ANTIMICROBIAL AND ANTIOXIDANT POTENTIALS OF ENDOPHYTIC ACTINOMYCETES ISOLATED FROM LEAVES OF *ASPHODELUS TENUIFOLIUS* (CAV.) (MARSA MATROUH, EGYPT)

Dina M. Fahmy[1*], Nagwa M. Sidkey[2], Mai Y. Elkawaga[2] and Doaa S. Zaid[1]

[1] Natural Product Unit, Department of Medicinal and Aromatic Plants, Desert Research Center, El-Matareya, Cairo, Egypt

[2]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt

[*]E-mail: dmfahmy@gmail.com

The study aimed to evaluate the antimicrobial and antioxidant abilities of four endophytic actinomycetes (strain Act_1, Act_2, Act_3, and Act_4) isolated from leaves of *Asphodelus tenuifolius,* as well as assessment of their phytochemical profile. Referring to morphological characters and 16S rDNA sequence analysis, actinomycete (strain Act2) was identified as *Streptomyces fenghuangensi*s (GIMN4.003T) with 96% identical sequence and accession number (NR1175021). The results showed variant inhibitory activity against pathogenic bacteria and fungi. Act_2 endophytic actinomycete isolate showed broad spectrum activity against all pathogenic tested organisms, recording notable MIC value 10 µg/ml against *Bacillus subtilus* and *Bacillus cereus,* while At_3 and At_4 showed strong impact on *Candida albicans.* All of the ethyl acatate extracts of actinomycete isolates revealed high antioxidant activity ranging from 45-82% using DPPH radical. Phytochemical screening of the isolates indicates the presence of phenolics, flavonoids, sterols, anthraquinones and naphthaquinones.

Keywords: *Asphodelus tenuifolius*, antimicrobial, antioxidant, *Streptomyces fenghuangensi*s, phytochemical screening

Endophytes are microorganisms that for the whole or part of their life live inside plant tissues by symbiotic, parasitic or mutualistic bond without causing overt bad effects (Stone et al., 2000). Based on these long-held associations, endophytes and plants have established good communication (Strobel, 2003). Medicinal plants synthesize chemical substances, providing raw material for pharmaceutical, cosmetic and health care industries (Berdy, 2012). Endophytic microorganisms in medicinal

plant most likely participate in metabolic pathways of its host and produce analogous or novel bioactive compounds, for example taxol (Strobel et al., 1999). Endophytic actinomycetes produce a diverse range of secondary metabolites that play an essential role in pharmaceutical industry. *Streptomyces* is the largest antibiotic-producing genus, producing antibacterial (Castillo et al., 2006), antifungal (Taechowisan et al., 2005), and antiparasitic drugs, in addition to a wide range of other bioactive compounds, such asimmuno-suppressants (Muramatsu and Nagai, 2013) and antitumors (Igarashi et al., 2007). It is reported that total number of bioactive metabolites produced by microorganisms are around 23,000 out of which 10,000 (45% of all bioactive metabolites) are produced by actinomycetes alone, and among this group of bacteria, 7600 (76%) compounds are reported from a single genus *Streptomyces* (Berdy, 2012). This clarifies their major importance in the world of pharmaceuticals.

Medicinal plants offer valuable therapeutic agents in traditional medicines. *Asphodelus tenuifolius* Cav. (family Liliaceae) is an annual, medicinal shrub, distributed widely in Northwestern coast Marsa Matrouh, Egypt. Seeds of *Asphodelus tenuifolius* are reported in traditional medicine to be diuretic, eaten with yoghourt and aids in healing wounds. The leaves are fried or boiled and are sometimes put in the sauce for couscous (Batanouny et al., 2005). Many previous researches focused on investigating endophytic actinomycetes in medicinal plants. As far of our knowledge no previous attempts have established on endophytic community in *Asphodelus tenuifolius*. This study aimed to isolate actinomycetes from leaves of *A. tenuifolius* and investigate their antimicrobial and antioxidant activities, and their bioactive metabolite.

MATERIALS AND METHODS

1. Isolation of Endophytic Actinomycetes

Endophytic actinomycetes were isolated from leaves of *Asphodelus tenuifolius*, collected from the Northwestern Coastal Region of Egypt, according to Saini et al. (2016).

The leaves of *Asphodelus tenuifolius* were sterilized using 5% (w/v) sodium hypochlorite. The sterilized leaves were cut into small pieces (0.5-1.0 cm) and placed on starch casein agar plates (SCA) supplemented with antifungal nystatin (50 µg/ml). Plates were incubated at 28^{o}C for 14 days under observation of the growth of endophytic actinomycetes. The obtained actinomycetes isolates were purified then stored at 4^{o}C for further investigations.

2. Screening and Extraction of Actinomycetes Cultures

The spore suspension of actinobacteria were extracted according to Devi et al. (2012). Briefly, 1 ml of each spore suspension was inoculated

into 300 ml starch casein broth in Erlenmeyer flasks, and incubated under shaking (200 rpm) at 37°C for 7 days. Each culture broth was filtered to separate spore and mycelial mass. Filtrates were centrifuged at 5000 rpm for 10 min. then the clear supernatants (culture broth) were transferred to sterile conical flaks and stored at -20°C for further screening.

The culture filtrates were extracted using ethyl acetate (3×200 ml) by partitioning in a separating funnel. The ethyl acetate extract (AtEAc) of each isolate was concentrated under reduced pressure at 45°C using rotary evaporator (Buchi, Switzerland). The AtEAc extracts were kept at 4°C until being used.

3. Characterization of Actinomycetes Isolates

Endophytic actinomycetes isolates were characterized based on colony morphology after 14 days of incubation. The morphological characteristics of the isolates were examined according to the Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). Scanning electron microscope (SEM) was used to obtain images of selected isolates. Living specimens were processed and examined by the procedures described previously (Ezra et al., 2004). 16S rDNA sequence analysis was executed to identify the active isolate

4. 16S rDNA Gene Amplification and Sequencing

The identity of the active actinomycetes was determined based on partial or nearly full length 16S rRNA gene sequence analysis. The genomic DNA of At_2 isolate was extracted by using a protocol of Thermo Scientific Gene JET Genomic DNA Purification Kit (ThermoK0721). 16S rRNA amplification was achieved by Polymerase Chain Reaction (PCR) technique using Maxima Hot Start PCR Master Mix kit (ThermoK1051). To 50 µl of the sample, 25 µl of Maxima Hot Start PCR Master Mix (2X), 1 µl (20 µm) of forward primer (AGA GTT TGA TCC TGG CTC AG), 1 µl (20 µm) of reverse primer (GGT TAC CTT GTT ACG ACT T), 5 µl Taq DNA polymerase, and 18 µl water, nuclease-free were added. The sample was gently vortexed, then subjected to perform PCR using the following thermal cycling conditions: initial denaturation/enzyme activation at 95°C for 10 min (1 cycle), denaturation at 95°C for 30 s (35 cycles), annealing at 65°C for 1 min (35 cycles), extension at 72°C for 1 min and 30 s (35 cycles), final extension at 72°C for 10 min (1 cycle).

PCR products were purified according to the protocol described in the manual of Gene JET PCR Purification Kit (ThermoK0701). The quality of the purified DNA was evaluated spectrophotometrically, by agarose gel electrophoresis. Finally, sequencing was achieved on GATC biotech Company (Germany) and sequenced with an automated capillary DNA sequencing system (ABI 3730).

5. Screening of the Antimicrobial Activity of Crude Extracts

Antimicrobial activity of the crude extract (culture filtrate) of actinomycetes was determined against pathogenic gram positive bacteria *(Bacillus subtilus, Bacillus cereus, Staphylococcus aureus*), gram negative bacteria (*Escherichia coli, Salmonella typhi* and *Klebsiella pneumoniaea)* as well as fungal pathogens (*Candida albicans* and A*spergallus niger*) using agar well diffusion method (Tawde et al., 2012).

Plates of Mueller Hinton Agar were inoculated with a volume of 0.1 ml of each of bacterial suspension (4×10^{6} CFU/ml) as well as fungal suspension (2×10^{6} CFU/ml. Fixed volumes (100 µl) of the crude extract were aseptically dropped into wells (5 mm diameter) made in culture media. Water without extract was used as control. Cefotaxime (30 µg/ml), Ampicillin sulbactam (20 µg/ml), and Fluconazol (100 µg/ml) were used as reference antimicrobial agents. Plates were incubated at 37°C for 24 h for bacteria and 28°C for fungi. The diameters of zones of inhibitions were measured.

6. Determination of Minimum Inhibitory Concentration of Ethyl Acetate Extract

Minimum inhibitory concentration (MIC) of Act_2EAc extract, which displayed the strongest antimicrobial activity was determined using broth dilution method (Jahan et al., 2011). Briefly, 100 µl of each tested organism, adjusted to 0.5 McFarland units (about 10^5 CFU/ml), was aseptically inoculated into tubes containing equal volumes of serially diluted Act_2EAc extract concentrations (5 to 200 µg/ml) in Muller Hinton broth medium. The tubes were incubated for 24 h at 37°C. The lowest concentration that produced no visible turbidity after full incubation period was regarded as MIC.

7. Free Radical Scavenging Assay

Free radical scavenging assay was carried out using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) reagent according to Yildirim et al. (2001). Dry extract of ActEAc was solubilized in 80% ethyl alcohol (1000 µg/ml). A volume of 3 ml from each extract mixed with 1 ml of 1 mM of DPPH radical alongside with ascorbic acid (1000 µg/ml) as positive control. The tubes were kept at room temperature in the dark for 30 min. The degree of disappearance of purple color was measured against blank (80% ethyl alcohol) at 517 nm. Data achieved in triplicate and results expressed in percentage.

$$Free\ radical\ scavenging\ activity = ((A\ blank - A\ sample)\ /\ (A\ blank))\ X100$$

Where, A blank is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound.

8. Phytochemical Analysis

Preliminary active compound profile was established for ethyl acetate extract (ActEAc). Tests were performed to detect the presence of saponines, naphthaquinones, glycoside, phenolics, steroids, anthraquinones, and flavonoids as described by Thomson (1997) and Harborne (1998).

RESULTS

1. Isolation of Endophytic Actinomycetes

Four endophytic actinomycetes (Act) were morphologically recognized in Starch casein agar solid medium spreading with leaf segments of *A. teniufolius* after 14 days of incubation (Fig. 1 and 2). During incubation, the green colour of leaves turned to brown and the colonies of actinomycetes emerged on the cut leaf margins. The colour of the spore mass was grey, and the strain produced white aerial mycelium and yellow to yellowish-white vegetative mycelium.

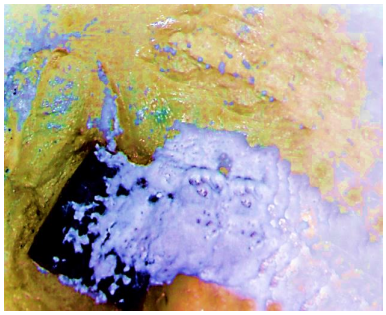

Fig. (1). Actinomycete emerge out of leaf margin on starch casein agar plate.

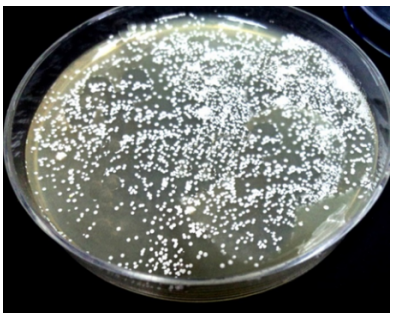

Fig. (2). Actinomycete growth on starch casein agar plate showing regular rounded colony with white aerial mass color and yellow substrate mycelium.

2. Antimicrobial Activity of Crude Extracts

Fermentation process was undertaken to propagate isolates and prepare the crude extract of each isolate. Antimicrobial potency of actinomycetes isolates was experienced against pathogenic bacteria and fungi. Data in table (1) reveal that 100 µl crude extract of Act_2 proved potent antimicrobial potential against all tested Gram-positive and Gram-negative bacteria, and fungal pathogen. Act_1, Act_3, and Act_4 recorded no inhibition effect against both Gram-positive and Gram-negative bacteria. Concerning fungal inhibition potential, Act_3 and Act_4 showed moderate antifungal activity. According to the attained data, the Act_2EAc extract showed relatively maximal activity against Gram positive *B. cereus* and *B. subtilis*

(recording MIC of 10 µg/ml for both), when compared with Cefotaxime (30 µg/ml) that record 30 and 20 µg/ml against both strains, respectively, and Ampicillin (20 µg/ml) that record no activity against *B. cereus* and *B. subtilis*. In contrary to Cefotaxime (30 µg/ml) and Ampicillin (20 µg/ml), Act_2EAc presented challenging pattern against Gram negative *E. coli*, *K. pneumoniaea,* and *S. typhi* recording MIC 125, 125 and 200 µg/ml, respectively. Comparatively, Act_2EAc extract exhibited potent antifungal effect against *C. albicans and A. niger* with MIC 25 and 75 µg/ml, respectively, when evaluated by control fluconazole (100 µg/ml) (table 2).

Table (1). Antimicrobial screening on crude extract of isolated endophytic actinomyctes from leaves of *A. tenuifolius* against pathogenic bacteria and fungi.

Pathogenic microorganism	Diameter of Inhibition Zone (mm)			
	Act_1	Act_2	Act_3	Act_4
Escherichia coli	-	20	-	-
Klebsiella pneumoniae	-	25	-	-
Salmonella typhi	-	25	-	-
Staphylococcus aureus	-	27	-	-
Bacillus subtilus	-	30	-	-
Bacillus cereus	-	30	-	-
Candida albicans	-	23	17	19
Aspergallus niger	-	25	-	-

Table (2). Minimum inhibitory concentration of ethyl acetate extract of Act_2 (Act_2EAc) against pathogenic microorganisms.

Extract	MIC (µg/ml)							
	E. coli	***K. pneumoniaea***	***S. typhi***	***S. aureus***	***B. subtilus***	***B. cereus***	***C. albicans***	***A. niger***
Act2EAc	125	125	200	75	10	10	25	75
Cefotaxime	30	R	R	30	30	R	-	-
Ampicillin	20	R	20	20	20	R	-	-
Fluconazol	-	-	-	-	-	-	100	100

R: resistance, Cefotaxime (30 µg/ml), Ampicillin sulbactam (20 µg/ml), Fluconazole (100 µg/ml)

3. Phytochemical Profile

Phytochemical screening achieved on ethyl acetate extracts (Table 3) showed the presence of phenolics and flavonoids in Act_1EAc, Act_2EAc,

Act_3EAc, and Act_4EAc. Anthraquinones were represented in Act_3EAc and Act_4EAc.

Table (3). Phytochemical screening of bioactive compound in Act_2EAc.

Biological active compounds	Act_1EAc	Act_2EAc	Act_3EAc	Act_4EAc
Anthraqinones	-	-	+	+
Fatty acids	-	-	-	-
Flavonoids	++	++	+	+
Glycosides	-	-	-	-
Naphthaquinone	-	-	-	-
Phenolics	+	+	+	+
Saponines	-	-	-	-
Sterols	+	+	-	-

Strongly present (++), present (+), absent (-)

4. Free Radical Scavenging Activity

Regarding to free radical scavenging potential of ethyl acetate extracts of endophytic actinomycetes, DPPH radicals, after 30 min of incubation, were scavenged by 68 to 82%. As shown in fig. (3), in comparison with ascorbic acid (1000 µg/ml) (92%). Act_2EAc succeeded in chelating maximally 82% of DPPH radical, displaying relatively potent radical chelation potential.

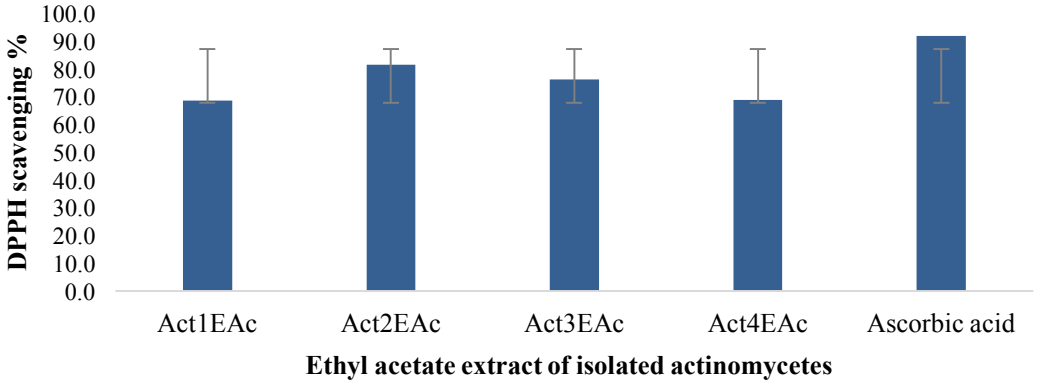

Fig. (3). DPPH scavenging activity of ethyl acetate extracts of isolated Streptomyces (ActEAc) and ascorbic acid as control, date represented as mean ± SD.

5. Identification of Actinomycete

Isolate Act_2 had morphological characteristics of the genus *Streptomyces*. SEM studies revealed a straight to flexuous mycelium without verticils. The aerial mycelium produced straight to flexuous spore chains. Act2 appeared to have characteristic streptomycete features (spore formation). Spores were rough to warty (Fig. 4). A partial 16S rRNA gene sequence was determined for isolate Act_2. Sequence comparison with representatives of the family Streptomycetaceae confirmed that strain Act_2 is closely related to members of the genus *Streptomyces*. It was 94–96% similar to the 16S rRNA gene sequences of *Streptomyces* species. As shown in table (4), high 16S rRNA gene sequence similarity was found with *Streptomyces fenghuangensis*, (GIMN4.003T, 96% similarity over 1613 bases), *Streptomyces nanhaiensis* (SCSIO 01248, 96% similarity over 1596 bases), and *Streptomyces radiopugnans* (R97, 96% similarity over 1591 bases).

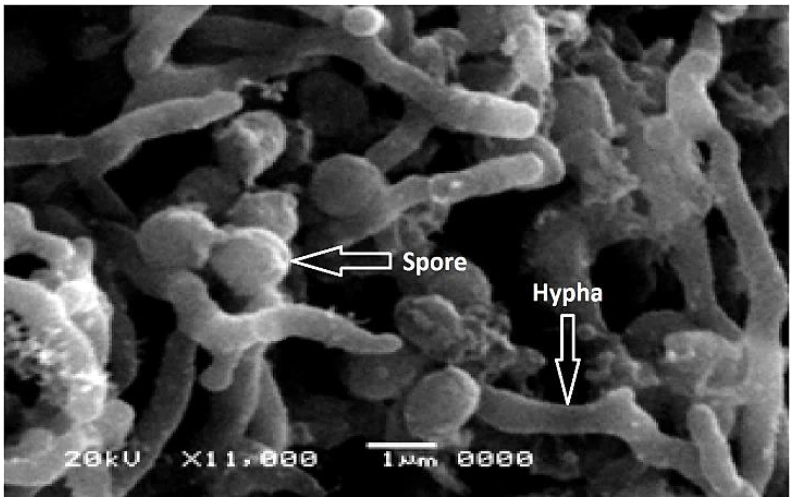

Fig. (4). Scanning electron micrographs of strain GIMN4.003T showing straight to flexuous (rectiflexibiles) spore chains of rough to warty spores after growth on Starch casein agar for 21 days at 28°C.

Table (4). Identification of endophytic actinomycete Act_2 isolated from *A. tenuifolius* leaves based on 5' partial 16S rRNA sequences.

Description	Max score	Total score	Query cover (%)	E value	Ident (%)	Accession
Streptomyces fenghuangensis strain GIMN4 003 16S ribosomal RNA gene partial sequence	1613	1613	84	0	96	NR 117502 1
*Streptomyces nanhaiensis*strain SCSIO 01248 16S ribosomal RNA gene partial sequence	1596	1596	84	0	96	NR 108633 1
Streptomyces radiopugnans strain R97 16S ribosomal RNA gene partial sequence	1591	1591	84	0	96	NR 0440131
Streptomyces atacamensis strain C6016S ribosomal RNA gene partial sequence	1568	1568	84	0	95	NR 108859 1
Streptomyces atacamensis strain C60 16S ribosomal RNA gene partial sequence	1550	1550	84	0	95	NR 1163561
Streptomyces barkulensis strain RC 1831 16S ribosomal RNA gene partial sequence	1535	1535	84	0	95	NR 1338691
Streptomyces megasporus strain NBBC 14749 16S ribosomal RNA gene partial sequence	1535	1535	84	0	95	NR 0411651
Streptomyces macrosporus strain NBRC 14748 16S ribosomal RNA gene partial sequence	1535	1535	84	0	95	NR 1124411
Streptomyces macrosporus strain A 1201 16S ribosomal RNA gene partial sequence	1520	1520	84	0	94	NR 026530 1
Streptomyces glaucosporus strain NBRC 15416 16S ribosomal RNA gene partial sequence	1500	1500	84	0	94	NR 0411811
Streptomyces thermolineatus strain NBRC 14750 16S ribosomal RNA gene partial sequence	1496	1496	84	0	94	NR 1124421
*Streptomyces thermolineatus*strain Al484 16S ribosomal RNA gene partial sequence	1491	1491	84	0	94	NR 0265291
Streptomyces morookaense strain CSSP70716S ribosomal RNA gene partial sequence	1487	1487	84	0	94	NR 115430 1

DISCUSSION

In this study, four strains of endophytic actinomycetes were isolated from leaves of *Asphodelus tenuifolius* that were collected from the Northwestern Coast, Egypt. Endemic plants in desert habitat exposed to difficult ecological conditions of high temperature, water scarcity and poor nutrition of sandy soil necessary for the development of plant. This desert habitat and its flora led to a significant variation in the endophytic microbial communities and their biological properties (Banerjee, 2011).

The study explored that, leaves of *A. tenuifolius* are inhabited by actinomycetes. In this context, the results are in line with that achieved by Saini et al. (2016), where out of the 50 endophytic isolates from different organs only one isolate yielded from leaf segment of *Syzygium cumini*. Similar trend was observed by other previous researchers, where Phuakjaiphaeo and Kunasakdakul (2015) reported that after 4 weeks of incubation at 30°C, only 4 out of 36 isolates of endophytic actinomycetes were obtained from leaf part of *Centella asiatica*. According to Saini et al. (2016), successful achievement of endophytic actinomycetes isolation from leaves of *A. tenuifolius* was strong evidence on success of the surface sterilization procedures. As he declared that, the epiphytic microbes are completely removed by 5% sodium hypochlorite, which is frequently used in plant surface sterilization procedures. Besides, Nystatin supplement (50

µgl/ml) in the starch casein agar, which inhibits the growth of fungi and other bacteria aids in establishing suitable settings to isolate and characterize endophytic actinomycetes.

In this work, the best antimicrobial action as evaluated by MIC value was achieved by Act_2EAc, which was the most active isolate against aggressive Gram positive *S. aureus, B. cereus* and *B. subtilus* with MIC 75, 10, 10 µg/ml, respectively. Referring to phytochemical screening data, Act_2EAc enclosed terpenoids, flavonoids and phenolics (Table 2), all of them reported to be antimicrobial agents (Mousa and Raizada, 2013). Consequently, antibacterial and antifungal potential may be attributed to the production of biologically active compounds in media. In harmony with Machavariani et al. (2014), who obtained 179 endophytic actinomycete isolates from leaf tissues of 20 medicinal plants. Out of these, 47 isolates were displaying antibacterial activity against *S. aureus* FDA209 P, 55 against *S. aureus* 209P/UF-2, 41 against *S. aureus* (MRSA) and 13 isolates were active against *E. coli*.

Research on the biological properties of actinomycetes has shown that these bacteria are prospective candidates to produce antifungal compounds, where Bachiega et al. (2005) reported that 20.3% of the actinomycete isolates studied was active against *C. albicans*. More recently, Gandotra et al. (2012) found that 33.3% of *Streptomyces* analyzed showed high degree of activity against *Candida* isolates. In accordance with former studies, 75% (3/4) of the actinomycete isolates (Act_4, Act_3 and Act_2) showed antifungal activity against *C. albicans* with variant potency (Table 1). Act_2EAc recorded MIC value of 25 µg/ml regarding inhibition of *C. albicans* growth. Compared to results reported elsewhere, this proportion is comparatively high, and shows good promise for discovering and production of bioactive compounds that inhibit *Candida albicans.*

The bioactive potential of the potent strains was extended by testing their chelation potential against DPPH radicals. In comparison with ascorbic acid, ethyl acetate crude extracts possessed promising antioxidant activity via chelation free radicals, particularly, Act_2EAc (Fig. 1). According to the phytochemical screening established in table (2), ActEAc presented a great proportion of phenolics and flavonoids as a part of the bioactive compounds pool. Moreover, ethyl acetate is often used as an extracting solvent with a significant selectivity in the extraction of low-molecular-weight phenolic compounds and high molecular-weight polyphenols. Conde et al. (2008) declared that ethyl acetate allowed the highest phenolic content and the selective removal of nonphenolic compounds. Thus, the antioxidant activity of ActEAc may be attributed to the presence of phenolic compounds in the extracts, whereas, there is a well-established significant correlation between phenolic compounds and antioxidant properties of medicinal plants (Baghiani et al., 2010 and Khennouf et al., 2010). The same pattern was seen in studies on endophytes as proved by Yadav et al., (2014), where the study

attributed the antioxidant power of endophytic fungi isolated from *Eugenia jambolana* to the natural phenolic and flavonoid compounds.

Act_2 as proved by 16S rRNA gene sequence analysis, was strongly identified as *Streptomyces fenghuangensis*, (GIMN4.003T). Moreover, morphological features of Act_2 as declared from scanning electron micrograph were identical to that described by Zhu et al. (2011). It is worth to mention, *Streptomyces fenghuangensis,* (GIMN4.003T), for the first time was isolated from medicinal plant whereas, it was isolated before from sea water by Zhu et al. (2011). Genus *Streptomyces* is a promising source of biological active materials with broad biological action as *Streptomyces* Tc052 and *Streptomyces* displayed antioxidant activity (Taechowisan et al., 2009). Likewise, *S. aureofaciens* CMUAc130 exhibited anti-inflammatory and antitumor potency (Taechowisan et al., 2007), also Taechowisan et al. (2005) declared that *Streptomyces aureofaciens* CMUAc130 presented antifungal activity. In 2014, Tanvir et al. reported that *Streptomyces albovinaceus* and *S. badius* are insecticidal.

In conclusion, data revealed four endophytic actionmycetes that were isolated from leaves of *A. tenuifolius*. Act2 was identified as *Streptomyces fenghuangensis* (GIMN4.003T). *Streptomyces fenghuangensis* (GIMN4.003T) exerted a promising antimicrobial and antioxidant activity that may be accredited to the presence of phenolics and flavonoids as shown by phytochemical profile investigation. Regarding the value of *Streptomyces,* more contributions in isolation of active compounds and studying their bioactivities is recommended.

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القدرات المضادة للميكروبات والأكسدة للنابوت الداخلي الأكتينومايسيتس المعزول من أوراق نبات الأسفوديلس تينوفيلس (مرسى مطروح، مصر)

دينا مصطفى فهمي[1]، نجوى محمود صدقي[2]، مي يونس الخواجة[2] ودعاء سعيد زايد[1]
[1] وحدة المنتجات الطبيعية، قسم النباتات الطبية والعطرية، مركز بحوث الصحراء، المطرية، القاهرة، مصر
[2] قسم النبات والميكروبيولوجى، كلية العلوم، جامعة الأزهر (فرع البنات)، القاهرة، مصر

الهدف من الدراسة تقييم النشاط المضاد للميكروبات والنشاط الإلتهامي (الكاسح) للشقائق الحرة لأربع من نابوت الأكتينوميسيت المعزولة من أوراق نبات الأسفوديلس تنيفولس، وتقييم المحتوى من المركبات الفعالة حيويًا. بالرجوع إلى الصفات الشكلية والتحليل الجزئي التتابعي للمادة الوراثية 16S rDNA للأكتينوميسيت Act_2، وجد أن Act_2 تعرف باسم *Streptomyces fenghuangensis* (GIMN4.003T) بنسبة تطابق ٩٦٪ والرقم الكودي له ١١٧٥٠٢١. وقد تم تقييم الجهد المضاد لكل العزلات ضد السلالات الممرضة من البكتيريا والفطريات، وأيضًا تقييم قيمة أقل تركيز مثبط (MIC) لأقوى عزلة. أظهرت النتائج مستويات منخفضة للنشاط التثبيطي لجميع السلالات ضد البكتيريا الممرضة ما عدا الأكتينوميسيت (Act_2) أظهر نشاطًا تثبيطيًا واسعًا ضد جميع السلالات الممرضة المختبرة مسجلة قيمة (MIC) ملحوظة ١٠ ميكروجرام/مل ضد كل من بكتيريا *Bacillus subtilus* و*Bacillus cereus*، بينما الأكتينوميسيت Act_3 وAct_4 أظهرا قدرة مثبطة جيدة ضد *Candida albicans*. كما برهنت النتائج على أن كل مستخلصات الإيثيل أسيتات لعزلات الأكتينوميسيت تملك نشاطًا مضادًا للأكسدة تتراوح قيمته بين ٤٥ و٨٢٪، وذلك بإستخدام الشق الحر DPPH. أظهر المسح الفيتوكيميائي لمستخلصات الإيثيل أسيتات لعزلات الأكتينوميسيت (ActAcE) عن وجود مركبات فعالة من الفينولات، الفلافونيدات، الإستيرولات، الأحماض الدهنية، الأنثراكينون والنفثاكينون.