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EXTRACELLULAR SYNTHESIS OF AG NANOPARTICLES USING *ESCHERICHIA COLI* AND THEIR ANTIMICROBIAL EFFICACY

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ABSTRACT
Microbe mediated synthesis of nanoparticles is gaining tremendous importance in present time due to its harmless and eco-friendly nature. The present study deals with synthesis of silver nanoparticles using the bacteria Escherichia coli extracellularly and evaluation of its antimicrobial activity. The nanoparticles were characterized by various means like UV Vis spectroscopy, TEM analysis, zeta potential analysis and FTIR analysis. The antimicrobial activity was tested by agar well diffusion method against Staphylococcus aureus (MTCC 87), Proteus mirabilis (MTCC 3310), Bacillus cereus (MTCC-1305) and Aspergillus niger (MTCC-9652). From the present study it can be concluded that the synthesized Ag nanoparticles exhibits significant antimicrobial activity against both Gram positive and Gram-negative bacteria and also against the tested fungus.

KEY WORDS
Antibacterial, Antifungal, Biosynthesis, Microbe, Silver

INTRODUCTION
Nanotechnology is a vast and emerging area that deals with the synthesis and application of nanoparticles [1]. Biosynthesis of nanoparticles are more preferred over physical and chemical method as it is eco-friendly and occur in ambient conditions [2, 3]. Microbe mediated synthesis can occur both extracellularly and intracellularly [4, 5]. The former is however simple and cheap. Silver nanoparticles are considered to be unique because of its wide application in different fields like food technology, agriculture, environmental technology and medicine [6, 7, 8]. Several workers have reported the therapeutic properties of Ag nanoparticles viz. antiviral [9, 10, 11, 12], antibacterial, anti-cancerous, anti-inflammatory [13, 14] etc. The present study aimed to synthesize Ag nanoparticles extracellularly using *Escherichia coli* and to evaluate its antimicrobial activity against some selected pathogenic microbes.

MATERIALS AND METHOD
Microorganisms: Microorganisms were procured from Microbial Type Culture Collection and Gene bank (IMTECH, Chandigarh, India). Ag nanoparticles were synthesized using *Escherichia coli* (MTCC 10312). The antimicrobial activity was carried out against *Staphylococcus aureus* (MTCC 87), *Proteus mirabilis* (MTCC-3310), *Bacillus cereus* (MTCC-1305) and *Aspergillus niger* (MTCC-9652).

Biosynthesis of Silver nanoparticles: *Escherichia coli* strain was freshly inoculated in a conical flask containing 100 ml of nutrient broth. The flask was incubated for 24 hours at 37° C. After incubation, the culture was centrifuged to remove the pellets. The supernatant was used for the synthesis of nanoparticles by mixing with $AgNO_3$ solution at 1 mM final concentration [15].

Characterization of Silver nanoparticles: The synthesized nanoparticle were characterized by UV Visible spectrophotometry and TEM analysis. The distribution was recorded using particle size analyzer.

FTIR analysis was performed for the detection of the organic functional groups which remain attached to the surface of nanoparticles. The stability of the synthesized nanoparticles were confirmed by zeta potential measurement [2].

Antibacterial activity:

Antimicrobial activity was tested by Agar Well Diffusion method [16]. Ampicillin was used as positive control. Diameter of the zone of inhibition was measured in cm and expressed as Mean ± Standard Deviation.

RESULTS AND DISCUSSION

It was observed that the colour of the reaction mixture (culture supernatant incubated with $AgNO_3$) changed from yellow to brown with time. This indicated the synthesis of nanoparticles. Due to surface plasmon resonance, the silver nanoparticles are reported to produce intense absorption peak in UV absorption spectra [15]. The UV-Vis spectra analysis showed an absorption peak at 255.5 and 313 nm (Fig. 1). TEM analysis revealed the shape and size of the nanoparticles. They were found to be spherical in shape and were less than 50nm in size (Fig. 2). The size distribution of Ag nanoparticles were found to be in average 297.7 nm (Fig 3). Furthermore, the particles carried a charge of −12.6 mV. The negative zeta potential signifies that the nanoparticles were stable. The FTIR spectra (Fig 4) shows peak at 3336.93 cm^{-1}, 1633.31 cm^{-1}, 1478.76 cm^{-1} indicating the presence of capping agents. The sharp peak at 3336.93 cm^{-1} is assigned for N-H stretching vibrations indicating H bonding and the presence of amines [17] and the band at 1633.31 cm^{-1} corresponds to alkanes. The peak at 1478.76 cm^{-1} also corresponds to amine group [18]. Microbes are considered to be suitable agents for synthesis of nanoparticles. Several workers have also successfully synthesized nanoparticles using microbes [15, 19, 20, 21, 22]. The different groups found in bacterial proteins like free amine may be responsible for formation and stabilization of Ag nanoparticles [23, 24]. Results of the antimicrobial activity of AgNPs are shown in table I**.** From the observations, it has been found that the synthesised AgNPs showed effective anti-microbial activity against the tested microbes such as *Staphylococcus aureus, Proteus mirabilis, Bacillus cereus* & *Aspergillus niger*. The maximum zone of inhibition has been found against *Proteus mirabilis* whereas the minimum against *Aspergillus niger*. The result is in accordance with the findings of other workers like Gurunathan et al. [25, 26]. These antimicrobial activity of Ag nanoparticles may be due to its ability to inhibit DNA replication [27]. They are also known to affect the membrane bound enzymes of the microbes [28, 29].

Table I: Antimicrobial activity of Ag nanoparticles

Sl. No.	Microbes	Zone of inhibition (in cm)						
		Day 1	Day 5	Day 12	Day 18	Day 22	Day 26	Ampicillin
1	*Proteus mirabilis*	2.1 ± 0.50	2.3± 0.50	2.7± 0.75	2.5± 0.50	2.5± 1.00	2.9± 0.00	1.3 ± 0.50
2	*Staphylococcus aureus*	1.8± 0.33	2.1± 0.50	2.0 ± 1.00	1.9± 0.50	1.9± 0.50	2.0± 1.00	2.4 ± 0.50
3	*Bacillus cereus*	1.7 ± 0.50	1.9± 0.50	1.8± 0.50	1.9± 0.50	1.9± 0.33	1.8± 1.00	2.8 ±0.50
4	*Aspergillus niger*	1.8 ± 0.50	1.7± 1.00	1.8± 0.50	1.8± 0.75	1.6± 1.00	1.7± 1.00	2.8 ±0.50

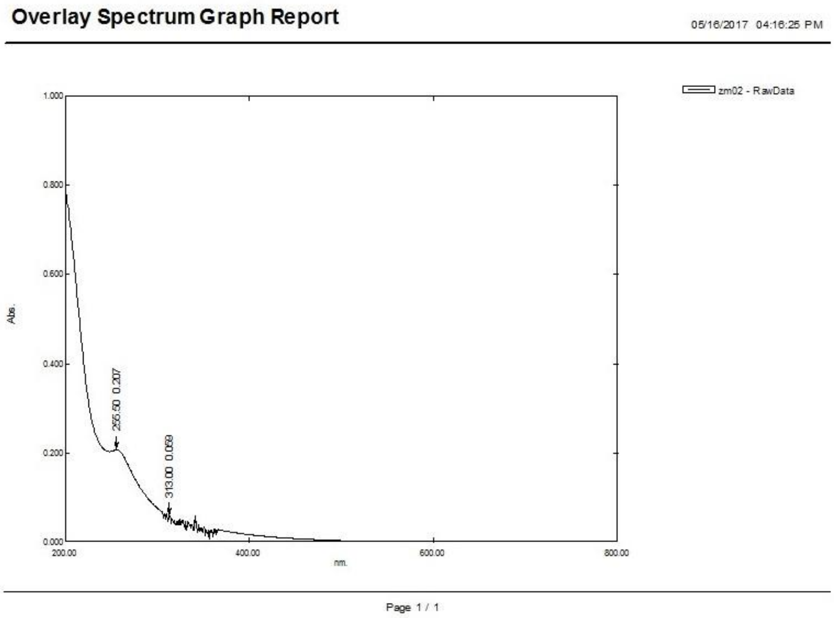
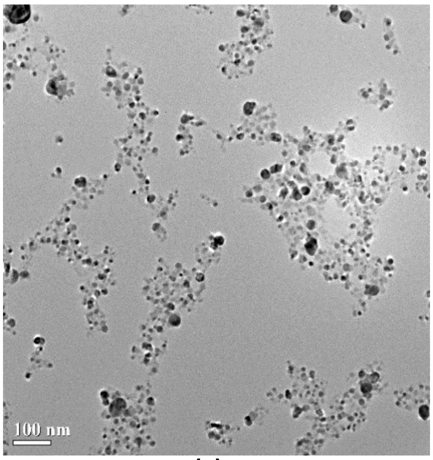
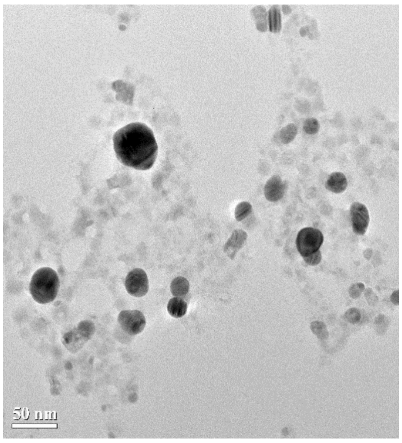


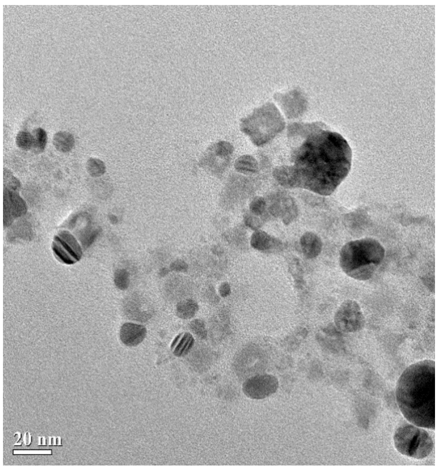
Fig 1: UV–Vis absorption spectrum of silver nanoparticles



(a)



(b)



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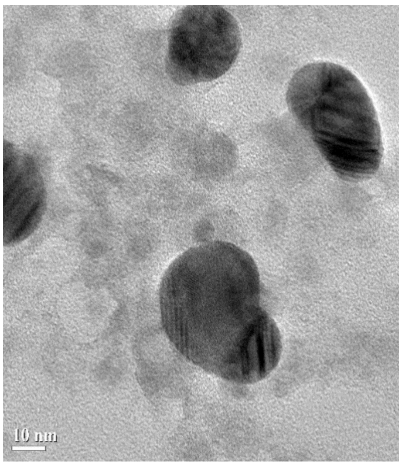

(d)

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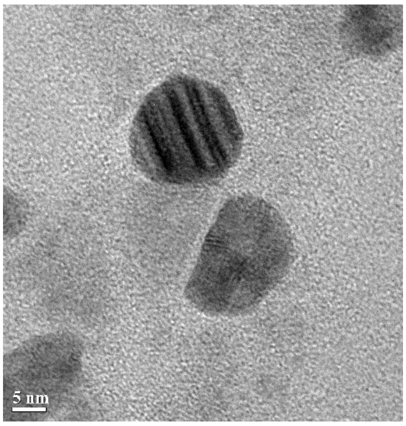
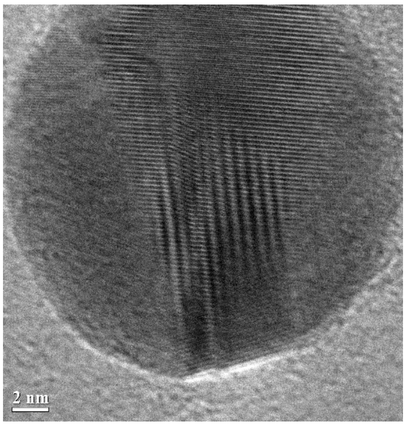
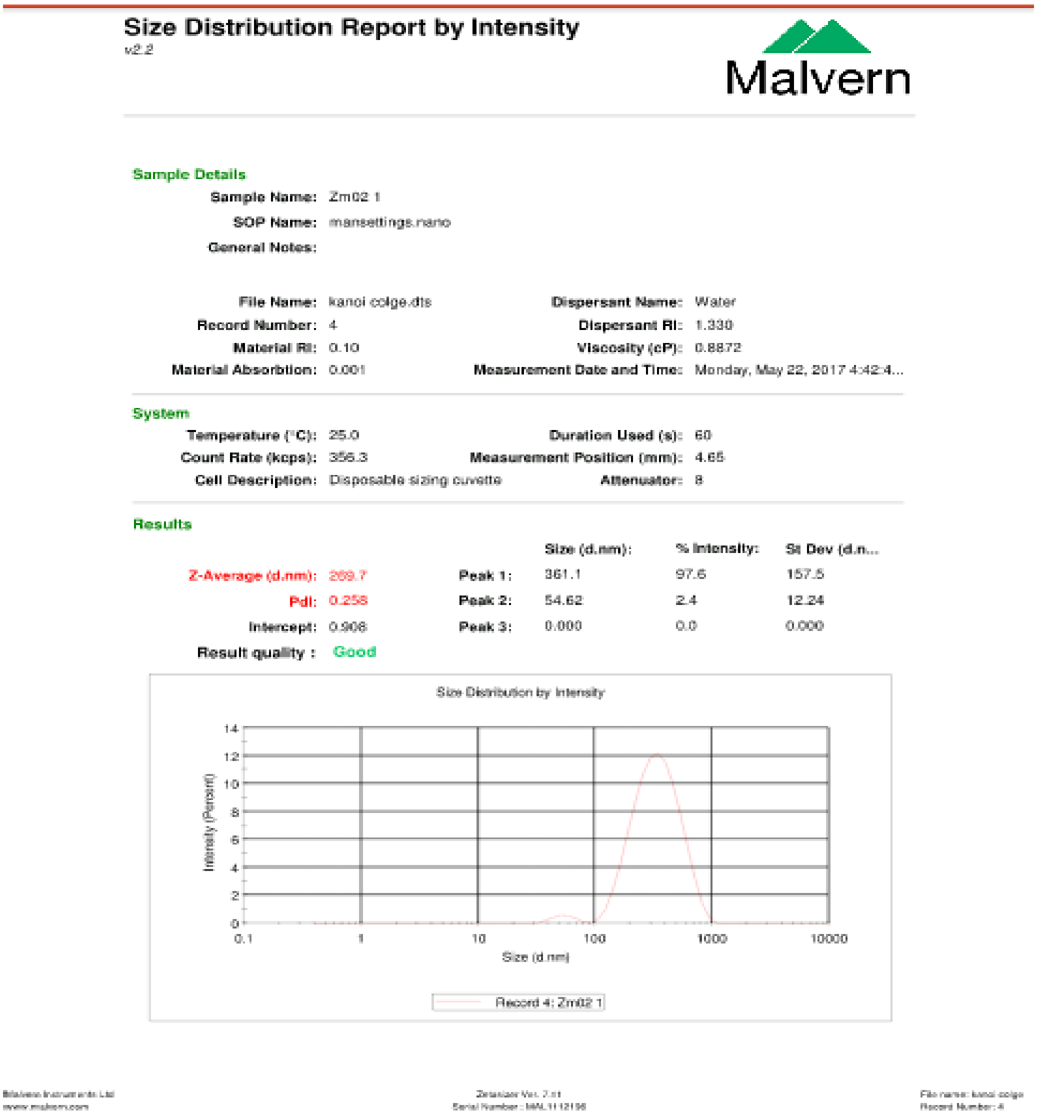




(e) **(f)**

Fig.2 (a-f): TEM images of Ag nanoparticles synthesized by *Escherichia coli*



Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

Sample Name:	Zm02 1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	kanoi colge.dts	Dispersant Name:	Water
Record Number:	4	Dispersant RI:	1.330
Material RI:	0.10	Viscosity (cP):	0.8872
Material Absorbtion:	0.001	Measurement Date and Time:	Monday, May 22, 2017 4:42:4...

System

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	356.3	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	8

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	269.7	Peak 1:	361.1	97.6	157.5
PdI:	0.258	Peak 2:	54.62	2.4	12.24
Intercept:	0.908	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

Fig. 3: Size distribution report of Ag nanoparticles

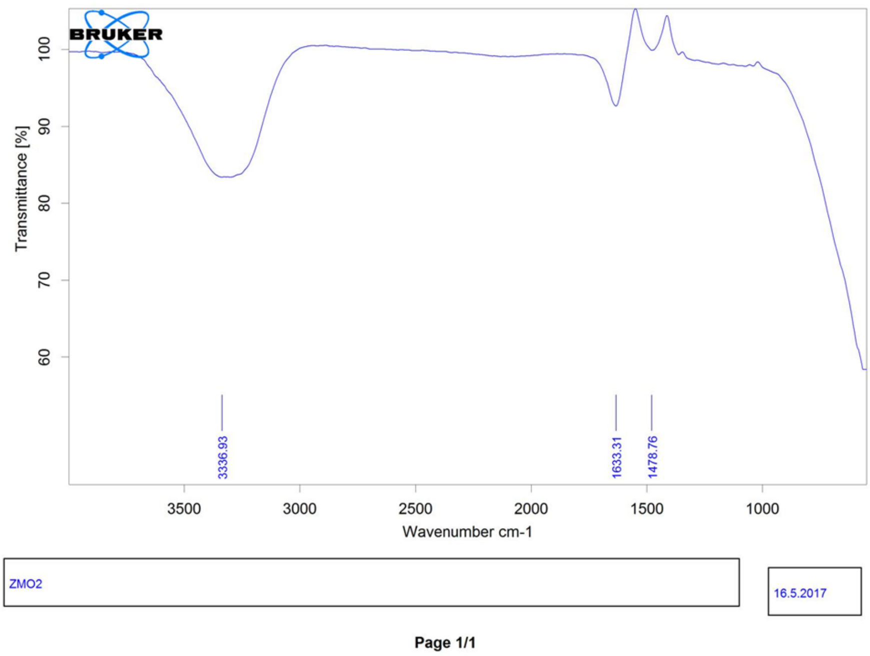


Fig 4: The FTIR spectra of the synthesized nanoparticles

CONCLUSION

The present study thus revealed that *Escherichia coli* can be effectively used to synthesize Ag nanoparticles. From the study it can also be concluded that the synthesized Ag nanoparticles exhibits significant antimicrobial activity against both Gram positive and Gram-negative bacteria and also against the tested fungus.

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