BIOLOGICAL STUDIES ON ACTIVE COMPOUNDS FROM *TRICHODERMA VIRIDE*.

BY

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Abstract

There has been some evidence to date suggesting that *Trichoderma* species. had several biological activities. To test antibacterial and antifungal activity, the secondary metabolites obtained by culturing the fungi *Trichoderma viride* RCMB 017002 on YES broth for 21 days were extracted using chloroform/methanol solvent system then screened by agar well diffusion method. Two fungal metabolites were isolated and purified from the chloroform/methanol (2:1) extract of *Trichoderma viride* broth culture using column chromatography. The chemical structures of the separated metabolites were elucidated using the spectroscopic methods FTIR, ^{1}HNMR and EI-MS analyses. The first compound was benzene diamine derivative produced from fraction 10 and showed good inhibitory activity against the tested bacteria, and tumor (against HepG2 & MCF-7) cells. Also, compound 1 showed moderate antiviral activity against herpes simplex type 1 virus along with no antifungal activities. However, compound 2 was with the known compound, 6-pentyl-α-pyrone that produced from fraction 17

with broad spectrum antimicrobial activity as well as moderate antitumor activities and weak antiviral activity. This report demonstrates the biological activities of new fungal metabolites from *Trichoderma viride* as potential drug that need further studies and development.

Key words: *Trichoderma viride*, Secondary metabolites, spectroscopic methods, antibacterial, antifungal, antiviral and antitumor.

Introduction

Antimicrobial agents have been in widespread and largely effective therapeutic use since their discovery in the 20th century. However, the emergence of multi-drug resistant pathogens now presents an increasing global challenge to both human and veterinary medicine. It is now widely acknowledged that there is a need to develop novel antimicrobial agents to minimize the threat of further antimicrobial resistance (Rachel, *et al.*, 2009).

Chemical pesticides have been the objects of substantial criticism in recent years, mainly due to their adverse effects on the environment, human health and other non-target organisms (Epstein and Bassein, 2003; Raju *et al.*, 2003). The potential use of microbe-based biocontrol agents as replacements or supplements for agrochemicals has been addressed in many reports (Hynes and Boyetchko, 2006).

Public awareness of the negative impact of synthetic fungicide and bactericide residues on human health and environment has prompted withdrawn of some chemical antibiotics from the market are the reasons why many research groups investigate potential strategies to develop alternatives to synthetic antibiotics (Gan-Mor and Matthews, 2003).

The development of newer drugs continues to rely heavily on the isolation of natural sources rather than applications based on rational drug design and combinatorial chemistry, thus fungal secondary metabolites are potential targets for the discovery of novel chemotherapeutic agents including antineoplastic and antiviral agents (Cragg and Newman, 2000).

Screening new organisms for antibacterial activity and searching for new antibacterial drugs is important due to the constant generation of new antibiotic-resistant strains of pathogenic bacteria (Janes *et al*., 2006).

Secondary fungal metabolites represent a diverse group of bioactive compounds characterized by their origin and biosynthetic pathways. In fungi they serve as regulators, chemical messengers in developmental processes, or as defense system for the survival of the organism against their environment (Schneider *et al*., 2008).

Fungal secondary metabolites may be considered a large and heterogeneous group of small molecules not directly essential for growth, but having an important role in signaling, development and interaction with other organisms (Song *et al.*, 2006; Mukherjee *et al.*, 2012).

Trichoderma spp. are well-studied filamentous fungi commonly found in the soil community that are widely marketed as biopesticides, biofertilisers and soil amendments, due to their ability to protect plants, enhance vegetative growth and used to protect and/or increase the productivity of various crops (Vinale *et al.*, 2008&2012; Lorito *et al.*, 2010).

One factor that contributes to the beneficial biological activities exerted by *Trichoderma* strains is the wide variety of secondary metabolites that they are able to produce (Sivasithamparam and Ghisalberti, 1998; Reino *et al.*, 2008).

The secondary metabolites of *Trichoderma viride* confer the biocontrol activity of this species (Chet, 1987; Ranković, 2005).

The accumulation of *Trichoderma* metabolites varies according to the species or the strain used and is related to the biosynthesis and biotransformation rates (Vinale *et al.*, 2009).

Therefore, *Trichoderma* is a potential source of bioactive molecules. Here, in this report the *Trichoderma viride* metabolites were screened for their antimicrobial properties followed by bioguided fractionations. This study is also intended to evaluate whether *Trichoderma viride* purified metabolites have the ability to produce antibacterial, antifungal, antitumor and antiviral agents.

Materials and Methods

Organisms:

The *Trichoderma viride* RCMB 017002 was obtained from the culture collection of the Regional Center for Mycology and Biotechnology. Also, the tested microorganisms, including nine bacteria and twelve fungi, were obtained from the culture collection of the Regional Center for Mycology and Biotechnology. These test microorganisms were challenged in this study to ascertain the antimicrobial properties of the *Trichoderma viride* RCMB 017002 extracts and its purified compounds. Of the bacterial isolates selected, included some Gram-negative organisms while the other Gram-positive organisms. Of the fungi examined, five were yeasts, with the remaining seven being filamentous fungi.

Cell lines:

HepG2 cells (human hepatocellular carcinoma cell line) and MCF-7 cells (human breast carcinoma cell line) were obtained from the American Type Culture Collection (ATCC). Vero cells (African green monkey kidney cell line) were obtained from European Collection of Cell Cultures (ECACC).

HSV-1 virus:

Herpes Simplex type 1 virus was used in this study to evaluate the antiviral activity of the tested metabolites. The virus strain used was GHSV-UL46 strain. Virus stocks were prepared by infecting Vero cell line (1 day after passage at a ratio of 1:4, infected to uninfected cells) in plastic flasks at 37°C **(Al-Salahi** *et al.*, **2015)**. The cells were checked by microscopy for cytopathic effects (CPE). At 75% CPE, the medium was decanted and freezed at -70°C. The virus was propagated and quantified in terms of the 50% tissue culture infective dose ($TCID_{50}$) by endpoint dilution (Flint *et al.*, 2000).

Media used:

Malt Extract Agar (MEA):

Ingredients in g/l: malt extract, 20.0; peptone, 1.0; glucose 20.0; agar, 20.0 and distilled water 1L. The pH medium was adjusted at 5.5. This medium was used for cultivation of *Trichoderma viride* RCMB 017002 and the tested pathogenic fungi.

Nutrient agar (NA): The medium was used to cultivate tested pathogenic bacteria. It contains (g/l) Beef extract, 3; Peptone, 5 and distilled water 1L.

Biosynthesis of fungal secondary metabolites:

For the biosynthesis of extracellular fungal secondary metabolites, a semi-synthetic medium of Yeast-Extract Sucrose (YES) liquid medium was used. The YES medium contains (g/l); Yeast extract, 20.0; sucrose, 150.0; distilled water, 1.0 L (Paterson and Bridge, 1994). For enhancement of secondary metabolite production, 1 ml trace element solution was added to 1 L YES medium. The trace element solution is prepared by dissolving 0.5 g magnesium sulphate, 0.5 g cupric sulphate and 0.5 g zinc sulphate in 100 ml distilled water. The pH was adjusted to 6.5 ±0.2 and then autoclaved at 121°C for 15 min. The spores were scrapped from the mycelium of *Trichoderma viride* RCMB 017002

after 10 days of growth at 25°C on MEA medium and suspended in sterile distilled water. Aliquots of 2 ml of spore suspension were used to inoculate 250 ml Erlenmeyer flasks, each containing 100 ml sterile YES liquid medium. The inoculated flasks were incubated at 28 °C for 21 day. The mycelium was then harvested by filtration. The filtrate was concentrated and used as concentrated crude extracellular secondary metabolites. The concentrated crude extract was sterilized by filtration and further dilutions were made from the stock (**El-Shiekh *et al.* 2014**).

Extraction of the secondary metabolites:

Yeast extract sucrose broths (2 L) obtained from fungal cultures was mixed with (2 L) chloroform / methanol (2: 1, v/v). The mixture was shaken vigorously in a separating funnel and left to settle down forming a dense lower organic layer containing the secondary metabolite. Extracted metabolites were then concentrated by using a speed vacuum device (Maxi Dry Plus) to a volume of 5 ml (**El-Shiekh *et al.* 2014**).

Purification of the secondary metabolites:

Two milliliters of crude extract (chloroform/methanol (2:1) extracted metabolites) were subjected to separation using column chromatography (1.5 cm diameter and 50cm long) packed with silica gel (G100) after activated at 80 ºC for 30min, then subjected to elution with graded series from chloroform and methanol. In order to stabilize and equilibrate the bed, the void volume of crude fungal extract was passed through the column. Thirty four fractions were obtained each contain 2ml. All fractions were stored at 0-4 ºC (**El-Shiekh *et al.* 2014**).

Antimicrobial activity:

Antibacterial and antifungal activities were expressed as the diameter of inhibition zones; well diffusion method was used. Holes (0.6 cm diameter) were digger in the agar using sterile cork borer in sterile malt agar plates for fungi and sterile nutrient agar plates for bacteria, which had previously been uniformly seeded with tested microorganisms. The holes were filled by fungal filtrates (100 µl). Plates were left in a cooled incubator at 4 °C for one hour for diffusion and then incubated at 37 °C for tested bacteria and 28°C for tested fungi (**Elaasser *et al.* 2011**). Inhibition zones developed due to active antimicrobial metabolites were measured after 24 h of incubation for bacteria and 48 h of incubation for fungi. The antibiotic ciprofloxacin (MAST Diagnostics Ltd., Merseyside, UK) and amphotericin B (Sigma-Aldrich, UK), were used as the positive control. The choice of ciprofloxacin was guided by the fact that it is a broad-spectrum antibiotic, thus having antibacterial properties for both Gram-positive and Gram-negative organisms.

Evaluation of the antiviral activity:

A plaque reduction assay was performed for the evaluation (**Harper, 2000**). Briefly, monolayers of Vero cells grown on 96-well culture plates were infected with Herpes Simplex type 1 virus. After incubation for 1h to allow viral adsorption, the inoculum was aspirated and the cultures were overlaid with maintaining medium [DMEM with 2% fetal calf serum] and 1% methylcellulose containing dilutions of the fungal metabolites. After an incubation time of 48h at 37ºC, the plates were fixed with formalin, stained with crystal

violet, air-dried and the number of plaques in each well of the antiviral activity according to the standard method (**Hill *et al.*, 1991**) was counted under a light microscope. Six wells overlaid with methylcellulose medium without the fungal metabolite were used as controls. The percentage of inhibition of plaque formation was calculated as follows: [(mean number of plaques in control - mean number of plaques in sample)/(mean number of plaques in control)] x 100.

Evaluation of the antitumor activity:

HepG2 and MCF-7 cells were grown as monolayers in RPMI 1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well microtiter plate incubated for 24h at 37ºC in a humidified incubator with 5%CO_2. The monolayers of the tumor cells were then washed with sterile phosphate buffered saline (PBS; 0.01 M pH 7.2) and simultaneously the cells were treated with 100 µl from different dilutions of fungal metabolites in fresh maintenance medium and incubated at 37ºC. A control of untreated cells was made in the absence of fungal metabolites. Six wells were used for each concentration of the test sample. Every 24 h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590nm using ELISA reader after well mixing. The percentage of viability was calculated as [1-(ODt/ODc)] x100% where ODt is the mean optical density of wells treated with the fungal metabolites and ODc is the mean optical density of untreated cells. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots (**Elaasser *et al.* 2011**).

Characterization of the purified active fraction:

In order to determine the chemical structure of the active compound, the following spectroscopic measurements were carried out:

1- Infra Red spectra:

Infrared absorption spectrum was carried out using anicum infinity series FTIR, Perkin-Elmer 1650 Spectrophotometer, at Micro Analysis Center, Cairo University.

2- Nuclear Magnetic Resonance (NMR):

Proton (^{1}H) NMR spectra was carried out using FT-NMR Braker Ac 200 spectrometer, at Micro Analysis Center, Cairo University.

3- Mass spectroscopy:

Electron impact mass spectrometric spectrum was carried out using direct inlet unit (DI-50) in the Shimadzu QP-5050 GC-MS at the Regional Center for Mycology and Biotechnology, Al-Azhar University.

Results and Discussion

The antagonistic properties of *Trichoderma* spp. in terms of antibiotic production have been described by **Ranković (2005)** and this has stimulated the investigations in this

field. Several species of the genus *Trichoderma* belong to the most profoundly studied and extensively used biological control agents mainly due to the production of secondary metabolites; this may not be the case for others (Harman *et al.* 2004; Reino *et al.* 2008). Subsequently, this study focused on detection, isolation and identification of significant secondary metabolites excreted by *Trichoderma viride* RCMB 017002 isolate with biological activities.

The antimicrobial activity of extracelluler secondary metabolites of *T. viride* RCMB 017002 metabolites against twenty one pathogenic bacteria, yeast and fungi is shown in Table 1. *Trichoderma viride* RCMB 017002 chloroform/ methanol (2:1, v/v) metabolites demonstrated antibacterial activity against all of tested bacteria (zone of inhibition ranged from 17 to 31 mm). Despite the amount of research devoted to elucidating the mechanisms by which *Trichoderma* exerts its antimicrobial activity, the knowledge regarding the involvement of the secondary metabolites is primarily considered to be the major mechanism for the bioactivity (Lorito *et al.*, 2010; Vinale *et al.*, 2012).

The active ingredients from the extract were purified on G100 silica gel column using bioguided fractionation resulting in two active purified fractions (fraction numbers 10 & 17) were responsible for the antimicrobial activity in *Trichoderma viride.* The active fraction 10 was confirmed its purity by TLC and designated as compound 1. However, fraction 17 was the second active fraction with its purity reached 98%) by TLC and designated as compound 2 (Fig. 1).

On the other hand, *Trichoderma viride* compound 1 exhibited high antibacterial activity hence zone of inhibition ranged from 17 to 28 mm when tested against the positive control (ciprofloxacin) and exerted their activities against 100 % (9/9) of the tested bacteria (Table 1). However, compound 2 exhibited lower antibacterial activity (ranged from 10-17 mm) also exhibited moderate antifungal activity against 91% (10/11) of the tested yeast and filamentous fungi and gave variable zone of inhibition (ranged from 8-15 mm) with *Candida dubliniensus* was the highly susceptible species followed by *Candida albicans*. Regarding the activity against the tested fungal species, the extract showed lower tendency to inhibit the tested yeast strains (4/5; 80%) along with no observed activity against the filamentous fungi tested. On the other hand, no antifungal activities were detected for compound 1 under these screening conditions. None of *Trichoderma viride* metabolites inhibited the growth of *Cryptococcus humicola* species examined under these screening conditions.

Similarly, Harris *et al.* (1993) reported the isolation and structure elucidation of several members of a new group of antibiotics from *T. viride* which have been termed viridiofungins; they belong to a family of the amino alkyl citrate antibiotics possessing several biological properties. They may act as inhibitors of squalene synthase, the first enzyme in the ergosterol biosynthesis pathway. Inhibitors of this enzyme have been described as potential antifungal agents (Nakayama *et al.* 2000).

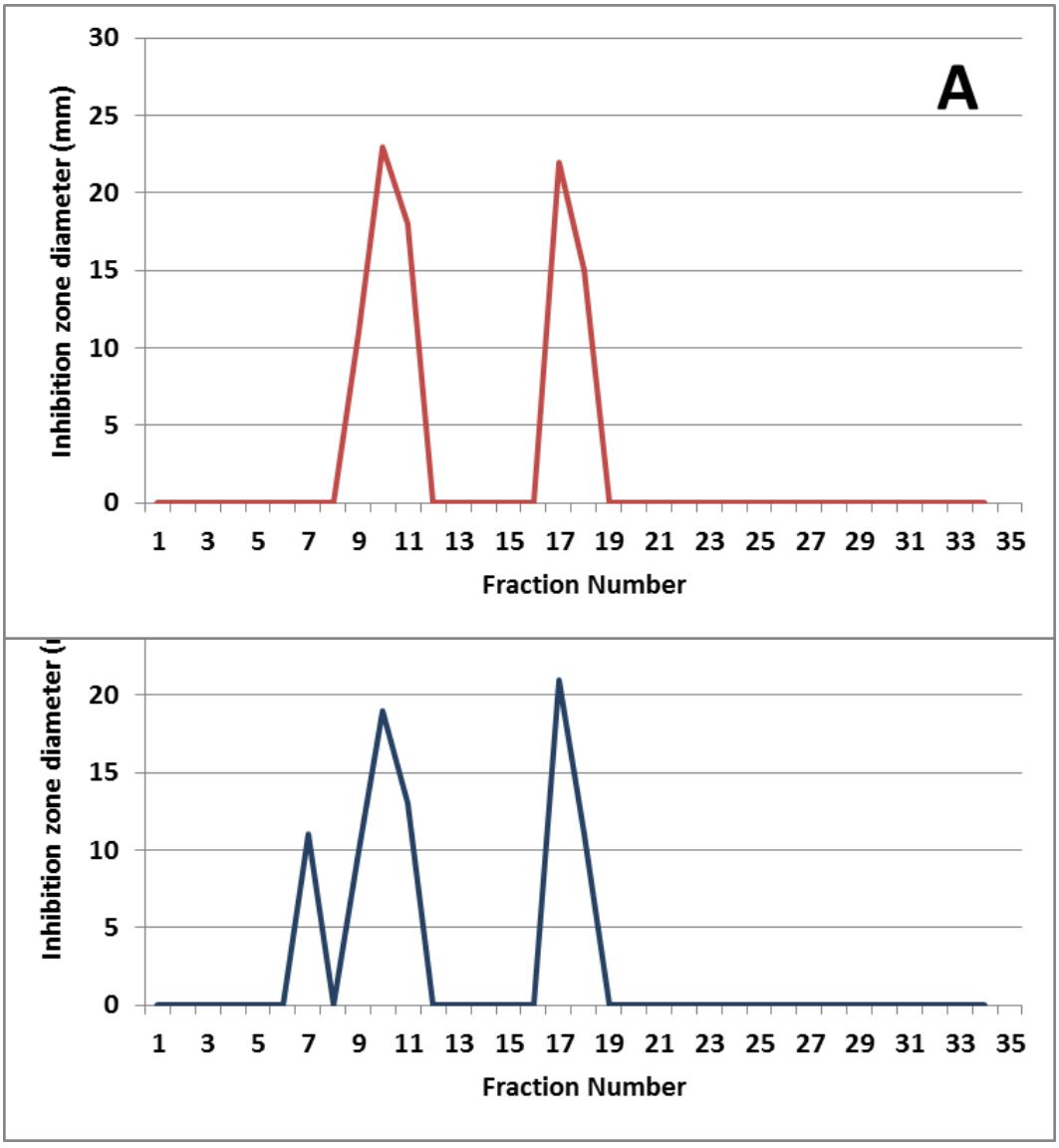

Fig. 1: The antimicrobial activities of the fractions obtained from chloroform/methanol extract of *Trichoderma viride.* **A,** The fractions were tested for their activities to inhibit *Bacillus thuringiensis;* **B,** Activities against *Pseudomonas aeruginosa*.

Table 1: Antimicrobial activities of *Trichoderma viride* metabolites against a range of pathogenic bacteria and fungi tested at 1 mg/ml.

Tested Microorganisms	*Trichoderma viride* **Extract**	Compound **1**	Compound **2**	Reference control[(2)]
Bacteria				
Acinetobacter baumannii	24	25	17	29
Bacillus thuringiensis	28	27	14	32
Escherichia coli	31	28	16	34
Klebsiella pneumoniae	23	20	12	30
Proteous vulgaris	18	21	10	27
Pseudomonas aeruginosa	21	19	11	26
Shigella dysenteriae	22	17	12	32
Staphylococcus aureus	17	20	14	27
Streptococcus pyrogens	19	20	13	25
Fungi				
Aspergillus flavus	0	0	9	25
Aspergillus fumigatus	0	0	11	27
Aspergillus niger	0	0	10	26
Candida albicans	11	0	14	30
Candida dubliniensus	12	0	15	28
Cryptococcus humicola	0	0	0	25
Fusarium oxysporum	0	0	11	23
Geotrichum candidum	9	0	12	29
Penicillium expansum	0	0	10	28
Rhodotorula glutinis	11	0	15	26
Syncephalastrum racemosum	0	0	11	27
Trichophyton cutamueum	0	0	8	26

[(1)] Antibacterial and antifungal activities were expressed as the diameter of inhibition zones (mm) using well diffusion method.

[(2)] Ciprofloxacin and Amphotericin B (1 mg/ml) acted as the positive control against the tested bacteria and fungi, respectively.

To investigate whether the tested fungal metabolites had an antitumor effect on MCF-7 and HepG2 cells, the cell lines were treated with various concentrations the fungal metabolites. The *in vitro* growth inhibitory rates (%) and inhibitory growth activity (as measured by IC_{50}) of the synthesized compounds were investigated in comparison with the well-known anticancer standard drug Vinblastine. Data generated were used to plot dose response curves presented in Figs. (2 & 3). However, the results revealed that the tested compounds showed high variation in the inhibitory growth rates and activities against the tested tumor cell lines in a concentration dependent manner (Figs. 2 & 3). Furthermore, the highest activity against hepatocellular carcinoma (HepG2) cells was detected for compound 2 hence it had the lowest effective concentration that inhibited 50% from the tumor cells (IC_{50} value 58.1 ± 4.5 µg/ml), then compound 1 (112.8 ± 9.4 µg/ml), followed by the extract (351.9 ± 23.7 µg/ml) (Table 2). Lower sensitivity against the tumor cells was detected for breast carcinoma (MCF-7) cell line (Fig. 3) showing higher IC_{50} values in the same trend of activity measured for HepG2 cell line (Table 2).

Previous report of Ahmed *et al.* (2010) confirmed the antitumor activity of *Trichoderma* metabolites against various tumor cell lines. Similarly, monorden that can be isolated from different fungal species has been reported as an anticancer agent and also acting as promoters of nerve regeneration (Pillay *et al.*, 1996; Arai *et al.*, 2003).

To determine the effects of fungal metabolites upon viral pathology, Vero confluent cells in 96-well plates were incubated at 37°C in an atmosphere of 5% CO_2 for 1 h in the presence or absence of HSV-1 virus. The inoculum was aspirated and the cultures were overlaid with maintaining medium with 1% methylcellulose containing two-fold serial dilutions of the fungal metabolite. Two days post infection, cell monolayers were stained with crystal violet, and plaques were scored by inverted light microscopy. The results shown in Fig. 4 indicated that the tested compounds inhibited the plaques formed from the virus infection in a concentration dependent manner. Compounds 1 exhibited moderate activity (reached 59.2% at the higher concentration tested) against Herpes Simplex type 1 virus infection in vero cells compared to reference drug. On the other hand, lower activity was exerted for the extract (3-36.8%). Moreover, the inhibitory activity of compound 2 was ranged from 4-28%.

Similarly, Vertihemipterin A isolated from the fungus *Verticillium hemipterigenum* BCC 2370 have been reported to possess an antiviral activity against HSV-1 (Seephonkai *et al.*, 2004).

Table 2. The antitumor activities of the tested compounds against breast and liver cancer cell lines compared with reference standard drug expressed as IC_{50} values

Tested compounds	IC_{50} values (µg/ml)	
	MCF-7	HepG2
Compound 1	193.1 ± 17.3	112.8 ± 9.4
Compound 2	102.3 ± 8.6	58.1 ± 4.5
Extract	406.2 ± 31.4	351.9 ± 23.7
Vinblastine (Reference Drug)	4.7 ± 0.5	3.8 ± 0.6

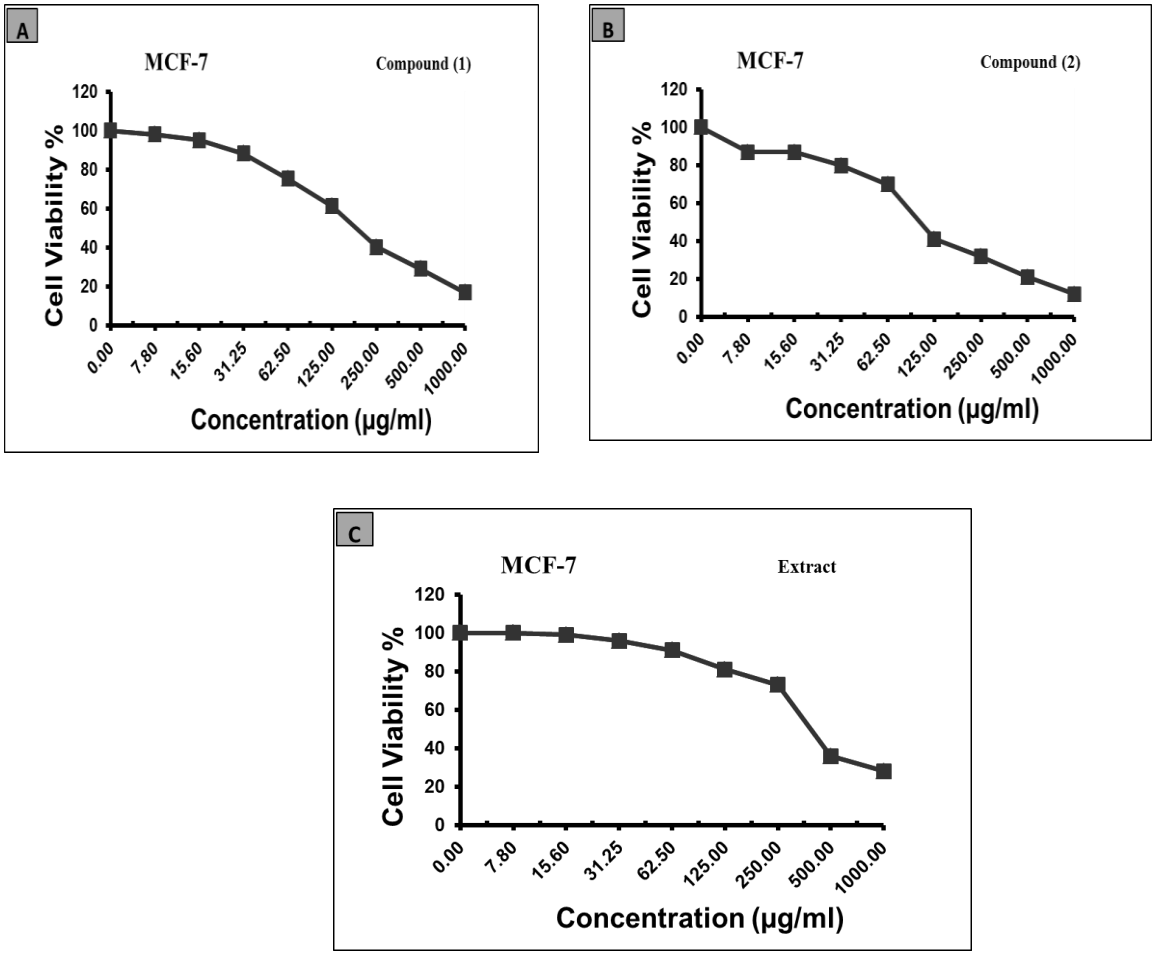

Fig. 2: The inhibitory activities of the two purified compounds and chloroform/ methanol extract produced from *T. viride* against breast cancer cell line (MCF-7).

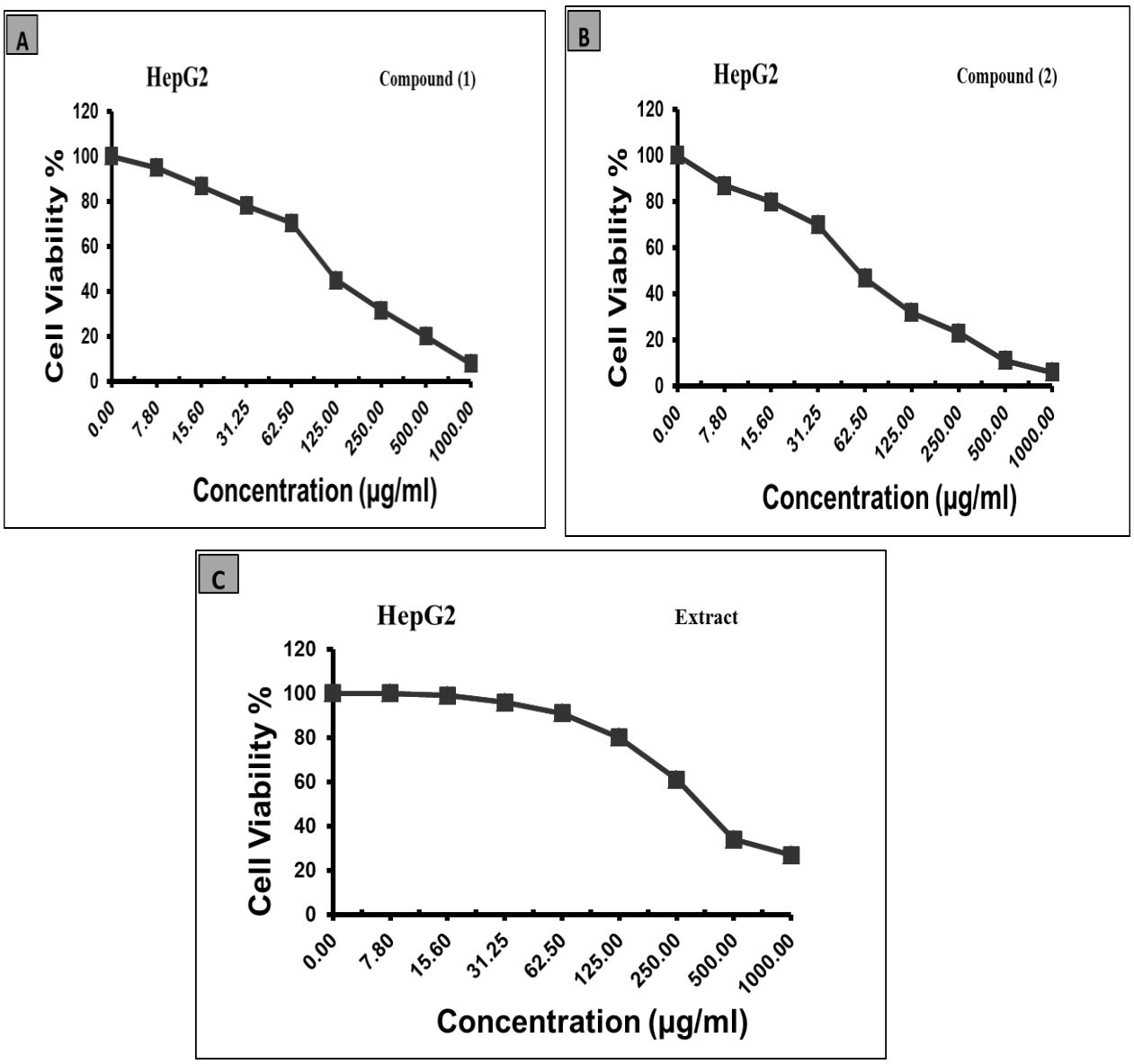

Fig. 3: The inhibitory activities of the two purified compounds and chloroform/ methanol extract produced from *T. viride* against liver cancer cell line (HepG2).

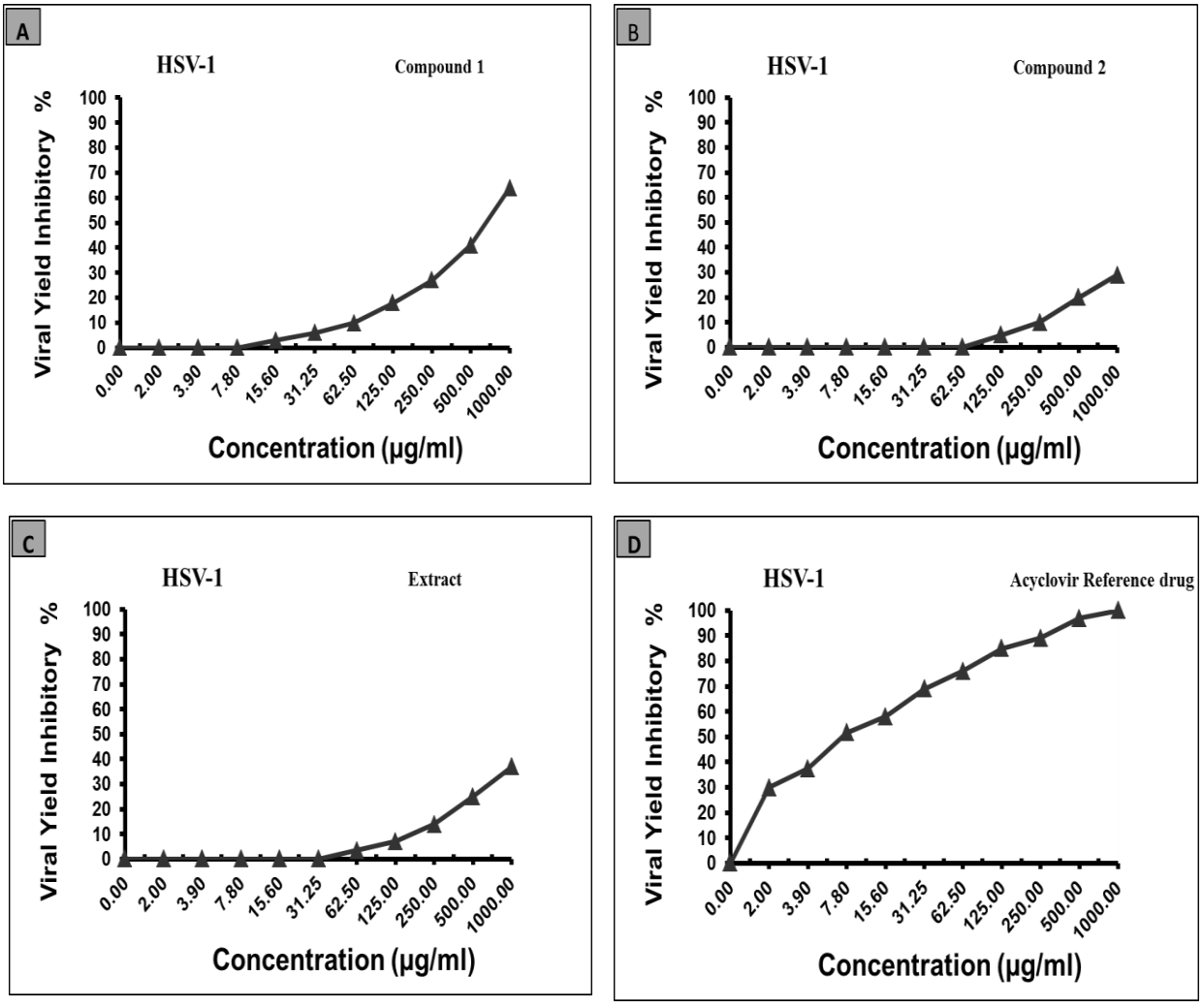

Figure (4): The inhibitory activity of the two purified compounds and chloroform/methanol extract produced from *T. viride* against Herpes Simplex type 1 virus compared with acyclovir reference drug using plaque reduction assay.

Characterization of the bioactive compounds:

Compound 1 (Fig. 5): The compound was isolated as brownish residue. Infra Red (IR) of this compound had three characteristic absorption bands at 3400cm^{-1} due to the presence of NH group of amine and at 2916 cm^{-1} for CH group of alkane as well as band at 1630 & 1450 cm^{-1} for the aromatic ring. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) showed only three characteristic signals 7.265 ppm integrating for aromatic ring, at 3.497 ppm integrating for CH_2 aromatic as well as peak at 1.616 ppm for -NH of amine. The mass spectrum of this compound confirmed these findings with the molecular peak at m/z 648.6 [M-H] with a characteristic fragmentation of m/z 44 (52.4%), 56.1 (33.2%), 72.15 (9.1%),

86 (100%), 114 (19%), 224.2 (2.2%), 254.3 (1.64%), 316.4 (2.5%), and 647.6 (3.6%). Consequently, the expected molecular formula is $C_{41}H_{59}N_7$ and named as 2[(4-ethyl, 4-propyl-diamine) 4-propyl-1-methylamine], 3-[4-(1,4-ethyl-diamine) diamine]-butane.

Compound 2 (Fig. 6): The compound (colourless oil) in its IR-spectrum showed bands at 2960, 2930, 1720, 1632 and 1556 cm^{-1}. The bands at 1720 and 1632 cm^{-1} indicated the presence of α-pyrone moiety. Absorption bands of saturated and α, β- unsaturated carbonyl group were also observed. The ^1H NMR spectrum (400 MHz, DMSO-d_6) showed only three characteristic one-proton double doublets at signals 5.95, 6.11 and 7.24 ppm corresponding to pyrone moiety. A methyl carbon singlet at δ 0.87 and a triplet at δ 2.45 integrating for methylene carbons adjacent to carbonyl group and a multiplet at δ 1.64 were attributed to methylene moiety attached to pyrone nucleus. A single sharp peak was obtained from mass spectroscopic analysis of the compound indicting its purity. The mass spectrum of this compound confirmed these findings with a protonated molecular ion peak at m/z 167.2 $[M^+H]^+$ with a characteristic fragmentation arranged according their relative abundance as m/z 95 (100%), 39 (78.4%), 81 (41.3%), 110 (32.8%), 123 (25.9%), 68 (25.1%),41 (15.7%), 166 (15.6%) and 53 (14%). Also, the mass spectrum revealed that the molecular weight of this peak is 166 Dalton. On the basis of spectral data and comparison with literature (**Claydon *et al.*, 1987**), the metabolite was identified as 6-pentyl-α-pyrone (Fig. 6).

Previous reports of **Claydon *et al.* (1987)** and **Evidente *et al.* (2003)** are in the same line of this study confirming the antimicrobial activity of the pyrone derivative produced by *Trichoderma.* Several other *Trichoderma* metabolites had been reported for their antifungal metabolites (**El-Hasan *et al.*, 2009; Shentu *et al.*, 2014**).

Previous pyranone derivative obtained from *Aspergillus candidus* had been reported for its antimicrobial, antiviral and antitumor activities (**Elaasser** *et al*.**, 2011).**

Similar inhibitory effects were demonstrated by fusaric acid and picolinic acid produced from the fungus *Fusarium oxysporum* on five malignant cell lines and on herpes simplex virus *in vitro* (Elaasser and El Kassas, 2013).

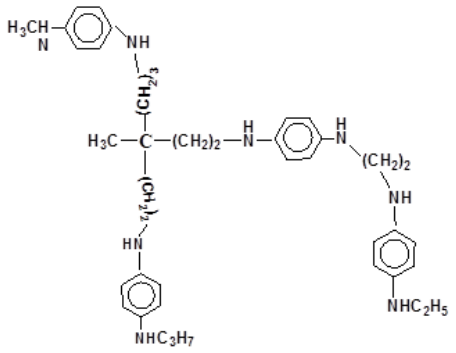

Fig. 5: The chemical structure of the active compound **1:** 1,1,1[(1-ethyl,4-propyl) benzene diamine], [(1, ethyl, 4-methyl) benzene diamine], 4[(1-ethyl, 4-methyl) benzene diamine], benzene diamine prop-3-ene.

Fig. 6: The chemical structure of the active compound **2:** 6-pentyl-α-pyrone.

In conclusion

This study reported the biological activities of new fungal metabolites from *Trichoderma viride* as potential drug that need further studies and development. The first compound was benzene diamine derivative produced from fraction 10 of *Trichoderma viride* chloroform/methanol (2:1) extract and showed good inhibitory activity against the tested bacteria, and tumor cells. Also, compound 1 showed moderate antiviral activity against HSV-1 along with no antifungal activities. However, compound 2 was pyrone derivative produced from fraction 17 with broad spectrum antimicrobial activity as well as moderate antitumor activities and weak antiviral activity.

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دراسات بيولوجية على مركبات نشطة منتجة من فطره *ترايكوديرما فيريدى*

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المركز الإقليمي للفطريات وتطبيقاتها – جامعة الأزهر

في هذا البحث تم دراسة منتجات الأيض الثانوية التي لها انشطه حيويه متعدده من فطره *ترايكوديرما فيريدى*، وذلك بعد تنميته على الوسط الغذائي السائل المكون من مستخلص الخميرة والسكروز وتحضينه لمده ٢١ يوم عند درجه حرارة ٢٨ درجه مئوية.

وتم استخلاص منتجات الايض الثانوية باستخدام الكلوروفورم والميثانول بنسبه ١:٢ وتم الكشف عن نشاط هذا المستخلص ضد واحد وعشرون من الكائنات الدقيقة الممرضة (بكتيريا وفطريات) باستخدام طريقه الانتشار عبر الأجار . وتم فصل وتنقيه - بطرق الفصل الكروماتوجرافي - عدد مركبين اثنين من المستخلص الفطري السائل بعد عمل تجزئه للمستخلص الفطري إلى (٣٤) جزءا.

وتم تعريف التركيب الكيميائى لهذين المركبين باستخدام طرق التحليل الطيفي (FTIR و HNMR و-EI mass). وتم تأكيد فعالية المركبين على الميكروبات الممرضة وكذلك اختبار هذين المركبين ضد نوعين من الخلايا السرطانيه المسببة لسرطان الكبد (HepG2) وسرطان الثدي (MCF-7). هذا بالإضافة إلى اختبار التأثير المضاد للفيروسات ضد فيروس الهربس البسيط من النوع الأول herpes simplex virus type1.

وكان المركب الأول مشتق من بنزين ثنائي الأمين وكان له نشاط جيد مضاد للبكتيريا المستخدمة وله أيضا نشاط جيد مضاد للأورام السرطانية للخلايا المستخدمة وله تأثير متوسط مضاد للفيروس المستخدم وليس له نشاط مضاد للفطريات . بينما المركب الثاني والذي تم تسميته ٦ بنتيل ألفا بيرون وكان له نشاط واسع المدى ضد الكائنات الدقيقة (بكتيريا وفطريات) وله نشاط متوسط مضاد لخلايا الأورام السرطانية المستخدمة وله تأثير ضعيف مضاد لخلايا الفيروس المستخدم في التجربة . وأكدت نتائج هذه الدراسة النشاط البيولوجي والفعالية لمنتجات الأيض من فطره *ترايكوديرما فيريدى* والذي يحتاج لمزيد من الدراسات والتطوير الدوائي لاستخدامه في العلاج.