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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(2): 427-445. **OPEN ACCESS**

Screening the Egyptian actinomycetes as candidates for anti-microbial compounds and identification of *Nocardiopsis dassonvillei* HBUM17483

Nermine Nasr Eldin Abed

Department of Botany and Microbiology Faculty of Science (For girls), Al-Azhar University, Nasr City, Cairo, **Egypt.**

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***Correspondence:** nermineabed67@gmail.com **Accepted:** 05 June 2017 **Published online:** 11 July 2017

The need for new antibiotics is increasing with the increase in antibiotics resistance. The present work focused on the isolation of antibiotic producing actinomycetes from sandy soil .Twenty-four actinomycetes isolates were isolated from sandy soil collected from different locations of Cairo- Egypt. The isolates were tested for their anti-microbial activity, one isolate showed good results against Gram (+ve), *Bacillus subtilis ,Staphylococcus aureus* and *Streptococcus pyogenes* and Gram (–ve) *Pseudomonas aeruginosa ,Klebsiella pneumonia* and *Campylobacter jejuni ,* no antimicrobial activity was observed against filamentous fungi like *Aspergillus fumigates, Syncephalastrumracemosum, Geotricumcandidum* and unicellular fungi *Candida albicans* .Morphological ,physiological and phylogenetic analysis (16S r RNA); in addition to biochemical studies and cultural characteristics that described by the International Keys of Bergey's Manual for identification of actinomycetes as well as chemical analysis of the cell wall ,were carried out for the active isolate under study, which proved that a 99% similarity with *Nocardiopsis dassonvillei* strain HBUM174832. The active metabolites were extracted by using ethyl acetate as a solvent. Compounds No.10 and No. 11 were the most active fractions against G-ve bacteria and G+ve bacteria. The separation, purification and characterization of the active metabolites were performed using Column & thin layer chromatography. The physicochemical studies of the purified active metabolites (10&11) have been investigated. The two purified metabolites (Compounds10&11) were found to belonging to 1, 3-2-hydroxyethyl, phenyl, prop an-2-one with M.W=178$M^+$and emperical formula $C_{11}H_{14}O_2$ and 2- hydroxyl-1-,3-,2- hydroxy-3-oxobutyl phenyl) hexan-3-one with M.W.= 279M^+-1and empirical formula $C_{16}H_{22}O_4$ respectively. The MICs values were determined and pre-luminary cytotoxicity were also assessed. It was concluded that Actinomycete isolate NO.A 18, identified as *Nocardiopsis dassonvillei* strain HBUM174832 has antibiotic producing potential that could be used in the development of new antibiotics for pharmaceutical applications.

Keywords: Actinomycetes, Antagonistic Activity, Antimicrobial Activity, Agar well diffusion method, concentration, Separation, Purification, Characterization *Nocardiopsis sp.*

INTRODUCTION

The appearance of multidrug resistant pathogenic strains caused substantial morbidity and mortality especially among the elderly and immune-compromised patients. To overcome this situation, there is an interest to improve or discover novel of antibiotics that have different mechanisms of action worldwide (Shawn, 1991; Busti et al.2006 and Saha et al. 2010).The continuous screening of secondary microbial products produced from potential bacterial taxa was important to discover novel chemicals for the

development of new therapeutic agents (Holt, 1989; Holt et al. 1994; Hemashenpagam, 2011 and Ibeyaima et al. 2016).Considerable research is being done in order to find a new chemotherapeutic agents isolated from soil that reduced efficiency or inactive against these isolates which has led to increase mortality among patients (Crowe and Olsson, 2001; Courtis et al. 2003 and Rice,2008). Soil microbial communities are among the most complex, diverse and important assemblages of organisms and they participate in various biological activities. Accordingly, they are an important source for the search of novel antimicrobial agents and molecules with biotechnological importance (Hackl et al. 2004; Tawiah et al. 2012; Abdulkhadir and Waliyu, 2012; Tiwari and Gupta, 2013 and Yunus et al. 2016).

Many groups of microorganisms like Gram-positive, Gram-negative bacteria and fungi have the ability of synthesizing antimicrobial agents and the top cultivable antimicrobial agent producers present in soils are the actinomycetes (Pandey et al. 2011 and Bizuye et al. 2013). Actinomycetes are gram positive, free-living, saprophytic bacteria, widely distributed in soil, water, and colonizing plants. Their population has been identified as one of the major group of soil population (Hozzein et al. 2004). The actinomycetes having high G+C content in their DNA. Actinomycetes population has been identified as one of the major group of soil population. Selvamohanal et al. 2009 have intensively pursued screening of actinomycetes for the production of novel antibiotics for many years. These searches have been remarkably successful and approximately two thirds of naturally occurring antibiotics including many of medical importance have been isolated from actinomycetes (Okami and Hotta,1988;Montalvo et al. 2005 and Selvamohannagar, 2016) .Antibiotics have been used in many fields including agriculture, veterinary and pharmaceutical industry(Selvakumar , 2010and Rai et al. 2016).

The present study intends at isolation, purification and identification of antimicrobial active compounds from actinomycetes from different localities of soil, Cairo, Egypt and determining the characterization of the active compound(s).

MATERIALS AND METHODS

Collection and processing of the soil samples.

Twenty four samples were used in this study and they were collected from5-15cm depth from different areas of sandy soil in Egypt making one sample (Sasdoun and Gharaibeh, 2003).The soil sample was diluted plating using starch nitrate medium(El-Nakeeb and Lechevalier,1963) and then incubated for four days at 28-30◦C.

Isolation of actinomycetes .

Isolation of actinomycetes was carried out using pour plate technique on starch- nitrate agar after serial dilution in distilled water (El-Nakeeb and Lechevalier, 1963) and then incubated at 28-30C for 5-6days. Dry colonies were purified by repeated streaking on starch-nitrate agar medium and stored at-20 C.

Screening of actinomycetes for antimicrobial activity .

Screening was carried out according to **Egorov, 1985**, Bizuye et al. 2013 and Abdul Wahab et al. 2015

Cultural characteristics of the isolated strain A18 .

The most potent actinomycetal isolate strain was selected after secondary screening ,and was studied and identified by morphological ,physiological and biochemical methods .Morphological methods consist of macroscopic and microscopic methods (Kawato and Sinobu, 1959; Bergey,1984 & Brergey and Holt,2000). The microscopic characteristics of a selected isolate was studied by using all media and methods of International Streptomyces project (ISP)as described by Shirling and Gottlieb (1966,1968,1969&1972) & Hensyl (1994).

Molecular Identification of the isolated strain A18.

Sequence data was analyzed in the Gene bank database by using the blast program available on the National Center for biotechnology Information Website (www.ncbi.nlm.nih.gov)Theunknown sequence was compared to all of the sequences in the database to assess the DNA similarities. Multiple sequence alignment and molecular phylogeny were evaluated using Bio Edit software (Hall, 1999).

Figure 1.16SrDNA universal primer seq.

Primer name	Nucleotide sequence (5'–3')	Target sequences	Techniques
27^{f}	AGAGTTTGATCCTGGCTCAG	V1, *16S rRNA* gene	PCR
1492^{r}	GGTTACCTTGTTACGACTT	V 9, *16S rRNA* gene	PCR

Scanning Electron Microscopy (SEM).
The isolateA18 was examined after dehydration, drying on the specimen stubs and coating by gold coating on SPI-Module sputter coater and then examined under SEM (JEOL JSM-5500LV) on high mode under 15KV at Regional Center for Mycology and Biotechnology (RCMB) Al-Azhar University Cairo Egypt.

Studies concerning the phylogenetic characteristic.
The preparation of total genomic DNA was conducted in accordance with the methods described by (Sambrook et al. 1989). PCR amplification of 16S r DNA gene of the local actinomycetes strain was conducted using two 16Sr DNA universal primer, which were, in accordance with the method described by (Edwards et al. 1989)as in Fig 1.Purification of PCR products for isolate under study was performed by using Gene JETTMPCR. Purification Kit (Fermentas) was performed in the Sigma company of scientific service from through Regional Center for Mycology and Biotechnology (RCMB) Al-Azhar University, Cairo- Egypt.

Sequences similarities and phylogenetic analysis .
Sequences data were analyzed in the Gene Bank database by using the BLAST program Available on the National Center for Biotechnology Information Website (www.ncbi.nlm.nih.gov). The unknown sequence was compared to all of the sequences in the database to assess the DNA similarities. Multiple sequence alignment and molecular phylogeny were evaluated using Bio Edit software **(Hall, 1999).**

Biosynthesis of active metabolites.
For the biosynthesis of active metabolites, a semi-synthetic medium of starch nitrate liquid medium was used. Aliquots of 2 ml of spore suspension was used to inoculate 250 ml Erlenmeyer flasks , each containing 100 ml sterile liquid medium. The inoculated flasks were incubated at 28 °C-30°C for 5-7 days (Perez et al. 1990).

Extraction of active metabolites .
The extraction process was carried out to clear filtrate using different solvents including diethyl ether, n-hexane, ethyl acetate, butanol and chloroform. The extraction lasted 48 hours during which the solvent was changed three times. The ethyl acetate extract was then evaporated on a rotary evaporator (BuchRotavap RE IV) under vacuum at 40°C. The crude ethyl acetate extract was subjected to biological activity test.

Fractionation and purification of the active metabolites .
The active extract among the previously prepared metabolites was subjected to fractionation using silica gel column chromatography. The column (1.5 x50) was packed with 20 gm. silica gel (G100; mesh 63-200 µm .The elution solvents started with ethyl acetate-methanol (90:10 v/v) followed by ethyl acetate-methanol (80:20 v/v , so on and finally 100% methanol. All fractions were stored at 4ºC till tested for biological activity (Barreto-Bergter et al. 2011).Each fraction was analyzed by thin layer chromatography (TLC) to check the purity and then the fractions in which the active compounds were found , collected and evaporated to the dryness then characterized biologically for activity. The samples were automatically spotted on TLC plates using, CAMMAG LINOMAT 5 application system. The developing processes were carried out using Toluene: Ethyl acetate: Formic acid (5: 4: 1, v/v/v) system.

Physicochemical properties and spectroscopic analysis of the active compounds .

Mass spectroscopy analysis , Infrared spectroscopy analysis(IR), Nuclear Magnetic Resonance (1H -NMR) and Spectroscopy analysis(U.V) were determined at the micro-analytical center - Faculty of Science, Cairo University from through the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University Cairo Egypt. Where, Mass spectroscopy analysis was done using electron impact ionization mode DI- 50 unit of Shimadzu GCMS-QP5050A , Infrared spectroscopy analysis (IR) was carried out using Perkin-Elmer 1650 FTIR spectrophotometer, The proton (1H) NMR spectra were carried out using FT-NMR Braker A© 200 spectrometer and the purified compound was dissolved in methanol and scanned in the range of 200-800 nm in UV- visible spectrophotometer (Spectron 1201 Milton Roy) at the micro-analytical center - Faculty of Science, Cairo University from through the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University Cairo Egypt.

Elemental Analysis .

The elemental analyses were determined at the micro-analytical center - Faculty of Science, Cairo University &from through the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University Cairo Egypt.

Predicting the chemical structure .

The spectroscopic data were employed to reach complete characterization of the empirical chemical structure and the suggested molecular formula of the active compound (s).

Determination of Minimum Inhibitory Concentration (MIC).

Minimum Inhibitory Concentration (MIC) of the antimicrobial agent has been determined by using the agar plate dilution technique (Betina, 1984and Singh et al. 2012).

Cytoxicity (cytopathic effect) of the two antimicrobial agent on the human tumor cell lines .

(HCT-116 cells (human colon carcinoma cells), HeLa cells (human cervical carcinoma cells), and HepG2 cells (human hepatocellular carcinoma) were treated with various concentrations of the tested compounds .The in vitro growth inhibitory activity of the tested compounds was investigated in comparison with the well-known anticancer standard drug Doxorubicin using crystal violet colorimetric viability assay. All assays were performed at least in triplicates and these results were calculated as an average of three determinations (Saha et al. 2010andAbdelfattah et al. 2016) at Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University Cairo Egypt

RESULTS AND DISCUSSION

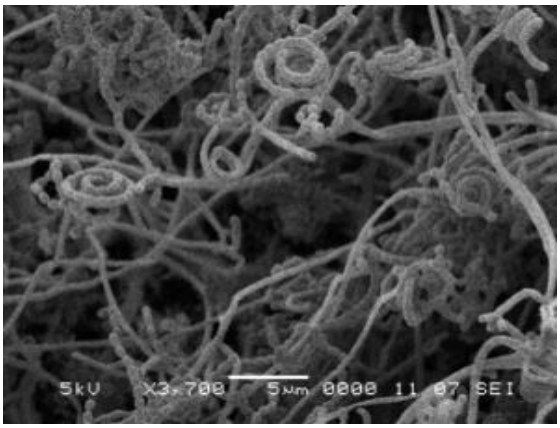
Twenty four different actinomycetes isolates were recovered from sandy soil samples that collected from different localities of Cairo, Egypt using starch nitrate medium. Among these isolates, it was found that, the isolate No. A18 exhibited various antimicrobial activities against Gram +ve and Gram –ve bacteria as shown in Table (1). Isolate No. A18 was showed high antagonistic activity against the tested organisms. Therefore, it was subjected to further studies.

Morphological & Cultural characteristics of the actinomycetes strain A18.

The most potent actinomycete isolate strain No. A18 showed the typical microscopic appearance of most species of the genus *Nocardiopsis* and spores were rod-shaped (0.46-1.5 mm) and smooth-surface.**(2)**

Morphology .

Determination of morphological traits and colours of the aerial and substrate mycelium, as well as of soluble pigments, was performed as described by Shirling & Gottlieb (1966, 1968, 1969 & 1972) and Hensyl (1994). Aerial mycelium of isolateA18 is long, moderately branched hyphae and, at the beginning of sporulation, more



or less

Fig. (2):Scanning electron micrograph of the Actinomycete isolate A18 , spiral shaped

spore chain and smooth spore surfaces showing zig-zag hyphae, and aerial mycelium dividing into rod-shaped spores (X4,500 and X 3, 700).

zig-zag shaped, they then divide into long segments, which subsequently subdivide into smaller spores of irregular size. Spores are elongated and smooth as shown in **Fig. 2.**

Cultural Characteristics .

The results in **Table (2**): showed that aerial hyphae were formed on all media except for Peptone yeast extract iron agar (ISP-6). Abundant and good growth was observed on starch casein agar and tyrosine agar (ISP–7). Moderate growth was also detected on yeast extract – malt extract agar (ISP–2), oat meal agar (ISP–3), inorganic salts-starch agar (ISP–4) and glycerol asparagine agar (ISP–5). No growth was detected on peptone yeast extract iron agar (ISP–6). The isolate A18 has aerial mycelium with yellowish white colour on(ISP– 1), (ISP–2),(ISP–3), (ISP–7) and starch casein agar medium and Light gray color on(ISP–4)and(ISP–5) media. While substrate mycelium was Yellowish brown on (ISP–1), pale yellow on (ISP–2) and (ISP–7), yellowish white on(ISP– 3) and yellowish gray on (ISP–4), (ISP–5) and starch casein agar. Depending on the medium used, the color of the substrate mycelium of isolateA18 is either yellowish white or yellowish brown or yellowish grey or pale yellow in accordance with Meyer, 1976 & 1994. The aerial mycelium varies from a sparse (hardly visible to the unaided eye) coating to a thick, farinaceous to woolly cover of the colonies on the following media: Tryptone yeast extract (ISP-1),Yeast extract/malt extract agar(ISP2),Oat meal agar(ISP3),Inorganic salts starch agar(ISP - 4),Glycerol asparagine agar (ISP – 5),Peptone yeast extract iron agar(ISP-6)and Tyrosine agar(ISP-7) .The color of the aerial mycelium depending on the media used, it was white or yellowish to grayish according to (color code W1-Co 7a, Cr7a –Co Gb on Prauser and Falta,1968;Prauser, 1976and Sindhuphak et al.1985 . Colonies of substrate mycelia have dense filamentous margins. Hyphae of the substrate mycelium fragment into coccoid elements after 3 to 4 weeks, depending on the medium used. Melanoid pigments are not produced on Peptone yeast extract iron agar(ISP6) ,tyrosine agar (ISP 7) and Starch caseinagar. Cell wall hydrolysate of the isolate A18 contains major amounts of meso-diaminopimelic acid (meso-DAP), no characteristic sugars could be detected and these results were also, reported previously by Lechevalier et al. 1977; Lechevalier,1989 and Castresana,2000. These data indicated that the actinomycete isolate A18 has cell wall chemo type III. The morphological characteristics and the chemotaxonomic analysis indicated that the actinomycete isolate A18 was belonging to the genus *Nocardiopsis*

Physiological characteristics.

Morphological & Biochemical characteristics of the actinomycetes isolate NO. A18 was given in the table 3, where the optimal growth of isolate A18 was observed at 28-37°C and at pH 7–7.5 and in the conc. of NaCl 0-5% (very good growth).No growth was observed at10- 20% NaCl orat45°C (Kroppenstedt and Evtushenko,2006).The isolate A18 was utilized L-Arabinose, mannose, glucose, rhamnose, maltose, mannitol, D-fructose, sucrose, D-xylose, Cellobiose and glycerol as sole carbon sources but it did not utilize in medium containing Trehalose, myo-inositol, Raffinose and lactose and this results were in agreement with (Meyer,1976 & 1994; Kroppenstedt and Evtushenko, 2006; Kampfer, et al. 2002 and Kampfer, 2012). Acid is produced from Larabinose, Galactose, Mannitol, Glucose, Sucrose, Rhamnose and D- xylose. But, it wasn't able to utilize Adonitol ,Dulcitol ,Erythritol, Lactose, Melibiose, Raffinose, I-inositol and Sorbitol and this results was in accordance with Meyer,1976 & 1994. Moreover, it was able to utilize Leucine, Histidine, Tryptophan, Serine, Peptone, Esculin, Arginin, Lysine, KNO_3, Glutamtic acid, Sodium nitrate and Potassium nitrate as anitogen source. Also, L-alanine, Gelatin, proline and serine were used as sole carbon as well as nitrogen sources, although proline and serine were weakly utilized, this results as mentioned by Yassin et al. 1997 ;Kampfer et al. 2002 and Ahmed et al. 2013. Meyer (1976 & 1994)**,** reported that *N. dassonvielli* IMRU 509T was not able to liquefy gelatin, while Yassin et al. reported in 1997 the opposite. The result on biochemical characterization indicated that melanin pigment production was not produced on either ISP medium 6 or 7or starch casein agar and although Gordon and Horan 1968reported the production of yellowish, greenish-yellow, or brown soluble pigment. The isolate A18 was able to decompose Tween 85, casein and Xanthine but it was not able to decompose Tyrosine, Tween 80,

urea and allantoin. The isolate A18 showed positive tests of the decarboxylation of Citrate,Malate,Succinate,Acetate and Pyruvate it showed Negative tests of the decarboxylation of

Table (1): The Antagonistic activities of the actinomycetes cultures isolated using starch nitrate medium.

Organisms \ Isolates	A9	A10	A12	A18
AspergillusFumigatus174832(RCMB02568)	-ve	-ve	-ve	-ve
Candidaalbicans (RCMB 05031)	-ve	-ve	-ve	-ve
Syncephalastrumracemosum(RCMB05925)	-ve	-ve	-ve	-ve
Geotricumcandidum(RCMB 05925)	-ve	-ve	-ve	-ve
Streptococcus Pyogenes(RCMB 010015)	10.4	10.8	11.0	23.7
Staphylococcus aureus(RCMB 010028)	12.2	12.0	10.2	18.3
Bacillus subitlis(010067)	8.0	7.3	-ve	18.0
Klebsiellapneumonia (RCMB 010052)	10.0	7.8	10.5	-ve
Pseudomonasaeruginosa(RCMB 010043)	9.0	-ve	11.4	-ve
Escherichia coli(RCM 010052)	13.3	9.8	9.0	14.8
Campylobacterjejuni (ATCC33291)	9.8	10.0	-ve	13.9

- ve=Negative**The data expressed as mean diameter of inhibition zone of pathogenic microbial growth.

Table(2): Cultural Characteristics of the actinomycete isolate A18.

Media	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
Tryptone yeast extract (ISP 1)	Good	Yellowish white	Yellowish brown	Yellowish brown
Yeast extract/malt extract agar(ISP2)	Moderate	Yellowish white	Pale yellow	----------
Oat meal agar(ISP3)	Moderate	Yellowish white	Yellowish white	----------
Inorganic salts starch agar (ISP – 4)	Moderate	Light gray	Yellowish gray	----------
Glycerol asparagineagar (ISP – 5)	Moderate	Light gray	Yellowish gray	----------
Peptone yeast extract iron agar(Isp6)	No Growth	---------	----------	----------
Tyrosine agar(ISP7)	Good	Yellowish white	Pale yellow	Yellowish white
Starch casein agar	Abundant	Yellowish white	Yellowish gray	Light yellow

Table (3) Morphological & Biochemical characteristics of the actinomycetes isolate NO. A18

Characteristics	Results	Characteristics	Results
Chemotaxonomic analysis: Cell wall Hydrolysis For: Diaminopimelic acid (DAP) Cell wall type Sugar Pattern	 Meso DAP III type Absent	Resistance to lysozyme	susceptible
Morphological characteristics: Spore chains	 Zig-zag arrangement	Production of : Phosphatase β-Galactosidase β-Glucosidase	 +ve -ve -ve
Spore surface	Elongated and Smooth	Growth in the Presence of: 0%NaCl 5.0%NaCl 10%NaCl 20% NaCl	 +ve +ve -ve -ve
Spore mass colour	White or yellowish to grayish	Growth at Temp.(optimal) 0-10◦C 28-37◦C 40-45◦C	 -ve +ve -ve
Substrate mycelial color	Yellowish brown to dark brown	PH 5-5.5 pH 6-6.5 PH7-7.5 PH 8-8.5 pH9-10	- ve -ve +ve -ve -ve
Diffusible Pigment Produced	Yellowish-brown or olive-colored to dark brown	Decarboxylation of: Citrate-Malate-Succinate-Acetate-Pyruvate.	 +ve
Melanin on tyrosine agar	-ve	Propionate-Lactate- oxalate.	-ve
Biochemical Characteristics Hydrolysis of: Starch Esculin Gelatin Elastin Arbutin Nitrate reductase Urease Adenin Hypoxanthine Xanthine	 -ve +ve -ve +ve +ve -ve -ve +ve +ve +ve	Acid production from Adonitol Ducitol Erythritol Glucose Inistol Lactose Mannitol Melibiose Raffinose Rhamnose Sorbitol Xylose L-Arabinose Galactose Sucrose	 -ve -ve -ve +ve -ve -ve +ve -ve -ve +ve -ve +ve +ve +ve +ve
Nitrogen source utilization:- Leucine Histidine Tryptophan Serine Peptone Esculin Arginin Lysine KNO3 Glutamtic acid Sodium nitrate	 +ve +ve +ve +ve +ve +ve +ve +ve +ve +ve +ve +ve	Carbon source utilization: L- Arabinose D-Mannose D-Glucose L-Rhamnose D-Mannitol D-Fructose D-Galactose Maltose Sucrose Lactose Raffinose Glycerol Trehalose	 + ve +ve +ve +ve +ve +ve +ve +ve +ve -ve -ve +ve -ve

Potassium nitrate	+ve	myo-Inositol D-Xylose Cellobiose nositol	-ve + ve + ve -ve
Decomposition of: Tyrosine Tween 80 Tween 85 Casein Xanthine Urea Allantoin	 *-ve* *-ve* *+ve* *+ve* *+ve* *-ve*	Utilization of the following Compounds as sole carbon and nitrogen sources:- L-Alanine Gelatin Proline Serine	 +ve +ve W W

Table (4): Similarity to the closest relative in Gen Bank and accession numbers of 16S rDNA Sequence producing alignment for isolate No A18

Acession	Description	Max Score	Total Score	Query coverage	E value	Max Ident.
EM_PRO:KF146896	*Nocardiopsis dassonvillei* strain BA6-3 16S ribosomal RNA gene, partial sequence.	1441	1955.1	99.2	99.2	0.0
EM_PRO:KF146893	*Nocardiopsis dassonvillei* strain BA3-2 16S ribosomal RNA gene, partial sequence.	1390	1955.1	99.2	99.2	0.0
EM_PRO:JX244108	Nocardiopsis sp. TRM46438 16S ribosomal RNA gene, partial sequence.	1426	1955.1	99.2	99.2	0.0
EM_PRO:JX244087	Nocardiopsis sp. TRM46386 16S ribosomal RNA gene, partial sequence.	1365	1955.1	99.2	99.2	0.0
EM_PRO:JN128291	*Nocardiopsis dassonvillei* strain HNS057 16S ribosomal RNA gene,partial sequence.	1400	1955.1	99.2	99.2	0.0
EM_PRO:JN128282	*Nocardiopsis dassonvillei* strain HNS048 16S ribosomal RNA gene, partial sequence.	1405	1955.1	99.2	99.2	0.0
EM_PRO:HF674979	Nocardiopsis sp. S5 partial 16S rRNA gene, strain S5	1435	1955.1	99.2	99.2	0.0
EM_PRO:FJ486426	*Nocardiopsis dassonvillei* strain HBUM174832 16S ribosomal RNA gene, partial sequence.	1446	1955.1	99.2	99.2	0.0
EM_PRO:FJ486358	*Nocardiopsis dassonvillei* strain HBUM174248 16S ribosomal RNA gene, partial sequence.	1444	1955.1	99.2	99.2	0.0
EM_PRO:EU841706	Streptomyces flavidofuscus strain HBUM174057 16S ribosomal RNA gene, partial sequence.	1447	1955.1	99.2	99.2	0.0

Figure (3): The nucleotide sequence of partial16S rDNAgene of isolate No. A18 *Nocardiopsis dassonvillei* strain HBUM174832.

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Score      Expect     Identities  Gaps        Strand
1984 bits(1074)       0.0        1246/1317(95%)      59/1317(4%)          Plus/Plus
Query 7    TGGCGGCCTGC-TGCACATGC-AGTCGAGCGGTAAGGCCCTTCGGGGTACACGAGCGGCG 64
           ||| ||| ||| | ||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct 5    TGGGGGCGTGCTTACACATGCAAGTCGAGCGGTAAGGCCCTTCGGGGTACACGAGCGGCG 64
Query 65   AACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTCCGGGATAAGCGGTGGAAACGCC 124
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 65   AACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTCCGGGATAAGCGGTGGAAACGCC 124
Query 125  GTCTAATACCGGATACGACCCGCCACCTCATGGTGGAGGGTGGAAAGTTTTTCGGTCAGG 184
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 125  GTCTAATACCGGATACGACCCGCCACCTCATGGTGGAGGGTGGAAAGTTTTTCGGTCAGG 184
Query 185  GATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTAACGGCCTACCAAGGCGATTACGGG 244
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 185  GATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTAACGGCCTACCAAGGCGATTACGGG 244
Query 245  TAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTGCG 304
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 245  TAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTGCG 304
Query 305  GGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGAAAGCCTGACGCAGCGACGCCGCGTG 364
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 305  GGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGAAAGCCTGACGCAGCGACGCCGCGTG 364
Query 365  GGGGATGACGGCCTTCGGGTTGTAAACCTCTTTTACCACCAACGCAGGCTCCCAGTTCTC 424
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 365  GGGGATGACGGCCTTCGGGTTGTAAACCTCTTTTACCACCAACGCAGGCTCCCAGTTCTC 424
Query 425  TGGGGGTTGACGGTAGGTGGGGAATAAGGACCGGCTAACTACGTGCCAGCAGCCGCGGTA 484
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 425  TGGGGGTTGACGGTAGGTGGGGAATAAGGACCGGCTAACTACGTGCCAGCAGCCGCGGTA 484
Query 485  ATACGTAGGGTCCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCGTG 544
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 485  ATACGTAGGGTCCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCGTG 544
Query 545  TCGCGTCTGCTGTGAAAGACCGGGGCTTAACTCCGGTTCTGCAGTGGATACGGGCATGCT 604
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 545  TCGCGTCTGCTGTGAAAGACCGGGGCTTAACTCCGGTTCTGCAGTGGATACGGGCATGCT 604
Query 605  AGAGGTAGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGA 664
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 605  AGAGGTAGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGA 664
Query 665  GGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCAT 724
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 665  GGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCAT 724
Query 725  GGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTG 784
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query 785  TGGGGACTTTCCACGGTTTCCGCGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGGAGT 844
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Query 964  CAGAGATGTGAGGTCATTTAGTTG-CGG-TGACAGGT-GTGCATGGCTGTCGTCAGCTCG 1020
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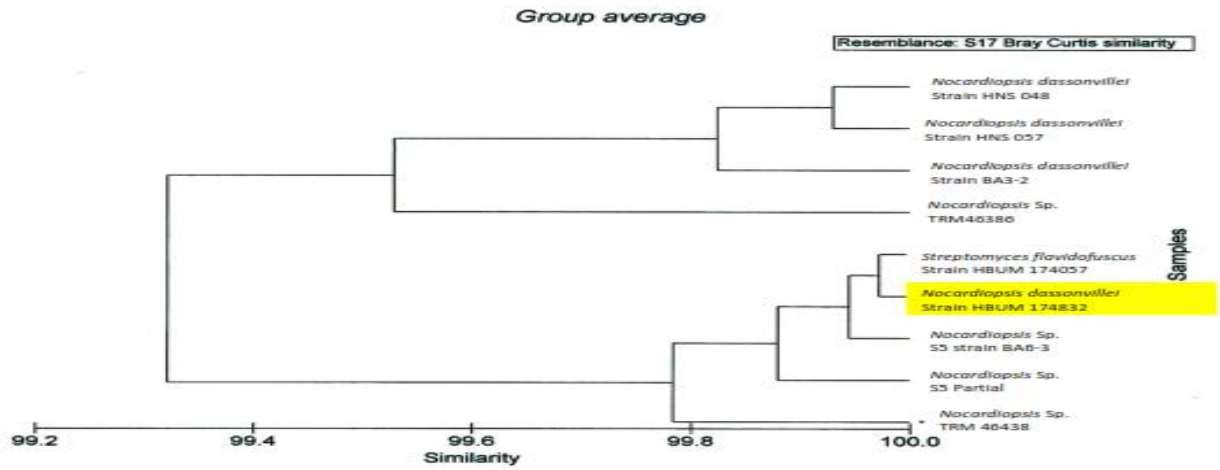

Figure. (4):phylogenetic tree high lighting the position of isolate No.A18 relative to the type strains of the other species within this genus

lactate, oxalate and propionate the same result s recorded by Kroppenstedt and Evtushenko,200 For chemotaxonomic and molecular studies, the isolateA18 was cultured in GYM broth medium (Shirling & Gottlieb,1966) and incubated at 28◦C-37◦C for 6 days at 200 r.p.m. Mycelia were harvested by centrifugation and washed twice with deionized water. We proposed on the basis of 16s r RNA sequence analysis and chemotaxonomic data that the isolate A18 belonged to the genus *Nocardiopsis* and represents, on the basis biochemical tests, a new species designated *Nocardiopsis*. The 16 S rRNA sequence of the isolate A18 was analyzed by a BLAST search and was aligned with those of *Nocardiopsis* reference species available in the GenBank database, which confirmed the identification of isolate A18 at the genus level (Hentschel et al. 2001;Webster et al. 2001; Imhoff and Stöhr, 2003; Montalvo et al. 2005; Nithyanand et al. 2011; Goodfellow, 2012; Sengupta et al.2015; Mohamed et al. 2015 and Girard et al.2012 & 2013).The nucleotide sequence of the 16S rRNA gene (1.272 kb) of the identified isolate proved a 99% similarity with *Nocardiopsis dassonvillei* strain HBUM 174832 as defined by Sun et al. (2010)as in Table 4 & Fig 3. The phylogenetic tree generated by a neighbor-joining method clearly revealed the evolutionary relationship of the isolate A18 with a group of Nocardiopsis species (Supplementary Figure S1).On the basis of the 16 S rRNA gene sequence analysis, the isolate A18 was designated as a species of *N. dassonvillei*strain HBUM174832.

Spectroscopic characteristic:-

Fractions No.10 and No. 11 were found to exhibit the most antimicrobial activities against (G –ve bacteria and G +ve pathogenic bacteria)as shown in the ultraviolet (UV) absorption spectrum of the two compounds (10& 11) which represented in Figs 5 & 6, compound No.10 recorded a maximum absorption peak at 281 nm whereas, compound No.11 recorded a maximum absorption peak at 282 nm **.**The purity of these compounds(10 and 11) were confirmed by the total ion chromatograms , from mass spectroscopic analysis of these compounds that separated in a single peak for pure compound as shown in figures (7&8), and Mass spectrometry analysis of the active substance (Mass spectrum) showed molecular ion peak for compound (10)at 173 m/z(M^+,7.37%) and the base peak at 65 (100%) that gave molecular weight of17 8M^+atomic mass unit ,whereas the compound (11) showed molecular ion peak at 264 (M+,0.02%); 144(M+,0.12%);130 (M+,0.18%) and base peak at149(M+,100%) that gave molecular weight of 27 9M^+-1 atomic mass unit. The infrared absorption spectrum(IR) of the purified active compound (10)exhibited the characteristic band at 3429 cm-1 was referred to stretching vibration of the OH group, bands at 2611 and 2931 cm-1 for CH stretching of CH_2 and CH_3 (asym.), respectively. The peak at 1631 cm-1 for carbonyl group of ketene ,finally, band at 968 for aromatic nucleus .Whereas, the infrared

Table (4): The Fractionation pattern of antimicrobial agents that produced by isolate NO.A18 using the mobile phase (Ethyl acetate) and their effect against different species of bacteria and fungi.

Fractions	*Fungi*		Gram Positive Bacteria		Gram Negative Bacteria	
	Aspergillus fumigatus (RCMB02568)	***Candida albicans (RCMB 05031)***	***Staphy lococcusAureus (RCMB010028)***	***Bacillus subitlis (010067)***	***Pseudomonas aeruginosa (RCMB010043)***	***Escherichia coli(RCM B010052)***
F1	-VE	-VE	-VE	-VE	-VE	-VE
F2	-VE	-VE	-VE	-VE	-VE	-VE
F3	-VE	-VE	-VE	-VE	-VE	-VE
F4	-VE	-VE	-VE	-VE	-VE	-VE
F5	-VE	-VE	-VE	-VE	-VE	-VE
F6	-VE	-VE	-VE	17.2	-VE	-VE
F7	-VE	-VE	-VE	-VE	-VE	-VE
F8	-VE	-VE	-VE	17.5	-VE	12.4
F9	-VE	-VE	20.4	23.4	-VE	16.3
F10	-VE	-VE	20.7	23.6	-VE	16.9
F11	-VE	-VE	20.9	24.1	-VE	17.2
F12	-VE	-VE	-VE	-VE	-VE	-VE
F13	-VE	-VE	-VE	-VE	-VE	-VE
F14	-VE	-VE	-VE	-VE	-VE	-VE
F15	-VE	-VE	-VE	14.1	-VE	-VE
F16	-VE	-VE	17.9	20.1	-VE	15.4
F17	-VE	-VE	18.1	19.6	-VE	15.1
F18	-VE	-VE	18.8	19.9	-VE	15.9
F19	-VE	-VE	19.2	20.2	-VE	15.9
F20	-VE	-VE	-VE	-VE	-VE	-VE
F21	-VE	-VE	-VE	17.0	-VE	13.4
F22	-VE	-VE	-VE	12.6	-VE	11.1
F23	-VE	-VE	-VE	-VE	-VE	14.3
F24	-VE	-VE	-VE	-VE	-VE	-VE
F25	-VE	-VE	-VE	-VE	-VE	-VE
F26	-VE	-VE	16.2	18.2	-VE	14.6
F27	-VE	-VE	16.5	18.4	-VE	14.1
F28	-VE	-VE	16.1	17.9	-VE	14.3
F29	-VE	-VE	-VE	-VE	-VE	-VE
F30	-VE	-VE	-VE	-VE	-VE	-VE
F31	-VE	-VE	-VE	-VE	-VE	-VE
F32	-VE	-VE	-VE	-VE	-VE	-VE
F33	-VE	-VE	-VE	-VE	-VE	13.1
F34	-VE	-VE	-VE	-VE	-VE	13.2
F35	-VE	-VE	-VE	-VE	-VE	-VE
F36	-VE	-VE	-VE	-VE	-VE	-VE

Negative= -ve

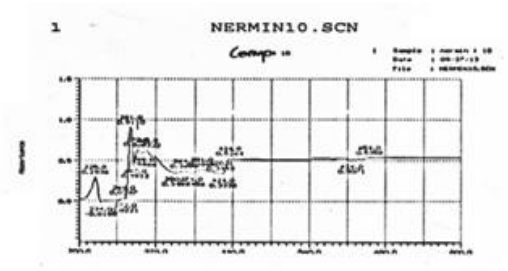

Fig.(5)

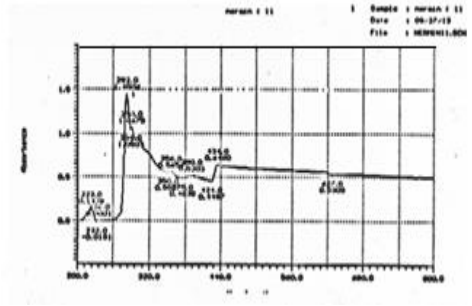

Fig. (6)

Figure (5 & 6): U.Vs pectrum of the purified active compounds(NO.10 & comp.NO.11)produced from isolate No. A18 *Nocardiopsis dassonvillei* strain HBUM174832

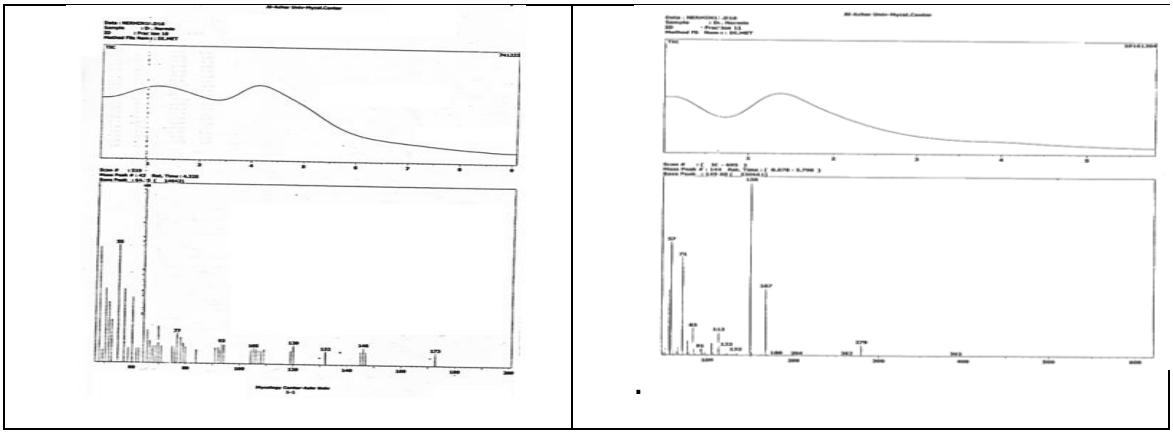

Figure (7): Mass spectrum of the purified active compound 10 produced from isolate No. A18 *Nocardiopsis dassonvillei*strain HBUM174832.

Figure (8): Mass spectrum of the purified active compound 11from isolate No. A18 *Nocardiopsis dassonvillei*strain HBUM174832

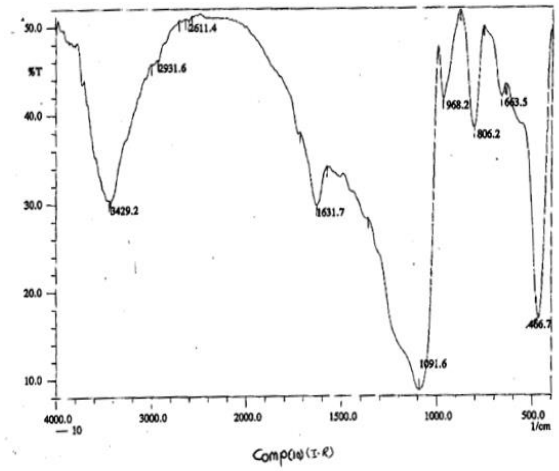

Figure (9): the I.R- spectrum of the purified active compound 10 produced from isolate No. A18 *Nocardiopsis dassonvillei* strain HBUM174832 .

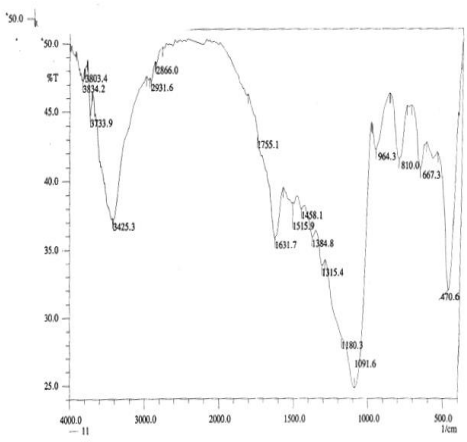

Figure (10): the I.R- spectrum of the purified active compound 10 produced from isolate No. A18 *Nocardiopsis dassonvillei* strain HBUM174832 .

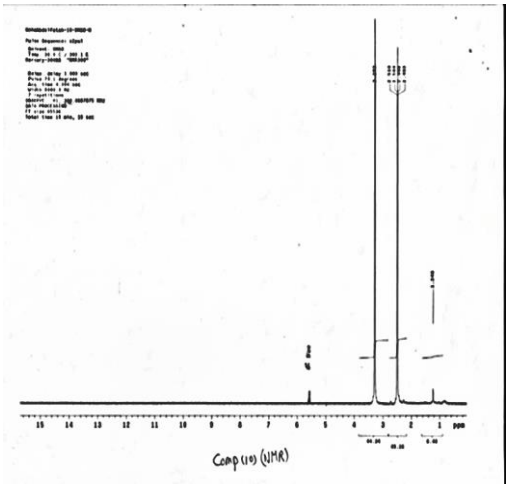

Figure (11): +1H-NMR- spectrum of the purified active compound 10 produced from isolate No. A18 *Nocardiopsis dassonvillei* strain HBUM174832 .

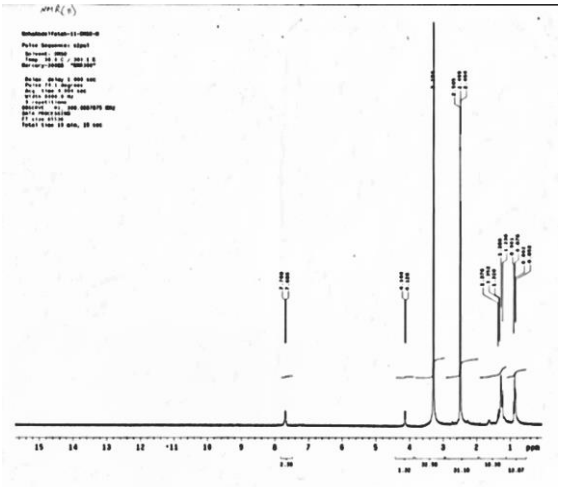

Figure (12): +1H-NMR spectrum of the purified active compound 10 produced from isolate No. A18 *Nocardiopsis dassonvillei* strain HBUM174832 .

Table (6): In vitro antimicrobial activity as MICS (µg /ml) of the purified active metabolites No 10 and No 11 produced by *Nocardiopsis* strain HBUM 174832 A18

Sample Tested microorganisms	Compound 10	Compound 11
	Minimum inhibitory concentration (µg/ml)	
Fungi		
Aspergillus fumigatus(RCMB 02568)	-ve	-ve
Candida albicans (RCMB 05031)	-ve	-ve
Syncephalastrumracemosum(RCMB 05925)	-ve	-ve
Geotricum candidum (RCMB 05098)	-ve	-ve
Gram Positive Bacteria:		
Staphylococcus aur(RCMB 010028)	32.25	7.81
Streptococcus pyogenes (RCMB 010015)	15.63	3.9
Bacillis subtilis (RCMB 010067)	3.9	1.95
Gram negativeBacteria:		
Pseudomonas aeruginosa (RCMB 010043)	-ve	-ve
Escherichia coli (RCMB010052)	62.5	32.5
Klebsiellapneumoniae (RCMB 0010093)	-ve	-ve

Negative=-ve

1-A- Evaluation of cytotoxicity against HCT-116 cell line (Compound10).	1-B- Evaluation of cytotoxicity against HCT-116 cell line (Compound11).
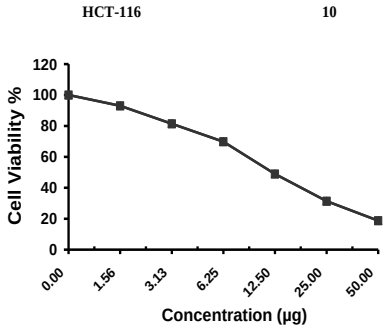	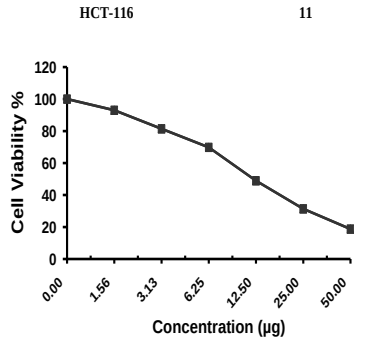
Figure (13): The inhibitory activity against colon carcinoma cells detected under the experimental conditions with IC50 = 23.8 µg.	**Figure (14): The inhibitory activity against colon carcinoma cells detected under the experimental conditions with IC50 = 21.3 µg.**
2-A--Evaluation of cytotoxicity against HepG-2 cell line Compound(10)	**2-B- Evaluation of cytotoxicity against HepG-2 cell line Compound (1 1)**
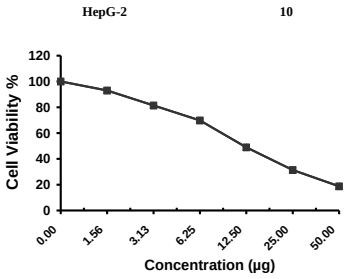	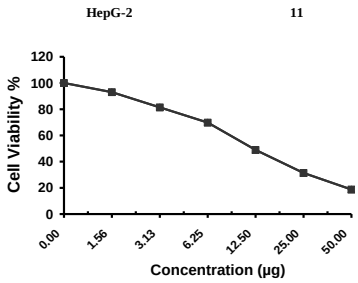
Figure (15): The inhibitory activity against Hepatocellular carcinoma cells detected under the experimental conditions with IC50 = 39.3 µg.	**Figure (16): The inhibitory activity against Hepatocellular carcinoma cells detected under the experimental conditions with IC50 = 44.5 µg.**
3-A -Evaluation of cytotoxicity against HELA cell line(compound 10)	**3-B-Evaluation of cytotoxicity against HELA cell line (compound 1 1)**
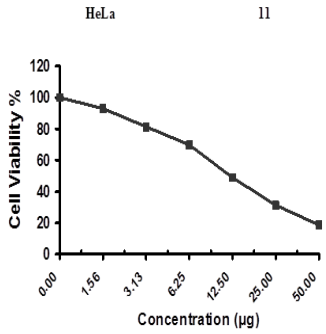	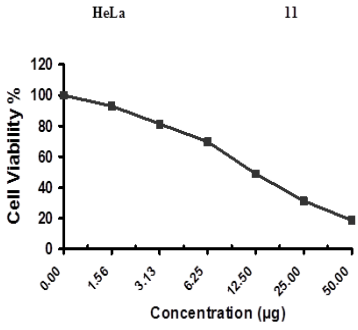
Figure (17):The inhibitory activity against Cervical carcinoma cells detected under theexperimental conditions with IC50 = 23.6µg.	**Figure (18): The inhibitory activity against Cervical carcinoma cells detected under the experimental conditions with IC50 = >50 µg.**

absorption spectrum(IR) of the purified active compound (11) exhibited characteristic absorption beaks at 3425.3cm-1 for the presence of-OH group (Figs 9 & 10). The peaks at 2866.0 cm-1 and 2931.6 cm-1 for the presence of CH_2 and CH_3asym., respectively. Adsorption band for carbonyl group at 1755.1 C=O and band at 1631.7Cm-1 for Benzene ring.

The proton nuclear magnetic resonance spectrum (H -NMR) of the active compound (10) was identified in DMSO as a solvent at 300 MHz (Fig11). The chemical shifts of the proton in the

active compound are shown as:-1.240 ($2H_e$,CH_{2d}),& 2.5-2.49($3H_b$,CH_3CO_b)and3.29($1H_a$,OH_a),6.5for ($4H_{,Ar}$). the^{+1} H NMR (Fig 12)was exhibited a rest protons in a compound 11 that appeared in high field region in between 0.8-0.9 for terminal $CH3_a$,in between 1.236-1.286 for 2H for one $CH2_b$, in between 1.328-1.286 for 2H for other $CH2_c$;in between 2.4-2.5 for 2H for other CH_2f,in between 4.14-4.126 1 H for OH_g ,in between 7.700-7.686 form,4H&Ar-H (Benzene ring)

Elemental analysis.

The elemental analysis of the compound (10) M.Wt=178M$^+$ showed that:C=74.23% ,H=7.93% and O=17.98% from which the empirical formula could be calculated for C_{11} H_{14} O_2 and also, from the fragmentation pattern of MS,+1H-NMR, UV,IR and elemental analysis of compound 10. It was found that, the active purified metabolite might be related to Family: Aromatic antibiotic and Sub family Benzene compounds. Whereas, The elemental analysis of the purified compound (11)279M$^+$-1 revealed that the detection of the following elements%(W/W):C=68.88%,H=7.95% and O= 22.94% from which the empirical formula could be calculated $C_{16}H_{22}O_4$ and from the fragmentation pattern of MS,+1H-NMR, UV,IR and elemental analysis it was found that, the active purified metabolite may be related to Family :Aromatic antibiotic and Sub family Benzene compounds . The results presented in Table 6 showed the MIC of the active compounds 10 & 11, the results indicated that the active compounds produced from *Nocardiopsis dassonvillei* strain HBUM 174832 A18, have antibacterial activity against both Gram- positive and Gram-negative bacteria (Betina, 1984 and Singh et al. 2012).

Evaluation of Cytotoxic activities against three tumor cell lines.

To investigate whether metabolites of the actinomycetal isolate strain A18 had an antitumor effect against three carcinoma cell lines**.** HCT-116 cells (human colon carcinoma cells), HeLa cells (human cervical carcinoma cells), and HepG2 cells (human hepatocellular carcinoma) were treated with various concentrations of the tested compound. The in vitro growth inhibitory activities of the tested compounds were investigated in comparison with the well-known anticancer standard drug Doxorubicin using crystal violet colorimetric viability assay. Data generated were used to plot a dose response curve and results revealed that all the tested compounds showed high variation in the inhibitory activity to the tumor cell line in a concentration dependent manner. Moderate inhibitory activity was observed for the compound under these experimental conditions. This results in accordance with (Xie et al. 2008 ; Zhang et al. 2013 & Aftab et al. 2015) .

Results indicated that compounds 10 &11 were found to be very active at 50 µg /ml against the tested human carcinoma cell lines with inhibition ratio values between 39.6% and 70 %. The results of cell viability in figures (13 -17) indicated that 23.6, 23.8 & 39.3 µg/ml was the effective concentration of compound10 that inhibited 50% from the HeLa, HCT-116, and HepG2 tumor cells. However, the results were showed that compound 11 was showed lower inhibitory activities with IC50 values of 21.3and 44.5 µg/ml HCT-116, and HepG2 tumor cells, respectively. Moreover, the Hela cell line was showed more resistant cells hence it was exhibited higher IC50 value of more than 50 µg/ml. This results in accordance with (Aftab et al. 2015).

CONCLUSION

Actinomycetes are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of about half of the discovered bioactive secondary metabolites notably antibiotics, anti-tumor agents, immunosuppressive agents and enzymes. Because of the excellent track record of actinomycetes in this regard, a significant amount of effort has been focused on the successful isolation of novel actinomycetes from terrestrial sources for drug screening programs in the past fifty years. The present study was an attempt to identify and pick out versatile actinomycete strain that display antimicrobial activity against a variety of microbial pathogens intrinsically. Our result indicate that we are able to identify two different antibiotics namely compd 10) M.W= 178M$^+$/$C_{11}H_{14}O_2$ /1, 3-2-hydroxyethyl, phenyl, prop an-2-one and antibiotic (compd11)M.W.= 279M$^+$-1 / $C_{16}H_{22}$ O_4/2- hydroxy-1 -,3-,2- hydroxyl-3-oxobutyl phenyl) hexan-3-one. Most microorganisms have developed resistance to existing antibiotics. So it has provoked the need of constant research like ours on the production of newer antibiotics to overcome the resistant microorganisms.

CONFLICT OF INTEREST

The present study was performed in absence of

any conflict of interest.

ACKNOWLEGEMENT
Author would like to Prof. Dr. Magda-El-Meleigy for her advises in revision for all research.

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