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In vitro Evaluation of Actinomycete Crude Extracts collected from Nile Delta (Egypt) for Antiviral Activity

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ABSTRACT

The use of microbial products in drug discovery is an ancient and well-established practice. Actinomycetes are known producers of pharmacological and anti-viral agents. This study aimed to screen the crude extract of Actinomycetes some Egyptian collection for their anti (Hepatitis A Virus (HAV-H10), Coxsackie B4 virus (COX B4) and Herpes Simplex Virus (HSV-1)) then, select the most potent actinomycete crude extract (having highest antiviral activity) for processes of purification, identification and mechanism of action. The maximum non toxic dose (MNTD) of each actinomycete extract was determined then antiviral activity against fast growing viral strains replicating in African green monkey kidney (VERO) cells was studied by the reduction in the number of plaques formed by the viruses. A total of 18 extracts Actinomycete isolates with antimicrobial potential against bacteria and fungi was screened for their antiviral activity. The results indicated that; Mnf-21kt extract showed the most promising antiviral activity against three virus strains while extracts from kfs-1ss and kfs-7ss showed inhibition activity against HAV-H10 and HSV-1 only. After purification of extract Mnf-21kt then the analysis of physico-chemical, elemental and spectroscopic analysis (UV, IR, H.NMR, Mass spectroscopy) indicated that; the active compound has the nature of nucleoside analog. On other hand, when the purified active substance was tested for its mechanism of action against HAV-H10, the result indicated that it has induced significant anti-infectivity and anti-replicative effect. The purified compound has promising broad spectrum antiviral activity in an in vitro system.

Key words: Actinomycete extracts, Antiviral, Cytotoxicity, Anti-infectivity, Virus therapy

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INTRODUCTION

Viruses cause numerous diseases in human, with viral-induced emerging and re-emerging epidemics representing a continuous major health risk to the public. Effective control of viral infection and disease has remained an unachieved goal, due to virus intracellular replicative nature and readily mutating genome, as well as the limited availability of anti-viral drugs and vaccines.

The use of microbial natural products in the manufacturing of drugs is an ancient and well-established practice that has yielded such familiar products as penicillin, and streptomycin[1]. *Streptomyces sp* are known as remarkable source of different natural metabolites, possessing a broad range of biological activities[2,3]. In addition to several antimicrobials, numerous compounds have proved to be potent anticancer (bleomycin, staurosporine, geldanamycin, novobiocin), immunosuppressant (FK506, rapamycin), anti-helmintic (avermectin) and antiviral (fativaricin A-1, rifampicin) agents[3,4,5,6,7,8]. Several Streptomyces-derived metabolites are characterized by multiple pleiotropic actions.

It is important to define the meaning of anti-infectivity, protective and anti-replicative effects as well as the possible molecular mechanisms related to each type of these effects.

Respectively, anti-infective activity may include one or more of the following activities:1- Direct inactivation of virus by the extract without affecting cell receptors or intracellular targets i.e., virucidal effect. This may be achieved by blocking the virus receptor molecules, or by virolysis if the inactivant has an enzymatic activity, or physically by antagonizing the net electric charge that lead to virus attraction to the host cells, or by increasing the size of the virion and prevention of the fitting into the receptor. 2- Induction of changes in cell membrane or cellular receptor of the virus. 3- Preventing the virus adsorption and/or uncoating. The last two activities are related to the protective function of a drug.

The protective activity of an extract may include one or more of the following activities: 1- Induction of changes at the cell membrane leading to inhibition of the virus adsorption and/or penetration. 2- Induction of changes in the cell lysosomes inhibiting virus uncoating. 3- Setting the intracellular biochemical mechanisms in such away, which resists the virus replication as in case of interferon [9].

Finally, the anti-replicative activity may include one or more of the following activities: 1- Inhibition of virus uncoating, or inhibition of cellular and/or viral translation mechanisms. 2- Inhibition of cellular and/or viral transcription and replication function. 3- Inhibition of viral

protein processing and/or capsid assembly or maturation.

The purpose of this study was to screen actinomycete extracts with antimicrobial potential (antibacterial and/or antifungal activity) for antiviral activity, select, purify, characterizes the most promising extract and evaluate their antiviral potential, with a long-term goal of discovering a new antiviral drug candidates.

MATERIALS AND METHODS:

Isolation of Actinomycete Cultures from Different Habitats:

Collection of samples:

Samples were collected from different localities at: Al_menofeya governorate sympolized as (Mnf-) kafr Tabloha sympolized as (Tb), kafr Tala sympolized as (Kt) and kafr Zorkan regions sympolized as (Kz), Cairo governorate sympolized as (Cai-) Al_Amereya-Nasser station region sympolized as (Nm), kafr El_sheikh Governorate sympolized as (Kfs-) at Paltim sympolized as (Pt), Sayedi Salem sympolized as (ss), Qaleen sympolized as (Ql) and Peala regions sympolized as (Pe). As shown in **figure (1):**

Preparation of samples:

The collected soil samples were sieved to remove various contaminant materials. Then the samples were air-dried, and mixed with $CaCO_3$ for 24 hours before plating[10] while water samples were collected in sterile glass bottles at a depth ranging from 20 to 30 cm and centrifuged at 5000 rpm for 15min. before plating on the isolation media.

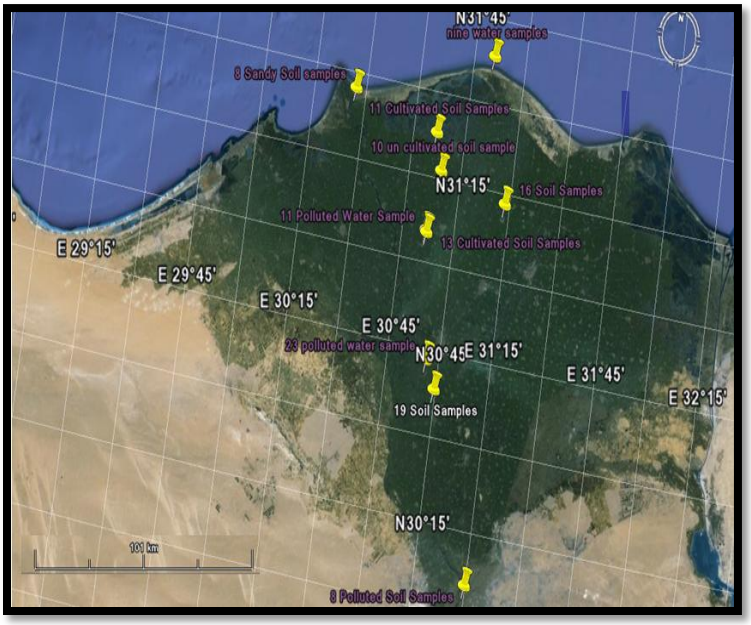

Figure 1: General map of the study area (Nile Delta of Egypt) showing the sampling points where different water and soil samples were collected.

Isolation of Actinomycete cultures:

Each sample was inoculated on media prepared with distilled water and media prepared with seawater. The isolation of actinomycetes colonies from different habitat was carried out by using two methods: 1- Spray and 2- Dilution method according to [10].

Production of Extracellular Bioactive Metabolites:

Spores from five to seven days old culture of the selected most Actinomycete isolates were used to inoculate 250 ml Erlenmeyer flask containing 100 ml of the fermentation medium (liquid starch-nitrate nutrient medium, pH = 7). The seeded flasks were incubated at 30 – 33 °C for 7 days at submerged conditions. After the incubation period, the fermentation broth was harvested (1.0 liters) and filtered through cotton wool then; the clear filtrate was exposed to the extraction process.

Extraction of Extracellular Bioactive Metabolites:

The extraction process was carried out using solvent system Ethanol: Chloroform 1:2 (v/v). The mixture was added to the filtered fermentation broth at ratio of (1:1 v/v); the organic phase was collected, evaporated under reduced pressure by using a rotary evaporator. The evaporation was continued until viscous syrup was obtained. The residual syrup was dissolved in a least amount of solvent and filtered through Whatman No.1 filter paper. These extracts were dissolved in DMSO at a concentration of 100 µg/ml as start concentration to be ready for assay.

Viruses:

Rapidly growing virus strains producing cytopathic effect (CPE) in VERO cell cultures within 3 days was used during this study. These were: Hepatitis A virus H-10 [11], Herpes Simplex Virus type 1 (HSV-1) [12] and coxsackie B4 (Cox-B4) [13,14]. The virus infection of VERO cells was assayed by quantal assay [15] to have 50% tissue culture infectious dose end point (TCID50%) and plaque formation unit (PFU) [16].

VERO Cell culture:

African green monkey kidney cells (Vero cell line) passages number 120 were propagated in Eagle minimum essential medium (EMEM) with Hank's balanced salt solution (HBSS) [17], supplemented with 10% Foetal bovine serum (FBS) and antibiotics (100IU penicillin and 100IU streptomycin /ml) solution and maintained in EMEM with Earl's balanced salt solution (EBSS) supplemented with 2% FBS and antibiotics solution.

Cytotoxicity Assay:

The maximum non-toxic concentration (MNTC) of each crude extract was determined according to [18]. After three days at MNTC, treated Vero cells did not show any morphological differences

when compared with control one.

Plaques Assay:

Anti infectivity effects of microbial extracts were done as described by [19] using 0.2ml of 1:1 mixture of 100 PFU for HAV-H10, 50 PFU for HSV-1 and 130 PFU for Coxsackie-B4 virus suspension in MM and MNTD of crude extracts in MM. On the other hand, protective effects against virus infection were performed as described by [20] where 0.2 ml of MNTD of purified compound were mixed with 2.8ml maintenance medium (MM) then added to each well of Vero cell. After incubation for 48 hrs MM was decanted and washed with HBSS and seeded with 0.2 ml/well containing 40 PFU of virus suspension in MEM without FCS. Finally, anti-replication activities were done as mentioned by[19] using 0.2 ml containing 100 PFU virus suspension in MM/well. After adsorption step, wells were washed twice with HBSS and overlaid with 1:10 purified compound in (v/v) 2% agarose 2x MEM.

RESULTS AND DISCUSSION:

Extract Cytotoxicity:

In this study, in vitro assays were established and employed to screen 18 actinomycete extracts for antiviral activity against three viral strains that are readily available in our laboratory. To properly test these extracts for antiviral activity, highly concentrated starting materials and broad dose-response studies provided the greatest amount of information. However, high concentrations of actinomycete crude extracts may be toxic to cell cultures. To address this, a set of experimental tests was performed to determine the safe and effective dose of these test extracts for the use in cell culture system.

The MNTD range was from 20 - 30 µg of the different microbial extracts as shown in (table: 1) except extracts Mnf-19kt, Mnf-21kt and Kfs-7Dk that showed low Cytotoxicity at 42.6, 40.8, 45.3 µg respectively. The concentration of 100 µg/ml was chosen as the maximum test concentration because drug-like molecules are typically sought to have the desired effect at a concentration less than or equal to 100 µg/ml [21]. In most drug development cases, drug candidates that require concentration higher than 100µg/ml are often discarded due to tolerance and cytotoxicity issues, as well as cost effectiveness. Also, because these are extracts and not purified compounds, the active molecule, if any, may be at a very low concentration within the extract and a concentration of 100µg/ml may allow for any molecule present to produce an antiviral effect. The fact that most extracts remained nontoxic throughout the 3-day experiment was promising.

At MNTD, treated Vero cells did not show any morphological difference when compared with control ones as shown in figure (1). All future experiments would rely on plaque assays that have an incubation time of up to 72 hrs. This time requirement falls well within the range that these extracts were shown to be nontoxic, thus validating the use of these extracts in future experiments that test for antiviral activity.

Anti infectivity effect of different microbial extracts against three viral strains:

The extracts were first tested for their ability to block viral attachment/entry into the cells. Viruses were pre-incubated with test extracts at their maximum safe concentration to allow any interactions to take place that may cause the neutralization of virus infectivity, possibly by binding to and blocking the virus itself from adhering to cells, or by blocking the cellular receptors that are utilized by the virus to enter the cells. This reduction of viral infectivity was determined by a reduced number of viral plaque formations relative to controls containing only virus.

The initial evaluation of these extract specimens demonstrated that some of these extracts have antiviral potential. Mnf-21kt, Kfs-7Dk, and Kfs-1ss. Mnf-21kt isolate extract showed the most promising antiviral activity against the tested virus strains as shown in the following results.

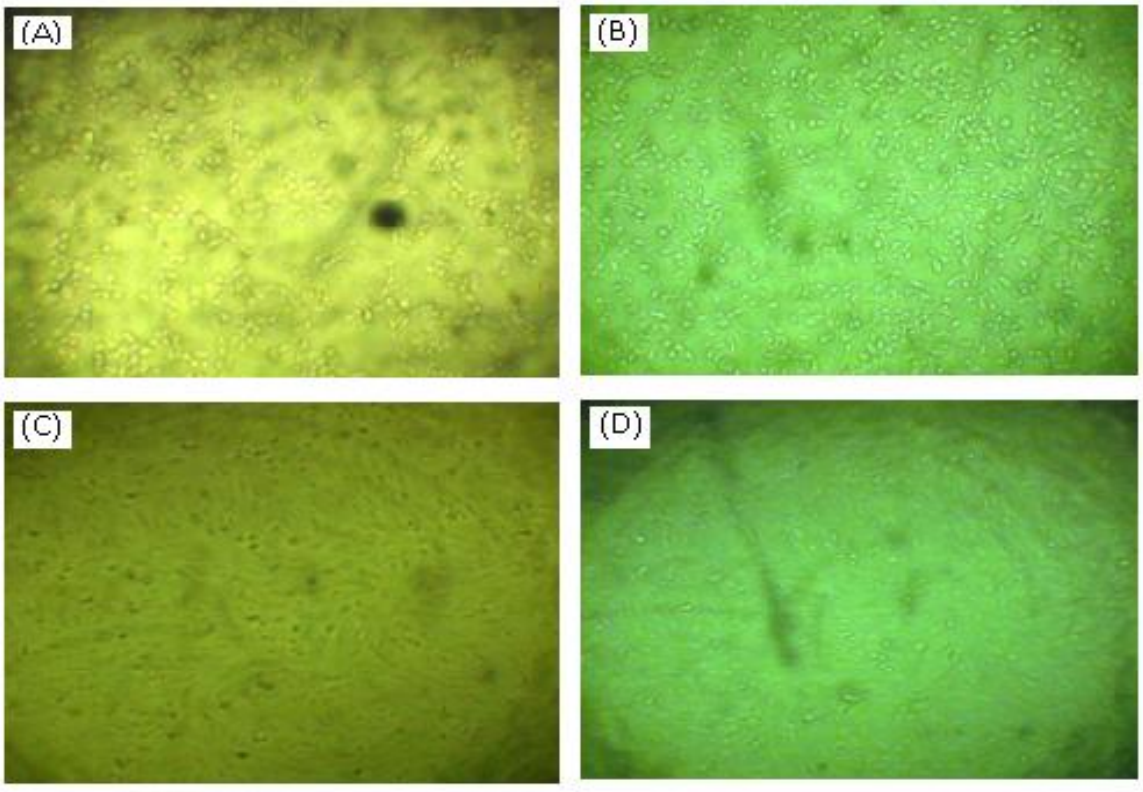

Figure 1: Slides A & B show a form of cytotoxicity of microbial extracts on Vero cells. Slide C & D showed no morphological changes in Vero cells.

Table 1: Summary of extract cytotoxicity.

Microbial Extract	Non Toxic Dose (µg/ml)	Microbial Extract	Non Toxic Dose (µg/ml)
Mnf- 3 Tb	25.8	Mnf-19kt	42.6
Mnf- 4 Tb	30.4	Kfs- 5pt	20.3
Mnf- 12Tb	23.3	Kfs-7Dk	45.3
Mnf- 13Tb	27.5	Mnf-14kt	25.4
Mnf- 18Tb	32.6	Kfs-19pt	33.1
Mnf- 19Tb	20.5	Kfs-1ss	24.4
Mnf- 21kt	40.8	Kfs-13pt	35.5
Mnf- 12kZ	22.2	Kfs- 2 pt	35.8
Mnf- 11Kz	25.3	Kfs-10pt	19.5

Anti infectivity effect of different microbial extracts against HAV-H10:

The result indicated that the most promising microbial extracts are: Mnf-19kt with about 80.06 ±0.58, Mnf-21kt with about 90.05 ± 0.58 and Kfs-7Dk with about 88.46 ± 0.32 % and Kfs-1ss with about 87.70 ± 0.58 of reduction in PFU infectivity as shown in table (2) and figures (2,3). For further tests the Mnf- 21kt should be employed as anti picornaviruses with positive single strand RNA viral mRNA activity.

Table 2: Summary of anti infectivity effect of different microbial extract against HAV-H10 (each value is mean of 3 replicates ± standard deviation (SD)):

Microbial Extract	PFU ± SD	% of reduction± SD	Microbial Extract	PFU ± SD	% of reduction± SD
Mnf- 3 Tb	128.00±0.88	1.43±0.30	Kfs- 5pt	128.00 ± 1.53	1.93± 0.58
Mnf- 4 Tb	130.33±0.33	0.33 ± 0.33	Kfs-7Dk	15.00 ± 1.53	88.46 ± 0.32
Mnf- 12Tb	111.67±1.20	14.11 ±0.59	Mnf-14kt	129.00 ± 5.51	1.34± 0.33
Mnf- 13Tb	89.00 ±0.58	31.90 ±0.59	Kfs-19pt	47.00 ±3.00	63.98 ±0.58
Mnf- 18Tb	47.33 ±2.19	63.89 ±0.59	Kfs-1ss	16.67 ±0.88	87.70 ±0.58
Mnf- 19Tb	77.00 ±2.08	40.97 ±0.58	Kfs-13pt	27.00 ±1.73	79.09 ±0.59
Mnf- 21kt	13.00 ±0.08	90.05 ±0.58	Mnf-11kZ	30.00 ±1.15	70.99±0.58
Mnf- 12kZ	40.33 ±2.60	69.02 ±0.58	Mnf-19kt	25.92±0.32	80.06 ±0.58
Kfs- 2 pt	118.67±4.10	8.98± 0.58	Kfs-10pt	94.67 ±4.84	27.12 ±0.59

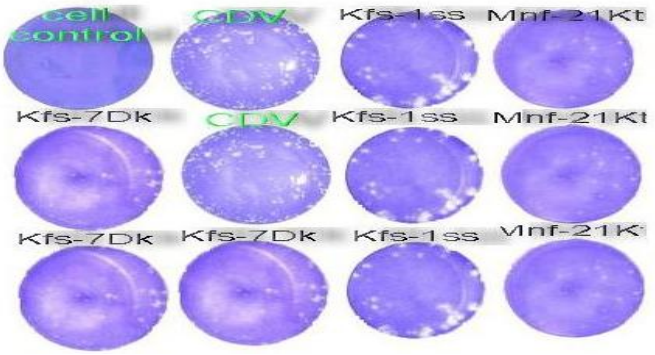

Figure 2: Plaques assay (Anti-infectivity effect) of most promising Mnf-21Kt, Kfs-1ss & Kfs-7Dk isolates on HAV-H10.

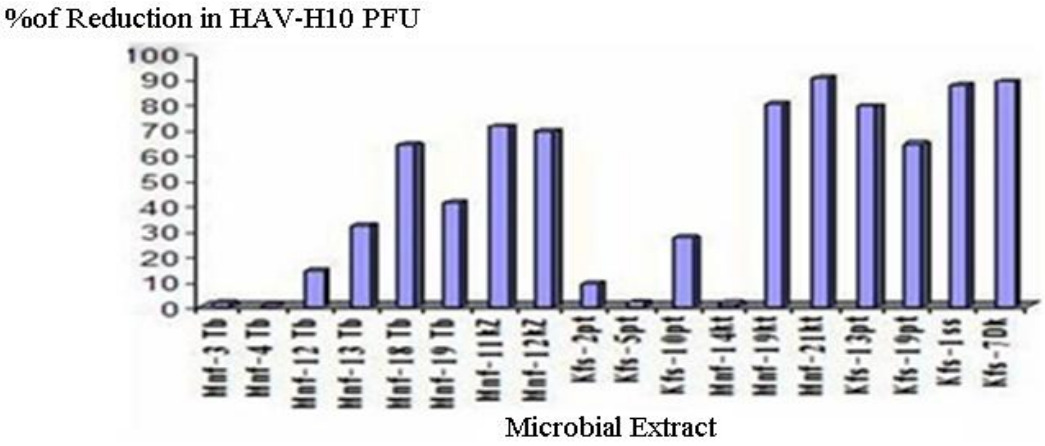

Figure 3: Mean percent of reduction in HAV-H10 PFU by anti-infectivity effect of different microbial extracts.

Anti infectivity effect of different microbial extracts against HSV-1:

The result indicated that the most promising microbial extracts are: Mnf-21kt with about 80.00 ± 0.58 and Kfs-1ss with about 72.00 ± 0.58and Kfs-7Dk with about 74.89 ± 0.59% of reduction in virus infectivity PFU [Table (3) and figures (4, 5)].

By comparison the antiviral activity of Mnf-21kt, Mnf-19kt, Kfs-1ss, and Kfs-7Dk against HAV-H10 (a picornavirus) was more that HSV-1 (a DNA virus). Mnf-19kt extract anti-infectivity against HAV-H10 is about 80% compared to anti-HSV of about 55%. There is a possibility that this extract affect a step in RNA use during virus replication.

Table 3: Summary of anti infectivity effect of different microbial extract against HSV-1 (each value is mean of 3 replicates ± standard deviation (SD)):

Microbial Extract	PFU ± SD	% of reduction± SD	Microbial Extract	PFU ± SD	% of reduction± SD
Mnf- 3 Tb	12.67 ±1.45	60.88 ±0.59	Kfs- 5pt	33.33 ± 2.03	33.11 ± 0.59
Mnf- 4 Tb	24.00± 2.08	52.00 ±0.58	Kfs-7Dk	12.55± 0.07	74.89 ± 0.59
Mnf- 12Tb	34.00 ± 2.08	32.00 ±0.58	Mnf-14kt	20.67 ± 1.76	58.89 ± 0.59
Mnf- 13Tb	19.67 ±0.88	60.89 ±0.59	Mnf-19kt	22.33 ± 1.20	55.11 ±0.59
Mnf- 18Tb	21.00 ±2.08	58.00 ±0.58	Kfs-19pt	38.67 ± 1.76	22.00 ±0.59
Mnf- 19Tb	40.67 ±1.20	18.89 ±0.59	Kfs-13pt	29.00 ± 0.08	42.00 ± .58
Mnf- 21kt	10.04±0.20	80.00 ± 0.58	Mnf-11kZ	43.67 ± 1.76	12.89 ±0.59
Mnf- 12kZ	50.00 ±.19	0.33 ±0.33	Kfs-1ss	14.00± 0.20	72.00 ± 0.58
Kfs- 2 pt	27.00 ± 1.15	46.00 ± 0.58	Kfs-10pt	49.33 ± 1.76	1.44 ±0.29

Anti infectivity effect of different microbial extracts against Coxsackie-B4:

The result indicated that; the most promising microbial extract are: Mnf-21kt with 64.0 ± 0.60, Kfs-1ss with 56.0 ± 0.58, Kfs- 2 pt with 49± 0.58%, Kfs- 13pt with 56± 0.59%, Mnf-14kt with 39± 0.60%, Mnf-11Kz with 31± 0.58% and Kfs-7Dk with 20.0 ± 02.% of reduction in PFU of virus infectivity. The result showed in table (4) and figures (6, 7).

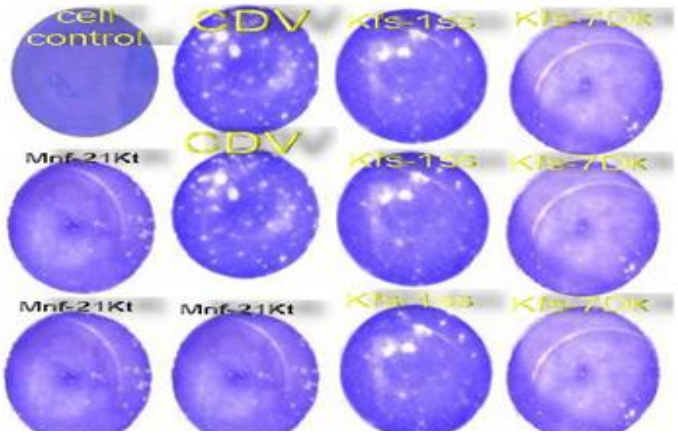

Figure 4: Plaques assay (Anti-infectivity effect) of Mnf-21Kt, Kfs-1ss & Kfs-7Dk isolates on HSV-1.

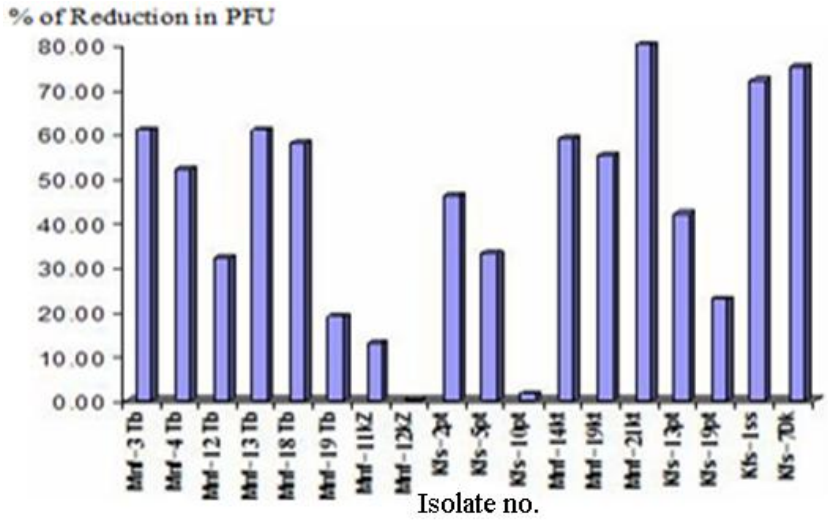

Figure 5: Mean percent of reduction in PFU of Anti-Infectivity effect of different microbial extracts on HSV-1.

Table 4: Summary of anti infectivity effect of different microbial extract against COX-B4 (each value is mean of 3 replicates ± standard deviation (SD)):

Microbial Extract	PFU ± SD	% of reduction± SD	Microbial Extract	PFU ± SD	% of reduction± SD
Mnf- 3 Tb	116.33 ± 3.18	3.0± 0.58	Kfs- 5pt	88.33 ±4.98	26.0±0.59
Mnf- 4 Tb	119.33 ± 0.67	1.0± 0.87	Kfs-7Dk	96.86± 0.22	20.0 ± 02
Mnf- 12Tb	118.66 ± 0.88	1.0± 0.89	Mnf-14kt	72.66 ±3.18	39.0 ±0.60
Mnf- 13Tb	117.66 ± 3.38	2.0± 0.58	Mnf-19kt	113.33 ± 3.84	6.0± 0.60
Mnf- 18Tb	115.00 ±2.89	4.0±0.58	Kfs-19pt	96.66 ±1.20	19.0 ±0.60
Mnf- 19Tb	103.66 ± 3.67	14.0± 0.59	Kfs-13pt	52.33 ±2.85	56.0 ±0.59
Mnf- 21kt	43.20 ± 0.88	64.0 ± 0.60	Mnf-11Kz	82.66 ±3.18	31.0 ±0.58
Mnf- 12kZ	120.00± 0.58	0.0± 0.33	Kfs-1ss	52.80±0.40	56.0 ± 0.58
Kfs- 2 pt	60.33 ± 4.98	49.7±0.58	Kfs-10pt	85.00 ±0.58	29.0 ±0.58

The anti-infectivity of Mnf-21kt (64.0%), Kfs-1ss (56.0%), Kfs- 2 pt (49%), Kfs- 13pt (56%), Mnf-14kt (39%), Mnf-11Kz (31%) and Kfs-7Dk (20%) and surprisingly Kfs-7Dk (20%) and Mnf-19kt (6%) against COX-B4 was less than against HAV-H10 which might be due to an RNA virus receptor and/or host cell RNA virus receptor differences. The almost equal inhibition of both HSV and COX-B4 virus infectivities may point to a "shared" motif either in virus receptor or host cell virus receptor.

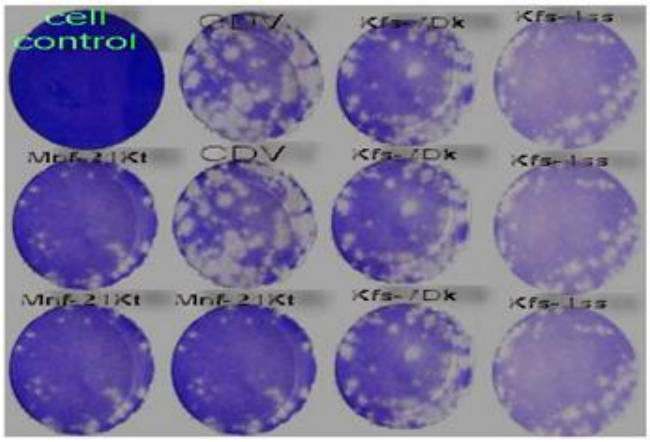

Figure 6: Plaques assay (Anti-Infectivity effect) of Mnf-21Kt, Kfs-1ss & Kfs-7Dk isolates against Cox-B4.

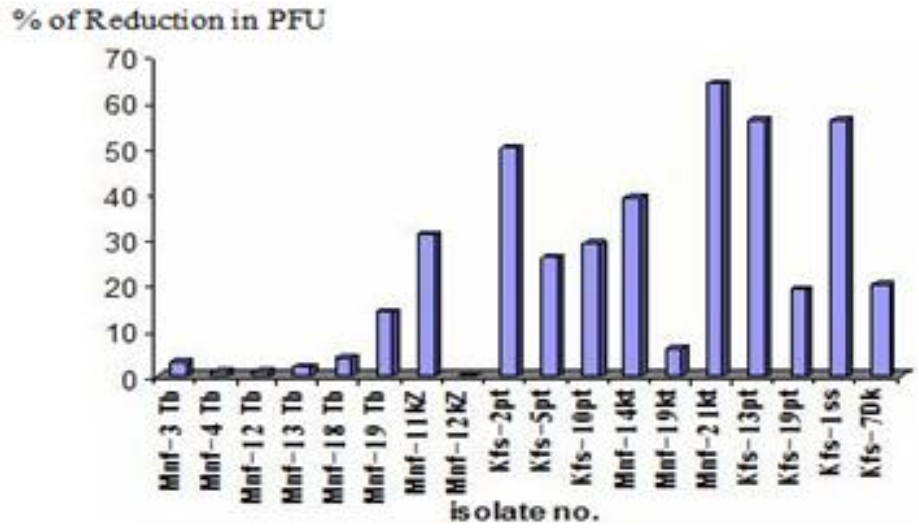

Figure 7: Mean percent of reduction in PFU of Anti-Infectivity effect of different microbial extracts against COX-B4.

The most potent antiviral isolate Mnf-21kt is subjected for further search of identification, production and purification of the active substance while the other two isolates undergo only identification.

Concerning identification of the most active actinomycete isolates that have antiviral activities. The chemotaxonomic, morphological and physiological properties of the actinomycete isolates

Mnf-21kt are consistent with assignment of *Streptosporangium corrugatum.* While the other two isolates are: the isolate kfs-7Dk may be *Streptomyces capillispirales* and the isolate Kfs-1ss may be *Streptomyces viridiviolaceus*. Majority of streptomyces and other actinomycets members produce a diverse array of antibiotics including nucleosides, aminoglycosides, β-lactams, macrolides, peptides, polyenes and tetracyclines[22]

CHARACTERIZATION OF PURIFIED ACTIVE SUBSTANCE OBTAINED FROM MNF-21KT EXTRACT:

After production, extraction, purification of active substance; the analysis of data obtained from spectroscopic characteristics, chemical, elemental analysis and physicochemical properties of the active substance produced by *Streptosporangium corrugatum* mnf21kt indicated that: this active antiviral compound may has nucleoside analogue (purine analogue) nature as shown in the following.

The spectroscopic characteristics of the purified active substance:

The ultraviolet (UV) absorption spectrum of the active substance in methanol using Perkin-Elmer Lambeda 15 UV/V spectrophotometer, exhibit maximum absorption peaks at 238nm (Figure: 8).

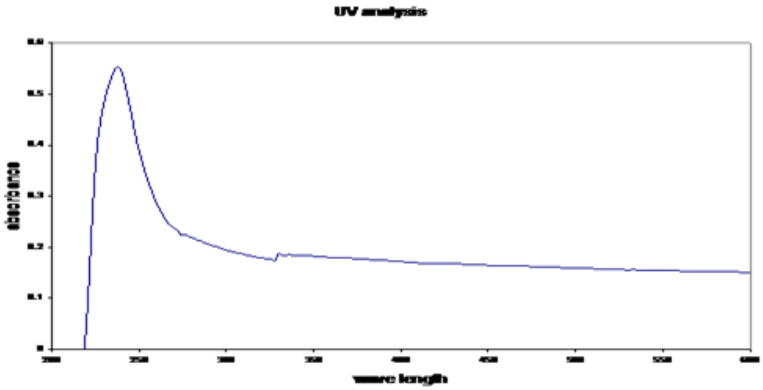

Figure 8: The UV-spectrum of the purified active substance.

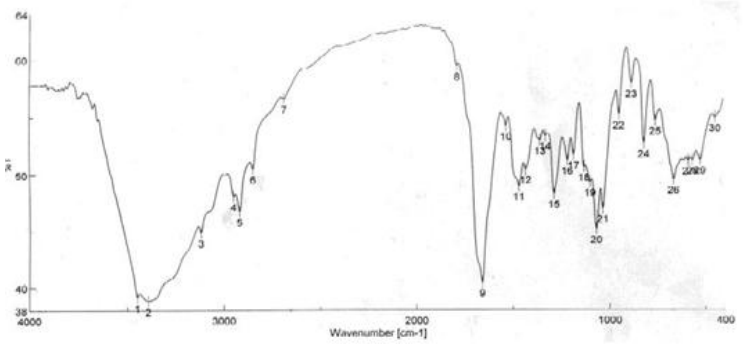

Figure 9: IR-spectrum bands of the purified active substance.

The IR spectra in KBr showed the following bands at: 3448 (broad band of OH), 3392, 3119 cm-1 (NH2, NH), 2952 cm-1(CH-aliph.) and other frequencies characteristic for the remaining part of the molecule (Figure: 9).

1HNMR spectra in methanol exhibited the following signals at δ = 9.0, 6.9 and 5.0 (s, 3H, OH which exchanged by D2O), 4.5 (d, 1H, NH which exchanged by D2O),4.32(t, 1H,CH-CH2OH), 4.0 (s, 2H, NH2 which exchanged by D2O),3.8(d, 1H, NH tetrahydropyrimidin which exchanged by D2O), 3.7(d, 2H, CH2-OH), 2.1 (s, 1H,CH-O), 1.8 (s, 1H,CH-N) 1.3 (s, 2H,CH2 tetrahydropyrimidin) which confirmed its structure (Figure: 10). The mass spectroscopy of purified active substance showed the maximum absorbance at 276.13. Dalton (Figure: 11).

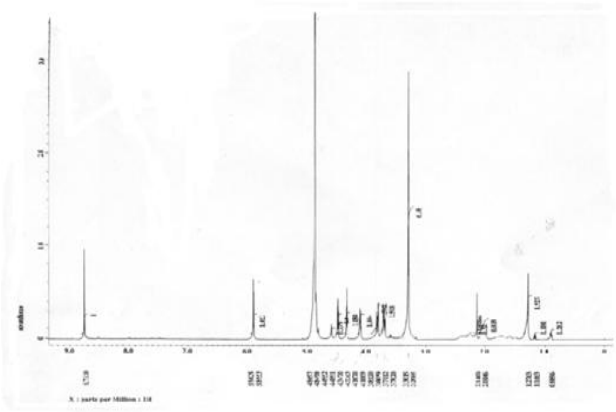

Figure 10: HNMR-spectrum peaks of the purified active substance.

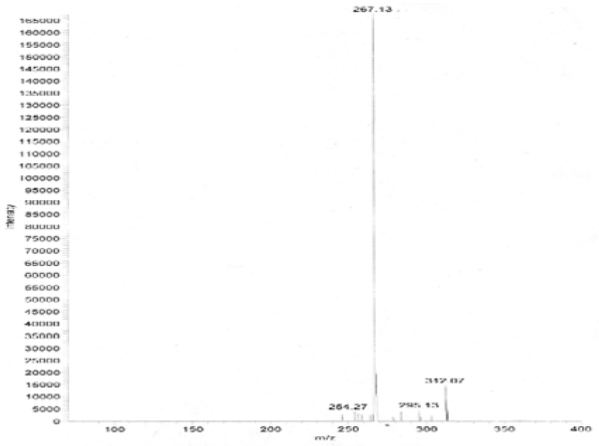

Figure 11: Mass-spectrum of the purified active substance.

Table 5 shows a summary of certain chemical reaction.

Chemical Test	Results	Remarks
Molish's Test	+	Presence of Sugar Moiety
Ninhydrin Test	+	Presence of Free Amino Ggroup
Nitroprusside Reaction	-	Absence of Sulfur
Ferric – chloride Reaction	-	Absence of Di-ketons Group
Fehling Reaction	-	Absence of Free Aldehyde or keto-Sugar Group
Meyer's Reaction	+	Absence of Nitro Group
Silver nitrate Reaction	-	Absence of Chloride
Millon's Reaction	-	Absence of Phenolic Group
Tollen's Reaction	-	Absence of Aromatic Aldehyde or Aromatic Di-ketone or AromaticAmines.

Chemical analysis of the purified active substance:

Classical color reactions have great significance to detect the presence of certain groups in the molecule of the investigated compounds, and enable to detect the active groups and nature of purified substance. The following color reactions were carried out on paper chromatograms of

the concentrated broth or on the substance in a solid form as shown in table (5). Elemental analysis of the purified active substance indicated that; it contains (% w/w): C= 44.28%, H= 6.32%, O=23.59% and N=25.82% So, the suggested chemical formula: ($C_{10}H_{17}N_5O_4$) for the active substance.

The analysis of data obtained from mentioned analysis above indicated that; this active antiviral compound may has nucleoside analogue (purine analogue) nature with the proposed formula as shown in Figure (12). Nucleoside analogues can either act by inhibiting viral reverse transcription (HIV), or inhibiting viral DNA or RNA polymerases synthesis. For example, lamivudine also used as mono-therapy in the treatment of hepatitis B virus infection[23]. The [$C_{10}H_{17}N_5O_4$] active compound have both anti-infectivity and anti-replicative effect but the main antiviral activity of this compound may be attributed to inhibition of a step during viral RNA replication.

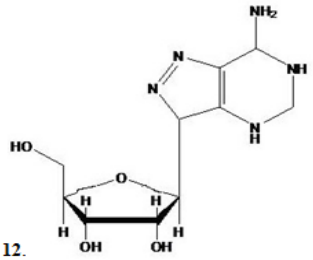

Figure: 12.

(2S,3S,4R)-2-(7-amino-4,5,6,7-tetrahydro-3*H*-pyrazolo[4,3,*d*]pyrimidin-3-yl)-5-(hyroxymethyl)tetrahydrofuran-3,4-diol

Determination of anti-infectivity, protective and anti-replicative effect of purified active substance against HAV-H10 by plaque assay.

The mean percent of reduction in PFU of HAV-H10 was carried out and the result indicated that; the purified active substance has anti-infectivity and anti-replicative effect only (the protective effect was not effective). Each value is mean of 3 replicates ± standard deviation. The results were recorded in table (6) and figure (13).

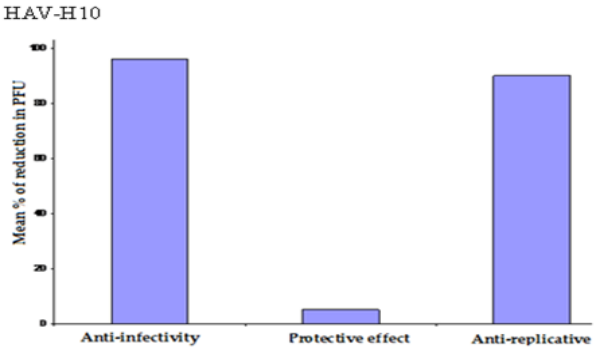

Figure 13: Anti-Infectivity, protective and Anti-Replicative effect of purified active substance using Vero cell culture against hepatitis A virus (HAV-H10).

Table 6: anti-infectivity, anti-replicative & protective effect of purified active substance against HAV-H10.

Assay	CDV (PFU/200µl)	Mean no. of plaques with active compound	Mean % of reduction in PFU
Anti-infectivity effect	120±1.9	5.0± 2.3	96 ± 2.1
Protective effect	110±2.8	100±4.5	9.1 ± 3.4
Anti-replicative effect	115±2.5	12±2.9	90 ±3.1

In the present study three bioactive crude extracts with varying degrees of antiviral activity against three viruses (HAV-H10, HSV-1 and COX-B4 virus) were obtained from the culture broth of actinomycetes isolates collected from Nile Delta (Egypt). The bioactive compound in purified form has nucleoside analog nature with strong antiviral properties against HAV-H10 a picornavirus. It's source actinomycetes culture broth had also anti-COX-B4 (64%) and anti- HSV (80%) anti-infectivity action. The difference in anti-infectivity of HAV-H10 and COX-B4 the two RNA picornaviruses may be due to difference of purines in the RNA genomes. This is also applies to the HSV-DNA genome purine motif. Further work is needed in order to achieve the long-term goal of discovering new compounds to be used as potential antiviral drug candidates.

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