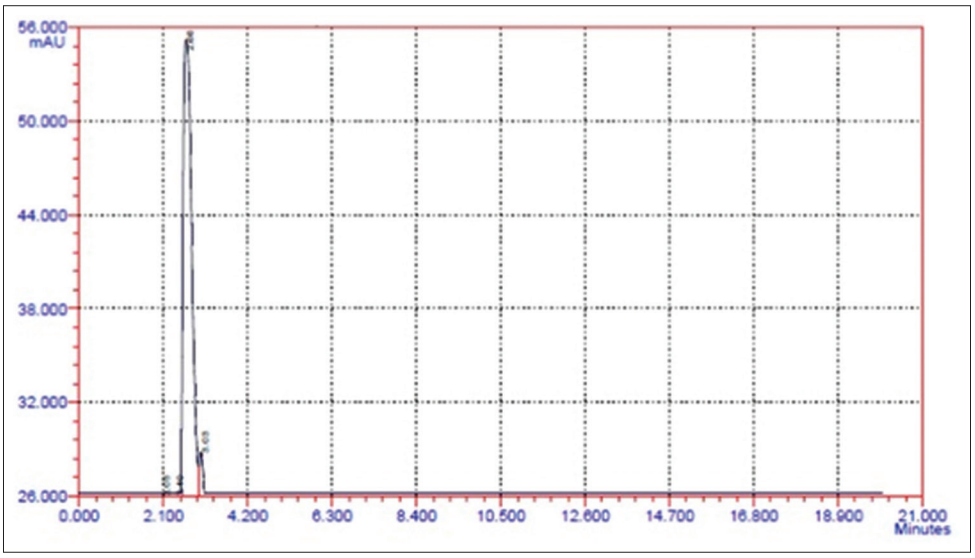

Figure 2: High-performance liquid chromatography analysis of silver nanoparticles

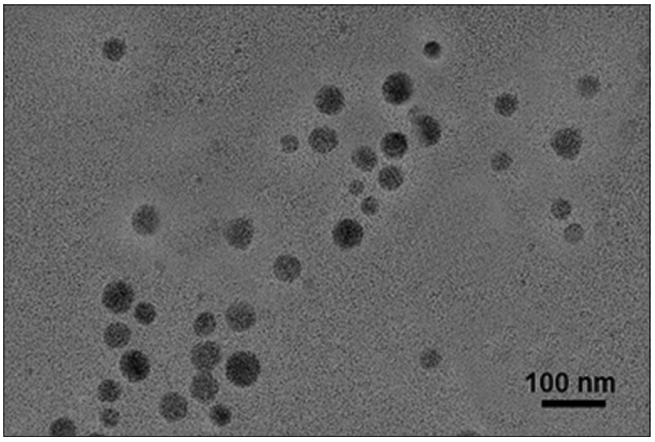

Figure 3: Transmission electron microscopy analysis of silver nanoparticles

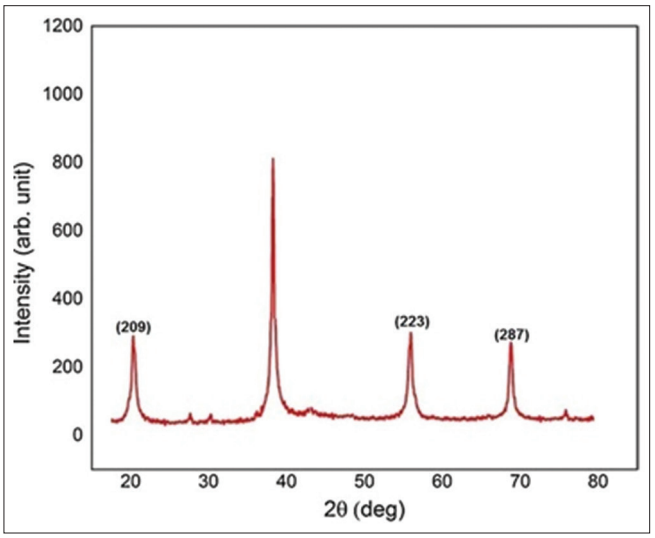

Figure 4: X-ray diffraction analysis of silver nanoparticles

various authors (Ali *et al*., 2011). HPLC analysis used to identify the major components in the collected plant extract. The peaks were produced in the various time durations for the confirmation of major components in the sample. The similar reports were given by various authors.[11] HPLC shows peaks in the range of 02.05, 02.40, 02.65, and 03.03, respectively. The results are shown in Figure 2. The standard thymol component was used to the identification of major component presence in the plant extraction. The biosynthesized silver nanoparticles were separated by centrifugation process and collected in the Eppendorf tube for the further applications.

TEM used for the morphological identification of biosynthesized nanoparticles (Figure 3). Based on TEM analysis, the shape and size of the biosynthesized silver nanoparticle were revealed.[12] This analysis mentions that the silver nanoparticles have 10 nm–20 nm or more and it confirmed the silver nanoparticle spherical in nature.[10]

Crystalline structure and diffraction properties of biosynthesized silver nanoparticles were characterized by X-ray diffraction method. The peaks were formed in various regions such as 209, 223, and 287 (Figure 4). According to the results, will confirmed that the silver nanoparticles presence in the sample. Nasiri and Nasiri[10] also reported that the XRD peaks were showed between 111 and 322, the pattern clearly showed that the presence of single-nucleotide polymorphism (SNP) in the sample. On the other hand, FTIR also carried out for the identification of functional groups in the silver nanoparticle which is produced from *C. copticum* plant extract, this analysis produces 10 different levels of bands (Figure 5).

Separated plant extract and silver nanoparticles used to identification of potential antimicrobial activity against of isolated organism. The silver nanoparticle and extract were loaded and placed in inoculated plate. Both plant extract and silver nanoparticle were form zone near by 21 mm and the results are given in Table 1. Kaur and Arora[13] also reported that *C. copticum* plant extract showed good antimicrobial activity against of *Enterobacteriaceae* family and some Gram-positive bacteria.

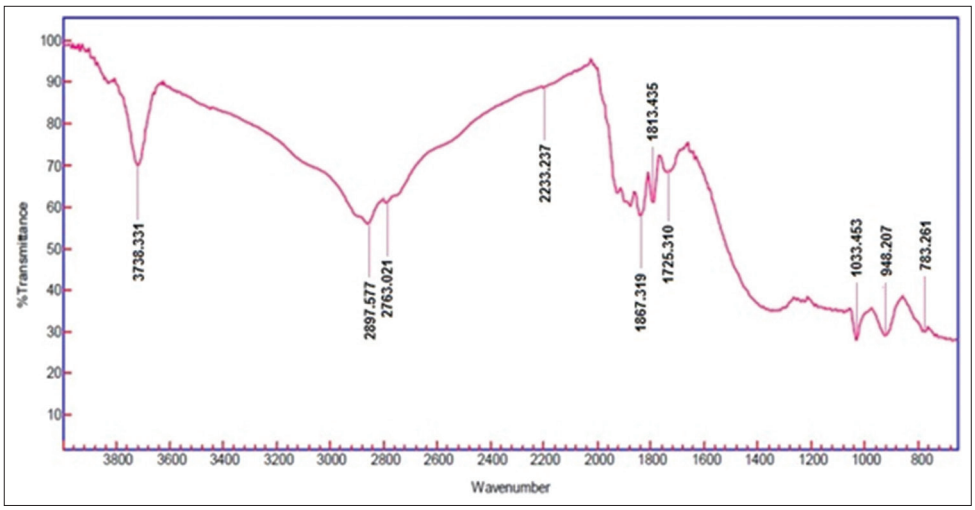

Figure 5: Fourier transform infrared analysis of silver nanoparticles

Conclusion

The green synthesis of SNPs was confirmed by TEM and XRD analysis. These analyses show the presence of silver nanoparticle and their size. In HPLC analysis, some major components were identified and the results are interpreted. The plant extract and silver nanoparticles separated and used to analysis of antimicrobial activity. The recorded results were showed that both plant extract and SNPs are able to control the microbial growth. In this work, both the plant extract and SNPs control the isolated organism.

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