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Biological Synthesis and Characterization of Silver Nanoparticles using Egg White and their Antimicrobial Activity

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The silver nanoparticles were synthesized by green synthesis process through the extract of egg white. This process is a simpler to handle, greater as a need to develop safe, cost effective, reliable, clean and eco-friendly nanoparticles. Formation of Ag Nanoparticles was confirmed by UV-Vis absorption peak at 400nm. Size of nanoparticles was noted from 8 to 50 nm with an average size of 24 nm. This was confirmed by TEM, SEM, XRD and FTIR analysis. Further the synthesized nanoparticles were evaluated for antimicrobial activity against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Result indicated that egg white mediated silver nanoparticles showed better antimicrobial potential for *Bacillus subtilis* (1.4cm) followed by *Pseudomonas aeruginosa* (1.1cm.) and *Escherichia coli* (0.8 cm.)

Key words: Egg white, Silver nanoparticles, Antimicrobial activity, SEM, TEM.

Nanotechnology is an important field of advanced research area in potential medical applications[1]. Many researchers in the field of nanotechnology have been looking at green synthesis process[2]. This process provides a wide range of resources for the synthesis of silver nanoparticles[3]. The rate of reduction of silver ions using biological agents is found to be much faster than physical or chemical methods wherein microbes are generally used as biological agents. Biological method is simpler to handle, greater as a need to develop safe, non-toxic, cost effective, clean and eco-friendly, in which no high pressure, energy, temperature, and toxic compounds for the preparation of nanoparticles were used[4,5] Silver nanoparticles have unique properties in nanotechnology industry. Owing to their size variation, more surface bulk atoms and high surface area that allows them to interact effectively with microbial membranes[6]. Silver ions very strongly interact and bind with certain protein having amino acids with additional carboxylic groups. Binding of silver ions with protein chain is mediated by sulfhydryl groups [7,8]. Silver Nano particles are used as anti-microbial agents. These ions have long been known to possess highly inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities[9]. It is generally conceived that silver ions interact with proteins by combining thiol (– SH) groups, which leads to the inactivation of the proteins [10]. Recent microbiological and chemical experiments have revealed that the interaction of silver ions with thiol groups play an essential role in bacterial inactivation [11, 12, 13]. Present study was aimed to synthesize and characterize silver nanoparticles using egg white as reducing agent and to study antimicrobial potential of nano size particles, keeping in view further extension of work to prepare any effective formulation for external use.

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MATERIALS AND METHODS

Source of materials

Fresh eggs were bought from a local market, silver nitrate ($AgNo_3$) and Muller Hinton agar, nutrient broth were supplied by (Titan Biotech Laboratory Chemicals, code-419), and (Himedia Laboratories, Mumbai). The bacterial strain *E. coli* (MTCC729), *Bacillus subtilis* (MTCC10518), and *Pseudomonas areruginosa* (MTCC4673) were obtained from Microbial Type Culture Collection Center (MTCC) located at the Institute of Microbial Technology (IMTECH) Chandigarh, India. The *Escherichia coli, Bacillus subtilis, and Pseudomonas areruginosa* were cultured in nutrient broth to study effect of silver nanoparticles on these bacterial strains.

Synthesis of silver nanoparticles

Egg white solution was fully dissolved in distilled water. The solution was magnetic stirred for 45 to 60 minutes. Cloudy white solution was obtained after stirring followed by setting the solution and filtration which resulted two layers i.e gauze and clear solution. Silver nitrate solution was added to clear solution and reaction was carried out at room temperature for 72 hours. The colour of solution was changed from white to brownish yellow. The solution was centrifuged at 15,000 r.p.m. for 15 minutes. The procedure was repeated twice. Finally greyish powder was obtained.

Characterization of Silver Nanoparticles

Techniques used for characterization of synthesized nanoparticles were UV-Vis spectra analysis, FTIR, XRD, SEM, and TEM.

Antimicrobial Activity

Antimicrobial activity of silver nanoparticles was studied using available method [3, 14].

RESULTS AND DISCUSSION

Present investigation was done to synthesize and characterize egg white derived silver nanoparticles, to study their potential as antimicrobial agent against *Escherichia coli, Bacillus subtilis, and Pseudomonas areruginosa.*

Characterization Techniques of synthesized Silver Nanoparticles

UV-Vis spectra analysis

Silver nanoparticles were first characterized using UV-Vis spectrophotometer which indicates the presence of silver Nano particles with the reduction of silver ion. As shown in Fig. 1 it was noted that the evolution of absorption peak occurred at 400 nm.

Fourier Transform Infrared Spectroscopy Analysis

The representative spectra of Silver nanoparticles are shown in Fig 2.Vibrational Assignments and functional Groups corresponding to the absorption peaks are shown. The absorption peaks at 1660.77, 1442.80, 1375.29 and 1327.07 represents the presence of NO2 which may be from AgNO3 solution. Strong interaction of water with the surface of silver[9] could be the reason for the O-H stretching mode peaks at 2883.68, 1660.77 and O-H in plane bending mode peaks at 1375.29, 1327.07.

Table 1. Antibacterial activity of silver nanoparticles synthesized with different concentrations using egg white

Name of Species	Concentration (µg/ml)	Zone of Inhibition (cm)	
		Ag Nanoparticles	*Vancomycin*
B.subtilis	2	1.1	2.5
	4	1.4	
	6	1.2	
E.coli	2	0.9	
	4	0.8	1.4
	6	0.6	
P.aeruginosa	2	1.1	
	4	1.0	2
	6	0.9	

XRD analysis

The egg-white extract-mediated synthesized silver nanostructure was confirmed by the characteristic peaks observed in XRD image which is shown in Figure: 3. Indexing process of powder diffraction pattern is done and XRD patterns were analysed to determine peak intensity, position and width. This data was used with the Scherer formula. The typical XRD pattern revealed that the sample contains mixed phase structures of silver nanoparticles. A number of strong Bragg reflections can be seen which correspond to the (111), (200), (220) (311) and (222) reflections of crystallites silver. Not any spurious diffractions

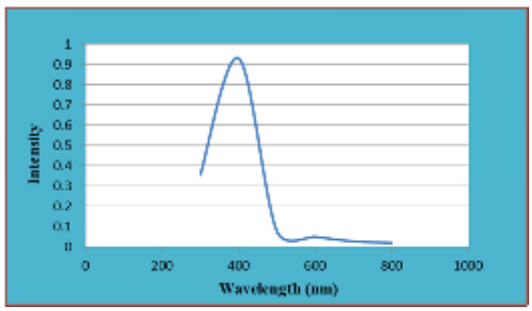

Fig. 1. UV-VIS spectrum of silver nanoparticles derived from Egg White

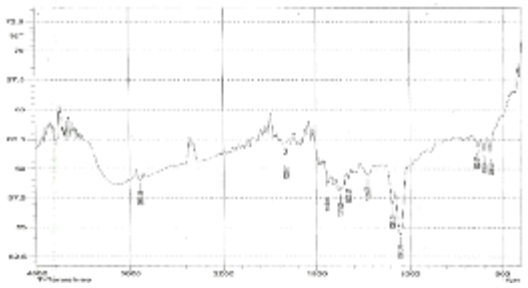

Fig. 2. FTIR spectra of silver nanoparticles synthesized by egg white

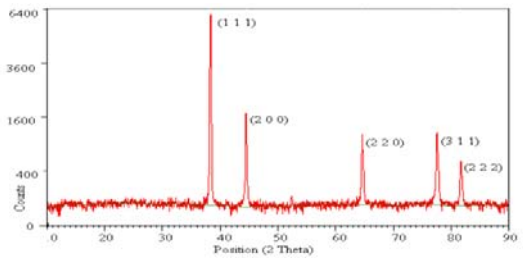

Fig. 3. XRD analysis of silver nanoparticles derived

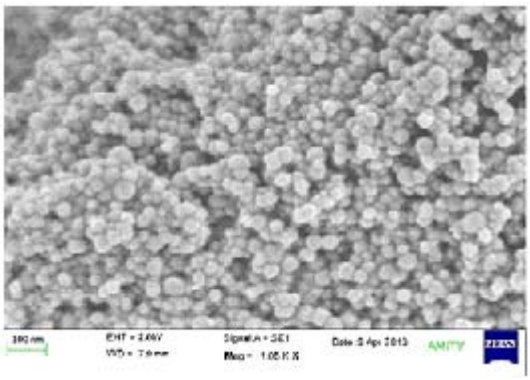

Fig. 4. The SEM image of silver nanoparticles synthesized from Egg White

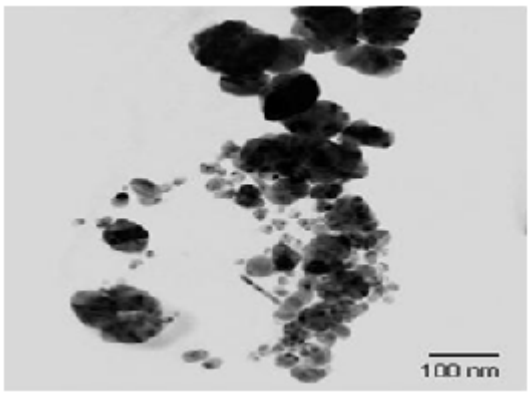

Fig. 5a. TEM image of the biosynthesized histograms silver nanoparticles

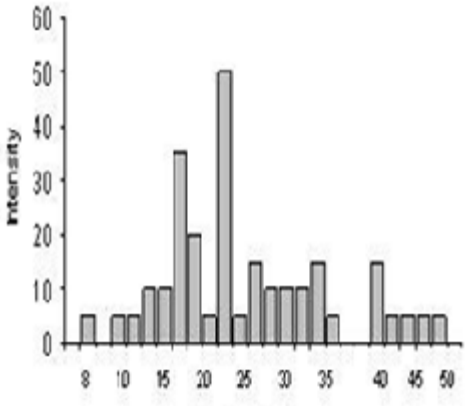

Fig. 5b. Intensity distribution for particle size of silver particles

were observed which confirmed that the main composition of the nanoparticles was silver and sample lacked any crystallographic impurities.

Scanning Electron Microscope Analysis

SEM analysis provided further insight into the morphology and size detail of nanoparticles. The size of nanoparticles was confirmed between 8 to 50 nm.

Transmission Electron Microscope Analysis

TEM was used to measure the size, shape, and morphology of the silver nanoparticles as shown in Figure 5a, the nanoparticles are agglomerated of small grains and some dispersed nanoparticles. It was observed that the particle size histograms of silver particles (in Figure 5b) show that the particles range in size from 8 to 50 nm with mean diameter 24 nm. It was confirmed that silver nanoparticles were synthesized using egg white as reducing agent through TEM method.

Antibacterial potential

Silver nanoparticles synthesized by using egg white were studied for antibacterial activity against *Bacillus subtilis, E. coli,* and *P.aeruginosa.* Silver nanoparticles showed effective inhibition at 4µg/ml for *B.subtilis*. As shown in table 1 the zone of inhibition was found 1.4 c.m. (Fig 6b) followed by *p. aeruginosa*at 2µg/ml showing zone of inhibition of 1.1c.m. However vancomycin showed inhibition of *B. subtilis* and *p.aeruginosa* with zone of inhibition of 2.5cm and 2cm respectively. The maximum zone of inhibition using silver nanoparticles observed in *E.coli* at concentration of 2ug/ml was 0.9c.m However vancomycin showed zone of inhibiton of 1.4cm.

Nowadays, green nanometal particles, especially silver, have drawn the attention of scientists because of their extensive application in the development of new technologies in the areas of electronics, material sciences and medicine at the nanoscale [15, 16, 17]. The bioreduction of the Ag+ ions could be associated with metabolic processes utilizing nitrate by reducing nitrate to nitrile and ammonium[18]. Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications. However, importance of biogenic silver nano particles as antimicrobials is much neglected in the literature[19]. *Gliricidia sepium* reduced silver nanoparticles were assayed[20] for antimicrobial activity against *E. coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538P (3 mm), *Klebsiella pneumoniae* (clinical isolate) (2 mm). In present study, egg white mediated silver nanoparticles also showed antimicrobial potential which may be used further in preparing any formulation or in any other application such as immobilization matrix, water purifier, textile impregnation etc. As per literature available, Ag-egg white protein bio-conjugates enhanced the efficacy of irradiation, and thus may be promising candidates for use during cancer radiation therapy[21]. Many studies are now available on biologically derived silver nanoparticles and hence to explore applications is the area to work upon. Toxicity study on green silver nanoparticles as antifungal and antibacterial agents is recommended so that such effective potential of green silver nanoparicles could be used in formulation for external use.

Fig. 6a. Antibacterial activity against *B.subtilis*

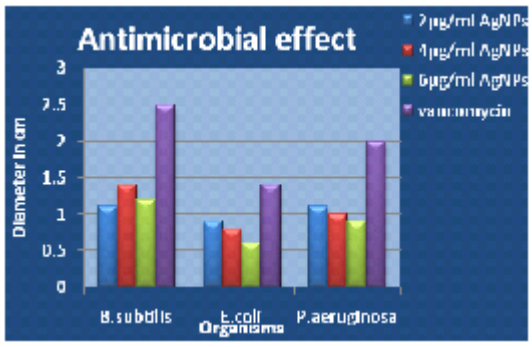

Fig. 6b. Antimicrobial activity by synthesized silver NPs

CONCLUSION

Present study demonstrated the synthesis of silver nanoparticles using Egg White. The synthesized silver nanoparticles were characterized by using UV- spectrophotometer, FTIR, XRD, TEM & SEM. Antibacterial activity was also observed against *B.subtili, E.coli and P.aeruginosa*. Further study is being conducted to explore the potential of these nanoparticles against fungi to prepare formulation for external use after conducting toxicity studies.

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