Biological Insight of Some Secondary Metabolites Produced by *Neosartorya fennelliae*

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ABSTRACT

Viral infections, diabetes, inflammations, and hepatic diseases are worldwide threats nowadays, so effective novel therapeutics are still urgently needed. Fungi always generated significant interest in drug discovery programmer's due to their immense potential to contribute to the discovery of new bioactive compounds. This study was carried out to investigate bioactive compounds from the secondary metabolites of *Neosartorya fennelliae.* The fractionation of fungal secondary extract to get pure compounds was carried out using Thin Layer Chromatography (TLC). Three active compounds were obtained, chemical characterization of the separated compounds lead to the suggested chemical names as Compound(1) as 4, 5-dihydro-4, 6-bis-(4-methoxyphenyl)-3(2H)-Pyridazinone, compound (4) as1-Hydroxy-2-(2'-methylprop-2'-enyl)-3-(prop-1"-enyl) anthraquinone and compound(10) as 5,7-dihydroxy-6-(3-methyl-2-butenyl)-8-(1-oxobutyl)-4-propyl-2H-1-Benzopyran-2-one. Compound (4) shows promising antiviral, anti-diabetes, and hepatoprotive followed by compound (10), while compound (1) has the best anti-inflammatory activity. So *Neosartorya fennelliae* considered as a potential source of bioactive compounds.

INTRODUCTION

Filamentous fungi produce a diverse array of secondary metabolites – small molecules that are not necessary for normal growth or development. Secondary metabolites have a tremendous impact on society; some are exploited for their antibiotic and pharmaceutical activities; others are involved in disease interactions with plants or animals. The presence of various phenolic compounds, polysaccharides, and terpenoids and other compounds, is the reason for their potent biological activities as anticancer, antioxidant, antimicrobial, antiaging, hepatic protective, hypoglycemic, hypocholesterolemic, and much more biological activities are discovered every day. The availability of fungal genome sequences has led to an enhanced effort at identifying biosynthetic genes for these important molecules (Chen *et al*., 2020).

Many active compounds derived from the fungal secreted secondary metabolites have been reported to have functional biological activity as either Anti-inflammatory and/ or antidiabetic (Zahran*et al*., 2019). Recently, many fungal species have attracted more and more attention as potential natural agents for the prevention and treatment of many diseases, such as cancer, cardiovascular diseases, inflammations, diabetes, viral and neurodegenerative diseases (Akpan *et al*., 2014; Jiao-Jiao *et al*. 2016).

Different *Aspergillus* species have proved their ability to produce plenty of secondary metabolites including butenolides, alkaloids, terpenoids, cytochalasins, phenalenones, ρ-terphenyls, xanthones, sterols, diphenyl ether and anthraquinone derivatives with diverse biological activities, such as anti-cancer, antifungal, anti-bacterial, anti-viral, anti-inflammatory, anti-diabetes, anti-trypanosomes and hepatoprotective. Three hundred and sixty-one secondary metabolites were reported from different *Aspergillus* species from January 2015 until December 2019(Zhou and Chen, 2011; Silveira *et al*., 2015; El-hawary *et al.,* 2020).

Viruses cause serious outbreaks in all continents leading to difficult symptoms and mortality and enormous economic burden for society especially nowadays. In addition, the constant emergence of new serotypes in virus groups that have a high mutation rate and low fidelity for viral replication adds challenges in combating against these viruses. There is a problem in using viral proteins as drug targets as it has a high rate of producing mutant resistant strains against them (De Palma *et al*., 2008). An antiviral drug has to fulfill a set of prerequisites when undergoing preclinical and clinical trials. A vital requirement is that the drug should be effective in inhibiting the virus infection without causing any cytotoxicity and with minimum side effects to the host cells. In addition, a drug should be able to completely inhibit the virus infection, partial inhibition leads to the generation of drug-resistant mutant strains. Due to these prerequisites, only a handful of synthetic antiviral drugs have made it past the clinical phase. Until today, the successful 'one bug–one drug' approach has been used for antiviral drug development. However, today the focus has shifted toward designing broad-spectrum antiviral, which can act on multiple viruses by targeting a common but essential viral function (Vigant *et al*., 2015).

Diabetes mellitus, a metabolic disorder, is one of the major causes of death among all health issues. Diabetes can be regulated by anti-diabetic drugs that control the activity of some metabolic enzymes. Fungi have been reported to be a potential source of developing remarkable anti-diabetic drugs. These drugs could control diabetes by inhibiting a major enzyme alpha-amylase that hydrolyzes carbohydrates into sugar (Khan *et al.,* 2019).

Also, the Liver is one of the most important organs in the human body. It has a fundamental rule in the regulation of many physiological processes, which related to different vital functions, such as metabolism, secretion, and storage. Many researchers analyzed its capacity to detoxify endogenous (waste metabolites) and exogenous (toxic compounds) substances of organisms, as well as for synthesize useful agents (Adewusi and Afolayan2010). The liver is also involved in the biochemical processes of growing, providing nutrients, supplying energy, and reproducing. In addition, it aids in the metabolism of carbohydrates and fats, in the secretion of bile, and in the storage of vitamins (Ahsan *et al.,* 2009). For these all, hepatic diseases continue to be a problem worldwide on public health. Thus, it is necessary to identify alternative pharmaceuticals for the treatment of hepatic diseases, with the aim of these agents being more effective and less toxic (Eduardo *et al.,* 2014). Plant extracts are vigorously investigated and widely employed as a protective treatment in acute and chronic liver diseases, while fungal extracts is not widely employed**.**

For all of the above, the aim of this work is to discover and investigate bioactive

compounds activity and its cytotoxicity and effectiveness as Antiviral, Anti-inflammatory, anti-diabetic and hepatoprotective compounds from the fungus *Neosartoria fennelliae.*

MATERIALS AND METHODS

Fungal Strain:

The alkalophilic fungus *Aspergillus fennelliae* was previously isolated and identified and some studies on its biological activation were carried out (Ragab *et al.,* 2014).

Media Used for The Purification and Renewal of Fungi:

Malt Extract Agar (MEA) Medium (Smith and Onions, 1983): The medium contains (g/L): malt extract, 20.0; peptone, 1.0; glucose 20.0; agar-agar, 20.0 and distilled water to 1L. The pH of the medium was adjusted to 10.5 by Na_2CO_3 10g/l, then autoclaved at 121°C for 20 min. This medium was used for the maintenance of fungi. This medium without agar (liquid medium) was used for secondary metabolites production.

Synthesis and Extraction of the Extracellular Secondary Metabolites:

For the biosynthesis of fungal secondary metabolites, a semi-synthetic malt extract liquid medium was used. The spores were scrapped from the mycelium of the tested fungi 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 malt extract liquid medium. The inoculated flasks were incubated at 28°C for 21 days.

At the end of incubation period, cultured medium was filtered using Whattman No. 1 filter paper in order to separate the mycelium and the filtrate. The cultured filtrate mixed with n-hexane in a separating funnel, shaken vigorously and left to settle down until complete separation. The hexane layer was separated and subjected to solvent system with an equal volume of chloroform: methanol (2:1, v/v) in a separating funnel. The aqueous and organic solvent phases were separated. The samples were extracted three successive times with chloroform: methanol (2:1), left for 15 min. to settle down, then concentrated by using a rotary evaporator (Buchi RV 4) to dryness and stored at 5°C till dryness.

Separation by Thin-layer Chromatography (TLC):

This is the rapid and inexpensive method for the identification of a number of compounds present in a crude sample and for the separation of fungal extracts into different fractions (Sasidharan *et al.,* 2011). The active extracts among the previously prepared extracellular metabolites were subjected to separation using silica column chromatography. The column (1.5 cm diameter and 50 cm long) was packed with 20 gm silica gel (G100; mesh 63-200 μm) after insertion of a stopper in the tapering lower end of the column. The silica gel was previously activated at 60ºC for 3 h. The extract obtained was fractionated by open column silica chromatography using elution solvents (10-ml volume each) started with ethyl acetate (100%), followed by gradient volume from mixture of ethyl acetate-methanol (90:10 v/v; 80:20 v/v; 70:30 v/v; 60:40 v/v; 50:50 v/v; 40:60 v/v; 30:70 v/v; 20:80 v/v & 10:90 v/v) ended by 100% methanol. Each active fraction was analyzed by thin-layer chromatography (TLC) to check the purity and then the fractions in which the active compounds were found were collected and evaporated to the dryness then characterized biologically or chemically for structure determination and nomenclature. Some of the collected fractions were combined based on similarities in TLC properties. TLC was performed on aluminum sheet plates precoated with silica gel G-60 (GF$_{254}$, layer thickness 0.2 mm, Merck, Darmstadt, Germany). Fractions having high activity were automatically spotted on TLC plates using CAMMAG LINOMAT 5 application system and the developing processes were carried out with two solvent systems consisting of Chloroform: Methanol (9:1; v/v) The plates were dried at room temperature then the purity of fractions was scanned.

Nomenclature of the Active Compound (s): Mass Spectroscopy and Infra-Red (IR) Spectrum: Previously carried out, obtained, and recorded at the Department of Chemistry, Faculty of Science, Cairo University. (Sabry, 2015) **Antiviral Activity Assay:**

1. Mammalian Cell Line:

Vero cells (derived from the kidney of African green monkey) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

The Vero cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, HEPES buffer and 50 μg/mL gentamycin. All cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were subcultured two times a week (Vijayan *et al*., 2004).

2. Virus Propagation:

The cytopathogenic HAV HM175 strain (ATCC VR-1402) of Hepatitis A virus and GHSV-UL46 strain for HSV-1were propagated and assayed in confluent Vero cells (Randazzo *et al.,* 2017). Infectious viruses were enumerated by determining the 50% tissue culture infectious dose ($TCID_{50}$) with eight wells per dilution and 20μl of inoculum per well using the Spearman-Karber method (Pinto *et al*., 1994).

3. Cytotoxicity Evaluation:

The Vero cell lines in the cytotoxicity assay were seeded in 96-well plates at a cell concentration of 2×10^5 cells per ml in 100 μL of the growth medium. Fresh medium containing different concentrations of the tested sample was added after 24 h of seeding. Serial two-fold dilutions of the tested compound (started from 3000 μg/mL to 2 μg/mL) were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, Jersey, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the tested sample. Control cells were incubated without test samples and with or without DMSO. The small percentage of DMSO present in the wells (maximal 0.1%) was not found to affect the experiment.

After the end of the incubation period, the viable cell yield was determined by an MTT colorimetric method (Mosman, 1983). In brief, the media was removed from the 96 well plates and replaced with 100 μl of fresh culture medium without phenol red then 10 μl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS, where PBS is phosphate buffered saline) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO_2 for 4 hours. An 85 μl aliquot of the media was removed from the wells, and 50 μl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm (Mosman, 1983) with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)]x 100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and tested compound concentration is plotted to get the survival curve of the Vero cell line after treatment with the specified compound. The 50% cytotoxic concentration (CC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose-response curve for each conc. using GraphPad Prism software (San Diego, CA. USA). The maximum non-toxic concentration [MNTC] of each compound was also determined and was used for further biological studies.

4. Evaluation of the Antiviral Activity:

Three samples had been submitted for evaluation of their antiviral activities against Hepatitis A virus and Herpes Simplex type 1 virus.

The antiviral screening was performed using a cytopathic effect inhibition assay at the Regional Center for Mycology and Biotechnology (RCMB, Al-Azhar University, Cairo, Egypt). This assay was selected to show specific inhibition of a biological

function, that is, a cytopathic effect in susceptible mammalian cells (Hu and Hsiung, 1989; Al-Salahi *et al*., 2015). In brief, monolayers of (2x105 cells/ml) Vero cells adhering at the bottom of the wells in a 96-well microtiter plate were incubated for 24 h at 37 °C in a humidified incubator with 5% CO2. The plates were washed with fresh DMEM and challenged with 104 doses of herpes simplex 1 or 2 viruses, and then the cultures were simultaneously treated with two-fold serial dilutions of the tested compound, starting from 500 µg/mL and going up to about 2 µg/mL (500, 250, 125, 62.5, …, 1.95 µg/mL) in a fresh maintenance medium; following this, they were incubated at 37 °C for 48 h. Infection controls, as well as an untreated Vero cell control, were made in the absence of tested compounds. Six wells were used for each concentration of the tested compound. Antiviral activity was determined by the inhibition of the cytopathic effect compared to a control, that is, the protection offered by the tested compound to the cells was calculated. Three independent experiments were assessed, each containing four replicates per treatment. Amantadine was used as a positive control in this assay system. After the incubation period, the viability of the cells was determined by MTT assay as described before in the cytotoxicity section (Mosman, 1983).

The viral inhibition rate was calculated as follows:

$$[(A - B)/(C - B)] \times 100\%$$

Where A, B, and C indicate the absorbance of the tested compounds with virus-infected cells, the absorbance of the virus control, and the absorbance of the cell control, respectively.

5- Data Analysis:

From these data, the dose that inhibited viral infection by 50% (EC_{50}) was estimated with respect to the virus control from the graphic plots, using the STATA modeling software. The percentages of viral inhibition in relation to each tested virus represent mean ± standard error of the mean values of three different experiments. EC50 values were determined directly from the curve obtained by plotting the inhibition of the virus yield against the concentration of the samples. The selectivity index (SI) was calculated from the ratio of CC_{50} to EC_{50} in order to determine whether each compound had sufficient antiviral activity that exceeded its level of toxicity (Al-Salahi *et al.,* 2015). This index is referred to as a therapeutic index, and it was also used to determine whether a compound warranted further study. Compounds that had an SI-value of 2 or more were considered to be active (Al-Salahi *et al.,* 2015).

Anti-inflammatory:

Membrane Stabilization:

Preparation of Erythrocyte Suspension: Whole blood was obtained with heparinized syringes from rats through cardiac puncture. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The blood was centrifuged for 10 minutes at 3000 g .

Hypotonic Solution-Induced Erythrocyte Haemolysis: Membrane stabilizing activity of the samples was assessed using hypotonic solution-induced erythrocyte haemolysis (Shinde et al., 1999). The test sample consisted of stock erythrocyte (RBCs) suspension (0.50 ml) mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate-buffered saline (pH 7.4) containing the extract (1000-7.81 µg/ml) or indomethacin. The control sample consisted of 0.5 ml of RBC mixed with hypotonic-buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g. In 96 well plates, the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated according to a modified method described by Shinde *et al*., (1999).

%Inhibition of heamolysis (membrane stabilization%) = 100 x {OD1-OD2/OD1}

Where: OD1 = Optical density of hypotonic-buffered saline solution alone OD2 = Optical density of test sample in hypotonic solution.

The IC50 value was defined as the concentration of the sample to inhibit 50% RBCs haemolysis under the assay conditions **In-vitro Antidiabetic Assay** (α-glucoidase inhibitory activity):

α-glucosidase (*Saccharomyces cerevisiae*) and 3, 5, di-nitro salicylic acid (DNS) were purchased from Sigma-Aldrich, Bangalore. P-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium carbonate (Na2 CO3), sodium dihydrogen phosphate, di-sodium hydrogen phosphate were purchased from Hi-Media.

α-glucosidase inhibitory activity of *B. vulgaris* subspecies *cicla* L. var. *flavescens* leaves different extracts and fractions were carried out according to the standard method with minor modification (Shai *et al.* 2011). In a 96-well plate, the reaction mixture containing 50 µl phosphate buffers (100 mM, pH = 6. 8), 10 µl alpha-glucosidase (1 U/ml), and 20 µl of varying concentrations of extracts and fractions (1000 to 7.81 µg/mL) was preincubated at 37°C for 15 min. Then, 20 µl P-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50 µl Na2 CO3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate Reader. Acarbose at various concentrations 1000 to 7.81 µg/mL) was included as a standard. Without a test, the substance was set up in parallel as control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula, Inhibitory activity (%) = $(1 - As/Ac) \times 100$, where, As is the absorbance in the presence of test substance and Ac is the absorbance of control.

The IC50 value was defined as the concentration of alpha-glucosidase inhibitor to inhibit 50% of its activity under the assay conditions.

Hepatoprotective Study in HepG2 Cell Line:
1-Principle:

HepG2 Cell lines are suitable for the in-vitro model system for the study of polarized human hepatocytes. HepG2 cell line with proper culture conditions displays robust morphological and functional differentiation with a controlled formation of apical and basolateral cell surface domain in models. The HepG2 cells were exposed to toxicant containing 1% CCl4 along with /without a tested sample of different concentrations or the medium alone is considered as control. At the end of the period, cytotoxicity was assessed by estimating the viability of the HepG2 cells by the MTT reduction assay.

2-Methods ()
Cell Line and Growth Media: The HepG2 cells of the human liver cell line were cultured in DMEM (Dulbecco's modified eagle's medium) contains 10% fetal calf serum, penicillin (100 U) and streptomycin (100µg).

3-Hepatoprotective Effect in HepG2 Cell Line Estimated by MTT Assay. The monolayer cell culture was trypsinated and the cell count was adjusted to 1.0 x105 cells/mL using a medium containing 10% newborn calf serum. To each well of the 96-well microtitre plate, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once, and 100µL samples with various drug concentrations were added to cells in wells of the microtitre plate. The plate was then incubated at 37°C in 5% CO2 atmosphere for 24 h. (Thirunavukkarasu *et al.*, 2014)

4-Experimental Design: (Hisayoshi *et al.*, 1999): Human liver HepG2 cells were exposed to a medium containing CCl4 (1%) with/without various concentrations from the tested compounds (6.25, 12.5, 25, 50,100, and 200 µg/mL). Then, cytotoxicity was assessed by estimating the viability of HepG2 cells by MTT reduction assay. The experimental groups were as follows:

Group 1: Control, untreated HepG2 cell line; Group 2: HepG2 cells with 1% CCl4; Group 3: HepG2 cells with 1% CCl4 and tested compound; Group 4: HepG2 cells with 1% CCl4 and silymarin standard drug, each treatment was repeated four times (i.e. 4 wells for each treatment).

5-MTT Assay: Following treatment with the abovementioned methods, after 24 h incubation, the medium was removed and 50 µl of 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) (5 mg/ml; Sigma, St. Louis, CA, USA) was added to each well. The plates were gently shaken then incubated in the dark at 37°C for an additional 4 h in 5% CO2 atmosphere. The reaction was stopped by the addition of 150 µl DMSO (Sigma) and the absorbance of samples at 570 nm was measured with a microplate reader (SunRise, Tecan, USA).

The tetrazolium salt (3-(4,5–dimethylthiazole-2-yl)-2,5 diphenyl tetrazolium bromide, MTT) is introduced into cells and reduced in a mitochondria-dependent reaction to yield a blue-colored formazan product. The product accumulates within the cell due to the fact that it cannot pass through the plasma membrane. On solubilization of the cells, the product is liberated and can be readily detected and quantified by a simple colorimetric method. The ability of cells to reduce MTT provides an indication of mitochondrial integrity and activity, which in turn may be interpreted as a measure of viability and/or the number of cells. The assay has therefore been adopted for use with cultures of exponentially growing cells. Determination of the cell's ability to reduce MTT to the formazan derivative after exposure to test compounds shows a hepatoprotective effect. The optical density of the formazan formed in the control cells was taken as 100%. The viability of HepG2 cells in other groups was presented as a percentage of the control cells. (Mosmann, 1983).

6-Data Analysis: The results were expressed as the percentage of viability was calculated as [(ODt/ODc)] x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells.

Hepatoprotective Percent = % Viability of treatment group – % Viability of negative control.

Statistical Analysis:

The statistical significance of the difference between mean values was determined by Student's unpaired t-test. Data were considered statistically significant at a significance level of $P < 0.05$. STATA statistical analysis package was used for the dose-response curve drawing in order to EC_{50} calculations.

RESULTS AND DISCUSSION

Nomenclature of the Active Compound (s):

Compound (1):

The expected molecular formula according to IR and mass spectrum is $C_{18}H_{18}N_2O_3$ and named as proposed: 4, 5-dihydro-4, 6-bis-(4-methoxyphenyl)-3(2H)-Pyridazinone. The suggested chemical structure is illustrated in figure1.
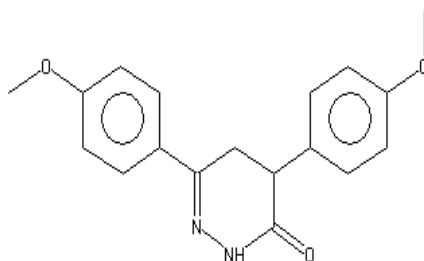

Fig. 1 The proposed chemical structure of the compound *N. f* (1) according to IR and mass spectrum.

Compound (4): The nuclear magnetic resonance spectrum of the compound that dissolved in CDCL$_3$ performed at 300 MHz was illustrated in figure (2). The expected molecular formula for the compound according to IR and mass spectrum is $C_{21}H_{18}O_3$ and the suggested chemical name is 1-Hydroxy-2-(2'-methylprop-2'-enyl)-3-(prop-1''-enyl) anthraquinone. The proposed chemical structure is illustrated in figure 2
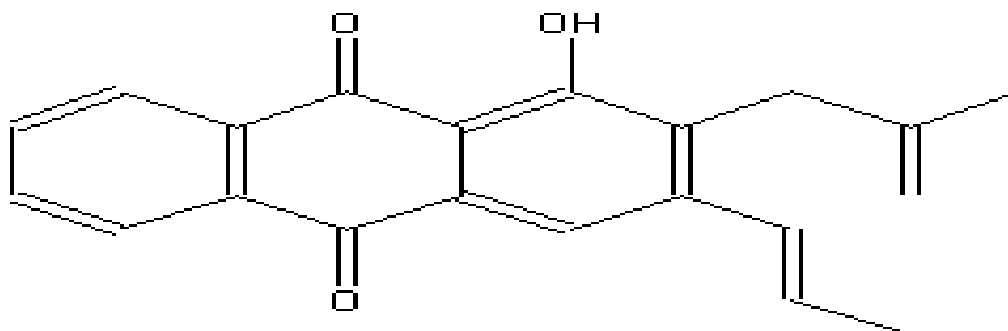
Fig. 2 The proposed chemical structure of the compound *N. f* (4) according to IR and mass spectrum.

Compound (10): The nuclear magnetic resonance spectrum of the compound performed at 300 MHz was illustrated. Consequently, the expected molecular formula for the compound is $C_{22}H_{14}NO_3$, and the suggested chemical name is5, 7-dihydroxy-6-(3-methyl-2-butenyl)-8-(1-oxobutyl)-4-propyl-2H-1-Benzopyran-2-one. The proposed chemical structure is illustrated in figure 3.
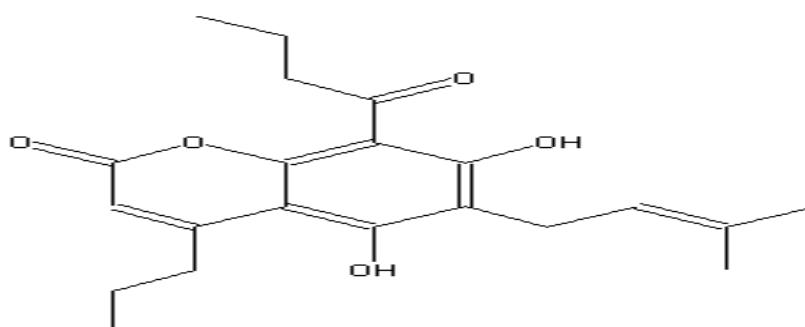

Fig. 3 The proposed chemical structure of the compound *N. f* (10) according to IR and mass spectrum.

Antiviral Activity:

The antiviral effects of the tested compounds on HAV and HSV-1 viruses when tested at maximum noncytotoxic conc. shows the results in Table (1) and figure (4) Compound (4) showed good antiviral effect against HAV and HSV-1 viruses with the values36.04and 25.12 % respectively with MNCC 52.7µg/ml, followed by compound (1) which had lower activity on the two viruses. Compound (10) showed no activity against the Herpes Simplex type 1 virus while it had low activity against HAV.

Table 1: Antiviral effects of the tested compounds on HAV and HSV-1 viruses

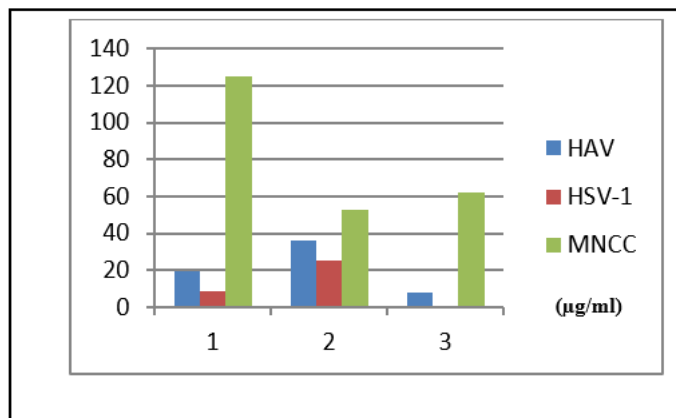| Compound Code	MNCC (µg/ml)	Antiviral effect (%)	
		HAV	**HSV-1**
(1)	124.8	19.28	8.6
(4)	52.7	36.04	25.12
(10)	62.5	7.8	0

Fig. 4: Antiviral effects of the tested compounds on HAV and HSV-1 viruses

This was similar to that reported by Moghadamtousi *et al.,* 2015 they indicated, that about 150 to 200 new compounds, including alkaloids, sesquiterpenes, polyketides, and aromatic compounds, are identified from various fungi annually. Numerous investigations demonstrated the potential of fungi as a promising source to develop new antiviral against different important viruses, including herpes simplex viruses, the human immunodeficiency virus, and the influenza virus. Various genera of marine fungi such as *Aspergillus*, *Penicillium*, *Cladosporium*, and *Fusarium* were subjected to compound isolation and antiviral studies, which led to an illustration of the strong antiviral activity of a variety of fungi-derived compounds.

Anti-inflammatory:

The compounds significantly displayed potent anti-inflammatory activity with an IC_{50} value of 116.6 µgfor compound 1and >1000 for compound 10.

Similarly, A new polyketide named curvularin was isolated from a marine fungus *Penicillium* sp. SF-5859, obtained from the Ross Sea, which suppressed the induction of cytokines and pro-inflammatory regulator, indicating a promising compound for anti-inflammatory activities (Ha *et al.*2017).Three new guaianes named graphostromanes D, F, and I compounds having anti-inflammatory activity were isolated from deep-sea *Graphostroma* sp. obtained from the Atlantic Ocean displayed potent anti-inflammatory activity with an IC50 value of 14.2 µM. (Niu*et al.,* 2018).
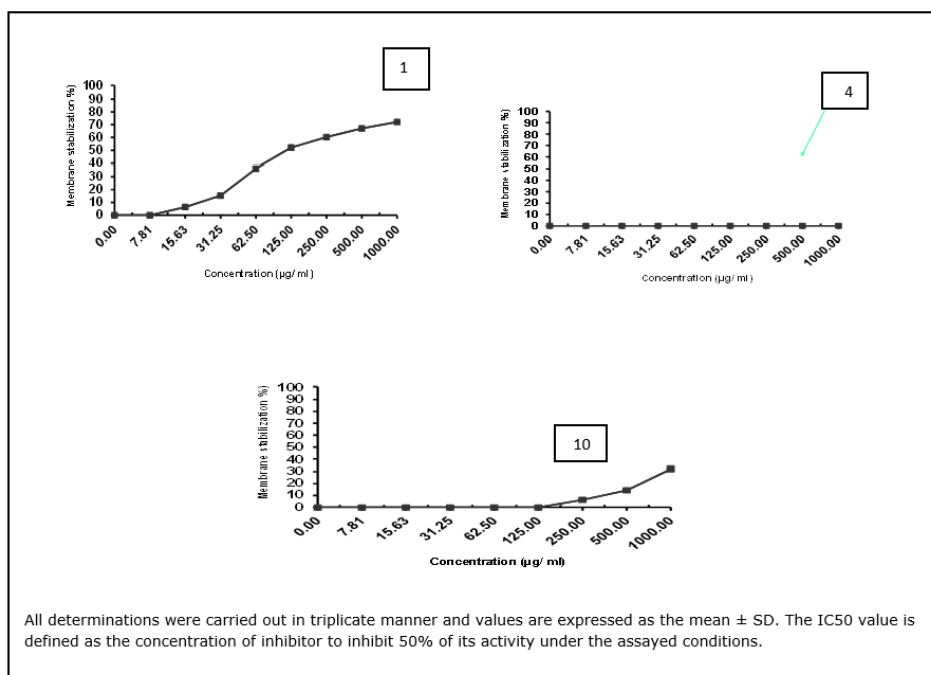
Fig. 5: Effect of compounds 1, 4 and 10 from *A. fennelliae* on anti-inflammatory activity

Anti-Diabetic Activity:

Similarly, it was reported by Singh and Kaur (2016) that endophytic *A. awamori* is capable of producing a peptide with alpha glycosidase inhibitory activity, that possesses dual (alpha glucosidase and alpha amylase) inhibitory activity, low IC_{50} values, with high stability under extreme conditions of pH and temperature, and is non-mutagenic in nature that can be commercially produced and exploited for better management of diabetes
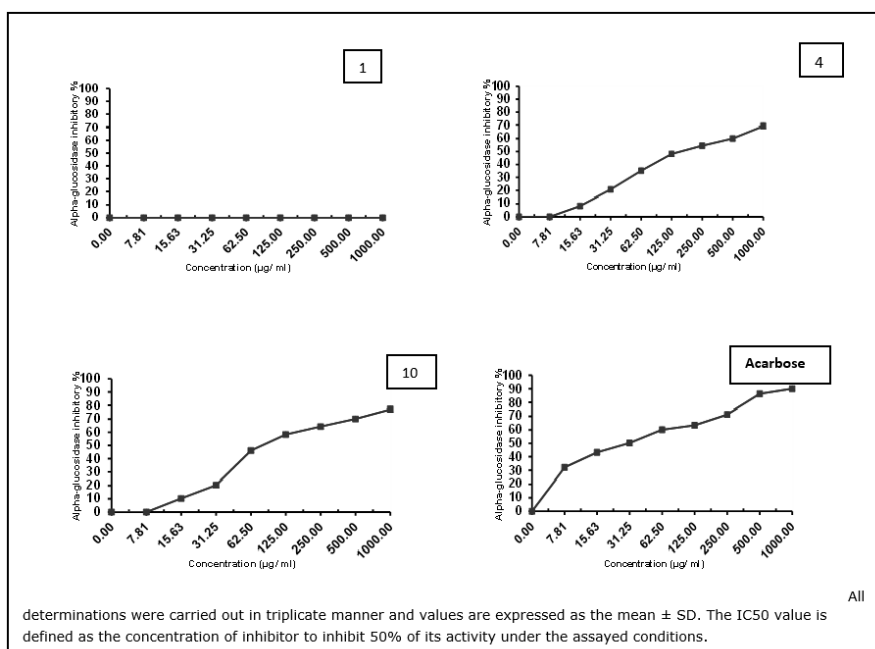

Fig.6: Effect of compounds 1, 4 and 10 from *N. fennelliae* on antidiabetic activity against Acarbose.

Hepatoprotective Activities:

The hepatoprotective activities were tested against HepG-2 intoxicated with carbon tetrachloride. The samples were tested at Non-cytotoxic conc. (200µg/ml) and the results in Table (2) as follows:

Table 2: Hepatoprotective activity of *Neosartorya fennelliae* pure compounds.

Compound code	Hepatoprotective effect %
(1)	20.7± 1.1
(4)	43.1±4.7
(10)	12.4± 1.2
Silymarin (standard drug)	88.4±3.8

The hepatoprotective activity showed moderate effectiveness of compound (4) with hepatoprotective effect % 43.1 followed by compound (1) and compound (10) recorded 20.7 and 12.4 respectively.

Previous evaluation of the pharmacological benefits of many anthraquinone compounds showed that most of them possess many biological activities of great importance made some of them is included in the pharmacopeia. Some of these important activities were antioxidant activity, antidiabetic, hepatoprotective activity, and cytotoxic activity (Abdel Hakim *et al*., 2019).

REFERENCES

Abdel Hakim F., Gad b H. A., Radwanc R. A., Ayoubd N., El-Shazlyb M. (2019). Chemical Constituents and Biological Activities of Cassia Genus: Review. *Archives of Pharmaceutical Sciences Ain Shams University,* 3(2): 195-227.

Adewusi EA, Afolayan AJ. (2010). A review of natural products with hepatoprotective activity. *Medical Plants Research Journal;* 4:1318–1334.

Ahsan MR, Islam KM, Bulbul IJ. (2009). Hepatoprotective activity of Methanol Extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *Global Journal of Pharmacol*, 3:116–122.

Al-Salahi R., Alswaidan I., Ghabbour H. A., Ezzeldin E., Elaasser M. and Marzouk M. (2015). Docking and Antiherpetic Activity of 2-Aminobenzo[de]-isoquinoline-1,3-diones. *Molecules,* 20, 5099-5111; doi: 10.3390/molecules 20035099.

Akpan OU, Bassey RB, Agba BS, EdeghaIA(2014) . Elevation of serum pancreatic amylase and distortion of pancreatic cyto-architecture in type 1 diabetes mellitus rats treated with *Ocimum gratissimum. Niger Medical Journal*; 55(1): 34.

Chen B., Sun Y., Luo F. and Wang C. (2020). Bioactive Metabolites and Potential Mycotoxins Produced by *Cordyceps* Fungi: A Review of Safety. *Toxins,* 12(6), 410; https://doi.org/ 10.3390/toxins12060410

De Palma A. M., Vliegen I., De Clercq E., Neyts J. (2008). Selective inhibitors of picornavirus replication. *Medicinal Research Reviews,* 28 823–884. 10.1002/ med.20125

Eduardo Madrigal-Santillán, Eduardo Madrigal-Bujaidar, IselaÁlvarez-González, María Teresa Sumaya-Martínez, José Gutiérrez-Salinas, Mirandeli Bautista, Ángel Morales-González, Manuel García-Luna y González-Rubio, J Leopoldo Aguilar-Faisal, and José A Morales-González (2014).Review of natural products with hepatoprotective effects. *World Journal of Gastroenterol*,20(40): 1478714804. doi: 10.3748/wjg.v20.i40.14787

El-hawaryS. S., MoawadA. S., BahrH. S., AbdelmohsenU. R. and Mohammed R. (2020).Natural product diversity from the endophytic fungi of the genus *Aspergillus .Royal Society of Chemistry Advances*, 10, 22058. DOI: 10.1039/d0ra04290k

Ha T. M., Ko W., Lee S.J., Kim Y-C, Son J-Y, Sohn J.H, Yim J.H and Oh H. (2017). Anti-inflammatory effects of curvularin-type metabolites from a marine-derived fungal strain Penicillium sp. SF-5859 in lipopolysaccharide-induced raw264. 7 macrophages. *Marine Drugs*,15(9):282

Hisayoshi K., Ke H., Aijun D., Yongkui J., Shigeo I.and Xinsheng Y. (1999). Antineoplastic agents III:Steroidal glycosides from *Solanum nigrum. , Planta Medica,* 65, 35-38.

Hu, J.M. and Hsiung, G.D. (1989). Evaluation of new antiviral agents I: In vitro prospective. *Antiviral Res*earch, 11, 217–232.

Khan R., Qousain Naqvi S. T., Fatima N. and Muhammad S. A. (2019). Study of antidiabetic activities of endophytic fungi isolated from plants. *Pure and Applied Biol*ogy, 8(2):1287-1295, http://dx.doi.org/10.19045/bspab.2019. 80071.

Moghadamtousi S. Z., Nikzad S., Abdul Kadir H. , Abubakar S. , and Zandi K. (2015). Potential Antiviral Agents from Marine Fungi: An Overview. *Marine Drugs Journal*; 13(7): 4520–4538. doi: 10.3390/md13074520

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunology Methods*; 65: 55-63.

Niu S, Xie C-L, XiaJ- M, LuoZ. H, Shao Z, Yang X.W. (2018). New anti-inflammatory guaianes from the Atlantic hydrotherm-derived fungus Graphostroma sp. MCCC 3A00421. *Science Rep*orts,8(1):530

Pinto, R.M., Diez, J.M. and Bosch, A. (1994). Use of the colonic carcinoma cell line CaCo-2 for in vivo amplification and detection of enteric viruses. *Journal of Medical Virology,* 44, 310–315.

Randazzo W., Piqueras J., Rodrıguez-Diaz J., Aznar R. and Sanchez G. (2017). Improving efficiency of viability-qPCR for selective detection of infectious HAV in food and water samples. *Journal of Applied Microbiology,* DOI:10.1111/jam.13519.

Sabry, S. M. (2015). Studies on the biological activities of some alkalophilic fungal isolates. Ph.D thesis. Faculty of science, AlAzhar University, Cairo, Eygpt.

SasidharanS, Chen Y, Saravanan D, Sundram K. M, Yoga Latha L. (2011). Extraction, Isolation and Characterization of bioactive compounds from plants' extracts. *African Journal of Traditional and Complementery Alternative medicine,* 8(1):1–10.doi: 10.21010/ajtcam. v14i3. 18

Shai LJ,Magano SR, Lebelo SL, Mogale AM (2011). Inhibitory effects of five medicinal plants on alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. *Journal of Medical Plant Research,* 5: 2863–2867.

Shinde U, Phadke A, Nari A, *et al.* (1999). Membrane stabilization activity a possible mechanism of action for the anti-inflammatory activity of Cedrusdeodara wood oil. *Fitoterapia,* 70:251–257.

Silveira M.L, Smiderle F.R., Agostini F., Pereira E.M., Bonatti-Chaves M., Wisbeck e., Ruthes A.C., Sassaki G. L., Cipriani T. R., Furlan S. A. and Iacomini M.(2015). Exopolysaccharide produced by *Pleurotussajor-caju*: its chemical structure and anti-inflammatory activity. *International Journal of Biological Macromolocules,* 75: 90-96. Doi: 10.1016/j.ijbiomac. 2015.01.023.

Singh B.and KaurA. (2016).Antidiabetic potential of a peptide isolated from an endophytic *Aspergillus awamori.*

Journal of Applied Microbiology, 120 (2):301-11. doi: 10.1111/ jam.12998.

Smith, D. and Onions, A.H.S. (1983). "The preservation and maintenance of living fungi". Kew, Surrey: Commonwealth Mycological Institute.

Thirunavukkarasu P., AshaS., RamanathanT., BalasubramanianT., ShanmogapriyaR., and Renugadevi G. (2014). *In vitro* hepatoprotective activity of isolated fractions of *Cressa cretica. Pharmaceutical Chemistry Journal,* Vol. 48, No. 2: 121-126.

Vigant F., Santos N. C., Lee B. (2015). Broad-spectrum antivirals against viral fusion. *Nature Reviews Microbiology,* 13 426–437. doi: 10.1038/nrmicro3475

Vijayan, P.; Raghu, C.; Ashok, G.; Dhanaraj, S.A.; Suresh, B. (2004). Antiviral activity of medicinal plants of Nilgiris.

*Indian Journal Medical Res*earch, 120, 24–29.

Zahran M., El-Hosainy A. and SabrySh. (2019). Bioefficacy of Exopolysaccharide production by *Cunninghamella echinulata. Egyptian Journal of Biomedical Sci*ence, 45, 52-71.

Zhang J.-J., Li Y, Zhou T., Xu D.-P., Zhang P, Li Sh. and Hua-Bin L. (2016). Bioactivities and Health Benefits of Mushrooms Mainly from China. *Molecules,* 21, 938; doi:10.3390/molecules21070938.

Zhou L. B; and Chen B. (2011). Bioactivities of water-soluble polysaccharides from Jison-grong mushroom: anti-breast carcinoma cell and antioxidant potential. *International Journal of Biological Macromolecules*, 48(1): 1-4. Doi:10.1026/ j.ijbiomac. 2010. 09. 004.

ARABIC SUMMARY

مركبات نشطة بيولوجيا من مستخلصات فطرة نيوسارتوريا فينيليا

شـــادية صبري

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تعد العدوى الفيروسية ومرض السكري والالتهابات وأمراض الكبد من التهديدات العالمية في الوقت الحاضر، لذا لا تزال هناك حاجة ماسة إلى علاجات جديدة فعالة. أَوَليت الفطريات دائمًا اهتمامًا كبيرًا في برامج اكتشاف الأدوية نظرًا لقدرتها الهائلة على المساهمة في اكتشاف مركبات نشطة بيولوجيًا جديدة. أجريت هذه الدراسة للتحقق من المركبات النشطة بيولوجيا من مستخلصات نواتج الأيض الثانوية لكائن نيوسارتوريا فينيليا. تم تجزئة المستخلص الثانوي الفطري للحصول على مركبات نقية باستخدام كروماتوجرافي الطبقة الرقيقة. تم الحصول على ثلاثة مركبات نشطة، و أدي التوصيف الكيميائي للمركب المفصول إلى الأسماء الكيميائية المقترحة كمركب (1) هو 4 ، 5-ثنائي هيدرو -4 ، 6-مكرر- (4-ميثوكسيفينيل) -3 (H2)- بيريدازينون ، مركب (4) كـ 1- (prop-1 " - enyl) -3- (2'-methylprop-2'-enyl) -2-Hydroxy-anthraquinone ومركب (10) كـ(3-5،7) -dihydroxy-6- ميثيل -2-بيوتينيل) -8- (1-أوكسوبوتيل) -4-بروبيل-12H-بنزوبيران-2-وان. يُظهر المركب (4) واعدًا مضادًا للفيروسات ومضادًا لمرض السكري والقوى الكبدية يليه المركب (10)، بينما يحتوي المركب (1) على أفضل نشاط مضاد للالتهابات. وبالتالي، تشير هذه الدراسة إلى فطر *Neosartorya fennelliae* كمصدر محتمل للمركبات النشطة بيولوجيًا.