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Biosynthesis and Antibacterial Activity of Silver Nanoparticles Using Medicinal Plants Associated Endophytic Bacteria From in Riyadh Region Saudi Arabia

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Abstract **Original Research Article**

Medicinal plants have been used by secondry communities to patronize various ailments. However, the capability of endophytes within these bio-prospective medicinal plants remains anonymous. The biosynthesis of silver nanoparticles (AgNPs) via the use of endophytic bacteria is a secure alternative for the chemical method. Plants were collected from Al Mamaria and several locations in the desert of Riyadh, Saudi Arabia. The microbial profile of the examined locations was analyzed using a 16S rRNA-based technique. Based on 16s rRNA and phylogenetic analysis, the selected isolates were identified. The bacterial extracts were tested for antibacterial activity and pharmacological characteristics. The 16S RNA-sequencing and phylogenetic analysis revealed that selected 24 isolates belongs to 4 phyla as following: Bacteroidota, Pseudomonadota formerly Proteobacteria , Actinomycetota, Bacillota (Firmicutes) have been isolated from different medicinal plants ((a)*Cactus* (b) *Artemisia* (c) *Artemisia monosperma* (d) *Alhagi* (e) *Zizyphus*(f) *Teucrium* (g) *Ducrosia* (h) *Capparis spinosa)*. Our 24 isolates selected from 121 isolates then purified by streaking on the same isolation medium based on dissimilarity in the color and shape of the colonies and then stored on slants at 4ºC and in 20% glycerol at -20ºC the rest of isolates stored for further analysis The dominated phyla was Actinomycetota , followed by *Firmicutes* . The *Streptomyces* genus was found in the majority of samples. The pharmacological characteristics of the bacterial extracts were assessed. Antibacterial testing revealed that 12 of 24 bacterial extracts were having antimicrobial activities against at least one of the microorganisms examined.

Keywords: Endophytes, 16S rRNA-sequencing, *streptomyces*, Nanoparticles.

INTRODUCTION

Many sorts of microbial populations, including bacteria, fungus, archea, and protests, have been discovered as endophytes in the plant's interior tissues. De Bary (1866) coined the word endophyte, which refers to microorganisms that live inside plant tissues for part or all of their life cycle without causing harm to the host plant (Schulz and Boyle. 2006).

Bacterial endophytes have been studied for over a century. Bacterial endophytes colonise plant tissues without causing disease and form beneficial relationships with their hosts through phytohormone synthesis, enzyme production, and nutrient mobilisation and translocation, such as phosphate (PO4-3) solubilization, nitrogen fixation, and ammonia (NH3) production (Hassan, 2017; Hassan *et al*., 2018; Naseem *et al.*, 2018).

Furthermore, many endophytes have a variety of applications, such as antimicrobial mechanisms that reduce pathogen-caused crop losses (St-Arnaud and Vujanovic, 2007; Abbamondi *et al*., 2016; Taktek *et al*., 2017; Hafez *et al*., 2020a; 2020b), and metabolites that are integrated into various biotechnological applications (St-Arnaud and Vujanovic (Hafez *et al*., 2017; Fouda *et al*., 2018; 2019a; 2019b).

Biotechnology and nanotechnology have been combined to develop nanotechnology, a new emergent interdisciplinary method (Porter and Youtie, 2009). At

the nanoscale level, this new technology created nanoparticles of numerous types (silver, copper, zinc, gold, and so on) (less than 100 nm). Nanoparticle production has become a major problem in recent decades due to its broad uses in a variety of industries (Dong *et al.,* 2017).

Because of their increased physicochemical properties relative to monometallic NPs, bimetallic and multimetallic nanoparticles receive more attention than monometallic nanoparticles, resulting in a wide range of applications (Vaseghi *et al.,* 2018).

For the creation of nanoparticles, three main methods can be used: chemical, physical, and biological methods (Khan *et al*., 2019). Chemical and physical approaches are often costly and have negative consequences for the environment and human health (Iravani, 2014). To overcome these restrictions, one of the proposed solutions is to use an unique bottom-up process for creating nanoparticles known as 'green synthesis,' which is regarded as an important tool and is receiving a lot of interest in current research. To reduce metal ions into metallic nanoparticles, this process relies on natural resources such as plants, fungi, bacteria, actinomycetes, yeast, and algae (Singh *et al*., 2018).

One of the most promising biological-based nanomanufacturing processes is microbial-mediated nanomaterial biosynthesis (Grasso *et al.,* 2020). Microorganisms can make nanoparticles by intracellular or extracellular synthesis, depending on where the nanoparticles are made, using enzymes or biomolecules produced by cell activities (Li *et al*., 2011). Microorganisms have several benefits over plants and algae for biosynthesis of nanoparticles, including the ability to scale up production and the ability to change culture conditions to obtain nanoparticles of desired shape and size (Yadav *et al.,* 2017).

Nanoparticles are currently widely used in a wide range of fields, including health, cosmetics, agriculture, and food science (Kingsley *et al.,* 2013). The manufacture of metal nanoparticles (MtNPs) using microbes and plants has recently been recognised as a cost-effective and environmentally friendly way for utilising microorganisms as nanofactories (Singh *et al*., 2016). Biological agents used in green nanotechnology provide a number of advantages over physical and chemical syntheses; in fact, these approaches are more expensive, use more energy, and use hazardous compounds that could have a negative impact on the environment (Patra and Baek, 2014).

In comparison to chemical and physical approaches for nanoparticle synthesis, the biological approach is less expensive, environmentally benign, non-toxic, and clean, and it can easily be scaled up to larger-scale synthesis (Singh *et al*., 2017).

Despite the large number of studies elucidating the green production of nanoparticles utilising microorganisms, little is known about the process and biochemical route involved in metal nanoparticle synthesis. Metal ions are reduced into nanoparticles in part by intracellular and extracellular microbial enzymes and secondary metabolites released by microorganisms. In reality, it has been discovered that when microorganisms are exposed to metal ion solutions, they respond to the stress by secreting enzymes and biomolecules with a reduction potential for metal salts, resulting in the metal ions being detoxified into less hazardous metal nanoparticles (Singh *et al*., 2018).

The activity of nanoparticles biosynthesized by endophytic bacteria is influenced by their size; it has been demonstrated that nanoparticles with a small size give a high surface/volume ration and ensure good activity. Temperature, pH, metal salt concentration, incubation length, agitation, nature and concentration of carbon and nitrogen sources in growth media should all be managed and tuned in order to produce homogeneous nanoparticles in size and form with satisfactory activity (Khandel *et al*., 2018).

The aim of this study is biosynthesis of silver Nanoparticles using Medicinal plants associated endophytic bacteria and assessing the antibacterial activity of biosynthezed Nanoparticles.

MATERIALS AND METHODS

1. Study area and Sample collection

Plants were collected from several locations in the desert of Riyadh, Saudi Arabia. Plant materials were obtained from these plants. In addition, soil samples were taken from the plant rhizosphere. All samples were collected in a sterile plastic bag and then transported to our King Saud University laboratory for bacteria isolation.

2. Isolation and purification of endopytic bacteria

Plant parts were surface sterilized to remove all the surface-living organisms. Started with carefully washed with tap water to remove attached clay; followed this, the plant parts were sequentially immersed in 5% aqueous solution of sodium chloride, tissues were then washed with autoclaved distilled water to wash away the residues and epiphytic organisms.

Following that, the explants were washed with distilled water. Finally, 1 gm of sterilised plant tissues were combined with 2 mL of autoclaved distilled water and ground in a mortar. Serial dilutions of the aqueous sterilised plant solution samples were generated by mixing 1 ml of the sample with 9 ml of sterilised distilled water (10^{-1}), stirring, and repeating the process until the concentration reached 10^{-5}. Each dilution was spread by smearing 100 microliters of the sample dilution onto the surface of isolation plates containing M1, MM, GM, and ISP2 media supplemented with nystatin (25 $g.mL^{-1}$) to

suppress fungal growth, The soil samples were also serially diluted by aseptically mixing 10 g of dirt in 90 mL of sterile distilled water (10^{-1}), mixing by shaking, and making tenfold dilutions up to 10^{-6}%. The agar plates were spread with 0.5 mL aliquots of each soil from dilutions 10^{-4}, 10^{-5}, and 10^{-6}. Each dilution was duplicated twice on two plates. After that, the plates were kept at 35 2 °C for 24 hours.

On a plate, the average colony count of bacteria produced was calculated. The results were represented as colony forming units per gramme of soil dry weight (CFU/g). Colonies were identified by their cultural and physical characteristics, and bacterial isolates were purified many times in the same isolation medium using the streaking method. These isolates' stock cultures were kept at 80 °C in cryotubes with 1.5 mL 20 percent (w/v) sterile glycerol solution (Wellington and Williams, 1978).

3. Identification of the isolated endophytic bacteria

Identification of strains morphologically was done by Electron microscope using Coverslip culture technique on both ISP2, as well as Hagem media agar **(**Yaminisudha *et al.*, 2015**)**.. 16S rRNA gene sequences were also used to identify the bacteria. Endophytic bacteria genomic DNA was isolated (Govindarajan *et al*., 2007), and 16S rDNA was amplified in PCR using the genomic DNA as template and bacterial universal primers 27 F (5'-GAGTTTGAT CACTGGCTCAG-3') and 1492 R (5'-TACGGC TACCTTGTTACGACTT-3') (Byers *et al*., 1998). After matching sequences with CLUSTAL X, (Kimura, 1980) by MEGA 5.0 programme (Tamura *et al.*, 2011). (Thompson *et al*., 1997). With 1,000 replications, a bootstrap analysis was performed (Felsenstein, 1985). The sequences were compared to sequences of the 16S rRNA gene from cultured species available on the EzTaxon-e server1 (Yoon *et al*., 2017). After matching sequences with CLUSTAL X, a phylogenetic tree was generated using the neighbor-joining (NJ) technique (Saitou and Nei, 1987) and Kimura's two parameter model (Kimura, 1980) by MEGA X software (Tamura *et al*., 2011). (Thompson *et al*., 1997). With 1,000 replications, a bootstrap analysis was performed (Felsenstein, 1985).

4.Characterization of the synthesized NPs

When the colour of the solution changed from colourless to brown, preliminary production of silver nanoparticles was confirmed (Manikprabhu and Lingappa, 2013). UV-visible spectroscopy was used to confirm the synthesis (T60UV-VIS Spectrophotometers). Transmission electron microscopy was used to determine the size and form of nanoparticles (TEM).

The TEM samples were generated by centrifuging the manufactured nanoparticles for 10 minutes at 10,000 rpm. The supernatant was discarded, rinsed, and resuspended in distilled water that had been sterilised. The prepared solutions were put onto copper grids for 1 minute, and the excess solution was blotted away. After that, the copper grid was viewed using TEM (JEM-100CX-II).

5. Screening of antimicrobial activities

In nutritional broth and potato dextrose broth, bacterial and fungal cultures were produced, respectively. Gram-negative and positive bacteria such as Staphylococcus aureus (ATCC 6538), Klebsiella pneumoniae (ATCC 10031), Bacillus subtilis (ATCC 10400), and Methicillin-resistant staphylococcus aureus (MRSA) were employed in the investigation (ATCC 33519). Candida albicans fungal cultures were utilized as target organisms (ATCC 6538). Using the paper disc diffusion method, the antibacterial properties of actinobacterial isolate extracts were investigated.

Each isolate's crude stock was put to sterile paper discs in the amount of 100 µl. They were aseptically transferred to the agar surface seeded with the test organisms after drying. For bacteria, the plates were incubated at 37°C overnight (Kirby Bauer method), and for fungi, they were incubated at 30°C for 72 hours (NCCLS M38- A2). A zone of inhibition around the well was looked for on the plates.

All the physiological and biochemical tests were carried out as described by Williams *et al.* (1983). Finally, interpretation of the results and taxonomical affiliation of the strains were concluded.

RESULTS

1. Isolation and Identification of bacterial strains

There is a diversity in the vegetation cover in the Al-Mamaria region in Riyadh, Kingdom of Saudi Arabia figure (1), where eight different species of medicinal plants were collected and identified, as following (*Capparis spinose* family of *Capparaceae*, *Alhagi* family of *Fabaceae*, *Artemisia* family of *Asteraceae*, *Ducrosia* family of *Apiaceae*, *Artemisia monosperma* family of *Asteraceae*, *Cactus* family of *Cactaceae*, *Ziziphus* family of *Rhamnaceae*, and *Teucrium* family of *Lamiaceae*.

Fig-1: Map of Kingdom of Saudi Arabia

Twenty Four bacterial isolates were successfully isolated from Medicinal plant samples, collected from the three different isolation sites located in Al-Mamaria region Riyadh Saudi Arabia. The bacterial isolates were chosen based on their morphological differences and were purified on the same isolation media used and then stored on slants at 4ºC and in 20% glycerol at -20ºC for further analysis (Figure 2).

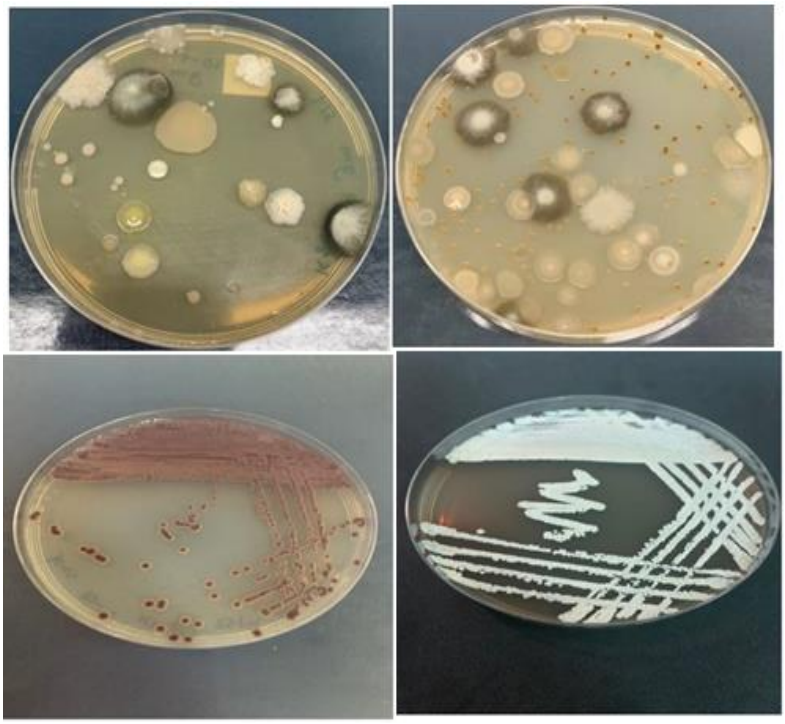

Fig-2: Isolation platesand de-replication of the selected strains in all tested sites of the AlMamria region used (ISP2) medium

The electron microscope micrograph of isolate (KH2) (Figure 3) Younger colonies are grey and have a smooth surface. The larger, older colonies are white in colour and and have velvet-like surface (due to spore formation). The colonies adhere to the surface of the agar, and are extremely tough in consistency. Gram-positive rods, which form branched filaments that produce coccoid spores terminally.

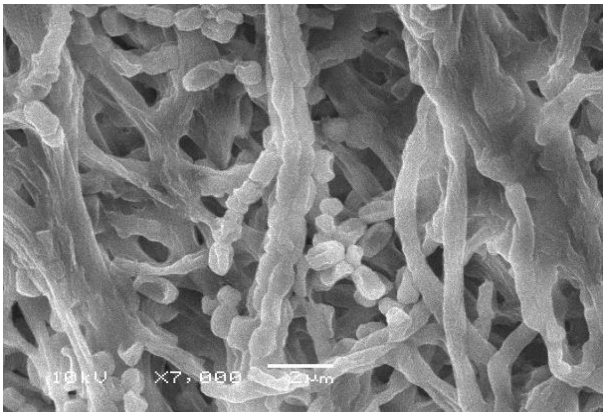

Fig-3: Electron micrograph of isolate KH2

The phylogenetic analysis of the 16S rRNA partial gene sequence of the 24 isolated strains was conducted as described in standard procedures from plant and soil samples; the 24 bacterial isolates were belonging to 12 species in 10 genera. *Streptomyces* (46.7%), *Micromonospora* (12.5), *Bacillus* (8.33%), *Nocardioides* (4.17%), *Sphingobacterium* (4.17%), *Stenotrophomonas* (4.17%), *Arthobacter* (4.17%), *Kocuria* (4.17%), *Microbacterium* (4.17%), *Rufibacter* (4.17%), *Belnapia*, (4.17%), and *Mycolicibacterium* (4.17%) were the most common genera. A total of ten bacterial strains were isolated and identified as belonging to the genera *Streptomyces* (10 species), three bacterial strains were identified as members of the genera *Micromonospora* (3 species), two bacterial strains were identified as members of the genera *Bacillus* (2 species), *Nocardioides* (1 species), *Sphingobacterium* (1 species), *Stenotrophomonas* (1 species), *Arthobacter* (1 species), *Kocuria* (1 species), *Microbacterium* (1 species), *Rufibacter* (1 species), *Belnapia*, (1 species), and *Mycolicibacterium* (1 species) **Table (1).**

Table-1: Sequence similarity of isolated strains based on 16S rRNA sequences compared to the GenBank database

Sample name	Most related similar strain						Score		Identities	
	Accession	Description	length	start	End	Coverage	Bit	E. Value	Match/ Total	PCt (%)
KH2	NR_040857.1	*Streptomyces galilaeus*	1484	3	1459	98	2691	0.0	1457/1457	100
KH3	NR-108988.1	*Nocardioides panzhihuensis*	1469	17	1462	99	2577	0	1431/1448	99
KH6	NR_118238.1	*Sphingobacterium detergens*	1483	8	1468	98	2538	0.0	1436/1465	98
KH12	NR_043350.1	*Streptomyces badius*	1496	4	1461	97	2693	0.0	1458/1458	100
KH13	NR_041577.1	*Stenotrophomonas maltophilia*	1538	12	1490	96	2687	0.0	1473/1481	99
KH16	KR080524.1	*Streptomyces alfalfae*	1487	18	1478	98	2699	0.0	1461/1461	100
KH17	NR_112257.1	*Streptomyces californicus*	1480	1	1460	98	2689	0.0	1459/1460	99
KH18	NR_041218.1	*Streptomyces flavoviridis*	1476	1	1454	98	2686	0.0	1454/1454	100
KH25	NR_125444.1	*Streptomyces thermolilacinus*	1476	2	1450	98	2671	0.0	1448/1449	99
KH29	NR_125444.1	*Streptomyces thermolilacinus*	1476	2	1458	98	2673	0.0	1454/1457	99
KH48	NR_115063.1	*Bacillus halotolerans*	1545	13	1496	96	2736	0.0	1483/1484	99
KH50	NR_043840.1	*Streptomyces variabilis*	1499	4	1472	97	2702	0.0	1467/1469	99
KH76	NR_043822.1	*Streptomyces* kanamyceticus	1463	15	1476	98	2551	0	1437/1464	98
KH93	KT989846.1	*Arthobacter flavus*	1426	2	1427	99	2516	0	1406/1426	99
KH94	NR_041218.1	*Streptomyces flavoviridis*	1476	1	1454	98	2686	0.0	1454/1454	100
KH101	KY694464.1	*Bacillus velezensis*	1508	16	1504	98	2726	0.0	1485/1489	99
KH108	LC113906.1	*Kocuria himachalensis*	1477	16	1474	98	2684	0.0	1457/1459	99
KH109	NR_029265.1	*Microbacterium arborescens*	1468	1	1456	99	2671	0.0	1453/1456	99
KH111	NR_116241.1	*Micromonospora tulbaghiae*	1435	1	1429	99	2639	0.0	1429/1429	100
KH114	NR-116241.1	*Micormonospora tulbaghiae*	1435	1	1432	99	2645	0	1432/1432	100
KH64	HG316123.1	*Rufibacter immobilis*	1454	3	1451	99	2501	0.0	1420/1452	98
KH90	NR_109297.1	*Belnapia rosea*	1473	24	1449	96	2615	0.0	1423/1426	99
KH97	NR_104690.1	*Mycolicibacterium madagascariense*	1470	8	1470	99	2558	0.0	1440/1466	98
KH112	NR_116241.1	*Micromonospora tulbaghiae*	1435	1	1435	100	2639	0.0	1434/1436	99

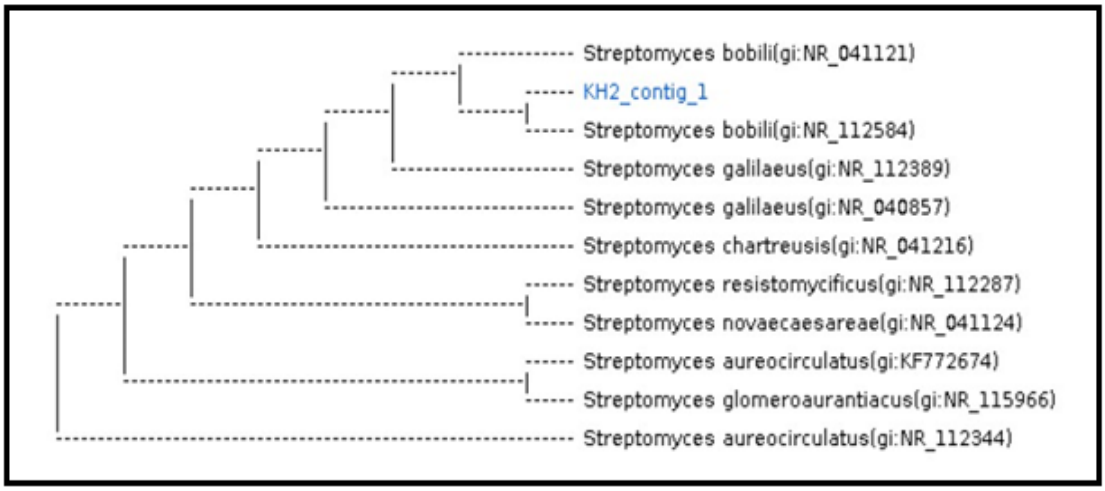

Fig-4: A neighbor joining tree of strain KH2 which show 100% similarity percentage to genus *Streptomyces bobili*

Preliminary comparison of the 16S rRNA sequence of strain (KH2) against the GenBank database indicated that 100% similarity to genus *Streptomyces bobili* NR_040857.1followed by *Streptomyces bobili* NR_041121. Bacteria is under *Streptomycetaceae* family.

2.Characterization of the synthesized NPs

Result from this study indicated that the AgNPs was successfully bio-synthesized from *Streptomyces alfalfa* KH16, which isolated from *Capparis spinosa* one of the medicinal plants collected. Characterization done for KH16 On the following Based on the change of color from light yellow to dark brown, which has widely been considered as an indicator for the synthesis of nanoparticles. Furthermore, there was a different absorption peak between the AgNO3 and the AgNPs. Indeed, a peak at 310 nm was observed for the AgNO3 alone. However, this peak disappeared in the AgNPs, and a new absorption peak of 420 nm appeared after incubation at 30°C for 24 h.

The results of TEM indicated that the biosynthesized AgNPs were spherical shape with the size from 25 to 50 nm with average 34 ± 3 (Fig. 5A and B).

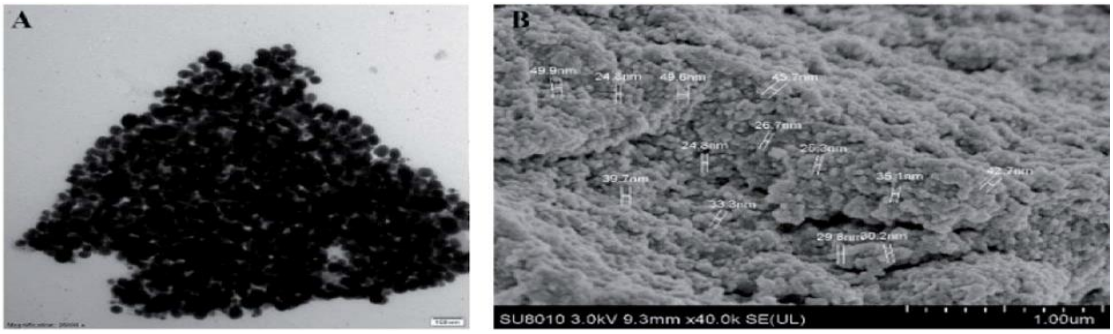

Fig-5: Characterization of the biosynthesized AgNPs from KH16 by the analysis of(A): transmission electron micrographs (B): scan electron micrographs

3.Screening the isolated actinobacterial strains for their antimicrobial activities

The antimicrobial activities of the selected 24 bacterial isolates were tested against some pathogenic Gram-positive, Gram-negative bacteria and yeasts. It was found that 12 isolates showed activities against the tested organisms as shown in table (2) and Fig. 6. It is obvious from the results that higher inhibition was recorded against Gram-positive bacteria while Gram-negative bacteria and yeast were less inhibited. According to the size of the inhibition zone the biologically active strains can be divided into three groups as shown in table (2):

Group 1: Slightly active strains which gave antimicrobial activities with inhibition zones from 10 to 20 mm .

Group 2: Moderately active strains which gave antimicrobial activities with inhibition zones ranging from 21 to 30 mm .

Group 3: Highly active group which contains organisms that have the ability to inhibit the growth of the tested strains with inhibition zones more than 30 mm. (Table 2, Figure 6).

Table-2: The antimicrobial activities of the biologically active bacterial isolates expressed as inhibition zones of growth against the used test organisms in mm.

Bacterial isolate	*B.subtilis*	*K.Pneumonia*	*C.albicans*	*S.aureus*
KH2	11	-	16	-
KH3	-	-	12	15
KH6	19	-	-	-
KH16	18	-	-	11
KH18	17	-	13	-
KH93	25	12	18	19
KH25	-	-	-	13
KH109	14	-	-	24
KH111	-	26	22	19
KH76	-	-	-	31
KH12	-	-	17	11
KH13	-	-	-	19

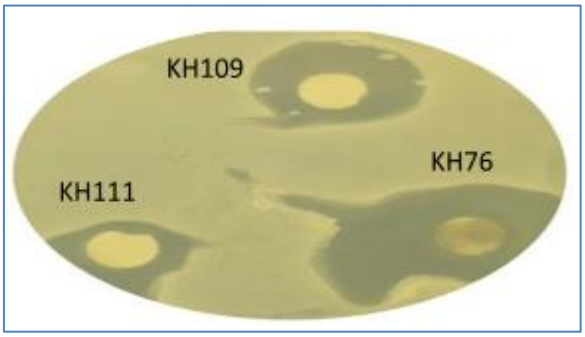

Fig-6: Inhibition zones of selected bacterial isolates KH111,KH76,KH109 against *S.aureus*

DISCUSSION

Generally, the symbiosis of endophytic bacteria and medicinal plants has a greater probability of producing chemical compounds than bacteria which live freely (Mearns-Spragg *et al*., 1998) .In this study we select total 24 Isolates belong to four phyla: Bacteroidota, Pseudomonadota formerly Proteobacteria , Actinomycetota, Bacillota (Firmicutes) have been isolated from different medicinal plants ((a)*Cactus* (b) *Artemisia* (c) *Artemisia monosperma* (d) *Alhagi* (e) *Zizyphus*(f) *Teucrium* (g) *Ducrosia* (h) *Capparis spinosa*).

Our 24 isolates selected from 121 isolates then purified by streaking on the same isolation medium (Fig.16) based on dissimilarity in the color and shape of the colonies and then stored on slants at 4ºC and in 20% glycerol at -20ºC the rest of isolates stored for further analysis. 2 Isolates (KH12, KH16) belongs to Actinomycetota and isolated from *C.Spinosa* while 12 isolates (KH2, KH3, KH17, KH18, KH25, KH50, KH76, KH93, KH108, KH109, KH111, KH64) belongs also to Actinomycetota but isolated from *Artemisia Monosperma*, Isolate KH6 belongs to phyla Bacteroidota and isolated from cactus, Isolate KH13 belongs to phyla Pseudomonadota and isolated from *Teucrium* ,while isolates (KH48, KH101) belongs to phyla Bacillato and isolated from *Zizyphus and Ducrosia* respectively.

Isolates (KH90, KH97) belongs to phyla Pseudomonadota and Actinomycetota respectively and isolated from *Alhagi.* Related findings have been observed in (Mancuso et al., 2016) study which record Phylogenetic characterization identified 33 phyla of Bacteria: 13 of which (Proteobacteria, Bacteroidetes, Verrucomicrobia, Actinobacteria) that isolated from the genera *Artimisia*. while in (Sarmiento-Vizcaíno et al., 2016) study researchers record isolation of endophytic *Streptomyces flavoridis* from the genera *Artemisia sieberi* which is comply with this study on the basis of genera whereas isolate (KH18) Streptomyces *flavoridis* isolated from *Artemisia Monosperma*, We can say that genera *Artimisia* a reservoir of actinobacterial species. Other related observations have been found in (Suvega and Arunkumar.2014) study whereas *C.spinosa* collected and bacterial isolation has been taken place where 14 endophytic bacterial strains were associated with it. *Belnapia rosea* isolated as endophytic bacteria from *ALhagi* which comply with this study on the genera basis.

Metallic nanoparticles such as zinc oxide nanoparticles and MgO nano-flowers showed high antibacterial activity against bacterial leaf blight disease (Ogunyemi *et al.*, 2019; Abdallah *et al*., 2019). Furthermore, silver nanoparticles (AgNPs) showed strong inhibition on Gram-positive and Gram-negative bacteria (Ibrahim *et al*., 2019; Sharma *et al*., 2009; Fouad *et al*.,2017). Endophytic bacteria and fungus are found in abundance on medicinal plants. Reviews of microbial secondary metabolites associated with medicinal plants have demonstrated that the secondary metabolites have interesting therapeutic effects (Cadamuro *et al*., 2021; Ancheeva *et al*., 2018; Xu *et al*., 2014). Because of their bioactive secondary metabolites, medicinal plants endophytic bacteria have pay the interest of pharmacological researchers, particularly when their host plants are also employed as native traditional/folk medicines (Bibi *et al*., 2020). Almost 1000 new natural products derived from mangrove associated microbes have been reported in the recent decade, with 850 derived from fungi and 120 derived

from bacteria (the majority of which are generated from endophytes (Ancheeva *et al*., 2018).

In agreement with the result of this study, previous reports showed that peaks of UV absorption of AgNPs were mentioned to range from 400 to 450 nm, where it was watched at 405, 410, 420, and 426 nm in different studies (Fouad *et al*., 2017; Ramteke *et al*., 2013;.Ibrahim *et al*., 2019)

The results of TEM indicated that the biosynthesized AgNPs were spherical shape with the size from 25 to 50 nm with average 34 ± 3 (Fig. 53A and B) which are consistent with the previous reports (Fouad *et al*., 2017; Singh *et al*., 2017; Lallawmawma *et al*., 2015).

The functional groups in AgNPs were characterized by the FTIR spectra, The peaks at 3385 and 2925 cm^{-1} may be due to –OH stretching from polysaccharides(Kiran *et al*., 2010). and C–H stretching of alkanes,(Aarthi *et al*., 2018) respectively. The peaks at 1732 and 1645 cm^{-1} could be denoted by the carbonyl stretching vibration,(Benelli *et al*., 2018) while the peaks at 1556 and 1359 cm^{-1} can be assigned to (C–N) and (C–C) stretching vibration of aromatic and aliphatic amines, respectively. The peaks at 1079 and 537 cm^{-1} could be assigned to (C–O) of an alkoxy group (Ghramh *et al*., 2018) and the CH2 groups, ,(Kiran *et al*., 2010) respectively.

The potential pharmacological effects of endophytic bacterial extracts were investigated in this work. Because their extracts have antibacterial activity, the bioassays suggested that these endophytic bacteria could be viable sources of bioactive secondary metabolites. The antimicrobial assays showed that 12 out of 24 bacterial extracts exhibited antimicrobial activity against at least one of the tested microbes. Several extracts showed antimicrobial activity against multiple tested microbes, for example, *Arthobacter flavus* KH93 exhibited antimicrobial activity against the four tested microbes which is partially consistent with (Munaganti *et al*., 2016) which showed that *Arthobacter* has strong antibacterial activity against *S.aureus* and *C.albicans* in combination with the variance of temperature and pH factors. Also *Micromonospora tulbaghiae* KH111 exhibited antimicrobial activity against 3 tested microbes which is in agreement with previous reports (Zhou *et al*. 2019) who identified three antimicrobial substances, fusolanone A-B and fusaric acid from the endophytic fungus *Fusarium solani* HDN15-410 from the plant *R. apiculata*. With MICs of 6.25–50 µg/mL, these compounds showed good antibacterial action against a wide range of pathogenic microorganisms, including *P. aeruginosa*, *Monilia albican*, *B. subtilis*, and *Vibrio parahaemolyticus.*

CONCLUSION

In this study, the AgNPs were biosynthesized successfully using endophytic bacteria isolated from medicinal plants (a)*Cactus* (b) *Artemisia* (c) *Artemisia monosperma* (d) *Alhagi* (e) *Zizyphus*(f) *Teucrium* (g) *Ducrosia* (h) *Capparis spinosa*. The selected isolates were identified based on 16s rRNA and phylogenetic analysis. The majority of identified samples were for the genus *Streptomyces* which belongs to Actinomycetota phyla followed by *Bacillus* which belongs to Bacillato phyla. The bacterial extracts of the evaluated for their pharmacological properties. The antimicrobial assays showed that 12 out of 24 bacterial extracts exhibited antimicrobial activity against at least one of the tested microbes.

The biosynthesized AgNPs was further confirmed and characterized by analysis of UV-visible spectroscopy, FTIR, TEM and EDS. Bacteriological test showed that the biosynthesized AgNPs had strong in vitro antibacterial activity against some pathogenic bacteria S.aureus strain and *K.pneumonia* strain.

Overall, this study indicated that the new biosynthesized AgNPs had great potential in protecting medicinal plants from infection of bacterial infections and provide a great insights for further studies on such kind of plants.

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