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Antimicrobial Activity of Silver Nano Particles Biosynthesized by *Lactobacillus* Mixtures.

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ABSTRACT

Biosynthesis method of nanoparticles acquires very important area due to their economic and ecofriendly benefits over chemical and physical methods of synthesis. The aim of the present study is the biosynthesis of silver using *Lactobacillus* mixtures and evaluating their antimicrobial. *Lactobacillus* mixture (*L.Acidophillus+L.plantarum*), was used as a source of *Lactobacillus*. AgNPs were biosynthesized by adding silver nitrate ($AgNO_3$) into cell free supernatant for each *Lactobacillus* mixture at concentration (1, 2, 3, 4 and 5 mM) in 1:1 ratio. AgNO3 and were used as precursor for synthesis AgNPs. Biosynthesis of AgNPs was firstly indicated by the color alteration from yellow into reddish brown for AgNPs .The characterization of biosynthesis nanoparticles achieved by UV-Visible spectrophotometry through which the maximum absorbance peak of nanoparticles was observed and it was (410nm), scanning electron microscope(SEM) was used to detect the size shape and distribution of nanoparticles, the shape was spherical and homogenous and the size was ranged between (30-100nm),The occurrence of Ag was analyzed by Energy Dispersive-x-ray Spectroscopy (EDS) analysis. Biosynthesized AgNPs showed antibacterial activity against multidrug resistant bacteria(MDR) of both gram positive and gram negative bacteria (*S.aureus,S.pyogenes, E.coli, E.faecalis, K.pneumoniae, S.typhi, A.baumannii, and P.mirabilis*)in addition to the antifungal activity against *A.niger and P.chrysogenum*.

Keywords: Biogenicsilver nanoparticles,*Lactobacillus*mixtures,Antimicrobial Activity,biosynthesis.

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INTRODUCTION

Nanotechnology is a modern field of science deals with synthesis and application of nanoparticles having a size of 1-100nm. Nanoparticles have been studied extensively because of their unique physicochemical characteristics including antibacterial properties catalytic activity, optical properties, electronic properties, and magnetic properties [1, 2]

Silver nanoparticles have received significant attention because of their antimicrobial activity and prevention the biofilm formation, as well as their unique biological properties, chemical, physical, and their applicability in electronics, and medicine [3, 4]

There are several number of chemical, physical, biological and hybrid methods available for synthesizing different types of nanoparticles, First and second methods which are expensive, energy consuming and potentially toxic to the environment [5]. Development of reliable, nontoxic, and eco-friendly methods for synthesis of nanoparticles are the most important to expand their biomedical applications. One of the options to achieve this goal is to use microbes to synthesize nanoparticles [6, 7]. The extracellular synthesis of silver nanoparticles using *Lactobacillus* species appears to be low cost effective and eco-friendly [8-10] and to develop new effective antimicrobial agents that overcome the multiple antibiotics resistance of microorganisms [11] *Lactobacillus* have probiotic functions, due to their antimicrobial and antioxidative properties ,adjusting the balance of intestinal flora, reducing blood cholesterol, inhibiting and reducing the risk of tumors and cancer, stimulating the immune system, stimulation of Vitamin C production and enhancement of digestion [12] Therefore the present study has been designed to biosynthesis of silver nanoparticles using *Lactobacillus* mixture species and studies their antimicrobial.

EXPERIMENTAL

Culture of *Lactobacillus*

The *Lactobacillus* mixture was obtained from pharmacy named vitalactic B, (L.*Acidophillus+ L.plantarum*) was used as a source of *Lactobacillus*. De Man Rogase and Sharp broth (MRS broth) was inoculated with *Lactobacillus* mixture and incubated under anaerobic condition using anaerobic jar with anaerobic gas back system at 37°C for 48 hrs. Colonies were picked up and confirmed as *Lactobacillus* depending on morphological and biochemical tests [13] .The second activation was worked from first activation at 37°C for 24 hrs [9].

Preparation of cell free supernatant of *Lactobacillus* mixture

After 24 hrs of incubation, the culture was centrifuged at 4500 rpm for 10 minute to prepare the cell free supernatant from *Lactobacillus* mixture. After centrifugation the cells were precipitated at the bottom of tube were discarded and cell free supernatants were collected for using in the synthesis of silver nanoparticles [9].

Biosynthesis of silver nanoparticles using cell free supernatant

$AgNO_3$ was precursor for biosynthesis of silver nanoparticles by *Lactobacillus*. $AgNO_3$ was added with concentration (1, 2, 3, 4 and 5 mM) to cell free supernatant which mixed well in ratio 1:1 .This step was prepared in dark condition to avoid oxidation of $AgNO_3$.The pH of the mixture was adjusted to (8.3). The resultant solutions were incubated in shaking incubator 150 rpm at 37° C for 24 hrs.

After incubation the color change was observed and the reaction mixture was centrifuged at 10000 rpm for 10 minute ,the supernatant was discarded and replaced with deionized distill water and re centrifuged three times at the same speed and time to remove remained supernatant ,the pellet deposit at bottom of tube which represent collection of nanoparticles then dried in oven at 40 °C for 18-24 hours. The dried powder was collected carefully and stored in sample vials for further analysis [9,14].

Characterization of silver nanoparticles

UV – Visible spectrophotometer Analysis

Color alteration of the mixtures was observed by measuring UV- visible spectrum of the mixture after periodically diluting the mixture with deionized distill water and subsequently measured by UV-Vis spectrophotometer [15].

Silver nanoparticle were analyzed using UV-Vis spectrophotometer (Shimadzu,1600) in the central laboratory of pharmacy college / kufa university.

Analysis by Scanning electron microscope (SEM)

Scanning electron microscope (Inspect S50. FEI) was used for characterization the morphological and size of nanoparticles in electron microscope unit, Faculty of Science/ Kufa University. Preparation the slides by adding small drop of suspension of biosynthesis nanoparticles on slide, left for drying and then analyzed by (SEM), The microscope worked at an accelerated voltage at 5-10 KV and different magnification, low vacuum, a spot size 4 and working distances5-10mm(15)

Energy Dispersive-x-ray Spectroscopy (EDS) analysis

Elemental analysis of single particle was carried out using Bruker EDS attached with SEM in electron microscope unit, Faculty of Science/ Kufa University. EDS performed for point analysis with accelerating voltage 10 KV, spot size 5, working distances 10mm, this analysis was used to detect presence of elements nanoparticles [14].

Antibacterial activity of nanoparticles

Antimicrobial activity of biogenic AgNPs was carried by agar well diffusion method against different kinds of pathogenic multidrug resistant bacteria of both gram positive and gram negative(table 1).Standardized suspension of each tested bacteria ($1.5x10^8$cfu/ml) by McFarland standard (0.5N) then swabbed separately onto sterile Muller-Hinton Agar (MHA) plates using sterile cotton swabs.

Agar was punched with sterilized cork borer 6 mm and 50µl from different concentration (200,300,400and 500µg/ml) of AgNPs was added into each well. One petridish sub cultured for each pathogenic bacterium and used as control and incubated for 24 hrs at 37°C, after incubation the inhibition zones were measured [16].

Antifungal activity of nanoparticles

Biogenic AgNPs was assayed against pathogenic fungi, *Aspergillus niger* and *Penicillium chrysogenum* using agar well diffusion method.

After making dilution from fungal growth of each tested pathogenic fungi, spread on sabouraud dextrose agar by cotton swab from dilution, 10^5. Agar was cut with sterilized cork borer 6 mm and 50µl from different concentration of AgNPs were added into wells as the same concentrations that used in antibacterial activity. One Petridis sub cultured for each fungus as control. After incubation for 3days at 28°C, the inhibition zones were measured [16].

RESULT

Biosynthesis of Silver nanoparticles

Lactobacillus mixture showed their ability in the extracellular biosynthesis for silver nanoparticles (AgNPs) using cell free supernatant and Silver nitrate($AgNO_3$) as a precursor for synthesis AgNPs it was added in a different concentrations to cell free supernatant of each *Lactobacillus* mixture and after shaking incubation for 24 hrs at 37°C , *Lactobacillus* mixture have the ability in changing the color (figure 1) of reaction

mixture from yellow to reddish brown which represents as indicator for biosynthesis the AgNPs by *Lactobacillus* mixture.

UV-visible spectrophotometer analysis

UV- Visible spectrophotometric is a proven technique for the analysis of nanoparticles. After 24 hours, color change was observed which indicated formation nanoparticles in the reaction mixture .The biosynthesis of nanoparticles can be confirmed by visual observation and measuring the surface plasmon resonance (SPR) band using UV-vis spectroscopy.

The absorption band of nanoparticles formed in the mixture has an absorption peak at 410 nm for AgNPs (figure 2) this evidence of the presence of surface plasmon resonance (SPR) of nanoparticles and a single SPR band indicates that nanoparticles have spherical shape.

SEM analysis of nanoparticles

Scanning electron microscope (SEM) was used to characterize the shape, size and distribution of nanoparticles .After biosynthesis of silver nanoparticles using cell free supernatant of each *Lactobacillus* mixture. The results showed well-dispersed nanoparticles and homogenous with diameter of (30-100nm) for silver nanoparticles, with variable shapes most of them present in spherical form (figure 3).

The concentration of AgNO3 which was added to the cell free supernatant also have effect on characterization of the formed nanoparticles, 4mM AgNO3 was the best concentration in the biosynthesis of AgNPs in comparison with other concentrations .

EDS analysis of nanoparticles

The occurrence of element was quantified by Energy Dispersive-x-ray Spectroscopy (EDS) analysis through observing the optical absorption peaks of silver and titanium elements. The presence of elemental silver which indicated the reduction of silver ions in reaction mixture by *Lactobacillus* supernatant . The EDS spectrum was recorded in the spot- profile mode, strong signals from the Ag atoms are observed while medium signals from oxygen and weaker signals from other atoms. The weight percentage of elemental constituents for AgNPs that was67.35%silver (figure 4). The peak of AgNPs was detected at 3Kev which is atypical absorption of metallic AgNPs. Depending on characterization of nanoparticles by the color change, Uv-visible spectrophotometer, SEM and EDS. The morphology, size, distribution and presence of metals nanoparticles were characterized and a consequence .AgNO3 (4mM) was used for further study.

Antibacterial activity

Antibacterial activity of AgNPs were biosynthesized by(LAB mix1)respectively were used to evaluate their ability in inhibition growth of multidrug resistant bacteria(MDR) (table 1).Method was used for detecting the antibacterial activity of nanoparticles using different concentration of AgNPs .After incubation the inhibition zone was measured in mm of each concentration of AgNPs ,results showed that AgNPs has the ability to inhibit the bacterial growth and appeared their antibacterial activity against G+ve and G-ve bacteria. The inhibition zone was greater in gram negative than in gram positive bacteria(table 2). The largest inhibition zone of AgNPs was (23mm)in *E.coli* with concentration (500µg/ml) while the largest inhibition zone in S. aureus was (13mm)with same concentration ,in addition the antibacterial activity in gram negative bacteria was also different in their sensitivity to AgNPs when exposed to the same concentration such as *E.coli, K.pneumoniae and E.faecalis*, the inhibition zone of these bacteria was (19, 15 and 12 mm) respectively by 400µg/ml of AgNPs. There is no antibacterial activity of AgNPs against *P. aeruginosa*. Also it was observed when increased the concentration of AgNPs the inhibition zone increased, (500µg/ml) showed large inhibition zone than 200µg/ml, 300µg/ml and 400µg/ml respectively (table2) and (figure5)..

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Antifungal activity

AgNPs that biosynthesized by (LAB mix1)was used to detect their ability in inhibition the growth of selected pathogenic fungi *A.niger* and *P.chrysogenum* by agar well diffusion method , the same concentration of AgNPs that used in antibacterial activity was used for antifungal activity against these pathogenic fungi and added into wells. After incubation for 3days at 28°C,the inhibition zone was measured the antifungal activity was observed increase with increasing concentration of AgNPs, the largest inhibition zone was(20mm)and(18mm)at500µg/ml for *A.niger and P.chrysogenum* respectively(Table 3),(figure 6)

Table 1: Pathogenic microorganism used in present study

Pathogenic bacteria	Pathogenic fungi
Pseudomons aeruginosa	*Aspergillus niger*
Proteus mirabilis	*Penicillium chrysogenum*
Salmonella typhi	
Klebseilla pneumoniae	
Escherichia coli	
Acinetobacter baumannii	
Enterobacter faecalis	
Staphylcoccus aureus	
Steptococcus pyogenes	

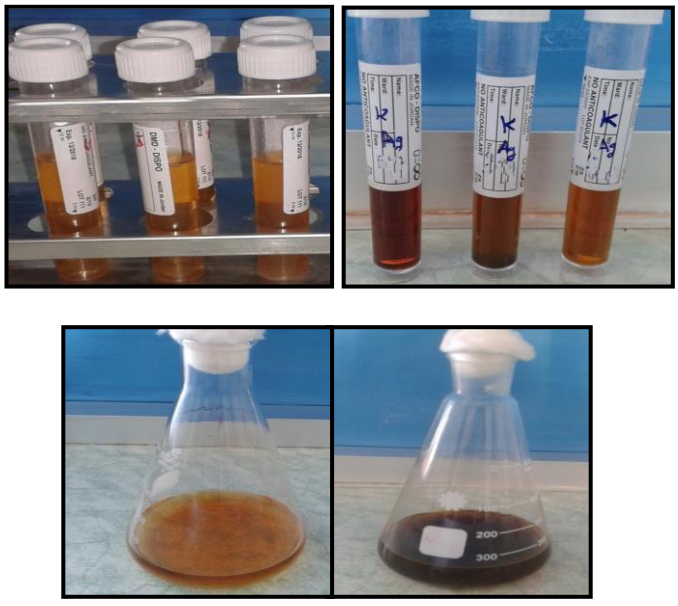

Figure 1: Biosynthesis of Silver nanoparticles.

(A) Original color (yellow) of supernatant without $AgNO_3$, (B) color change (reddish brown) of supernatant after adding (2 , 3 and 4 mM)of $AgNO_3$ and incubate the reaction mixture at 37° C for 24 hrs in shaking incubator (150 rpm), (C) Biosynthesis of AgNPs after adding 4mM of $AgNO_3$ into supernatant and incubation by shaking incubator at 37°C for 24 hrs

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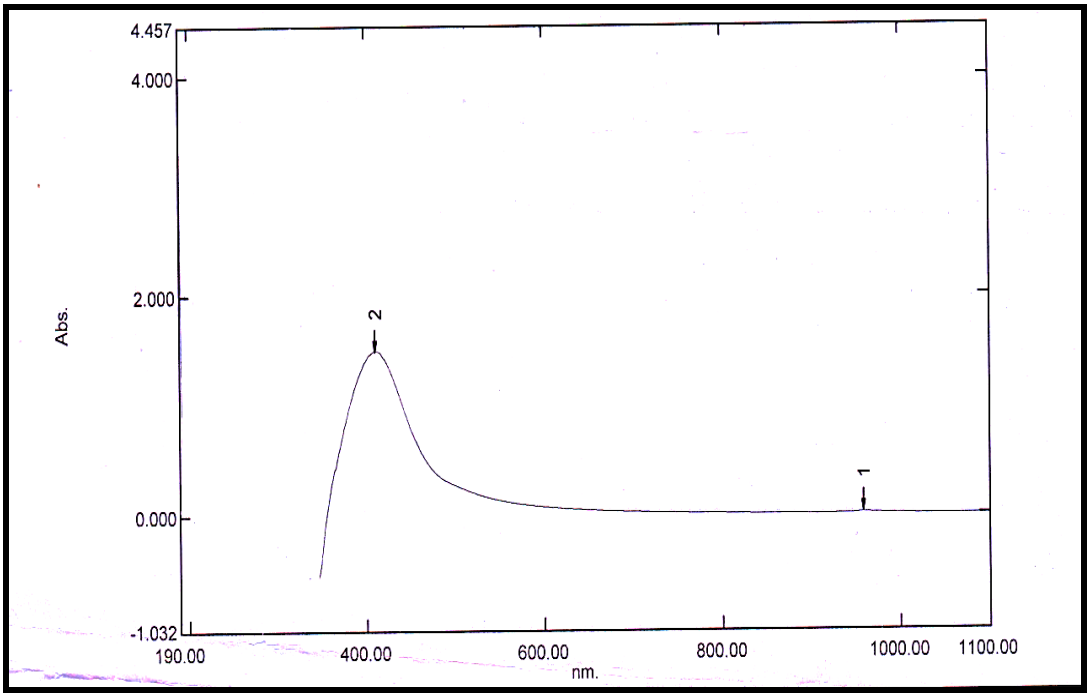

Figure 2: UV–visible absorption spectrum of biogenic silver nanoparticles The absorption spectrum of silver nanoparticles exhibited a strong broad peak at 410 nm.

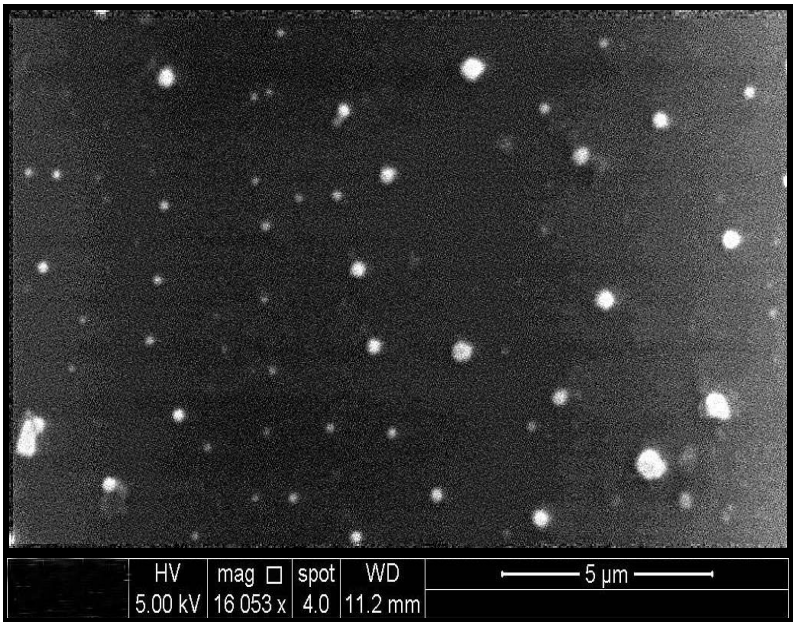

Figure 3: SEM micrograph of silver nanoparticles. The shape of AgNPs was spherical and homogenous, size between (30-100nm).

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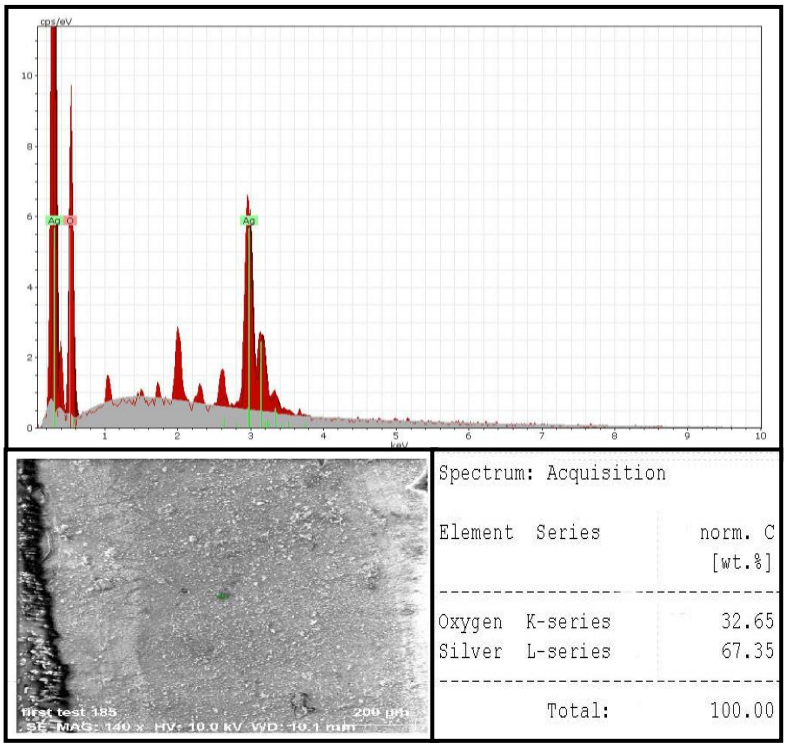

Figure 4: EDS analysis of biogenic silver nanoparticles: Illustrated strong signals from the Ag, medium signal from O2, the optical absorption peak of Ag was observed at 3Kev, the weight percentage of silver (67.35%).

Table 2: Zone of inhibition of pathogenic bacteria in mm by silver nanoparticles

Pathogenic bacteria	Concentration of AgNPs			
	200 µg/ml	**300µg /ml**	**400 µg /ml**	**500µg/ml**
E.coli	12	16	19	23
K.pneumoniae	0	14	15	19
E.feacalis	0	12	12	14
S.typhi	12	15	16	18
A.baumannii	0	12	13	17
P.mirabilis	0	13	15	16
P. aeruginosa	0	0	0	0
S.pyogenes	11	11	12	12
S.aureus	11	12	12	13

Table 3: zone of inhibition of pathogenic fungi in mm by silver nanoparticles

Pathogenic fungi	**AgNPs concentration**			
	200 µg/ml	**300 µg/ml**	**400 µg/ml**	**500 µg/ml**
A. niger	12	12	13	20
P.chrysogenum	11	13	14	18

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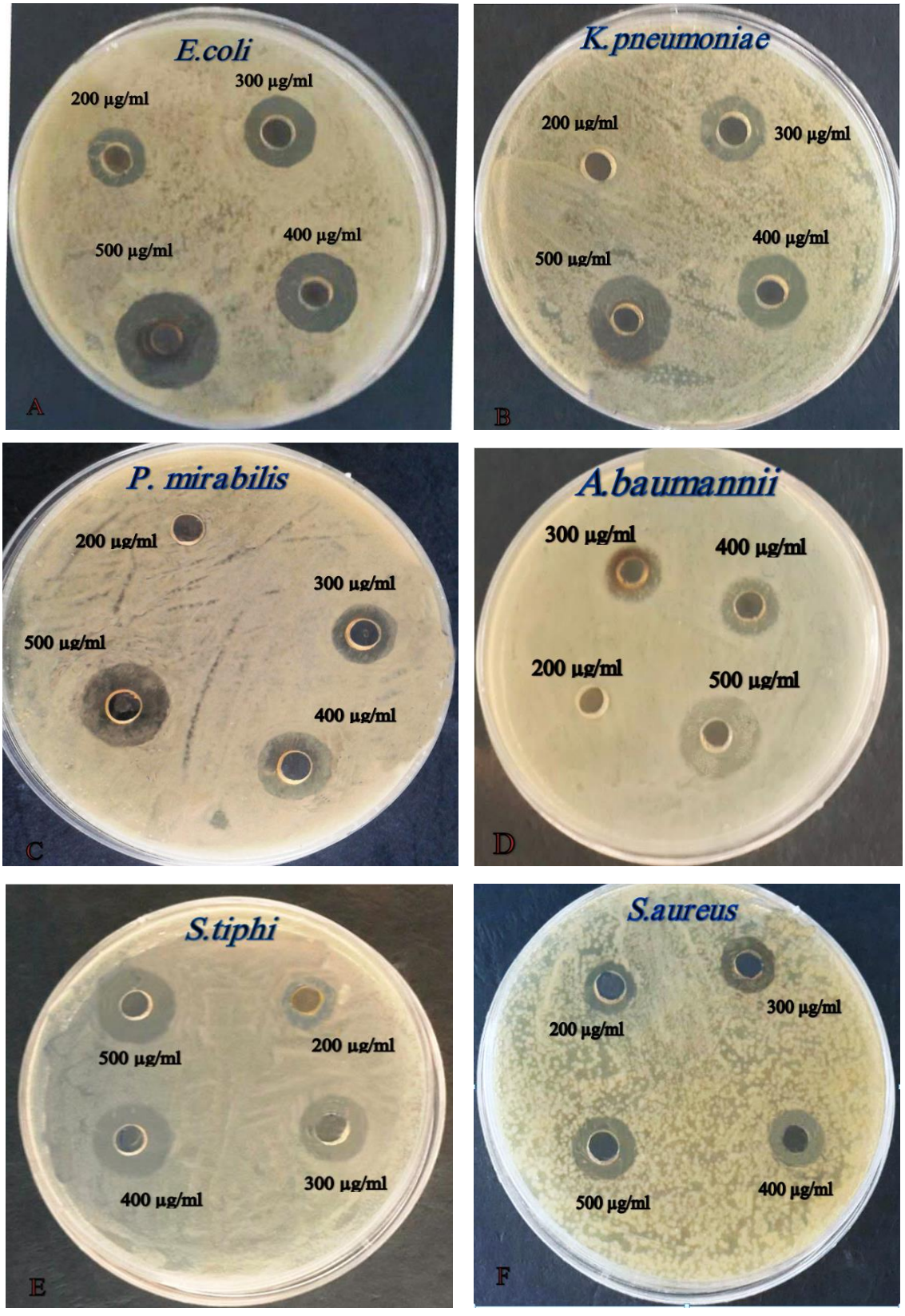

Figure 5: Antibacterial activity of silver nanoparticles

Against :A) *E.coli* (B)*K.pneumoniae,(C) P.mirabilis,(D) A.baumannii ,(E)S.typhi,(F) S.aureus* with concentration(200,300,400 and 500µg/ml), cultured on MHA at 37°C for 24 hrs.

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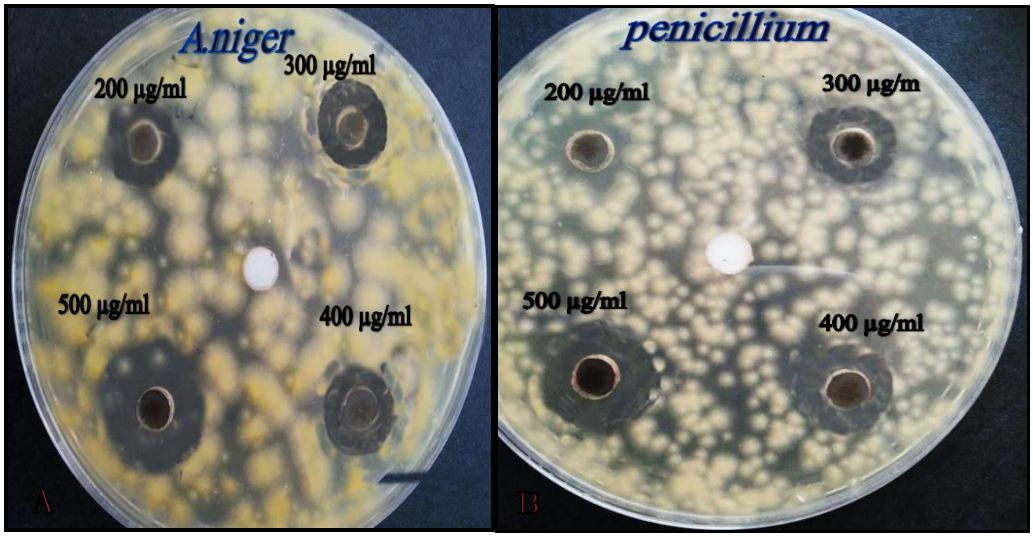

Figure 6: Antifungal activity of silver nanoparticles
Against :(A)*A.niger*(B*) P.chrysogenum* with concentration(200,300,400 and 500µg/ml)

DISCUSSION

In this study, silver nanoparticles were biosynthesized by supernatant of *Lactobacillus* mixtures (*L.acidophillus+L.plantarum*).Morphological and biochemical characterization were confirmed as *Lactobacillus*. When reaction mixture containing supernatant of *Lactobacillus* mixture and different concentration of $AgNO_3$ was added under dark conditions and adjustment of pH to 8.3, the medium color was changed from yellow to reddish brown (figure 1). The form of a brown color in solution is a suggestion of the creation of silver nanoparticles in the mixture due to reduction of Ag+ ions to Ag metal by the reducing agents (enzymes, proteins, amino acid, polysaccharides etc) in the cultural supernatants, The color displayed by metallic nanoparticles is a result of the coherent excitation of entire free electrons within the conduction band, leading to surface plasmon resonance (SPR) [14,17, 18].

Cell free culture is the simplest and easiest techniques for the size-controlled synthesis of silver nanoparticles. The supernatant can be modified and maintained, where the components inside the cell would try to preserve constant environment such as heatshock proteins and need more steps of purification that why, supernatant used for the biosynthesis of silver nanoparticles instead of whole cells itself [19].

Not all the organisms are capable of synthesis of silver nanoparticles; the precise way leading to the development of silver nanoparticles by all organisms is yet to be elucidated. The organisms which contain the "Silver resistance machinery" can synthesize silver. The reduction of silver ions by combinations of biomolecules found in extracts "such as enzymes, proteins, amino acids, polysaccharides and vitamins is environmentally benign and chemically complex". [20].

Nicotinamide adenine dinucleotide (NADH-) and NADH-dependent enzymes play a key role in the biosynthesis of nanoparticles. The reduction seems to be initiated by electron transfer from the NADH by NADH-dependent reductase as electron carrier [21]. *Lactobacillus* is known to produce nitrate reductase only above pH 6 which may be responsible for bioreduction of Ag+ to Ag0 and the consequent formation of AgNPs [22].

There is a possibility that the aldehydic group present in the extra polysaccharides secreted by the *Lactobacillus*sp might be involved in the reduction of silver ions to zero valent silver (Ag0) [23]

The size and shape of the nanoparticle can also be affected by controlling the environment (pH and temperature), at room temperature silver nanoparticles of 50 nm are synthesized whereas at 60°C nanoparticles of 15 nm are synthesized. Similarly at acidic pH the size of the nanoparticle ranged 45 nm

whereas at pH 10 the size is just 15 nm. "At acidic pH and lower temperatures there will be less nucleation for silver crystal formation on which new incoming silver atoms deposit to form larger sized particles. But as the pH and temperature increase, the dynamics of the ions increase and more nucleation regions are formed due to the availability of –OH ions and increased temperature.The conversion of Ag+ to Ag0 increases followed by increase in the kinetics of the deposition of the silver atoms". [19].

Alkaline condition of the silver nanoparticle synthesis will be faster than in acidic conditions. In other words, synthesis enhances as the pH increases towards alkaline region and reaches the maximum at pH 10 after which the AgNPs synthesis decreased due to the alkaline ion (-OH) is very much required for the reduction of metal ions, also under alkaline conditions the ability of the enzyme responsible (not only nitrate reductase) for the synthesis of silver nanoparticles increases [24].

Characterization of silver and Titanium nanoparticles

The Characterization of biogenic nanoparticles was performed by UV-visible spectroscopy, scanning electron microscope (SEM) and Energy Disperse Spectroscope (EDS) analysis. The preliminary confirmation of the extracellular biosynthesis of silver nanoparticles was gained by the contrast color change which belongs to the SPR phenomenon. The our results was also observed with the UV spectroscopic study of the colloidal solution, the values of maximum absorption peak of the silver nanoparticles was focused at 410 nm (figure 2)

The results shed the plasmon resonance is sharp and point to little aggregation of the particles in solution. The absorption of brown color due to excitation of surface plasmon vibration in particles, surface plasmon absorption strongly depends on the particle size, shape dielectric medium and chemical surrounding the UV-Vis absorption spectra of nanoparticles dispersed in water [25]

The increase in intensity could be due to increasing number of nanoparticles formed as a result of reduction of silver ions present in the aqueous solution. This peak intensified shows that a lot of silver nanoparticles are reduced from $AgNO_3$. The information that silver nanoparticles peak stayed close to 400 nanometer signpost that the particles were well distributed in the solution and there was not much aggregation [26].

SEM determine the size and shape of biogenic nanoparticles, experimental results showed well-dispersed nanoparticles with diameter of 30-100nm, with varying shapes most of them present in spherical form (figure 2) these results related with [27].

The difference in shape and size of nanoparticles that synthesized by biological systems is public. If the biological process of silver nanoparticles is to be a possible alternative to the chemical method, then greater control over particle size and polydispersity would be essential [26].

EDS analysis detect the presence of elemental silver which indicated the reduction of silver ions to silver metal in reaction mixture, the weight percentage of silver was 67.35% for (figure 4),The absorption peak was detected at 3Kev which is a typical absorption of metallic AgNPs, our finding agree with results of [15]

The morphology of a material is really crucial. The toxicity is greatly dependent upon the size and shape of the metal. Among the noble metals, nano-sized Ag has antimicrobial effects. Since, they are not only toxic (to microbes), but also serve as slow releasing metal ion particles. Hence, in this case, if there concentration exceeds beyond the acceptable limits (even in nano form), they become noxious. Moreover, "the small particle size, a large surface area and the ability to generate reactive oxygen species play a major role in toxicity of NP. The interaction with the cells is size-dependent and seems to depend also on the shape of the particles". [28].

Nano-Ag looks to be more toxic than Ag+ ions towards *E. coli*. Nature of the material compounds/elements and the amount used (concentration) also have emotional impact the toxicity [29].

Antimicrobial activity of nanoparticles

The occurrence and rise the cases of multiple antibiotic-resistant microbes, Nanoparticles are at the present reflected an alternate solution to antibiotics and look to have a high potential to solve this problematic. AgNPs were considered for the most part attractive for the construction novel agents of antimicrobials [30, 31]. The AgNPs were tested to evaluate their antibacterial activity against MDR of Gram-positive and gram negative bacteria and antifungal activity by agar well diffusion method. The results appeared AgNPs have the antibacterial against tested pathogenic bacteria except *p. aeruginosa* (table 2), (figure 5) and antifungal activity (table 3) and (figure 6).There were large inhibition zone in G-ve in comparison with G+ve bacteria, the maximum inhibition zone in *E.coli* was 23mm with concentration (500µg/ml) of AgNPs while the maximum inhibition zone in *S. aureus* was 13mm in the same concentration , this variance was perhaps credited to the difference of the peptidoglycan layer of the bacterial cell between G+ve and G-ve bacteria. The G-v cell envelope comprises of outer membrane, tinny peptidoglycan layer, and cell membrane. Whereas, G+ve envelope made up of lipoteichoic acid comprising thick peptidoglycan layer and cell membrane [32, 33]. The thick peptidoglycan layer of G+ve may protect formation of pits but increased cell membrane permeability can be indication for the construction of Ag-NPs pits in the cell walls [3,4]. Beside this, there is variances in the sensitivity of tested pathogenic bacteria to AgNPs when exposed to the same concentration(400µg/ml) of AgNPs such as *E.coli, K.pneumoniae* and *E.faecalis*, the inhibition zone of these bacteria was 19,15 and12 mm respectively ,this may be return to the differences in intrinsic susceptibility of bacterial species depends on the concerted activity of several elements, what has been named as intrinsic resistome [35]. As well as when increase concentration of silver nanoparticles appeared increase in antibacterial activity, these results similar with [34]. The mode of action of the inhibitory activity of Ag ions on microbes is incompletely known. It is stated that the + charge on the silver ion is the cause for antimicrobial activity as it can interest the negatively charged cell membrane of microbes through the electrostatic interaction. This attraction probably overpowers other aspects, such as size and shape that can impact the bacterial cell death [36].

The steady relief of silver ions from silver nanoparticles is a critical action of silver nanoparticles that should be interested prior to synthesis [37]. Ions of silver interact with protein and nucleic acid (negatively charge) initiating structural alterations and deformation in the cell wall, in the membrane and in the nucleic acids of cells. Silver ion bind with electron donor functional groups (such as phosphates, hydroxyls, thiols, imidazoles and indoles). The AgNPs also destruction membranes and lead to the release of ROS, producing free radicals with powerful bactericidal action [38, 39] .Silver ions are acknowledged to prevent function of number of enzymes such as NADH dehydrogenase II in the respiratory system, which is a candidate for the site of making of reactive oxygen species [40]. Small sized nanoparticles showed more antibacterial activity than large size particles because small sized particles affect a large surface area of the bacteria. Bactericidal activity of AgNPs of smaller dimensions (<30 nm) was found to be optimal against *S. aureus* and *K. pneumonia* [41].

Several studies proposed that AgNPs interfere with bacterial replication by binding with their nucleic acids. The interaction of silver ions with sulfhydryl (–SH) groups of proteins that cause the DNA unwinding, and contact with hydrogen bonding processes are also been demonstrated lead to cell division was inhibited [42] .Ribosomes may be denatured by silver ions or small AgNPs as a consequence prevent protein synthesis. Translation and transcription can be inhibited by the binding the AgNPs with the genetic material of the bacterial cell [43]. The nanoparticles can modify the signal transduction in bacteria. Nanoparticles can dephosphorylate the peptide substrates on tyrosine residues, which cause signal transduction inhibition and thus the inhibition of growth [44]. The inhibition of tested pathogenic fungi (*A.niger* and *P.chrysogenum*) by AgNPs increased with the increase of the silver nanoparticles concentration ,the maximum inhibition zone was in 500µg/mlAgNPs (table 3)and (figure 6) due to the ability of AgNPs to saturate and adhere to the fungal hyphae (Kim et al.,2009) also suggested that Ag-NPs inhibit the normal budding process, probably through the destruction of membrane integrity [45, 46]. pH-Dependent biosynthesized AgNPs plays important role in the antibacterial activity by these nanoparticles, the smallest nanoparticles synthesized in alkaline pH showed more antibacterial activity than large particles which are synthesized in acidic pH [47]. Hexagonal AgNPs display the highest antibacterial effect when compared to other NPs shapes, this was attributed to the specific surface areas and facets reactivity [48]. Organisms generally possess mechanisms for detoxifying metals. This includes exclusion from the cell, isolating the metal in the cytoplasm by concentrating it in granules, precipitating it in the cell wall, or transforming it (e.g., by oxidation or reduction) into a harmless form in the organism. Microbes are able to adsorb or complex metals because of the presence of a large number of

chemical sites to which these metals may bind. Metal accumulation in organisms is a very rapid process, with over 90 percent occurring within 10 minutes of the first contact with the metal ions. Organisms that are capable of complexing metal with extracellular polymers and cell wall polymers can survive in aqueous environments that are heavily laden with levels of metallic ions that would be toxic if retained in the cytoplasm [49].

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