

Production of Bioactive Natural Products for Drug Research Using Food Waste as a Resource and New Isolated Microorganism

Shimaa I. A. Mohamed¹ , Sahar Abd Al-Daim² , Ahmed Nageh³ , Rania Abdel-Razik^{1,*} 

¹ Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, ElBuhous St. 12622, Dokki, Giza, Egypt

² Environmental Virology Lab 176, Water pollution Research department, Environment and Climate Change Institute, National Research Centre, ElBuhous St. 12622, Dokki, Giza, Egypt

³ Centre of Scientific Excellence for Influenza Viruses, National Research Centre, ElBuhous St. 12622, Dokki, Giza, Egypt

* Correspondence: rania122bc@gmail.com; Ra.abdel-Razik@nrc.sci.eg;

Received: 9.07.2024; Accepted: 15.07.2025; Published: 20.12.2025

Abstract: Natural substances obtained from fungi have the potential to treat several ailments, including cancer and viral-causing diseases. The newly isolated fungal strain was obtained from soil and cultured using Carrot peel juice. FTIR analysis was performed for fungal extracts. Additionally, antioxidant scavenging activity for extracts was measured using DPPH analysis. Using the MTT assay, the cytotoxic effects of extracts on MCF-7 and normal cells HSF were assessed. Finally, we evaluated the antiviral activity of the extracts against SARS-CoV-2. The results indicated that the isolated strain belonged to the genus *Talaromyces*, a member of the *Trichocomaceae* family of fungi. FTIR results revealed the different functional groups between the tested samples. The fungal extract produced by shaking showed the highest antioxidant activity with IC₅₀ (63.90 µg/ml). The anti-proliferative assay indicated that only the fungal extract under shaking conditions exerted a significant inhibitory effect on MCF-7 cells. The obtained result showed moderate antiviral activity against SARS-CoV-2, with a selectivity index of 3.084 under shaking conditions, 17.1 under static conditions, and 6.03 under control conditions. Finally, fungi can be grown on wastes for the production of metabolites with various biological activities. It yields promising anticancer and antiviral agents that warrant further study.

Keywords: cancer; fungi; antiviral; antioxidants; MCF-7.

© 2025 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The authors retain copyright of their work, and no permission is required from the authors or the publisher to reuse or distribute this article, as long as proper attribution is given to the original source.

1. Introduction

Cancer is a fatal condition with an increased tendency to spread to other organs [1]. Breast cancer is thought to be the main cause of death from cancer. Radiation, chemotherapy, and surgery are frequently used in the treatment of cancer [2]. There is a significant chance of cancer recurrence even with the surgical treatment's excellent cure rate [3,4]. Finding new therapeutic medications with potent cytotoxic effects is therefore imperative [5]. When reactive oxygen species (ROS) are produced in excess, the body's natural antioxidants are unable to control their production. This imbalance leads to oxidative stress, a major health issue [6]. Therefore, administering exogenous antioxidants would help meet the body's antioxidant requirements to prevent oxidative stress and preserve its redox state. The commercial synthesis

of natural antioxidants from conventional food sources could threaten human nutrition and contribute to rising food costs on the global market [7].

Large amounts of peel waste are generated by fruit and vegetable businesses and household kitchens, leading to financial and nutritional losses, as well as environmental problems. Between 25 and 30 percent of the final product is wasted just in the preparation of fruits and vegetables. Pomace, peels, rinds, and seeds are among the most often used waste materials. These materials are rich in bioactive compounds, including vitamins, lipids, polyphenols, enzymes, and carotenoids. These bioactive chemicals are being used in a variety of industries, including food to create edible films, probiotics in the food industry, and other industries to create valuable products. In microbiological research, microorganisms are cultivated in a lab setting by adding suitable culture medium and creating a favorable atmosphere [8]. Most experiments use commercially available media, such as MacConkey agar, Cetrimide agar, and Nutrient agar, although these are generally considered costly [8]. Numerous media and substrates have been shown to be effective for isolating and culturing organisms [9]. Certain fruits and vegetables, such as cabbage, carrots, and gooseberries, have been used as substitutes for nutritional agar to grow both bacteria and fungi [10,11]. Microorganisms metabolize simple and complex sugars found in fruit and vegetable bio-waste.

Furthermore, using fruit waste to produce high-value products such as probiotics, edible films, carbon dots, nanoparticles, and biosorbents will be an ecologically responsible and sustainable approach to creating new business opportunities. Research and technological improvements are lacking in most of these medicines, which are still in early development. Therefore, to maximize the economic potential of horticulture waste through early investment, research, and the formation of industrial consortia must be pursued. It will also encourage the use of horticultural waste in the manufacturing of goods with value addition [12].

Citrus (C) fruit peels contain very valuable bioactive chemicals [12]. Moreover, fruits and their peels have long been used to treat stomach problems, infections, and skin irritation. [13,14]. Notably, *C. reticulata* and *C. unshiu* peels are sold as crude medications in Japan under the brand name "Chimpi." Similar to this, dried peels of *Citrus aurantium* are used to make the well-known folk remedy "Touhi." [12, 15]. Citrus fruits, such as "tangerines," are among the most widely consumed foods in many nations worldwide. In China, citrus peel, known as "Chenpi," has been used as a medication to cure digestive and respiratory conditions. In addition, tangerine peels are used as aromatic spices in baked goods and drinks in Western nations [16-18]. Chinese medicine reportedly uses citrus fruit peels to treat high blood pressure, coughing, stomachaches, and muscle aches [19]. In Chinese and Indian traditional medicine, Wampee (*Clausena lansium*) peels are applied topically to relieve stomachaches and bronchitis [20,21].

Numerous viruses that cause epidemics in humans have been transmitted from person to person and from animals to humans. These infections include the hepatitis C virus, coronavirus, Ebola virus disease, and human immunodeficiency virus (HIV). They have an impact on human societies' economic development, wealth, and growth by causing numerous serious infectious diseases [22].

The emergence and widespread spread of viral illnesses can be attributed to shifts in global habitats and the development of transportation infrastructure. Certain viral infections, such as those caused by coronavirus, seriously impair societal, economic, and human situations. They cause millions of fatalities and numerous severe illnesses, which have an impact on human society's ability to progress and succeed economically. SARS-CoV-2, also

known as the severe acute respiratory syndrome coronavirus, is the virus that causes coronavirus disease 2019 (COVID-19). It appears to have a zoonotic origin; rodents and bats are the hosts, while other species, such as civets, raccoon dogs, camels, and possibly pangolins, act as intermediate hosts during transmission [23,24]. Consequently, the need for novel antiviral agents is ongoing.

In our current study, we decided to cultivate the isolated fungi using carrot peel juice under different conditions and to assess the production of phytochemicals, focusing on their antioxidant, antiviral, and anticancer effects in relation to their functional groups.

2. Materials and Methods

2.1. Microorganism and maintenance.

The microorganism used in this study was a new strain of *Talaromyces verruculosus* (PP659686), isolated from soil. A soil sample from the top 10-20 cm of the ground was collected from the herbarium of the National Research Centre (NRC, Giza, Egypt). To make tenfold serial dilutions, 10 mg of the soil sample was suspended in 50 mL of sterile water. Distribution across the surface of potato dextrose (PDA) agar plates was used to isolate using a suitable dilution of the soil samples. The plates were inspected after 7 days of incubation at 30°C to determine whether any colonies had developed. Then, individual colonies were selected, transferred to an agar plate containing the same media, and allowed to develop for an additional 7 days. A portion of the terminal colonial development of a single unique colony was transferred to pure PDA media slants and kept at 4°C with bimonthly subcultures, and the cultures were identified by 18S rRNA.

2.1.1. Molecular identification.

DNA isolation for extraction, the Gene JET Genomic DNA purification kit 98 (Thermo Scientific # k0721) was used on the bacterial isolates' genomic DNA. The 18S rRNA fragments were partially amplified by PCR at Sigma Company of Scientific Services, Egypt (www.sigma-co-eg.com), using Maxima Hot Start PCR Master Mix (Thermo K1051). The assigned sequences were compared using a BLAST search against the sequences in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov) to perform phylogenetic analysis. PCR amplification of the 18S rRNA gene was carried out using specific primers ITS5 5' (TCC GTA GGT GAA CCT GCGG) 3' and ITS4 5' (TCC TCC GCT TAT TGA TAT GC) 3'.

2.1.2. Preparation of inoculum.

Seven-day-old fungal slants were used to prepare a 10⁷ CFU/mL spore suspension, which was then used to inoculate the production medium.

2.2. Chemical analysis.

2.2.1. Chemicals.

Unless otherwise indicated, analytical-grade chemicals were obtained from Sigma-Aldrich and used as supplied, requiring no additional purification. The deionized water used to prepare all solutions had a resistivity of at least 18.2 MΩ · cm.

2.2.2. Fermentation process and secondary metabolites production.

Carrot peel juice (500 ml in each flask) was used as the basal medium for production. The production medium was sterilized by autoclaving at 121°C and a pressure of 1.5 atm for 15 min. The previously prepared inoculum of the used isolate was added to the culture medium and incubated at 150 rpm; another flask was incubated statically at room temperature for 72 h. A production medium without any inoculum was used as a negative control. At the end of the fermentation period, chloroform was added to each flask for extraction, and the mixture was evaporated to dryness.

2.2.3. Extraction and determination of secondary metabolite products.

Each flask containing free fungal cells received 100 ml of chloroform/100 ml of medium at the conclusion of the fermentation process. To be sure that none of the secondary metabolite products remained, the extraction was done twice. To produce the semi-solid residue (test material), chloroform extracts were combined, dried with anhydrous sodium sulphate, and evaporated to dryness in vacuo at 50°C. The sample was then analyzed.

2.2.4. Fourier transform infrared analysis.

For FT-IR analysis, a few milligrams of the secondary metabolite's dry powder were used, and a Jasco FT/IR-6100 type A was used. After adding potassium bromide (KBr) to the sample, it was crushed into a 10 mm disc. At last, the spectra were obtained between 400 cm⁻¹ and 4000 cm⁻¹ wavenumbers.

2.2.5. Determination of antioxidant activity.

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging analysis was performed to evaluate antioxidant activity using a spectrophotometer (Jasco V-630, UV/Visible), as described by [25]. The IC₅₀s were determined for each sample. Briefly, eight-fold serial dilutions (10, 25, 50, 100, 250, 500, 750, 1000 µg/ml) of the stock solution 10 mg/ml were prepared to determine the scavenger percentage. Finally, 517 nm was used to calculate the absorbance. The following formula was utilized to determine the antioxidant (%):

$$\text{Scavenging (\%)} \text{ of DPPH radicals} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (1)$$

Where A₀ represents the DPPH solution's absorbance, and A₁ represents the sample's absorbance.

2.3. Biological analysis.

2.3.1. Cell propagation and maintenance.

The appropriate conditions were used to culture MCF-7 (cancer cell line) and HSF (normal human skin fibroblasts) cell lines, which were purchased from the ATCC (American Type Culture Collection). The cells were propagated in DMEM (Lonza, Belgium) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 g/ml streptomycin sulphate at 37°C in a humid incubator with 5% CO₂. The cells were collected after being trypsinized with 0.025% trypsin and 0.02% EDTA, followed by two washes in DPBS. Cells were divided for continued cultivation when the cell density reached about 80%. When the cells were in logarithmic growth phase, the experiments began.

2.3.2. Anti-proliferation assay.

The impact of three fungal extracts on the proliferation of MCF-7 and HSF normal cell lines was examined using the MTT assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (Sigma-Aldrich Corp, St Louis, MO, USA) [7]. Basically, the extract was serially diluted (0, 7.81, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL) for 48 h on seeded cells on a 96-well plate with a flat bottom. Doxorubicin (Dox, Mr=543.5) was used as a reference cytotoxic drug, with 100% inhibition at concentrations of 0.37, 0.75, 1.5, 3, and 6 µg/ml. The vehicle utilized to dissolve the evaluated crude extracts was dimethyl sulfoxide (DMSO) from Sigma-Aldrich Corp (St Louis, MO, USA), and the final concentration on the cells was less than 0.2%. The optical density (570 nm) was measured by a microplate reader, and DMSO was utilized as a blank. The formula used to calculate the percentage of cell viability was:

$$Cell\ viability(\%) = 1 - \left(\frac{OD\ Treatment - OD\ blank}{OD\ control - OD\ blank} \right) * 100 \quad (2)$$

2.3.3. Virus isolation and propagation.

SARS-CoV-2, an Isolated strain of viruses: hCoV-19/Egypt/NRC-03/2020, a highly pathogenic strain of SARS-CoV-2 (GISAID accession number: EPI_ISL_430819), was isolated in VERO-E6 cells (ATCC No. CRL-1586) from an oropharyngeal swab specimen used in this study that was taken from an infected human in Egypt on March 18, 2020.

2.3.4. Cytotoxicity concentration 50% determination by crystal violet assay.

With certain adjustments, the cytotoxicity was assessed in accordance with [26]. Fresh media was added to all wells in row 2 of a U-shaped 96-well plate, and 180 µl was added to wells in row 1. Row 1 from (A1 to H1) received 20 µl of the prepared stock solution of the plant extract, resulting in a concentration of (1:10) from the stock plant extract, followed by a serial dilution. Columns 2 through 10 were made with a 2-fold serial dilution. The VERO-E6 monolayer sheets were treated at 90% confluency with 50 µl of each dilution, and they were then incubated for 72 hours. Following a fixation step with 10% formaldehyde, the cells were incubated for 3 hours at room temperature. Following washing, the cells were visible by dissolving the stain in pure methanol after being stained with (0.5%) crystal violet solution. An ELISA reader was used to measure the optical density of the 96-well plate, and the following formula was used to determine the cytotoxicity:

Cytotoxicity is calculated as follows:

$$\left(\frac{OD\ of\ untreated\ cells - OD\ of\ treated\ cells}{OD\ of\ untreated\ cells} \right) * 100 \quad (3)$$

2.3.5. Inhibitory concentration 50% determination.

2.4×10^4 Vero-E6 cells were seeded into each well of 96-well tissue culture plates and incubated overnight at 37°C in a humidified environment with 5% CO₂. After giving the cell monolayers one wash with 1x PBS, they were exposed to viral adsorption for one hour at room temperature (RT) using hCoV-19/Egypt/NRC-03/2020 (Accession Number on GSAID: EPI_ISL_430820). The test chemicals were added to the cell monolayers at different concentrations using 100 µL of DMEM. After incubation for 72 hours at 37°C in a 5% CO₂ incubator, the cells were fixed for 20 minutes with 100 µl of 4% paraformaldehyde and stained

for 15 minutes at room temperature with 0.1% crystal violet in distilled water. Next, 100 μ L of 100% methanol was used to dissolve the crystal violet dye. Using an Anthos Zenyth 200RT plate reader (Anthos Labtec Instruments, Heerhugowaard, Netherlands), the optical density of the color was determined at 570 nm [27]. The compound's IC₅₀ is the amount needed to reduce the viral-induced cytopathic effect (CPE) by 50% compared to the virus control. With a few minor adjustments, the protocol was followed exactly as it had been previously reported [28,29].

2.4. Statistical analysis.

Three different experiments' sets of mean values are shown, along with their standard errors of the mean (mean \pm SEM). GraphPad Prism v5 was used to process all data, and Dunnett's multiple-comparison test was used to analyze the results of the one-way analysis of variance (ANOVA) post hoc test. * For every test, a value of P <0.05 was considered statistically significant.

3. Results and Discussion

At some time in their lives, 1 in 8 women will be diagnosed with aggressive breast cancer [30,31]. With over 520,000 fatalities annually from cancer, it is the leading cause of death for women globally [32,33], with over 40,000 of those deaths occurring in the United States only [34]. Further study is required to reduce the human and societal toll of this disease, despite progress in survival and treatment over the past few decades. Microorganisms are essential for advancing medicinal therapy and the chemistry of natural products [35]. Since the discovery of penicillin, they have been considered a rich source of distinctive bioactive chemicals [36]. In the environment, fungi are heterotrophic, eukaryotic microorganisms that frequently coexist symbiotically. Humans have been using fungi for a very long time for a variety of purposes, making beer, wine, leavened bread, and soy products, as well as for medical purposes and daily use [37].

3.1. Molecular identification of the isolated organism.

The molecular identification of the isolated organism by PCR indicated that it belongs to the genus *Talaromyces*, a member of the *Trichocomaceae* family of fungi. The genus *Talaromyces* belongs to the *Trichocomaceae* family of fungi (Figure 1). The genus's members, first identified in 1955 by American mycologist Chester Ray Benjamin, produce soft, cottony fruit bodies called ascocarps, which have cell walls composed of closely woven hyphae. Frequently, the fruit bodies are yellow or are encircled with granules with a yellowish hue. The genus was estimated to have 42 species in 2008, although many more have since been identified.

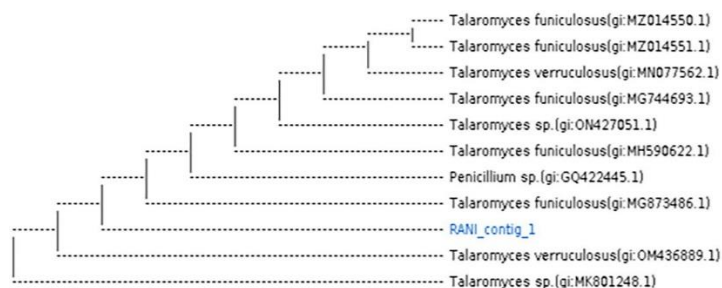


Figure 1. Phylogenetic tree of the isolated fungal strain *Talaromyces verruculosus* according to gene sequencing.

3.2. *Fourier-transform infrared spectroscopy analysis of chloroform fungal extracts.*

FTIR analysis of three samples revealed the presence of distinct functional groups; the FTIR image is not shown. All spectra of chloroform extracts (control, static, and shaking) show a medium sharp band at 719.37-729.29 cm^{-1} , revealing the methylene group (Table 1) ($-(\text{CH}_2)_n$ ($n \geq 3$)), 1229.26 cm^{-1} carbonyl group [38]. Moreover, a peak at 601.03 cm^{-1} indicated the presence of C-Br stretching and a halogenated compound, only in the shaking fungal extract [39]. The band at 2848.44 - 2940.91 cm^{-1} was attributed to the presence of methylene C-H asymmetric and symmetric stretch found in lipids in all tested samples [40]. On the other hand, the band in the case of shaking fungal extract was observed at 1721.02 cm^{-1} , corresponding to aliphatic carbonyl compounds such as ketones, carboxylic acids, and α, β -unsaturated esters [41]. Finally, the aromatic OH group was observed at 3382.82 cm^{-1} , corresponding to the presence of phenol only in the shaking fungal extract. The results revealed differences in functional groups among the three tested samples, which correlated with differences in their biological activities, including antioxidant, antiviral, and anticancer activities.

Table 1. FTIR spectra of three chloroform fungal extracts (control, shaking, and static) using carrot peel juice as a basal medium.

Control	Shaking	Static	Functional group		Reference
Wavenumber (cm^{-1})					
	601.03		C-Br, C-I	Halogenated derivative	[24]
	719.37-729.29		$-(\text{CH}_2)_n$	W, Methylene	[27]
	1229.26		C-O	carbonyl group	[23]
	1365.65-1389.26		CH_3	gem-Dimethyl or "isopropyl group"	[29]
	1469.62-1471.62		Asymmetric CH-bending	m, R- CH_3 , R- CH_2	[30]
	1721.02		C=O stretching	m, aliphatic ketone, carboxylic acid, α, β -unsaturated ester	[26]
	2848.44		Symmetrical CH stretching	m, Alkane lipid	[31]
	2916.16		Methylene C-H asym./sym. Stretch	s, alkanes, lipid	[31]
	2954.43		Asymmetry CH_3 stretching	alkanes and lipids	[32,25]
	3382.82		Aromatic (-OH)	b, phenol	[33]

3.3. *Antioxidant activity.*

The antioxidant capacity of the chloroform extract of three tested samples (negative control, static, and shaking flasks) was determined to show how fast the DPPH) methanol solution decolorized diphenylpicryl hydrazine (yellow-colored product) concentration-dependently (Figure 2). IC_{50} values were calculated as shown in Table 2. The data revealed that the fungal extract shaken at 150 rpm and room temperature has the highest antioxidant capacity, with an IC_{50} value of 63.90 $\mu\text{g/ml}$ and a DPPH scavenging effect of $93.66 \pm 0.43\%$ at 1000 $\mu\text{g/ml}$. On the other hand, the negative control and the fungal extract at static conditions showed lower antioxidant scavenging activity, with IC_{50} values of 346.11 and 219.19 $\mu\text{g/ml}$, respectively. Antioxidants and scavengers are used to combat reactive oxygen species (ROS), which are known to be responsible for the development of a number of human malignancies [42]. The ability of antioxidants to donate hydrogen has been proposed to have an impact on DPPH [43].

Table 2. Antioxidant scavenger capacity of chloroform fungal extracts.

Sample	IC ₅₀ (µg/ml)	Percentage (%) at 1000 µg/ml
Control	346.11	79.84 ±0.57
Static	219.19	77.50± 0.27
Shaking	63.90	93.66 ±0.43

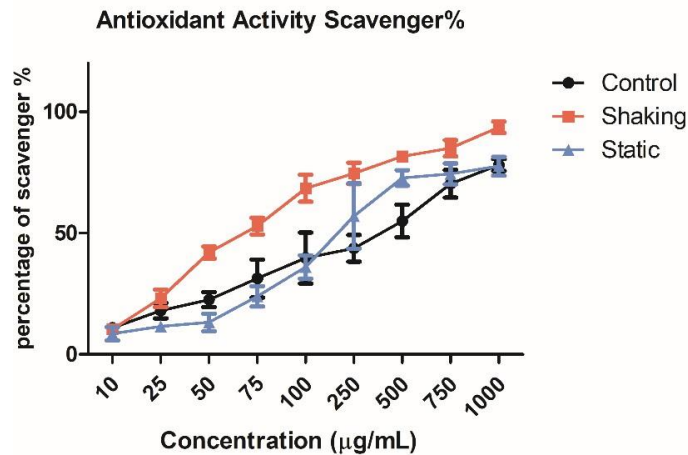


Figure 2. Antioxidant scavenger activity of chloroform fungal extracts (negative control, Shaking and static conditions). All experiments were performed in triplicate. Data are expressed as mean ± SEM.

3.4. Anti-proliferative activity.

In this assay, we investigated the *in vitro* cytotoxicity of a fungal extract at different cultivation conditions using carrot peel juice as a basal medium. Using MTT assay, the results indicated that only the chloroform fungal extract at shaking condition (150 rpm) induced significant cytotoxic activity against human breast cancer (MCF-7) cells at different concentrations (0, 7.81, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL) for 48 h treatment with an IC₅₀ value of 107.68 µg/mL. The reference drug, doxorubicin, has an IC₅₀ of 1.2 µg/mL against the MCF-7 cell line. After being exposed to DMSO for 48 hours, MCF-7 vitality was not significantly affected. On the other hand, the fungal extract at static conditions showed less cytotoxic activity than under shaking conditions, with an IC₅₀ value of 947.43 µg/mL. Carrot peel juice was used only as a negative control and did not exhibit a significant anti-proliferative effect on the treated MCF-7 cell line. Moreover, the extracts did not exhibit cytotoxicity against HSF normal cells, indicating that they are safe. Additionally, the anticancer activity of the three tested extracts was examined using the MTT assay.

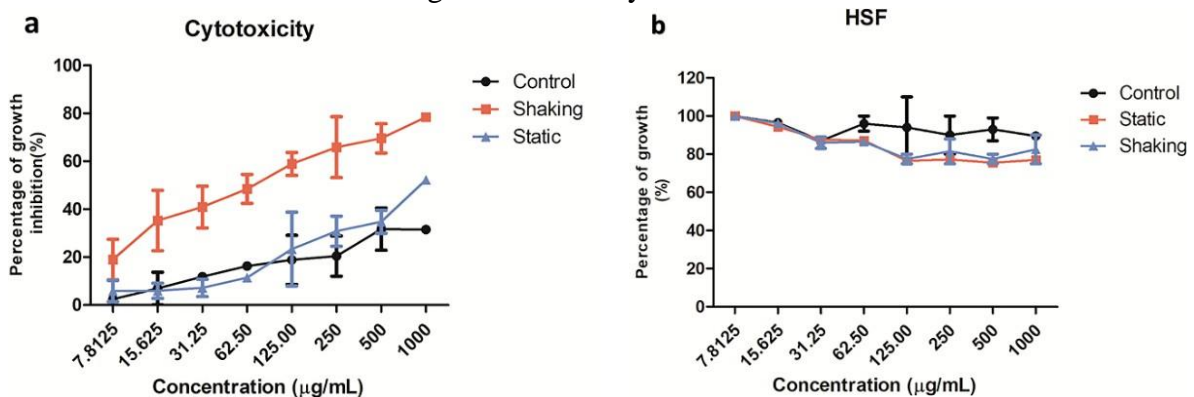


Figure 3. Cytotoxic effects of chloroform fungal extracts at different conditions (shaking, static) and carrot peel juice as a negative control on (a) MCF-7; (b) HSF normal human skin fibroblast after treatment for 48 h. All experiments were performed in triplicate. Data are expressed as mean ± SEM.

Only the shaking fungal extract exhibited a significant anticancer activity against the breast cancer cell line MCF-7 (Figure 3). Numerous endophytic fungi produce anticancer agents with an outstanding capacity to inhibit tumor growth. For instance, *Fusarium oxysporum*, *Catharanthus roseus*, and *Catharanthus roseus* all develop vinblastine from their hosts [44]. According to a study, CHO-K1, MCF-7, and HepG-2 cell lines showed reduced proliferation after treatment with fungal vinblastine [45]. According to a different study, when vinblastine and indibulin were given together at 50 and 150 nM, respectively, cell proliferation in MCF-7 was decreased by 53% and 71%, resulting in combination indexes (CI) of 0.67 and 0.5 [46].

3.5. Antiviral activity.

Results of antiviral test by using crystal violet assay of tested compounds showed that compounds Control and Shaking showed moderate antiviral activity against (hCoV-19/Egypt/NRC-03/2020) SARS-CoV-2 virus, with selectivity index (SI) equal = 6.03 and 3.084, respectively (Figure 4a, b, c). The compound Static showed promising antiviral activity against the same virus, with a selectivity index (SI) of 17.1, as shown in Table 3. Recently, several studies have been published on the efficacy of microbial secondary metabolites against SARS-CoV-2. The antimicrobial natural compound Aurasperoneas, which was isolated from *Aspergillus niger* in the Red Sea tunicate *Phallusia nigra*, shows remarkable effectiveness on SARS-CoV-2 in vitro with an IC₅₀ of 12.25 μM [47]. Additionally, Neoechinulin A, isolated from *Aspergillus fumigatus* MR2012 in the Red Sea, has a target comparable to Mpro and an IC₅₀ of 0.47 μM against SARS-CoV-2 [48].

Table 3. CC₅₀, IC₅₀, and SI of samples on VERO-E6 cells, against SARS-CoV-2 using the crystal violet assay.

NO.	Sample code	CC ₅₀ μg/ml	IC ₅₀ μg/ml	SI (CC ₅₀ / IC ₅₀)
1	Control	583.4	96.81	6.03
2	Static	300.7	17.94	17.1
3	Shaking	500	162.1	3.084

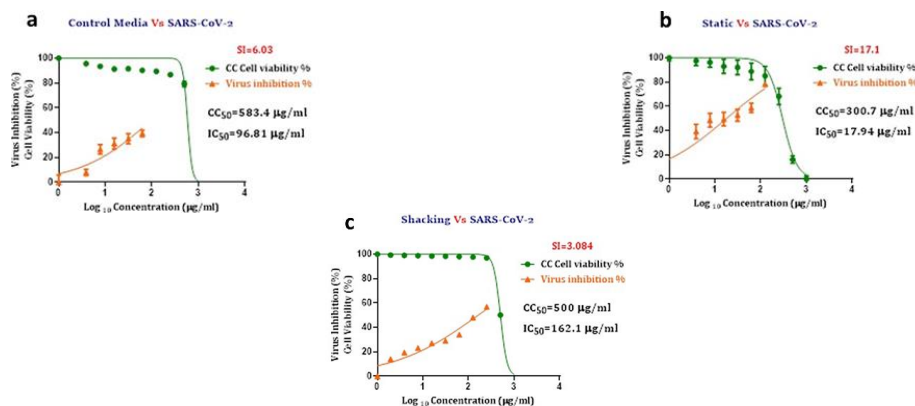


Figure 4. Antiviral activity by using the crystal violet assay of tested compounds showed that compounds (a) Control and (c) Shaking showed moderate antiviral activity, while (b) Static showed promising antiviral activity against (hCoV-19/Egypt/NRC-03/2020), SARS-CoV-2 virus. All experiments were performed in triplicate. Data are expressed as mean ± SEM.

4. Conclusion

Our current study evaluated and demonstrated the efficiency of using food waste, specifically carrot peel juice, as a basal medium for the production of biologically active

metabolites by the fungus *Talaromyces* isolated from soil. The chloroform fungal extracts under static and shaking conditions showed the presence of various chemical components, including alcohols, phenols, alkanes, alkenes, carboxylic acids, ethers, ketones, alkyl halides, and halogen compounds. The fungal extract produced by shaking showed marked antioxidant scavenger activity up to 93.66%. The chloroform fungal extract under shaking conditions exhibited a significant inhibitory effect on MCF-7 growth, with no cytotoxicity toward normal HSF cells, indicating the safety of the isolated extracts. Additionally, the extracts exhibit promising antiviral activity against SARS-CoV-2. Finally, our work provides a basis for additional research employing isolated fungal secondary metabolites against different human cancer cells, the SARS-CoV-2 virus, and in vivo models, as well as for prospective treatments.

Author Contributions

Conceptualization, R.A. and Sh.I.; methodology, R.A. and Sh.I.; investigation, R.A. and Sh.I.; formal analysis, Sh.I.; data curation, Sh.I.; resources, R.A.; writing—original draft preparation, R.A., Sh.I., S.A., and A.N.; writing—review and editing, R.A. and Sh.I.; visualization, R.A. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The cell line study protocol was approved by the Institutional Review Board (National Research Centre), Approval number 044121223, date of approval 26.12.2023).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

No funding.

Acknowledgments

The National Research Centre, Egypt, is acknowledged by the authors for its assistance in making this work possible.

Conflicts of Interest

The authors declare there are no conflicts of interest.

References

1. Siegel, R.L.; Miller, K.D.; Wagle, N.S.; Jemal, A. Cancer statistics, 2023. *CA. Cancer J. Clin.* **2023**, *73*, 17-48, <https://doi.org/10.3322/caac.21763>.
2. Baskar, R.; Lee, K.A.; Yeo, R.; Yeoh, K.-W. Cancer and Radiation Therapy: Current Advances and Future Directions. *Int. J. Med. Sci.* **2012**, *9*, 193-199, <https://doi.org/10.7150/ijms.3635>.
3. Uramoto, H.; Tanaka, F. Recurrence after surgery in patients with NSCLC. *Transl. Lung Cancer Res.* **2014**, <https://nanobioletters.com/>

- 3, 242, <https://doi.org/10.3978/j.issn.2218-6751.2013.12.05>.
4. Rao, K.V.; Faso, A. Chemotherapy-induced nausea and vomiting: optimizing prevention and management. *Am Health Drug Benefits*. 2012 Jul;5(4):232-40. <https://doi.org/10.5772/intechopen.96194>.
 5. Pradhan, P.; Sharpe, L.; Menzies, R.E. Towards a Stepped Care Model for Managing Fear of Cancer Recurrence or Progression in Cancer Survivors. *Cancer Manag. Res.* **2021**, *13*, 8953-8965, <https://doi.org/10.2147/CMAR.S294114>.
 6. Fratta Pasini, A.M.; Cominacini, L. Potential Benefits of Antioxidant Phytochemicals on Endogenous Antioxidants Defences in Chronic Diseases. *Antioxidants* **2023**, *12*, 890, <https://doi.org/10.3390/antiox12040890>.
 7. Mohamed, S.I.A.; Jantan, I.; Nafiah, M.A.; Seyed, M.A.; Chan, K.M. Lignans and polyphenols of *Phyllanthus amarus* Schumach and Thonn induce apoptosis in HCT116 human colon cancer cells through Caspases-dependent pathway. *Curr. Pharm. Biotechnol.* **2021**, *22*, 262-273, <https://doi.org/10.2174/1389201021666200612173029>.
 8. Jadhav, P.; Sonne, M.; Kadam, A.; Patil, S.; Dahigaonkar, K.; Oberoi, J.K. Formulation of Cost Effective Alternative Bacterial Culture Media Using Fruit and Vegetables Waste. *Int. J. Curr. Res. Rev.* **2018**, *10*, 6-15, <https://doi.org/10.7324/IJCRR.2018.1022>.
 9. Geris, R.; Teles de Jesus, V.E.; Ferreira da Silva, A.; Malta, M. Exploring Culture Media Diversity to Produce Fungal Secondary Metabolites and Cyborg Cells. *Chem. Biodiversity* **2024**, *21*, e202302066, <https://doi.org/10.1002/cbdv.202302066>.
 10. Mwandira, W.; Mavroulidou, M.; Joshi, S.; Gunn, M.J. Fruit and vegetable waste used as bacterial growth media for the biocementation of two geomaterials. *Sci. Total Environ.* **2024**, *947*, 174489, <https://doi.org/10.1016/j.scitotenv.2024.174489>.
 11. Gamit, T.; Hajoori, M.; Maisuria, N. A Review: Formulation of Alternative Culture Media. *Int. J. Life Sci. Agric. Res.* **2023**, *2*, 206-212, <https://doi.org/10.55677/ijlsar/V02I08Y2023-01>.
 12. Adhikari-Devkota, A.; Kurauchi, Y.; Yamada, T.; Katsuki, H.; Watanabe, T.; Devkota, H.P. Anti-neuroinflammatory activities of extract and polymethoxyflavonoids from immature fruit peels of *Citrus*' Hebesu'. *J. Food Biochem.* **2019**, *43*, e12813, <https://doi.org/10.1111/jfbc.12813>.
 13. Gao, Z.; Gao, W.; Zeng, S.-L.; Li, P.; Liu, E.-H. Chemical structures, bioactivities and molecular mechanisms of citrus polymethoxyflavones. *J. Funct. Foods* **2018**, *40*, 498–509, <http://doi.org/10.1016/j.jff.2017.11.036>.
 14. Matsumoto, T.; Nishikawa, T.; Furukawa, A.; Itano, S.; Tamura, Y.; Hasei, T.; Watanabe, T. Antimutagenic Effects of Polymethoxy Flavonoids of *Citrus unshiu*. *Nat. Prod. Commun.* **2017**, *12*, 1934578X1701200108, <http://doi.org/10.1177/1934578X1701200108>.
 15. Shilpa, V.; Shams, R.; Dash, K.K.; Pandey, V.K.; Dar, A.H.; Ayaz Mukarram, S.; Harsányi, E.; Kovács, B. Phytochemical Properties, Extraction, and Pharmacological Benefits of Naringin: A Review. *Molecules* **2023**, *28*, 5623, <https://doi.org/10.3390/molecules28155623>.
 16. Ihara, H.; Yamamoto, H.; Ida, T.; Tsutsuki, H.; Sakamoto, T.; Fujita, T.; Okada, T.; Kozaki, S. Inhibition of Nitric Oxide Production and Inducible Nitric Oxide Synthase Expression by a Polymethoxyflavone from Young Fruits of *Citrus unshiu* in Rat Primary Astrocytes. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 1843-1848, <http://doi.org/10.1271/bbb.120215>.
 17. Guo, J.; Tao, H.; Cao, Y.; Ho, C.-T.; Jin, S.; Huang, Q. Prevention of Obesity and Type 2 Diabetes with Aged Citrus Peel (*Chenpi*) Extract. *J. Agric. Food Chem.* **2016**, *64*, 2053-2061, <https://doi.org/10.1021/acs.jafc.5b06157>.
 18. Yang, M.; Jiang, Z.; Wen, M.; Wu, Z.; Zha, M.; Xu, W.; Zhang, L. Chemical Variation of Chenpi (Citrus Peels) and Corresponding Correlated Bioactive Compounds by LC-MS Metabolomics and Multibioassay Analysis. *Front. Nutr.* **2022**, *9*, 825381, <https://doi.org/10.3389/fnut.2022.825381>.
 19. Tang, K.; He, S.; Zhang, X.; Guo, J.; Chen, Q.; Yan, F.; Banadyga, L.; Zhu, W.; Qiu, X.; Guo, Y. Tangeretin, an extract from *Citrus* peels, blocks cellular entry of arenaviruses that cause viral hemorrhagic fever. *Antivir. Res.* **2018**, *160*, 87-93, <https://doi.org/10.1016/j.antiviral.2018.10.011>.
 20. Hyun, J.M.; Jo, Y.J.; Kim, J.E.; An, H.J.; Choi, Y.H.; Hyun, C.-G.; Lee, N.H. Tetramethyl-O-scutellarin isolated from peels of immature Shiranuhi fruit exhibits anti-inflammatory effects on LPS-induced RAW264.7 cells. *Trop. J. Pharm. Res.* **2017**, *16*, 2197-2205, <https://doi.org/10.4314/tjpr.v16i9.22>.
 21. Phachonpai, W.; Riyamongkol, P.; Mann, D.; Tongun, T. The potential of *Clausena lansium* (Lour.) peel extract consumption on hyperglycemia, hyperlipidemia and augmented oxidative stress in type-2 diabetic model rats. *J. Appl. Pharm. Sci.* **2023**, *13*, 181-188, <https://doi.org/10.7324/JAPS.2023.112499>.
 22. Morens, D.M.; Fauci, A.S. Emerging Pandemic Diseases: How We Got to COVID-19. *Cell* **2020**, *182*, 1077–

- 1092, <https://doi.org/10.1016/j.cell.2020.08.021>.
23. Amoutzias, G.D.; Nikolaidis, M.; Tryfonopoulou, E.; Chlichlia, K.; Markoulatos, P.; Oliver, S.G. The Remarkable Evolutionary Plasticity of Coronaviruses by Mutation and Recombination: Insights for the COVID-19 Pandemic and the Future Evolutionary Paths of SARS-CoV-2. *Viruses* **2022**, *14*, 78, <https://doi.org/10.3390/v14010078>.
 24. Guo, Y.-R.; Cao, Q.-D.; Hong, Z.-S.; Tan, Y.-Y.; Chen, S.-D.; Jin, H.-J.; Tan, K.-S.; Wang, D.-Y.; Yan, Y. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. *Military Med. Res.* **2020**, *7*, 11, <https://doi.org/10.1186/s40779-020-00240-0>.
 25. Pitz, H.d.S.; Pereira, A.; Blasius, M.B.; Voytena, A.P.L.; Affonso, R.C.L.; Fanan, S.; Trevisan, A.C.D.; Ribeiro-do-Valle, R.M.; Maraschin, M. *In Vitro* Evaluation of the Antioxidant Activity and Wound Healing Properties of Jaboticaba (*Plinia peruviana*) Fruit Peel Hydroalcoholic Extract. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 3403586, <https://doi.org/10.1155/2016/3403586>.
 26. Yan, K.; Rawle, D.J.; Le, T.T.; Suhrbier, A. Simple rapid in vitro screening method for SARS-CoV-2 antivirals that identifies potential cytotoxicity-associated false positives. *Viol. J.* **2021**, *18*, 123, <https://doi.org/10.1186/s12985-021-01587-z>.
 27. Gordon, D.E.; Jang, G.M.; Bouhaddou, M.; Xu, J.; Obernier, K.; White, K.M.; O’Meara, M.J.; Rezelj, V.V.; Guo, J.Z.; Swaney, D.L.; Tummino, T.A.; Hüttenhain, R.; Kaake, R.M.; Richards, A.L.; Tutuncuoglu, B.; Foussard, H.; Batra, J.; Haas, K.; Modak, M.; Kim, M.; Haas, P.; Polacco, B.J.; Braberg, H.; Fabius, J.M.; Eckhardt, M.; Soucheray, M.; Bennett, M.J.; Cakir, M.; McGregor, M.J.; Li, Q.; Meyer, B.; Roesch, F.; Vallet, T.; Mac Kain, A.; Miorin, L.; Moreno, E.; Naing, Z.Z.C.; Zhou, Y.; Peng, S.; Shi, Y.; Zhang, Z.; Shen, W.; Kirby, I.T.; Melnyk, J.E.; Chorba, J.S.; Lou, K.; Dai, S.A.; Barrio-Hernandez, I.; Memon, D.; Hernandez-Armenta, C.; Lyu, J.; Mathy, C.J.P.; Perica, T.; Pilla, K.B.; Ganesan, S.J.; Saltzberg, D.J.; Rakesh, R.; Liu, X.; Rosenthal, S.B.; Calviello, L.; Venkataramanan, S.; Liboy-Lugo, J.; Lin, Y.; Huang, X.-P.; Liu, Y.; Wankowicz, S.A.; Bohn, M.; Safari, M.; Ugur, F.S.; Koh, C.; Savar, N.S.; Tran, Q.D.; Shengjuler, D.; Fletcher, S.J.; O’Neal, M.C.; Cai, Y.; Chang, J.C.J.; Broadhurst, D.J.; Klippsten, S.; Sharp, P.P.; Wenzell, N.A.; Kuzuoglu-Ozturk, D.; Wang, H.-Y.; Trenker, R.; Young, J.M.; Caverio, D.A.; Hiatt, J.; Roth, T.L.; Rathore, U.; Subramanian, A.; Noack, J.; Hubert, M.; Stroud, R.M.; Frankel, A.D.; Rosenberg, O.S.; Verba, K.A.; Agard, D.A.; Ott, M.; Emerman, M.; Jura, N.; et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* **2020**, *583*, 459–468, <https://doi.org/10.1038/s41586-020-2286-9>.
 28. Scudellari, M. How the coronavirus infects cells—and why Delta is so dangerous. **2021**.
 29. Naqvi, A.A.T.; Fatima, K.; Mohammad, T.; Fatima, U.; Singh, I.K.; Singh, A.; Atif, S.M.; Hariprasad, G.; Hasan, G.M.; Hassan, M.I. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. *Biochim. Biophys. Acta - Mol. Basis Dis.* **2020**, *1866*, 165878, <https://doi.org/10.1016/j.bbadis.2020.165878>.
 30. Algotiml, R.; Gab-Alla, A.; Seoudi, R.; Abulreesh, H.H.; El-Readi, M.Z.; Elbanna, K. Anticancer and antimicrobial activity of biosynthesized Red Sea marine algal silver nanoparticles. *Sci. Rep.* **2022**, *12*, 2421, <https://doi.org/10.1038/s41598-022-06412-3>.
 31. Salvo, E.M.; Ramirez, A.O.; Cueto, J.; Law, E.H.; Situ, A.; Cameron, C.; Samjoo, I.A. Risk of recurrence among patients with HR-positive, HER2-negative, early breast cancer receiving adjuvant endocrine therapy: A systematic review and meta-analysis. *Breast* **2021**, *57*, 5–17, <https://doi.org/10.1016/j.breast.2021.02.009>.
 32. O’Reilly, D.; Sendi, M.A.; Kelly, C.M. Overview of recent advances in metastatic triple negative breast cancer. *World J. Clin. Oncol.* **2021**, *12*, 164–182, <https://doi.org/10.5306/wjco.v12.i3.164>.
 33. Jia, S.; Liu, Z.; Zhang, J.; Zhao, C.; Zhu, L.; Kong, J.; Han, H.; Shang, Y.; Shen, D.; Duan, X. Can internal mammary lymph nodes irradiation bring survival benefits for breast cancer patients? A systematic review and meta-analysis of 12,705 patients in 12 studies. *Radiat. Oncol.* **2021**, *16*, 42, <https://doi.org/10.1186/s13014-021-01772-y>.
 34. Cipriano, É.; Mesquita, A. Emerging Therapeutic Drugs in Metastatic Triple-Negative Breast Cancer. *Breast Cancer: Basic Clin. Res.* **2021**, *15*, 11782234211002491, <https://doi.org/10.1177/11782234211002491>.
 35. Bounous, V.E.; Andronico, N.; Govone, F.; Giorgi, M.; Actis, S.; D’Alonzo, M.; Biglia, N. New challenges in treatments for menopausal symptoms in breast cancer survivors. *Gynecol. Reprod. Endocrinol. Metab.* **2023**, *4*, 62–71, <https://doi.org/10.53260/grem.234023>.
 36. Barzkar, N.; Sukhikh, S.; Babich, O. Study of marine microorganism metabolites: new resources for bioactive natural products. *Front. Microbiol.* **2024**, *14*, 1285902, <https://doi.org/10.3389/fmicb.2023.1285902>.
 37. Haidari, H.; Melguizo-Rodríguez, L.; Cowin, A.J.; Kopecki, Z. Therapeutic potential of antimicrobial peptides for treatment of wound infection. *Am. J. Physiol. Cell Physiol.* **2022**, *324*, C29–C38,

- <https://doi.org/10.1152/ajpcell.00080.2022>.
38. Kaddouri, Y.; Bouchal, B.; Abrigach, F.; El Kodadi, M.; Bellaoui, M.; Elkamhawy, A.; Touzani, R.; Abdellatif, M.H. New N-Alkylated Heterocyclic Compounds as Prospective NDM1 Inhibitors: Investigation of In Vitro and In Silico Properties. *Pharmaceuticals* **2022**, *15*, 803, <https://doi.org/10.3390/ph15070803>.
 39. Lochaiwatana, Y.; Poolthong, S.; Hirata, I.; Okazaki, M.; Swadison, S.; Vongsavan, N. The synthesis and characterization of a novel potassium chloride-fluoridated hydroxyapatite varnish for treating dentin hypersensitivity. *Dent. Mater. J.* **2015**, *34*, 31-40, <https://doi.org/10.4012/dmj.2014-102>.
 40. Tsai, J.-C.; Lo, Y.-L.; Lin, C.-Y.; Sheu, H.-M.; Lin, J.-C. Feasibility of rapid quantitation of stratum corneum lipid content by Fourier transform infrared spectrometry. *J. Spectrosc.* **2004**, *18*, 401015, <https://doi.org/10.1155/2004/401015>.
 41. Al-Otibi, F.O.; Alrumaizan, G.I.; Alharbi, R.I. Evaluation of anticandidal activities and phytochemical examination of extracts prepared from *Vitex agnus-castus*: a possible alternative in treating candidiasis infections. *BMC Complement. Med. Ther.* **2022**, *22*, 69, <https://doi.org/10.1186/s12906-022-03552-x>.
 42. Butler, M.S. The Role of Natural Product Chemistry in Drug Discovery†. *J. Nat. Prod.* **2004**, *67*, 2141-2153, <https://doi.org/10.1021/np040106y>.
 43. Khalighi-Sigaroodi, F.; Ahvazi, M.; Hadjiakhoondi, A.; Taghizadeh, M.; Yazdani, D.; Khalighi-Sigaroodi, S.; Bidel, S. Cytotoxicity and antioxidant activity of 23 plant species of leguminosae family. *Iran. J. Pharm. Res. IJPR* **2012**, *11*, 295-302.
 44. Granado-Serrano, A.B.; Angeles, M.M.; Laura, B.; Luis, G.; and Ramos, S. Quercetin Modulates NF- κ B and AP-1/JNK Pathways to Induce Cell Death in Human Hepatoma Cells. *Nutr. Cancer* **2010**, *62*, 390-401, <https://doi.org/10.1080/01635580903441196>.
 45. Lee, C. and Shim, S.H. Endophytic fungi inhabiting medicinal plants and their bioactive secondary metabolites. *Nat Prod Sci* **2020**; *26*: 10-27. <http://dx.doi.org/10.1016/B978-0-12-819654-0.00011-9>.
 46. El-Sayed, E.R. Discovery of the anticancer drug vinblastine from the endophytic *Alternaria alternata* and yield improvement by gamma irradiation mutagenesis. *J. Appl. Microbiol.* **2021**, *131*, 2886-2898, <https://doi.org/10.1111/jam.15169>.
 47. ElNaggar, M.H.; Abdelwahab, G.M.; Kutkat, O.; GabAllah, M.; Ali, M.A.; El-Metwally, M.E.A.; Sayed, A.M.; Abdelmohsen, U.R.; Khalil, A.T. Aurasperone A Inhibits SARS CoV-2 In Vitro: An Integrated In Vitro and In Silico Study. *Mar. Drugs* **2022**, *20*, 179, <https://doi.org/10.3390/md20030179>.
 48. Alhadrami, H.A.; Burgio, G.; Thissera, B.; Orfali, R.; Jiffri, S.E.; Yaseen, M.; Sayed, A.M.; Rateb, M.E. Neoechinulin A as a Promising SARS-CoV-2 M^{Pro} Inhibitor: In Vitro and In Silico Study Showing the Ability of Simulations in Discerning Active from Inactive Enzyme Inhibitors. *Mar. Drugs* **2022**, *20*, 163, <https://doi.org/10.3390/md20030163>.

Publisher's Note & Disclaimer

The statements, opinions, and data presented in this publication are solely those of the individual author(s) and contributor(s) and do not necessarily reflect the views of the publisher and/or the editor(s). The publisher and/or the editor(s) disclaim any responsibility for the accuracy, completeness, or reliability of the content. Neither the publisher nor the editor(s) assume any legal liability for any errors, omissions, or consequences arising from the use of the information presented in this publication. Furthermore, the publisher and/or the editor(s) disclaim any liability for any injury, damage, or loss to persons or property that may result from the use of any ideas, methods, instructions, or products mentioned in the content. Readers are encouraged to independently verify any information before relying on it, and the publisher assumes no responsibility for any consequences arising from the use of materials contained in this publication.