Study of Analytical Characterization and Antibacterial Activity of Silver Nanoparticles Synthesized by Using Pineapple Juice as a Reducing Agent

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Abstract

In the past years, various phytosynthetic processes have been utilised of silver nanoparticles for the low cost and environmentally friendly comparing to the chemical and physical methods. These unique metal nanoparticles are used in several applications which are including pharmaceuticals, antimicrobial and biomedical applications. Wherefore, the object of this work is biosynthesizing AgNPs from extracts of pineapple juice as reducing studying their characteristics by Ultraviolet-Visible (UV-vis) Spectrometer, Atomic Force Microscope (AFM), SEM Scanning Electron Microscope and Transmission Electron Microscope (TEM) The UV-Vis spectra results show a strong resonance centered on the surface of silver nanoparticles (AgNPs) at 410 nm. the presenting study is involved the preparation of (AgNPs) from natural source, which confirmed by UV-Visible spectral analysis in 410nm and the synergism effects with some antibiotic to increase the sensitivity to some pathogenic bacterial isolate as *E. coli*. In addition, our study is presented results of the minimum inhibitory concentration (MIC) that MIC for AgNPs is 25 while other antibiotics are (32,64,128). Finally, the synergism activity shows a significant difference in the sensitivity of some antibiotics used against *E. coli* bacteria, especially with levofloxacin and increased the inhibition form 419 mm to 1657.2 mm.

***Keywords**: AgNPs, pineapple juice, UV-Vis, E. coli, synergistic role.*

Introduction

Nanoparticles (NP) are atomic or molecular aggregates with dimensions between 1 nm and 100 nm [1,2]. NPs great Have a great scientific interesting because have a bridge between bulk material and atomic or molecular structure [3]. There are different processes to prepare silver nanoparticles (AgNPs), such as conventional temperature assisted process, controlled reaction at high temperatures, and microwave assisted process [4]. Green method for the synthesis of AgNPS with plant extracts plays a very important role in nanotechnology without any harmful chemicals (5). Recently, some plants were used to synthesize silver nanoparticles including Ziziphus mauritiana [6] and Cyperus sp. [7]. The Green synthesized is by using pineapple juice. Pineapple [Ananas comosus (L.) Merr.], is the third most important fruit crop in the tropical and subtropical regions of the world, it is only preceded by banana and citrus [8]. Pineapple has an important source of sugars, organic acids and the most important is considerating as an antioxidant, such as ascorbic acid, flavonoids, and other phenolic compounds related to antioxidant activities [9,10]. UV-vis spectroscopy is rapid, easy, sensitive, selective technique for various types of NPs and the measurements of this technique are consuming short times [11, 12].

Experimental section

Materials: Research chemicals were supplied by MERCK Company-Germany.

Instruments: UV-Vis spectroscopy (Shimadzu, Japan), Atomic force microscope (AFM); (SPM AA

3000, USA); Transmission electron microscope (TEM); (Philips CM 100, Holland) and (Scanning Electron Microscope (SEM) FEI Nova NanoSEM 450.

Synthesis of silver nanoparticles using pineapple juice

Only, 440 ml of pineapple juice aqueous was added to 240 ml distilled water in conical flask, the solution was heated to 50 -60 °C. 10 ml of aqueous solution was added to 1mM $AgNO_3$ with stirring. the color of the solution will change to orange after 15 min. This indicates for the formation of $AgNP_S$.

Antibacterial activity

Isolate

E. oli isolates were obtained from microbiology laboratories at the Faculty of Science, Anbar University. the isolation was confirmed by using some of the biochemical investigations that included with growth of the nutritious broth and Macconky agar. In addition to carrying out some special tests including Gram stain and some biochemical tests after that the isolation was diagnosed using VITEK 2.

Sensitivity test

A number of antibiotics were used by Bioanalyse company. Where they were followed the method of spreading around the tables on Mueller Hinton Agar fertilized with *E. coli* bacteria [13].

The minimum inhibitory concentration (MIC).

The values of the minimum inhibitory concentrations (MIC) were determined by broth dilution technique in microtiter plates and this technique is based on the Clinical and Laboratory Standards Institute (CLSI) guidelines [14] for each compound: Ag+ ion, AgNPs, chitosan, and the antimicrobial drugs (Gentamycin,Amikacin, Doxycycline, Levofloxacin, Trimethoprim,Cefixime) against Grum-positive bacteria E.coli. The MIC investigations, the bacterial suspension 0.5 McFarland (approximately 108 colony forming units per mL) were prepared from E. coli bacteria in Mueller Hinton agar, at 37 °C, for 24 h. It was added 150 µL of bacteria suspension and 150 µL of the antibacterial agent solution in a polystyrene microtiter plate, both solution was diluted in Mueller Hinton broth. The concentrations of the compound are ranged between (0.03-64 µg mL-1). The plates were incubated for 24 h and the bacteria growth was determined by turbidity and color change in the system by adding the non-enzymatic marker (resazurin aqueous solution 0.01 g L-1). Resazurin turns from dark blue to colorless under the metabolism of bacteria [15].

Results and Discussion

Characterization

The formation of silver particles (AgNPs) was confirmed after 15 min at 50-60 °C by the color change of the solution and the surface plasmon resonance (SPR) band which obtained by UV-Visible spectrum with 410 nm [13]. The optical absorption spectra are normally determined by a UV-visible spectrophotometer. The function of UV-visible absorption is based on measuring the intensities of two transmitted beams, one beam transmitted from the sample and another transmitted from the reference cavity**.** UV-Visible spectral analysis of the bio-synthesized AgNPs from pineapple juice and their color were checked in this test to confirm the formation of AgNPs [16]. The color change of the mixture solution from yellow to the brown color at peak 410 nm,and that agree with results of [6, 7].

The atomic force microscope (AFM) is used to differentiation the NPs. It supplies the ability of 3D visual image and both qualitative and quantitative information about different actual features such as size, roughness, morphology and surface texture **[17].** $AgNP_S$ that formed from pineapple juice as reducing agent have smooth a surface and small particles diameter. distribution, the average particle of AgNPs is measured by AFM images was (32 nm),

SEM analysis in most of measurements s that specialized with nano particles. This study gave image for synthesized silver nano particles was shown high-resolution. Average of size particles around between 20 -30 nm with spherical shape.

Transmission electron microscopic (TEM) is one of the most popular characterization techniques for NPs **[18].** In this technique, a real image of NPs is taken; different magnifications can be used to show more detailed or general shape of NPs. These images are containing a

lot of information regarding shape and size distribution, and even crystallographic structure and characteristics of nanoparticles. TEM images data clearly shows that the structure and nature of reducing agent play an important role to reduce the Ag ion into Ag atom. This method provides highly stable water-soluble. Mono dispersion and Spherical Shape only particles, average size between (18- 30) nm ([19].

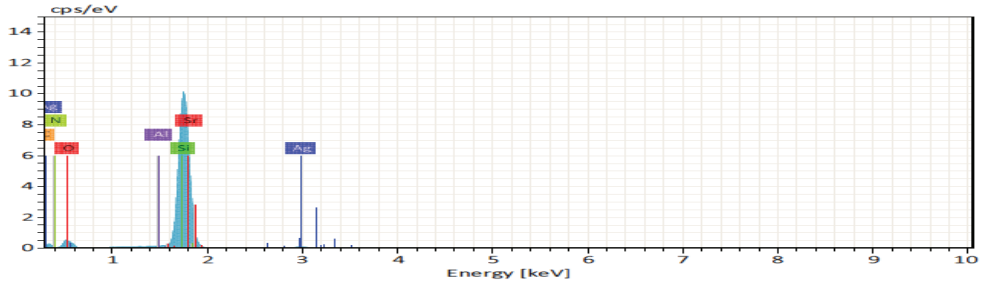

Figure 1. EDX images of AgNP

The EDX spectrum of the biosynthesized AgNPs (figure 1) shows finding silver element as an indicator for the formation of silver nanoparticles from the. Also, it exhibits the C, O, N, Al Si, and Sr elements have been presented in the used sample. Si element has been presented from deposition of AgNPs on the bottom

Biological Applications of AgNPs

AgNPs have been used in applications of AgNPs in different biological and biomedical applications, example antibacterial, antifungal, antiviral, anti-inflammatory, anti-cancer, and anti-angiogenic. In our study, we focused on the efficacy of AgNPs as an antibacterial agent using a number of common antibacterial against E. coli bacteria.**Antibacterial Activity of AgNPs**

One of the most typical model organisms is E. coli which is used in medical and biology researchs as well as drug mechanisms and drug targets **studies [20–22]. The nature** of *E. coli* is Gram-negative bacteria which has the same structure of any cell by using **the minimum inhibitory concentration**.

The MIC value of AgNPs tested against E. coli was found to be high (in the range of (32 -128) (µg/ml) table (1).

(Table 1) Antibiotics and (AgNPs) **MIC (µg/ml) determination by micro-titer plate**

Isolate	Cefixime	Trimethoprim	Levofloxacin	Doxycycline	Amikacin	Gentamycin	(AgNPs)
E.coli	128	128	32	-	64	32	25

Therefore, we investigated the mechanism of synergistic influence of combination between AgNPs and antibiotics against *E. coli*. The synergism was presented with *E. coli* when AgNPs combined with Gentamycin, Amikacin, Levofloxacin, Trimethoprim, Cefixime, if found the highest value of **MIC** at Trimethoprim, Cefixime. AgNPs were effective in inhibiting bacterial growth Therefore, it can be said that AgNPs are broad spectrum agents whose performance is not blocked by the drug-resistant mechanisms of the bacteria. [23]

The combination test between antibiotics and silver nanoparticles (Synergistic role)

Ten microliters colloidal AgNPs was injected into wells in media which containing antibiotics. The plates were cultured by bacteria and then incubated at 37C° for 18-24 h. The diameter of inhibition zone was measured by the ruler compared with the control; antibiotics and AgNPs alone. AgNPs have utilised in medicine field, and in this work, it was used the synthesised AgNPs as antibacterial agent through simple technique called well

diffusion assay. The potential of anti-bacterial properties for AgNPs was shown against *E. coli*. The synthesised AgNPs has ability to inhibit *E. coli* growth with various types of antibiotics. The empty spaces were significantly increased with using AgNPs-antibiotics as shown in table 2.

Table 2. Zone of inhibition for E. *coli* growth by synergistic effect between antibiotics and AgNPs.

E. coli	CEFTRIAXONE	CEFIXIME	Trimetho	DOXYCYCLINE	GENTAMICIN	AMIKACIN	LEVOFLOXACIN	
78.6	38.5	0	0	616	707.175	254.6	419	E. Coli without (AgNPs)
78.6	95	78.5	66.144	962.543	855.7	855.7	1657.2	E. Coli with (AgNPs)

The increasing of inhibition zone relates with the combination of the Ag nanopoarticle with antibiotics which increase methods of killing bacteria *in vitro* and that is called synergistic effect between AgNPs and antibiotics [24]. The role of silver nanoparticles biosynthesized from natural organic compounds in fruits extracts may be play an important agent against microbes [7]. referred to that silver nanoparticle which produced in the green chemistry method is considered potential nano-drug against human microbes' pathogens *in vitro*.

Conclusion

In this study, we used green method to synthesis AgNPs by using pineapple extract as reducing and stabilising agent which proved by UV-Visible spectral analysis, EDX, SEM, and AFM analyses. we tested activity of synthesized Ag NPs as anti-bacterial against E. coli by using well diffusion assay technique. The study showed that Ag NPs were anti-bacterial very active with different type of antibiotics against E. coli and we found sliver nanoparticles has the synergism effect with some antibiotic such as (Gentamycin, Amikacin and Doxycycline, Levofloxacin, Trimethoprim and Cefixime) in increase its activity towards *E. coli*.

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