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EFFECT OF DIFFERENT BIOSYNTHESIS METHODS FOR SILVER NANOPARTICLES ON THEIR ANTI-BACTERIAL ACTIVITY

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ABSTRACT

Background: silver nanoparticles (AgNPs) have attracted significant interest in the field of biomedical applications and there are many methods for AgNPs synthesis. Biosynthesis of AgNPs is the most common method in in the last period. Current study aimed to determine effect of different biosynthesis methods for AgNPs on their antibacterial activity.

Methods: AgNPs biosynthesis by three methods include; extract of white rot fungal strain *Ganoderma enigmaticum* (A), leaves of corn plant *Zea mays* (B) and root of *Rhazya stricta* plant (D). Characteristics of biosynthesis AgNPs are detected by UV-Vis spectrophotometry (UV-Vis), Field emission scanning electron microscope (FE-SEM), Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). Antibacterial activity of biosynthesized AgNPs tested against five isolates of *Escherichia coli* (*E. coil*) and 5 isolates of *staphylococcus aureus* (*S. aureus*) isolated from patients diagnosed with urinary tract infection (UTI) by disk diffusion method.

Results: AgNPs biosynthesis by plant extracts have the best inhibition effect on bacteria isolates in compared with fungal extract when AgNPs biosynthesized by Corn plant (B) have the highest antibacterial effect against *E. coil* and *S. aureus* (19.55±1.95 and 25.33±2.15 respectively) followed by AgNPs biosynthesis by *Rhazya stricta* plant (D) (15.71±1.21 and15.61±0.43 respectively). In compared with *E. coil*, *S. aureus* more effected by most antibacterial agents that used in this study but statically significant appeared toward B only (p=0.047).

Conclusion: activity of biosynthesis AgNPs different according to materials which used for producing AgNPs in biosynthesis method.

Keyword: AgNPs, biosynthesis, E. coil, S. aureus, Antibacterial, Ganoderma enigmaticum, Rhazya stricta, Zea mays

I. INTRODUCTION

Antibiotic resistance is forcing scientists all across the world to look for new medicines that are more active. In recent years, the usage and search for medicines derived from plants and other natural items such as edible mushrooms has grown [1,2,3]. Silver and its compounds have a significant toxicity to microbes but a low toxicity to mammalian cells, as we now know. Scientists are looking for new approaches to find antibacterial medicines that are resistant to antibiotics as a result of advancements in nanotechnology [4]. AgNPs are useful for treating infectious illnesses, and they may be synthesized using a variety of chemical and physical techniques. These approaches are both expensive and harmful to the environment [5]. These facts have prompted the search for innovative, simple, and environmentally friendly alternatives that are not harmful to human or animal health. The advancement of green synthesis methodologies has ushered in a revolution in the realm of AgNP synthesis [6]. Simple, low-cost, ecologically safe, and therapeutically efficacious bio-fabricated nanoparticles were developed. Plants, plant extracts, fungi, yeasts, bacteria, and tiny biomolecules (e.g. vitamins, amino acids, or polysaccharides)

are used in the green synthesis to execute the reduction method [7,8,9]. Overall, plant extracts were reacted with an aqueous solution of silver nitrate, resulting in the fast production of chemically stable nanoparticles. Surprisingly, the utilization of microbe extract has resulted in the production of NPs with predictable size, shape, and purity [10]. The purpose of this work is to provide a green approach for synthesis of AgNPs utilizing *Rhazya stricta* (*R.stricta*) roots, *Zea mays* (*Z. mays*) leaves, and *Ganoderma enigmaticum* extract (*G. enigmaticum*).

Harmal is another name for *R.stricta*, which is found in Iraq and South Asia. Oil of Harmal is thought to be a good source of d-tocopherol, a kind of vitamin E [11,12,13]. It is used to treat a variety of ailments, including diabetes, mellitus, sore throat, obesity, metabolic, cardiovascular, neurodegenerative, and tumor diseases. Anti-carcinogenic, anti-oxidant, anti-microbial, anti-dramatist, anti-hypertensive, anti-depressant, anti-inflammatory, anti-pyretic, antifungal, and herbicidal properties have been discovered [14,15,16]. On MFC7 cell lines, AuNPs produced with *R.stricta* extract had the highest anti-cancer activity. *Z. mays*, on the other hand, is a glabrous, erect shrub with alkaloids, flavonoids, and phenolic chemicals [11,12,17]. *Z. mays* is used to manufacture popcorn, chips, corn oil, corn starch, and a range of other goods all over the world [18,19]. Polyphenols (chlorogenic acid, caffeic acid, rutin, ferulic acid, morin, quercetin, naringenin, and kaempferol), anthocyanins, flavonoids, flavonols, and flavanols have all been discovered in the *Z. mays* plant and its various parts, including the kernel, leaves, and roots, and are responsible for the plant's antioxidant, anti-inflammatory, and other medicinal properties *G. enigmaticum* is a kind of edible mushroom that may be found in Iraq and other Asian countries. Proteins, vitamins, polysaccharides, and other interesting materials are abundant in *G. enigmaticum*, and these fungi have recently been exploited for nanoparticle production [21,22,23]. To assess the influence of the material utilized in the manufacturing of nanoparticles on their efficiency as an antibacterial agent, three biological techniques were used in the current investigation.

II. MATERIALS AND METHODS

Chemicals & reagents

Sigma-Aldrich supplied silver nitrate that was 99.0 percent pure (St. Louis, MO, USA). The reaction was done with deionized water, and the purification and filtering was done with Whatman filter sheets (125 mm) [21].

Isolation and Identification of Microorganisms

Five isolates from each one of *E. coil* and *S. aureus* isolated from patients diagnosed with urinary tract infection in Al-Diwaniya Teaching Hospital/Iraq. The identification of bacteria isolates was done depending on morphological features of the colonies on MacConkey agar plats that confirmed by subculture on Eosin Methylene Blue agar (for *E. coli* isolates) and on Mannitol salt agar (for *S. aureus*) that incubated for 24 hours at 37˚C then the result confirmed by biochemical tests and microscopically examination with Gram's stain [23].

Biosynthesis of silver nanoparticles

In the current study, there are three green methods used for AgNPs synthesis include; extract of basidiomycetes white rot fungal strain *G. enigmaticum* (A), leaves extract of corn plant (B) and root extract of *Rhazya stricta* plant (D).

AgNPs biosynthesis by Basidiomycetes white rot fungal strain *Ganoderma enigmaticum* (A): *G enigmaticum* strain were supplied from Baghdad's Directorate of Agricultural Research, Ministry of Science and Technology The white rot fungal strain was cultured in malt extract broth with a glucose concentration of 10 g/l and a malt extract concentration of 5 g/l. The flasks were incubated at 32 oC in the orbital shaker at 200 rpm with the final pH of the mixture being 6.0. The mycelium was separated by filtering after 5 days of incubation, and the supernatant was mixed with the same amount of AgNO3 solution (1 mM) (made in deionized water) and incubated on a shaker at 200prm in the dark at 32 °C[21].

AgNPs biosynthesis by Corn plant *Zea mays* (B): The full Z. mays grain was acquired at a local market in Al-Diwaniya city, Iraq. The newly growing leaves were gathered and chopped into little pieces in clean containers (1cm). About 25 g of leaf bits were then placed in a 300 mL tapered flask, along with 100 mL of distilled water, and heated for 15 minutes while stirring constantly. Before being utilized for the AgNPs synthesis, the aqueous extract of corn leaves was cooled in the lab, kept, and filtered at 3-4oC. The synthesis of AgNPs was carried out utilizing leaves extract and the green synthesis method. In a 500 mL conical flask containing 200 mL of 1 mM AgNO3, 20 mL of leaves extract was added and swirled constantly at room temperature until the solution became

reddish brown. To regulate the form and size of the nanoparticles, a 1:10 ratio of leaves extract to AgNO3 was maintained with the use of lower concentrations of leaves extract and AgNO3 [24].

AgNPs biosynthesis by *Rhazya stricta* plant (D): R. stricta was discovered in agricultural areas in the Diwaniyah district and recognized in the Baghdad University College of Science's Taxonomy lab. Plant roots were collected in clean containers, washed three times with deionized water, and dried. 150 g of dried roots were steeped in 80% methanol for 3 days at room temperature before being filtered using Whatman filter paper (125 mm). A rotary evaporator was used to evaporate the solvent. The extract yielded 15 g after drying. Ten grams of dried extract were liquefied in 18 mL methanol and kept at 4 oC to make AgNPs. At the same time, 1mM AgNO3 was generated in deionized water. 6 mL of methanol-extracted plant roots were added drop by drop in 100 mL of AgNO3 with continuous shaking (one mM). A magnetic stirrer was used to heat the process for 2 hours at 60 degrees Celsius. The synthesis of AgNPs was detected by a change in color in the mixture. For 20 minutes, the sample was centrifuged at 1200rpm. The supernatant was removed to get rid of any unorganized debris. To remove any residual enzymes or proteins, the material was rinsed three times with distilled water. After sonication, the material was dried in a vacuum drier overnight at 50 oC [25].

Characterization of silver nanoparticles

UV-Vis spectroscopy analysis: In the region of 350–600 nm, an ELICO SL-159 Spectrophotometer was used to perform UV-Vis spectral analysis. The generation of pure Ag ions was monitored by UV-Vis spectrum detection [26].

Fourier transform infrared spectroscopy: An FT-IR spectrometer was used to investigate the biomolecules that decrease Ag+. FT-IR spectroscopy is an experimental method for evaluating functional groups, molecular structure, and chemical bonding of organic and inorganic materials [25] by producing an infrared absorption spectrum (Perklin Elmer Spectrum 100).

Field emission scanning electron microscopy: The size and shape of AgNPs were studied using FE-SEM, and the elemental conformation of AgNPs was evaluated using energy dispersive spectroscopy [26].

X-ray diffraction: X-ray diffractometer (XRD; D8 Advance; Bruker, Billerica, MA, USA) was used to assess the structural characterisation and crystalline nature of vacuum-dried AgNPs [26].

Antibacterial activity

The disk diffusion method was used to evaluate AgNPs and extracts for antibacterial activity against *S.aureus* and *E. coli*. Individual disks were soaked in distilled water (negative control), standard antibiotic cefipime (positive control) with the commercial name maxipime (glaxosmithkline), extracts (E1,E2,E3), and AgNPs solution with additions A, B, and D. 50g/disk of each drug was used to ensure bactericidal activity was found. After that, the plates were incubated at 37°C for 24–48 hours to determine the zones of inhibition for both bacterial strains [27].

Experimental Design

A: AgNPs biosynthesis by extract of white rot fungal strain *G. enigmaticum*
B: AgNPs biosynthesis by leaves extract of corn plant
D: AgNPs biosynthesis by root extract of *R. stricta* plant.
E1: Extract fungal strain *G. enigmaticum.*
E2: Leaves extract of corn plant *Z. mays* .
E3: Root extract of *R. stricta* plant.
PC: Standard antibiotic cefipime (positive control).
NC: Distilled water (negative control)

Statistical Analysis

All computations were done with Microsoft Excel 2010 and the Statistical Package for Social Sciences (SPSS) version 20 computer software. All of the studies' findings were reported as the mean value of AgNPs' impact on five isolates from each bacterial species, plus standard deviation (SD). One-way analysis of variance was used to look for significant differences between mean values, and only the findings with a p value of 0.05 were considered statistically significant.

III. RESULTS

Characteristic of Biosynthesized AgNPs

The biosynthesis of AgNPs was detected by observing a color change in the reaction solution after 1 hour of incubation and successive increases in the color intensity from light yellow to orange brown throughout the reaction. A variety of characterisation methods, as detailed below, were used to identify the production of AgNPs.

1. UV-Vis spectrophotometer

The shift in hue was used to track the creation of silver nanoparticles. The UV–visible spectra of silver nitrate solutions challenged with extracts of the fungal strain G. enigmaticum (A), corn *Zea mays* plant (B), and *Rhazya stricta* plant (C) are shown in Figure 1. (C). After 18 hours, a distinctive surface Plasmon absorption band at 429 nm was found in G. enigmaticum extract (A), whereas no absorption band was observed in controls negative. In the case of *Zea mays* plant extract (B) and *Rhazya stricta* plant extract (C), an absorption band at 457nm and 425nm, respectively, was found after 24 hours, which is the typical surface plasmon resonance peak of AgNPs, confirming their production. After 72 hours of incubation, there was no change in intensity, suggesting that the silver ions had completely disappeared.

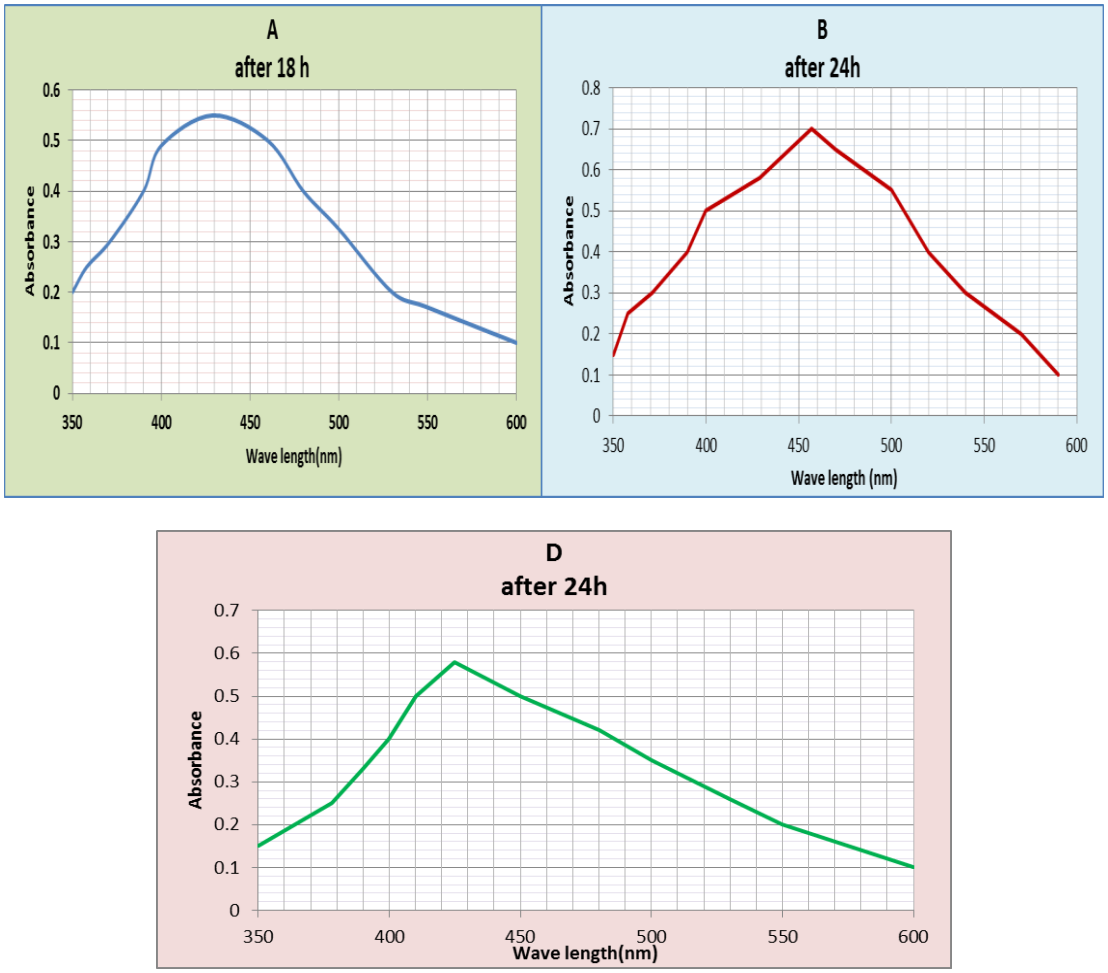

Figure (1): UV-visible absorption spectra of biosynthesized AgNPs by extract of *G. enigmaticum* after 18 h (A), *Zea mays* plant after 24 h (B) and *Rhazya stricta* plant after 24 h (D)

2. Fourier transform infrared spectroscopy

The decrease of silver ions and stability of AgNPs in solution is due to biomolecules. The biomolecules in cell free extract might be peptides, proteins, or carbohydrates. FT-IR was used to examine the interaction between protein and AgNPs, as shown in Figure 2. On A, absorption peaks were detected at 3410.6, 2519.12, 2130, 1778.11, and 740.02 cm1. whereas absorption peaks located at 3950.3, 3411.19, 2679.8, 2225.13, 2008.4 and 1300 cm^{-1} were observed for B. In addition, absorption peaks located at 3900.15, 2804.09, 2406.11, 2007.8, 1433.2 cm^{-1} were observed for D. A peak at 3950.3, 3900.15, 3410.6 and 3411.19, cm^{-1} The presence of a hydroxyl (–OH) group

linked to the N–H bond of amino groups suggested stretching of the N–H bond. Stretching of –CH functional groups might explain the absorption peaks at 2804.09, 2679.8, 2519.12, and 2406.11 cm-1. C–O stretching in the carboxyl connected to the amide linkage in amide I is shown by the peaks at 2225.13, 2130, 2007.8, and 2008.4 cm-1. The existence of C–N stretching in amide was revealed by absorption bands at 1778.11, 1300, and 1433.2 cm1. The two bands at 740.02 cm1 might be indicative of aromatic and aliphatic amines' –O– stretching vibrations.

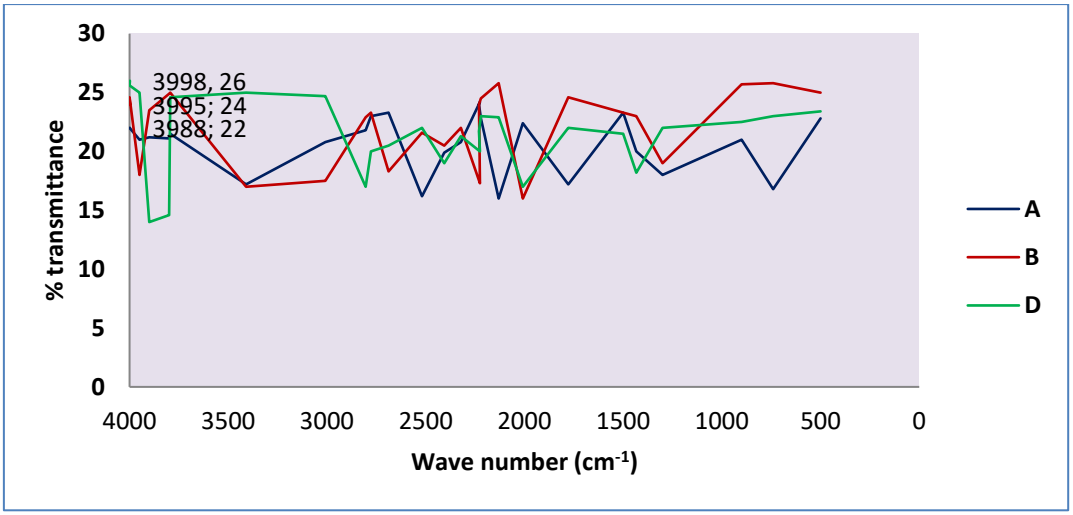

Figure (2): FT-IR spectra of biosynthesis AgNPs

3. FE-SEM

The FE-SEM examination of the above-mentioned material indicated the formation of nanoparticles, which were confirmed to be silver by EDX. The FE-SEM image showed the formation of a cluster of spherical beadlike structures of AgNPs that were firmly aggregated, as shown in Figures 3 and 4. EDX examination found a peak in the silver region, confirming the synthesis of silver nanoparticles. The optical absorption peak is observed at approximately 3.5, 4.2, and 4.8 keV for AgNPs biosynthesis by fungal strain *G. enigmaticum* (A), AgNPs biosynthesis by Corn plant (B), and AgNPs biosynthesis by *Rhazya stricta* plant (D), respectively, which is typical for the absorption of metallic silver nanoparticles due to surface Plasmon resonance [37]. Other Na, Cl, and O peaks were discovered, which might be attributed to protein or enzyme emissions in the culture supernatant (Figure 4).

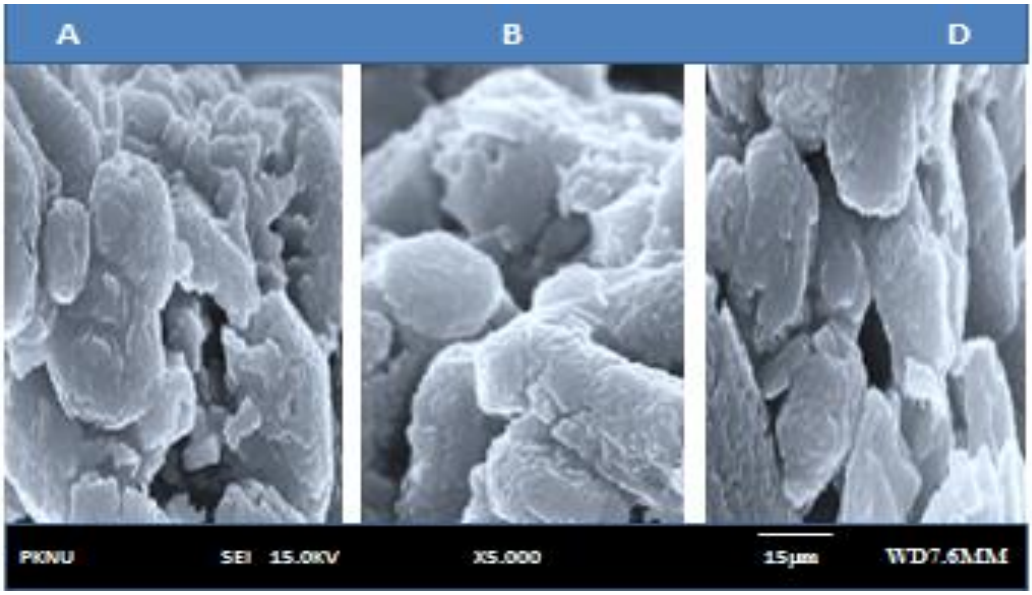

Figure (3): Scanning electron microscopy image include; AgNPs biosynthesis by fungal strain *G. enigmaticum* (A), AgNPs biosynthesed by Corn plant (B) and AgNPs biosynthesis by *Rhazya stricta* plant (D).

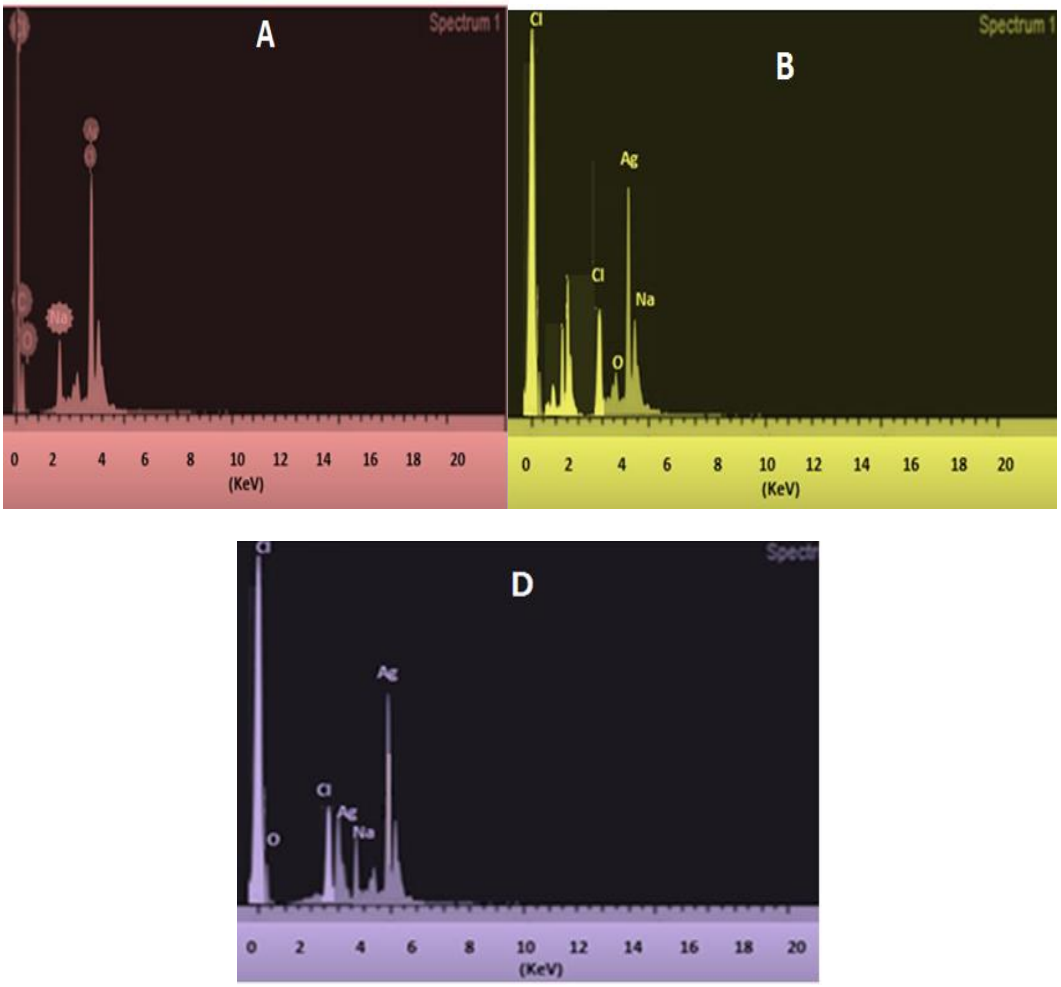

Figure (4): Energy dispersive X-ray image of AgNPs synthesized by fungal strain *G. enigmaticum* (A), AgNPs biosynthesed by Corn plant (B) and AgNPs biosynthesis by *Rhazya stricta* plant (D).

Antibacterial activity of biosynthesis nanoparticles

Antibacterial activity of biosynthesis AgNP tested against 5 isolates of *E. coil* and 5 isolates of *S. aureus* which are isolated from UTI. Mean of Zone of inhibition (mm) are calculated for each test, and result showed that AgNPs biosynthesis by plant extracts have the best inhibition effect on bacteria isolates in compared with fungal extract methods when AgNPs biosynthesized by Corn plant (B) have the highest antibacterial effect against *E. coil* and *S. aureus* (19.55±1.95 and 25.33±2.15 respectively) followed by AgNPs biosynthesis by *Rhazya stricta* plant (D) (15.71±1.21 and15.61±0.43 respectively). AgNPs biosynthesis by white rot fungal strain also have antibacterial activity against *E. coil* (12.80±2.11) and *S. aureus* (14.85± 1.87) but less than AgNPs biosynthesis by plant in B and C **(table 1)**. Plant extract and fungal culture filtrate, E1, E2 and E3, also displayed antibacterial activity against *E. coil* (4.22±1.71, 4.19±2.02 and 5.02±0.88 respectively) and *S. aureus* (4.81±1.14, 4.17±0.55 and 5.11±1.52) but very low in compared with positive control cefipime (PC), B and C **(Figure 5).**

In compared with *E. coil*, *S. aureus* more effected by most antibacterial agents that used in this study but statically significant appeared toward B only (p=0.047). Biosynthesis AgNPs in B more effected after PC on bacterial isolates especially on *S. aureus* (25.33±2.15) flowed by A (14.85± 1.87) whereas AgNPs in D inhibit *E. coil* (15.71±1.21) with slight increase over *S. aureus* (15.61±0.43) **(Figure 6)**.

Table (1): Antibacterial activity of biosynthesis AgNP against *E. coil* and *S. aureus*

Biosynthesis AgNP 50µg/disk	Zone of inhibition (mm)		P value
	E. coil	*S. aureus*	
	Mean ± SD	Mean ± SD	
A	12.80±2.11[a]	14.85± 1.87[a]	0.211
B	19.55±1.95[b]	25.33±2.15[b]	0.047*
D	15.71±1.21[c]	15.61±0.43[c]	0.721
E1	4.22±1.71[d]	4.81±1.14[d]	0.645
E2	4.19±2.02[d]	4.17±0.55[d]	0.811
E3	5.02±0.88[d]	5.11±1.52[d]	0.758
PC	28.08±1.52[e]	29.00±2.10[e]	0.803
NC	0.00±0.00[f]	0.00±0.00[f]	1.00

(*) mean significant differences ($p<0.05$) and Values with different superscript letters are also refer to significantly different at $P < 0.05$.

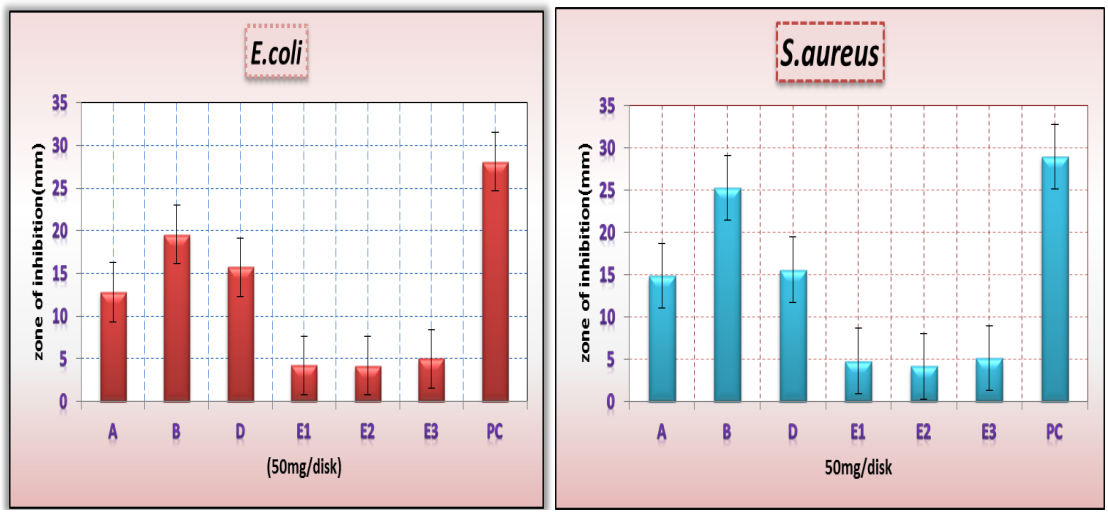

Figure (5): Antibacterial effect of biosynthesis AgNPs against *E. coil* and *S. aureus*

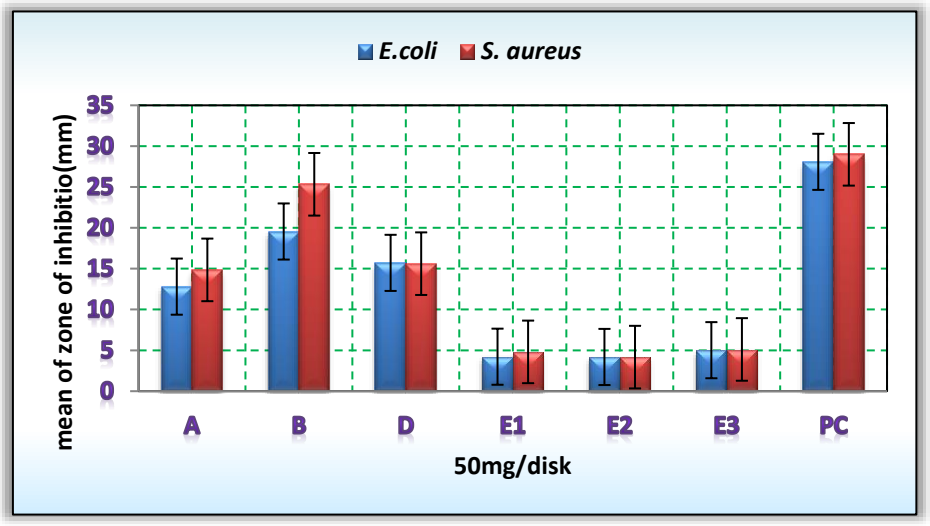

Figure (6): Antibacterial effect of biosynthesis AgNP against *E. coil* and *S. aureus*

IV. DISCUSSION

In the last decade, there has been dramatically grown scientific interest in Biosynthesis of nanoparticles using different capping and reducing agents found in biological sources such as plant parts, extracets, microorganisms, and/or larvae. The traditional green synthesis technique is both cost-effective and environmentally beneficial due to the absence of hazardous and poisonous ingredients. In this study, AgNPs were produced biologically by the fungal strain G. enigmaticum (A), the Corn plant (B), and the *Rhazya stricta* plant (C) (D). After 1 hour of incubation, a measured color change in the reaction solution and successive increases in the intensity of the color from light yellow to orange brown showed biosynthesized AgNPs. The production of AgNPs in the reaction mixture was clearly shown by the development of brown hue in the reaction solution [28,29]. Techniques such as UV-Vis spectroscopy, FE-SEM, EDS, and FT-IR were used to characterize the biosynthesized AgNPs [30,31]. These methods are used to determine particle size, nature, properties, crystallinity, and surface area, among other things. The bio-transformation from an ionic state to silver particles was demonstrated by this surface Plasmon vibration, which was identified by a widened peak in the UV–Vis spectrum [32]. Shehzad *et al.*, (2018) found that when *R. stricta* root extract was added to an aqueous solution of AgNO3, the color changed from pale yellow to orange brown, indicating that the silver ion was reduced. In addition, increasing the concentration of root extracts in the synthesis process causes the particle size to rise. Increasing the quantity of plant extract resulted in a noisy and widened peak in the UV–Vis spectrum [24]. The production of silver nanoparticles was also confirmed by current EDX analysis, which revealed a peak in the silver area. For AgNPs biosynthesis by fungal strain *G. enigmaticum* (A), AgNPs biosynthesis by Corn plant (B), and AgNPs biosynthesis by *Rhazya stricta* plant (D), the optical absorption peak is observed at approximately 3.5, 4.2, and 4.8 keV, respectively, which is typical for the absorption of metallic silver nanoparticles due to surface Plasmon resonance. Other Na, Cl, and O peaks were also identified, which might be attributable to emissions from proteins or enzymes in the culture supernatant [33]. According to Kalainila *et al*., (2014), the EDX spectrum displays a distinct silver signal coupled with a faint oxygen and chlorine peak, which might be connected to bio-molecule binding and interaction with AgNPs' surface [34].

The bio-compounds involved in the reduction of ionic and capping of decreased AgNPs produced by fungal strain *G. enigmaticum* (A), Corn plant (B), and *Rhazya stricta* plant were confirmed by FTIR analysis (D). The existence of carboxyl (–C=O), hydroxyl (–OH), and amine (N–H) groups in the extracts was verified by the spectrum of the existing extracts, which also indicated their role in the reduction of silver ion to metallic AgNPs. Current extracts contain proteins that cap AgNPs by binding to their carboxyl or amino groups [35]. Proteins from the extracts might attach to AgNPs via free amino or carboxyl groups, according to the researchers[35,36]. The existence of residual extract in the sample is owing to the spectra's similarity, with only minor peak position changes. Thus, the functional groups of biocompounds contained in the extracts of the fungal strain *G. enigmaticum* (A), Corn plant (B), and *Rhazya stricta* plant play a major role in the reduction and capping of AgNPs, according to these findings. SEM and EDX investigation of different kinds of AgNPs yielded similar results earlier [37,38,39].

The production of AgNPs by the fungal strain *G. enigmaticum* (A), Corn plant (B), and *Rhazya stricta* plant (D) has an antibacterial impact against *E. coil* and *S. aureus*, according to these findings. The actual mechanism of AgNPs' antibacterial activity against harmful microorganisms is unknown. Few studies have shown that the bactericidal effects of NPs may be due to electrostatic interaction between negatively charged bacterial cells and positively charged NPs [40]. In addition, many potential mechanisms for AgNPs' beneficial antibacterial action have been postulated, including enzyme degradation, cellular protein inactivation, and DNA breakage [41,42]. Biosynthesized AgNPs of a smaller size could have attached to the surface of the bacterial cell membrane and disrupted its power functions, such as respiration and permeability, and then easily penetrated to the inside of the bacteria and caused further injury, possibly by interacting with sulfur- and phosphorus-composition mixtures, as DNA causing in cell lyses [43,44]. Ovington reported in 2004 that nano-crystalline silver yields a probable antimicrobial effect by releasing a cluster of highly responsive silver cationes and radicales in the cell surface or pathogen body, which could be a possible explanation for AgNPs' antibacterial activity in the current study [45].

S. aureus is now more affected by antibacterial agents than E. coil. The differential in sensitivity to AgNPs between Gram negative and Gram positive bacteria might be due to differences in membrane width and molecular structure. The peptidoglycan-based cell wall of Gram-positive bacteria is significantly thicker than that of Gram-negative bacteria [46,47]. Pal et al. discovered that the form and size of nanoparticles affects their antibacterial efficacy against Gram-negative bacteria like E. coli. They demonstrated that the observed interaction between silver NPs of various shapes and E. coli was similar, with varied inhibitory effects. They hypothesized that AgNPs with the same surface area but different shapes may have different effective surface areas in terms of active facets [48]. For E.

coli and S. aureus, Sadeghi et al. observed a variety of antimicrobial properties of nano-silver forms (nanorods, nanoparticles, and nanoplates). Due to the increased surface area of AgNPs, SEM examination revealed that both strains were damaged and severely inhibited by Ag-nanoplates [49].

In present results, AgNPs biosynthesis by plant extracts have the best inhibition effect on bacteria isolates in compared with fungal extract methods when AgNPs biosynthesized by Corn plant (B) have the highest antibacterial effect against *E. coil* and *S. aureus* (19.55±1.95 and 25.33±2.15 respectively) followed by AgNPs biosynthesis by *Rhazya stricta* plant (D) (15.71±1.21 and15.61±0.43 respectively). This may be related to shape, size, and concentration of nanoparticles and capping agents of AgNPs biosynthesized by plant extracts especially AgNPs biosynthesized by Corn plant (B) [50,51]. AgNPs have also been shown to exhibit antibacterial action against both gram-negative and gram-positive bacteria in previous investigations. The antibacterial activity of AgNPs is widely understood to be size and dosage dependent. The findings were backed up by earlier research [52,53], which found that AgNPs had a greater antibacterial activity against gram-negative bacteria than gram-positive bacteria. The antibacterial activity of NPs was decreased as their size increased [35].

V. CONCLUSION

AgNPs biosynthesis by plant extracts have the best inhibition effect on bacteria isolates in compared with fungal extract methods when AgNPs biosynthesized by Corn plant have the highest antibacterial effect against *E. coil* and *S. aureus* followed by AgNPs biosynthesis by *Rhazya stricta* plant.

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